SEROLOGICAL AND MOLECULAR EVALUATION OF TISSUE-DWELLING PARASITES (SARCOCYSTIDAE) IN HARVESTED WILDLIFE IN THE CANADIAN ARCTIC

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By

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ABSTRACT

Country foods are integral to Inuit cultural identity and food security. However, the harvest and consumption of wildlife pose risks of transmission of zoonotic parasites, such as *Toxoplasma gondii*, of concern for Inuit communities in the Canadian Arctic. The main aim of this thesis was to determine seroprevalence and tissue prevalence of *Toxoplasma gondii* and related tissue-dwelling coccidian parasites in harvested wildlife in the Canadian Arctic. To address these objectives, I used multiple serological techniques to detect antibodies against parasites and molecular approaches to detect DNA in tissue samples. The overall seroprevalence for *T. gondii* in caribou from Nunavik, Québec was 18% (95% CI: 11.5-28%) and 1% (95% CI: 0.2-4.6%) in belugas from the Eastern Beaufort Sea, Northwest Territories. On magnetic capture DNA extraction and qPCR for *T. gondii* in heart and brain of caribou and belugas, DNA of *T. gondii* was detected in only one beluga heart. This suggests that the risk of zoonotic transmission of *T. gondii* from harvesting and consuming caribou and belugas is low, but not zero. Antibodies to *Neospora caninum* were detected in 5% (95% CI: 0.6-8%) and DNA was detected in tissues of 1.5% (95% CI: 0.3-7.9%) of caribou harvested in Nunavik. Furthermore, DNA of *Sarcocystis* spp. was detected in both heart and skeletal muscle from caribou (85%, 95% CI: 76.4-91.2%), and muscle of beluga (59%, 95% CI: 47.9-69.2%). Phylogenetic analysis of *Sarcocystis* spp. from caribou were linked to terrestrial canids, and were similar to sequences reported in Europe, possibly reflecting translocations of *Rangifer* sp. In contrast, *Sarcocystis* sp. from belugas indicated a potential marine life cycle. Finally, I compared in-house ELISAs with a commercial ELISA kit in previously tested polar bear samples for *T. gondii* to optimize these tests in wildlife, emphasizing the need for improved testing protocols, known controls, and statistical approaches to test comparison when gold standards are lacking. In conclusion, this thesis contributes to our understanding of tissue-dwelling coccidian parasites in wildlife harvested for human consumption, offers insights into arctic trophic dynamics, and highlights the importance of a One Health concept for human and animal health in the Canadian North.

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“With every job when it’s complete, there is a sense of bitter-sweet.”

-M.P.

DEDICATION

In loving memory of my dad, Eliseo,

Whose love propelled me to reach such great heights.

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LIST OF ABBREVIATIONS

bp: base pairs
BLAST: basic local alignment search tool
CDC: Center for Disease Control and Prevention
cELISA: competitive ELISA
CFAP: Centre for Foodborne and Animal Parasitology
CI: confidence interval
DDN: data-display network
DFO: Department of Fisheries and Oceans
Df: degrees of freedom
DH: definitive host
DNA: Deoxyribonucleic acid
EBS: Eastern Beaufort Sea
ELISA: enzyme-linked immunosorbent assay
FITC: fluorescein isothiocyanate
FJMC: Inuvialuit Fisheries Joint Management Committee
g: gram
HF: heart fluid
IFAT: Immunofluorescence assay
IgG: immunoglobulin G
IH: intermediate host
ISR: Inuvialuit Settlement Region
ITS: Internal Transcribed Spacer
K: Kappa value
LNUK: Local NUK
Max.: maximum
MAT: modified agglutination test
MC-qPCR: magnetic capture quantitative PCR
mg: milligram
Min.: minimum
ML: maximum likelihood
n: sample size
neg: negative
ND: no dilution
NLD: New Life Diagnostics
NUK: Nunavimmi Umajulivijiit Katujaqatigininga
OD: optical density
PCR: polymerase chain reaction
pos: positive
PV: Parasitophorous vacuole
RNUK: Regional NUK
RPM: rotation per minute
RT: room temperature
SD: standard deviation
SLE: St. Lawrence Estuary

S/P: sample to positive
µg: microgram
µL: microliter
ºC: Celsius

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Harvested wildlife as traditionally-consumed country foods in the Canadian North

Inuit country foods refers to the marine and terrestrial wildlife, fish and plants that are locally or regionally harvested (Inuit Tapiriit Kanatami, 2017). For the Inuit people, these country foods hold immense significance, serving as a fundamental aspect of their cultural identity, ensuring food security, and providing substantial dietary value (Kuhnlein and Receveur, 1996; Sheikh *et al.*, 2011) (Fig. 1.1). The sharing of traditional food continues to be a cherished cultural practice, even in modern times, and is recognized as a mechanism to maintain food security (Searles, 2002; Chan *et al.*, 2006). Country foods contribute to nutrition as they are important sources of energy, proteins, vitamins and minerals for Inuit (Kenny, Hu, *et al.*, 2018). These country foods are considered less expensive than market foods. The ownership of hunting gear among Inuit families, as well as the practice of cost-sharing for hunting expenses with other families, contributes to reducing the overall costs associated with acquiring traditional country food (Chan *et al.*, 2006).

Species diversity in country food varies regionally, but generally consists of approximately 14 marine mammals, 14 land animals, 70 birds, 48 fish and shellfish, and 48 plants (Kuhnlein and Receveur, 2007). Some of these country foods hold significance as ecological and cultural “keystone” species. The concept of ecological keystone species, although subject to debate, refers to species that demonstrate vital importance for ecosystem function (Cottee-Jones and Whittaker, 2012). This means that these species have a strong influence on numerous others within their ecosystem, playing a crucial role in the structure and dynamics of their community. Cultural keystone species are defined as culturally significant species that greatly shape the cultural identity of a people. Their importance is evident in the fundamental roles they play in diet, materials, medicine, and/or spiritual practices (Garibaldi and Turner, 2004).

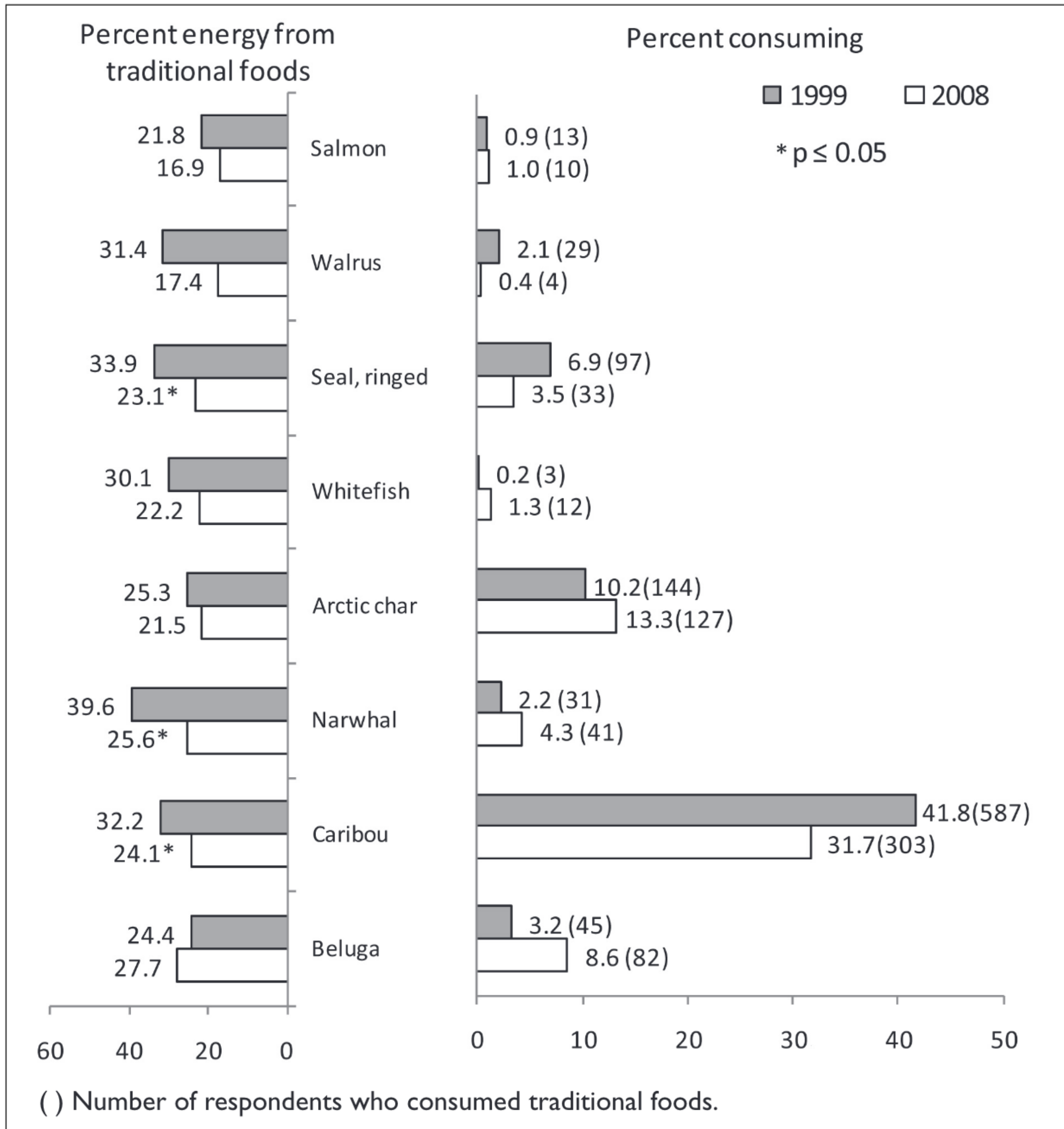

Fig. 1.1 *Percent of energy (left) contributed from top traditional foods and percent of consumption (right) among Inuit consumers from surveys from 1999 and 2008.*

Note: From "Changing dietary patterns and body mass index over time in Canadian Inuit" by Sheikh, N. Egeland, G.M. Johnson-Down, L. Kuhnlein, H.V. 2011. International Journal of Circumpolar Health 70(5), pp. 511–519. Copyright 2011 by Sheikh et al. Published by Taylor & Francis.

1.1.1 The hosts: Caribou (*Rangifer tarandus*)

1.1.1.1 Biology and ecological importance

In the Canadian North, caribou are both ecological and cultural keystone species. Caribou belong to the order Artiodactyla, even-toed mammals that walk on their toenails; more specifically, they are members of the family Cervidae or the deer family (Hummel and Ray, 2014). They are the most abundant land-based mammal in the North, characterized by large social aggregations and extensive migrations.

Caribou have an important role in the food chain within the tundra, where they share their habitat with musk oxen (*Ovibos moschatus*), and boreal forest, inhabited by other cervids (moose, elk, deer), wood bison, mountain sheep and goats. Among the various predators, the wolf (*Canis lupus*) stands as the primary predator of caribou, with species co-evolved together alongside each other. This relationship has significantly influenced much of the caribou's behavior, shaping their survival strategies and migration patterns (Feldhamer, Thompson and Chapman, 2003; Musiani *et al.*, 2007). In addition to wolves, other predators that coexist with caribou include grizzly bears (*Ursus arctos*), black bears (*Ursus americanus*), mountain lions (*Puma concolor*), golden eagles (*Aquila chrysaetos*), lynx (*Lynx canadensis*) and coyotes (*Canis latrans*) (Feldhamer, Thompson and Chapman, 2003; Hummel and Ray, 2014).

Caribou fulfill a multifaceted role in the ecosystem, extending beyond their significance in the food chain. Their abundant populations serve as a vital source of fertilizer, contributing to the nutrient cycling for the tundra soil. Moreover, caribou have an indirect yet essential role in the nutrient cycles of aquatic ecosystems. By maintaining adult mosquito populations, they provide aquatic mosquito larvae that serve as food for fish and birds (Hummel and Ray, 2014).

The species *Rangifer tarandus* encompasses both the North American caribou and the Eurasian reindeer. These animals have a circumpolar range, inhabiting the tundra and boreal forests. High arctic island and forest herds are mostly sedentary. Tundra herds are typically migratory (Chester, 2016). Classification schemes used by different management agencies are not always consistent with one other. Classification of caribou by ecotypes (Festa-Bianchet *et al.*, 2011) (Fig. 1.2) is one option, described as follows:

- Boreal forest caribou are one of the most widespread mammals in Canada, inhabiting the boreal forest from Newfoundland to the northeast of British Columbia and the Northwest Territories. They are sedentary, commonly found as single individuals and small groups.
- Mountain caribou live in the mountains of western North America, with a few populations residing in eastern Canada. They can have both sedentary and migratory behaviour, migrating up to 100 km between seasonal ranges.
- Migratory Tundra caribou include the largest caribou herds in North America, forming groups of hundreds or thousands of individuals. They are gregarious during calving and migrate between seasonal ranges that are often hundreds of kilometers apart. Distribution spans from the coastal tundra of Alaska to the tundra of northeastern Labrador.
- Peary caribou (*R.t. pearyi*) are considered a separate subspecies, adapted to the High Arctic islands. Their coat color and small size differentiate them from other caribou. Typically, group sizes are small. Migratory behaviour varies from annual migrations between seasonal ranges on different islands to year-round occupation of relatively small home ranges.

For this thesis, I focused on the Leaf-River herd, a migratory tundra caribou residing in Nunavik, northern Québec. This herd boasting an estimated population of approximately 332,000 caribou in 2015, has, however, exhibited fluctuation in numbers over time. The leaf-River herd partakes in one of the most extensive known terrestrial migrations among mammals, profoundly shaping the landscape of the northern Québec tundra and taiga. This migration holds significance for various communities, including Inuit, Cree, and Naskapi peoples from Québec; as well as non-aboriginal residents and non-residents within the same province, who engage in the harvest of the Leaf-River caribou (Taillon, Brodeur and Rivard, 2016).

1.1.1.2. Caribou as country food in the Canadian North

Caribou is the country food most commonly consumed in the Canadian North (Fig. 1.1), with an average annual consumption ranging from 29.6 to 122.8 kg/person (Kenny and Chan, 2017). It is ranked among the top five dietary sources of energy for Inuit diet, accounting for approximately 5.6 to 11.2% of total energy intake. Additionally, caribou is a significant source of protein, fats; various vitamins such as Thiamin, Riboflavin, B12; and minerals including Fe, Mg, Zn, Cu (Kenny, Fillion, *et al.*, 2018; Kenny, Hu, *et al.*, 2018).

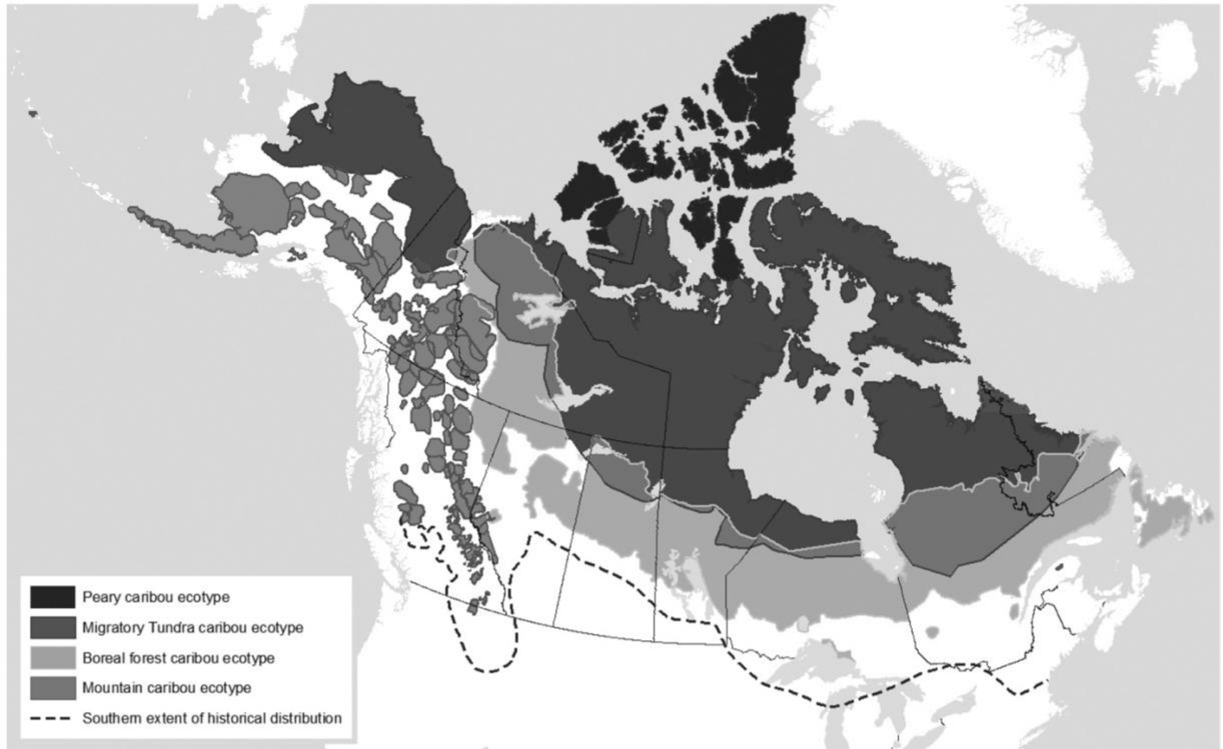

Fig. 1.2 *Distribution of ecotypes of caribou in North America*

Note: From "Conservartion of caribou (Rangifer tarandus) in Canada: an uncertain future" by Festa-Bianchet, M. Ray, J.C. Boutin, S. Côté, S.D. Gunn, A. 2011. Canadian Journal of Zoology. 89(5), pp. 419–434. Copyright 2011 by Festa-Bianchet et al. Published by Canadian Science Publishing

1.1.2 The hosts: Beluga whale (*Delphinapterus leucas*)

1.1.2.1 Biology and ecological importance

Belugas are members of the Order Cetacea, a group of entirely aquatic, streamlined, nearly hairless, mammals. Specifically, they are classified under the suborder Odontoceti, the toothed whales (Chester, 2016). Belugas are endemic to high latitudes of the Northern Hemisphere and have a circumpolar distribution in the Arctic and Sub-arctic regions (Fig. 1.3). For this thesis, my focus was on the Eastern Beaufort Sea (EBS) population situated within the western Canadian Arctic. Renowned as one of the largest beluga populations in Canada, this group comprises an estimated count of approximately 32500 whales. The EBS beluga embark on a spring migration from Alaska (Fraker 1979,) traversing to their summer habitat within the Canadian Beaufort Sea. During this phase, they form a dense congregation within the Mackenzie Estuary, peaking during late June and early July (Harwood *et al.*, 1996). During the fall, they migrate back to their wintering ground in the Bering Sea (Citta et al. 2016, Storrie *et al.*, 2022)

During the winter, belugas tend to inhabit partially open waters along the edges of the pack ice. In the summer, they migrate to shallow coastal bays, estuaries, and inlets to feed. Belugas forage near the sea bottom feeding on a diverse range of fishes and invertebrates such as squids, octopi, crabs, shrimp, and marine worms (Feldhamer, Thompson and Chapman, 2003; Würsig, Thewissen and Kovacs, 2018). Notably, Arctic cod (*Boreogadus saida*) and Capellin (*Mallotus villosus*) are the most consumed fish species by belugas.

Belugas natural predators are polar bears (*Ursus maritimus*) and killer whales (*Orcinus orca*). Belugas comprise approximately 15-19% of the diet of polar bears (Thiemann, Iverson and Stirling, 2008).

1.1.2.2. Beluga whale as country food in the Canadian North

Belugas hold importance for the Inuit as they serve as a vital source of food and play a central role in social activities. In certain communities, particularly in the Western Arctic, beluga hunting has been practiced for generations and continues in the present days (Friesen and Arnold, 1995; Harwood *et al.*, 2014). Among these communities, beluga hunting is considered one of the most social of all the hunting activities, involving not only the hunt itself, but also the processing and sharing of the harvested resources (Tyrrell, 2008).

Belugas are the most consumed marine mammals among the Inuit and are highly valued for their nutritional and economical contributions. Their skin, blubber, meat, and internal organs are utilized as food for both people and sled dogs, offering sustenance and nourishment (Binnington *et al.*, 2017; Waugh *et al.*, 2018). In terms of nutrition, belugas are a significant source of protein, as well as vitamins such as A, B12, D, E. They are also a great source of selenium (Kenny, Hu, *et al.*, 2018).

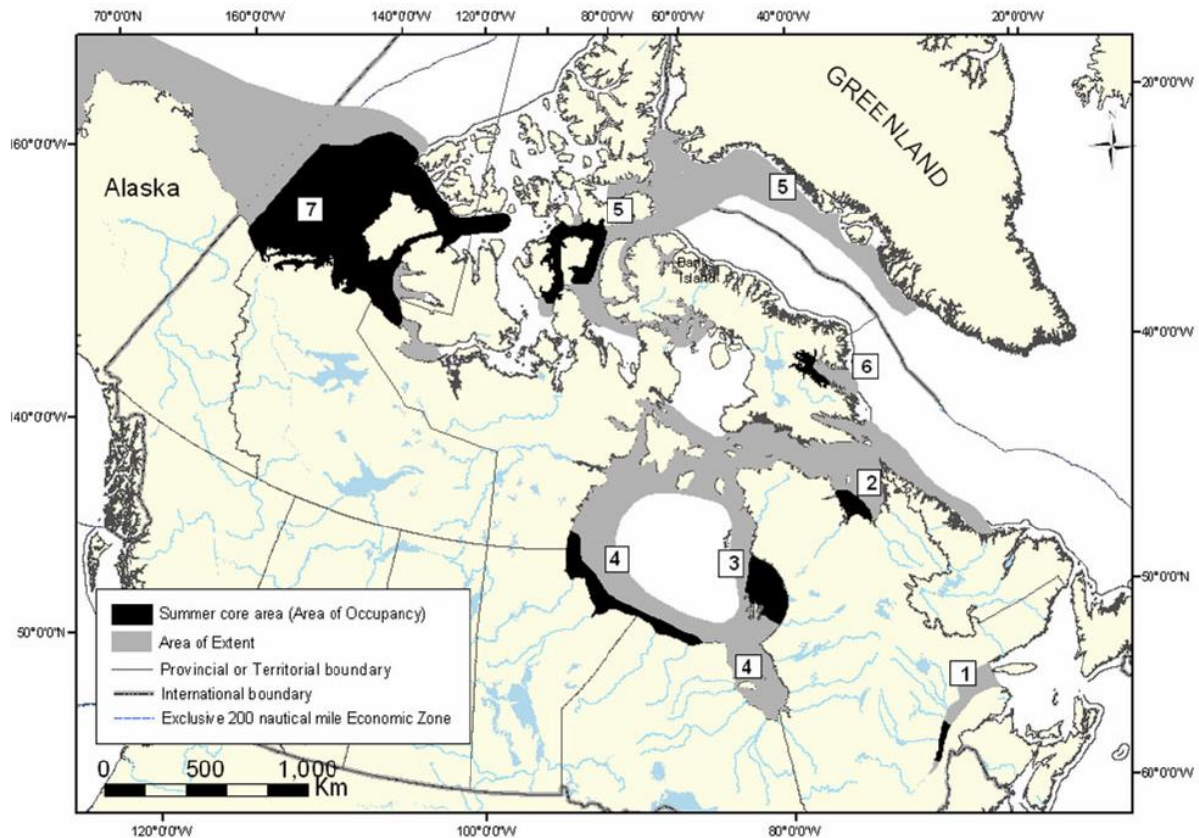

Fig. 1.3 *Location of the Canadian beluga populations*

Note: 1, St. Lawrence Estuary; 2, Ungava Bay; 3; Eastern Hudson Bay; 4, Western Hudson Bay; 5, Eastern High Arctic; 6, Cumberland Sound; 7, Eastern Beaufort Sea. 2004. From "COSEWIC assessment and update status report on the beluga whale *Delphinapterus leucas* in Canada" by Committee on the Status of Endangered Wildlife in Canada. Copyright 2004 by His Majesty the King in Right of Canada.

1.1.3 The hosts: polar bear (*Ursus maritimus*)

1.1.3.1 Biology and ecological importance

Polar bears, as part of the order Carnivora, are meat-eating mammals that primarily prey on other animals (Chester, 2016). These animals belong to the bear family, Ursidae, and stand out as the only species of bears that spend a significant portion of their lives in saltwater habitats (Berta, Sumich and Kovacs, 2006). Distributed throughout the circumpolar regions of the Northern Hemisphere, polar bears inhabit the Arctic Ocean's Sea ice and adjacent landmasses (Fig. 1.4). Researchers have recognized 19 subpopulations of polar bears, which can be categorized into four ecoregions based on similarities in life history and sea ice dynamics (Amstrup, Marcot and Douglas, 2013). For this thesis, I focused on the western Hudson Bay polar bear subpopulation, located in the seasonal ice ecoregion. This specific subpopulation is identified by an estimated populace of around 740 polar bears (McCall, Derocher and Lunn, 2015). These bears showcase a strong affinity to their terrestrial summering areas along the expanse of the Western Hudson Bay, spanning across the regions of Nunavut and Manitoba. During November and December, polar bears return to the sea ice, with the exception of pregnant females which remains on land until February and March, after having given birth (McCall, Derocher and Lunn, 2015; Lunn *et al.*, 2016).

Polar bears primarily reside on the sea ice, when available. However, in areas where the ice melts entirely during the summer or recedes far from the coastlines into deeper waters of the Arctic Basin, polar bears may spend several months onshore or on the pack ice to find suitable living conditions (COSEWIC, 2018; Würsig, Thewissen and Kovacs, 2018).

Polar bears occupy the top position in the Arctic food chain (Thiemann, Iverson and Stirling, 2008; Würsig, Thewissen and Kovacs, 2018). Their diet showcases a diverse array of animals, with ringed seals (*Pusa hispida*) as predominant prey (Feldhamer, Thompson and Chapman, 2003; Thiemann, Iverson and Stirling, 2008). Additionally, they also consume bearded (*Erignathus barbatus*), harp (*Pagophillus groenlandicus*) and hooded (*Cytosphora cristata*) seals. Polar bears are known to kill larger animals such as walruses (*Odobenus rosmarus*) and belugas. Their diet is versatile and includes other sources such as carcasses of dead whales and caribou, nesting seabirds and their eggs, mussels, crabs, and even human refuse (Feldhamer, Thompson and Chapman, 2003; Berta, Sumich and Kovacs, 2006).

Polar bears hold a vital role in the global perspective, serving as a barometer for critical Arctic environmental concerns, such as climate change and pollution. They act as sentinels for the ever-changing Arctic environment, providing crucial insights into the health of this delicate ecosystems (Amstrup, Marcot and Douglas, 2013; Atwood *et al.*, 2016; COSEWIC, 2018).

1.1.3.2 Polar bears in the Canadian North

Polar bears play a significant role in the culture, traditions, and economy of northern communities (Vaudry, 2016). Inuit from Alaska, Canada and Greenland have a deep connection with polar bears, and they harvest these animals for subsistence purposes. The meat from harvested bears is consumed as part of Inuit sustenance (COSEWIC, 2018). Beyond their nutritional value, different parts of the polar bear hold traditional significance. Inuit craftsmen skillfully utilize various bear parts, such as skulls, teeth, bones, and claws, for creating tools, clothing, and even traditional medicine (COSEWIC, 2018).

In Nunavut and the Northwest Territories, guided sport hunting of polar bears is permitted (Dowsley, 2009). Polar bear hunting is carefully managed in Canada. Most provinces and territories, excluding Manitoba, allow regulated harvests, with wildlife management boards determining the total allowable harvest or harvest levels for each management unit. These recommendations are then shared with the responsible federal, provincial, or territorial jurisdiction to ensure proper conservation and management of polar bear populations (COSEWIC, 2018).

Unfortunately, especially in areas where human settlements overlap with polar bear habitats along coastal regions, bears are occasionally killed in defense of life and property (Atwood *et al.*, 2017; Würsig, Thewissen and Kovacs, 2018).

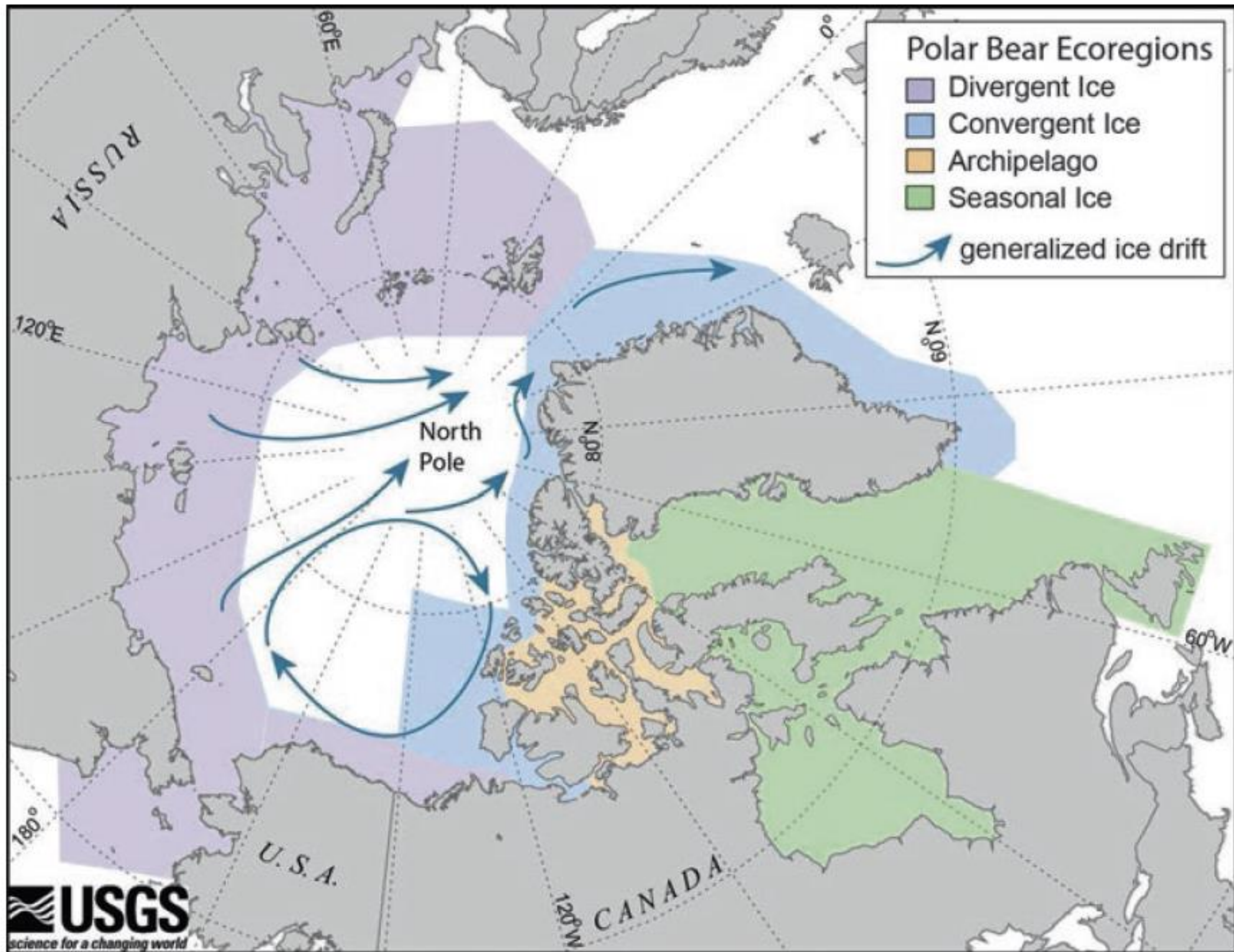

Fig. 1.4 *Map of four polar bear ecoregions defined by grouping recognized subpopulations.*

Note: From "Human–Polar Bear Interactions in a Changing Arctic: Existing and Emerging Concerns, in A. Butterworth (ed.) Marine Mammal Welfare" by Atwood, T.C., Simac, K., Breck, S.W., York, G. Wilder, J. Cham. 2017. Springer International Publishing (Animal Welfare), pp. 397–418. Copyright 2017 by Springer International Publishing AG.

1.2 The Parasites: Family Sarcocystidae

1.2.1 Phylum Apicomplexa

The Phylum Apicomplexa comprises single-cell, intracellular protozoan parasites that are renowned for their distinctive apical complex, a combination of structures crucial for invading and establishing themselves within the host cells (Fig. 1.5). At the anterior tip of these parasites, one or two polar rings encircle the region. Additionally, the apical complex contains conoids and microtubes, which likely play an important role in locomotion. Two essential components of the Apical complex are the rhoptries and micronemes. Rhoptries are responsible for the adhesion to and penetration of host cells, facilitating the parasite's entry into its host. Micronemes extend anteriorly into the rhoptries or connect to a common duct system with the rhoptries, eventually reaching the cell surface at the apex. The life cycles of these parasites are highly complex, involving both asexual and sexual reproduction phases, the latter unique among protozoan parasites of veterinary importance (Roberts *et al.*, 2013).

The phylum Apicomplexa includes two distinct classes: Conoidasida and Acoboidasida, each with its own unique characteristics. The Conoidasida class includes gregarines and coccidias, which are distinguished by the presence of conoids in their sporozoites. Among the families within this class, some hold significant implications for both public and animal health, including Cryptosporidiidae, represented by *Cryptosporidium* spp., Hepatozoidae encompassing *Hepatozoon* spp., Eimeriidae consisting of *Eimeri*a, *Isospora*, *Cystoisospora*, *Cyclospora* spp., and Sarcocystidae which includes *Toxoplasma*, *Neospora*, *Sarcocystis* and *Besnoitia* spp. (Roberts *et al.*, 2013).

The Acoboidasida class lacks conoids and includes haematozoea like the malarial parasites represented by *Plasmodium* spp., and piroplasms such as *Babesia* and *Theileria* spp. (Roberts *et al.*, 2013).

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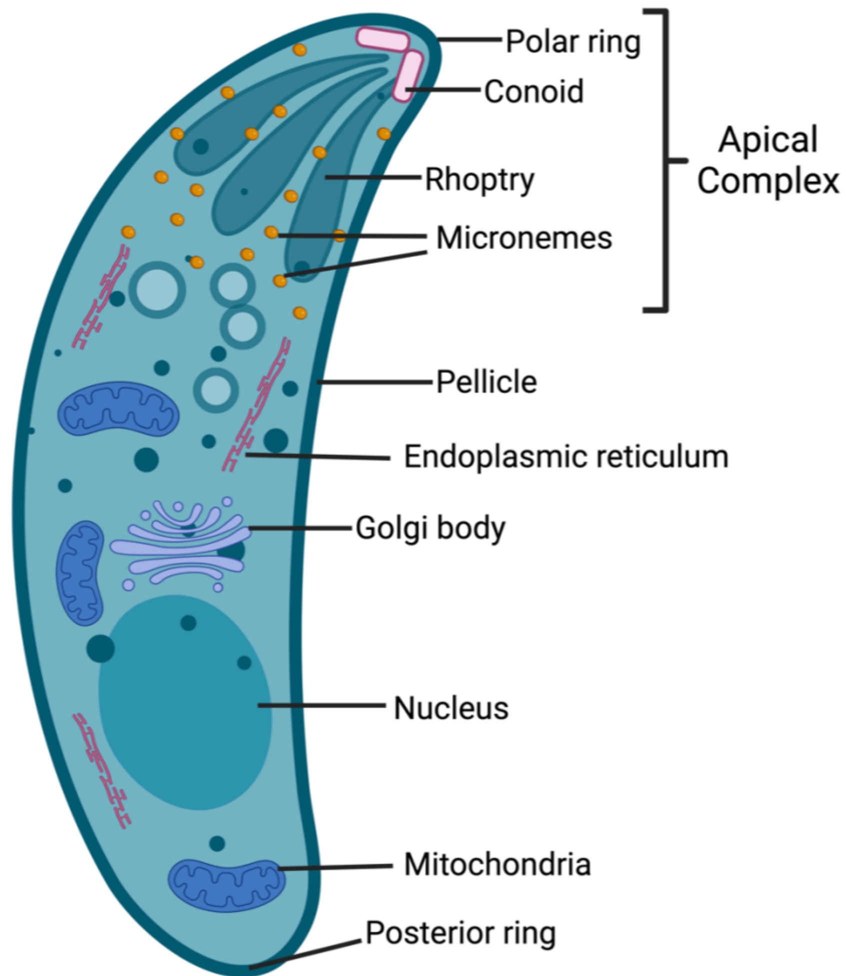

Fig. 1.5 *Apicomplexan structure of a sporozoite or merozoite.*

Note: Adapted from "Phylum Apicomplexa: Gregarines, Coccidia, and Related Organisms, in *Gerald D. Schmidt & Larry S. Roberts' foundations of parasitology*" by Roberts, L.S., Janovy, J., Nadler, S. and Roberts, L.S. 2013. Ninth edition. New York: McGraw Hill, pp. 120. Copyright 2013 by McGraw-Hill Companies, Inc. Created in BioRender.com

1.2.2 Family Sarcocystidae

The Sarcocystidae are characterized by parasites that exhibit a predator-prey heteroxenous life cycle, involving both intestinal and tissue stages. Within this family, sexual reproduction takes place during the intestinal phase within the definitive hosts, primarily mammalian carnivores and birds. Asexual reproduction, in turn, occurs in vasculature and tissues of their vertebrate intermediate hosts (Roberts *et al.*, 2013).

1.2.2.1 Life cycle of the Sarcocystidae

In the general life cycle of these parasites, oocysts are typically shed in the feces of the definitive hosts (DH), where they may sporulate in the environment or are already sporulated when excreted. Intermediate hosts (IH) can get infected when they ingest sporulated oocysts in fecal-contaminated food, water, or soil (Fig. 1.6). Once inside the IH, asexual reproduction takes place. The oocyst walls are disintegrated by digestive enzymes, freeing the sporozoites in the intestine. These sporozoites then penetrate enterocytes of the intestinal epithelium and become surrounded by a membrane derived from the host cell, resulting in the development of the parasitophorous vacuole (PV). Within the PV, the sporozoites replicate through endodyogeny, a process where two daughter cells form inside the mother cell, resulting in tachyzoites. After several rounds of replication, the tachyzoites rupture the host cell's membrane, releasing new tachyzoites that go on to infect new host cells, such as neural, macrophage, fibroblast, vascular endothelial, muscle, liver cells. Within a few days of infection, some tachyzoites differentiate into bradyzoites, leading to the formation of the tissue cysts that persist in several organs (Dubey, 2010; Roberts *et al.*, 2013; Dubey *et al.*, 2016; Attias *et al.*, 2020; Lindsay and Dubey, 2020).

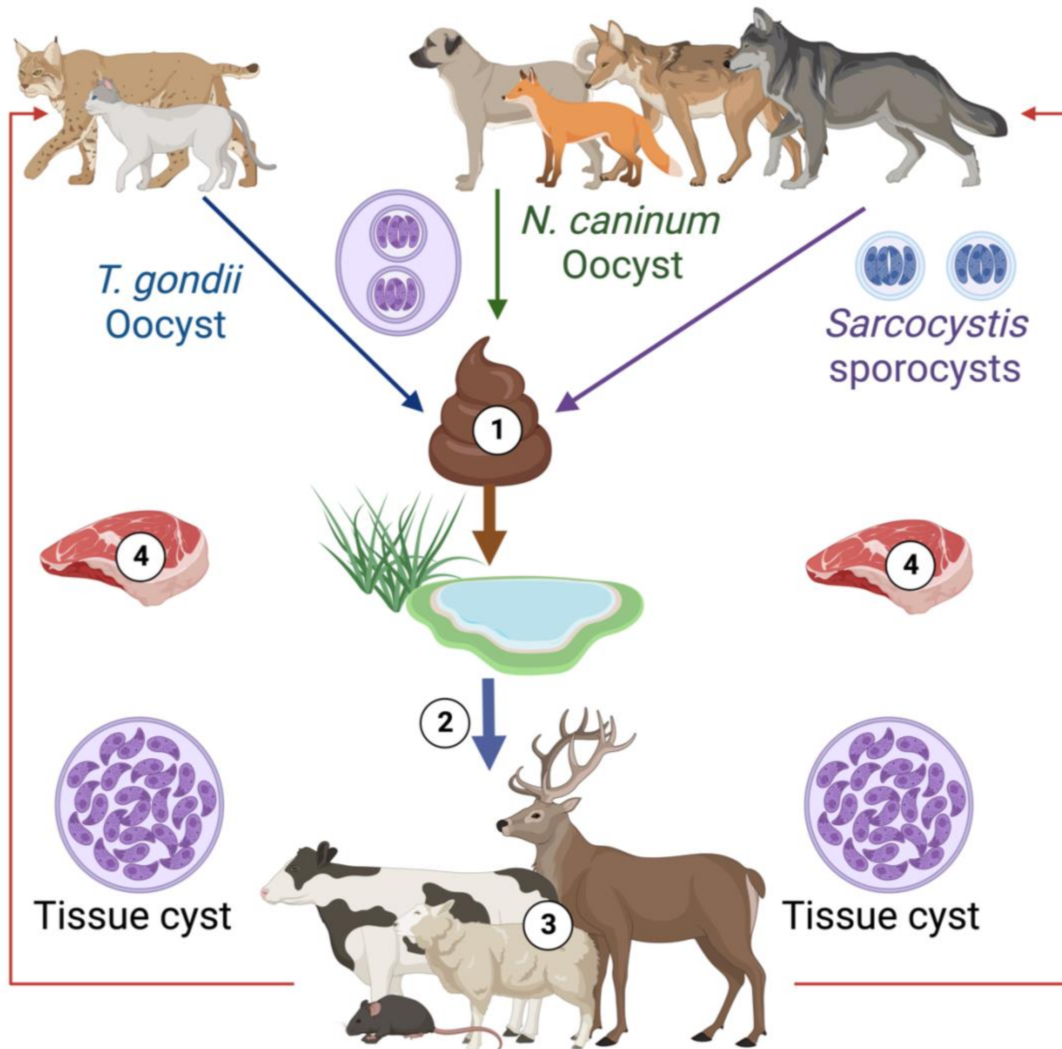

Fig. 1.6 *General life cycle of Sarcocystidae parasites.*

Note: For *Toxoplasma gondii* and *Neospora caninum*, oocysts are shed in feces by definitive hosts (DH, felids, and canids respectively), sporulate and become infective in the environment, whereas for Sarcocystis spp., sporulation occurs in the intestine of the DH and sporocysts are immediately infective in the intermediate host (IM), 2. IH ingest sporulated oocysts or sporocysts in food, water or soil. 3, Sporozoites are released, divide rapidly, and tachyzoites disseminate to somatic tissues of the IH, and form tissue cysts. 4, DH becomes infected by ingesting prey species with bradyzoites within tissue cysts. Created in BioRender.com

The completion of the life cycle occurs when tissue cysts are ingested by a new DH (Fig. 1.7). The cysts are transported through the stomach, where the proteolytic enzymes dissolve the cyst wall releasing the bradyzoites. These bradyzoites then infect the epithelial cells of the small intestine, leading to schizogony, where nuclear divisions precede the individualization of each cell, forming the merozoites. Following several divisions, the merozoites undergo gametogony, differentiating into gametes. The macrogametes (female) are generated from a single merozoite, and exhibit distinct characteristic, such as a single nucleus, several micropores, endoplasmic reticulum, numerous mitochondria, double-membraned vesicles, and wall-forming bodies. The microgametes (male) are formed by a schizogonic division, and they consist of a compact nucleus in the periphery and two flagella. Sexual reproduction occurs when microgametes actively move to the periphery of the macrogamont, where their membranes fuse, and only the nucleus of the microgamont enters the macrogamont. After fertilization, a protective wall develops around the zygote, resulting in the formation of the oocyst.

Subsequently, the oocyst is released into the intestinal lumen and is excreted in the feces of the DH. Depending on the parasite species, sporulation can occur in the lamina propria of the intestine of the DH, or externally in the environment. The oocyst initially contains one large nucleus with one or two prominent nucleoli and several granules. As sporulation progresses, the nucleus elongates and becomes parallel to the longitudinal axis of the sporont. The elongated nucleus divides into two nuclei, one at each pole of the sporont. These nuclei undergo a second transverse division, and the sporont cytoplasm divides transversely into two sporoblasts. Each sporoblast contains two nuclei which move towards the opposite poles of each sporoblast. The sporoblasts become surrounded by an eosinophilic wall, transforming into sporocysts. Ultimately, each oocyst contains two sporocysts, and as oocyst maturation continues, each sporocyst harbors four sporozoites, likely formed by a third nuclear division. The cycle continues when a new host becomes infected by ingesting contaminated food, water, or soil carrying the sporulated stage of the parasite (Dubey, 2010; Roberts *et al.*, 2013; Dubey *et al.*, 2016; Attias *et al.*, 2020; Lindsay and Dubey, 2020b).

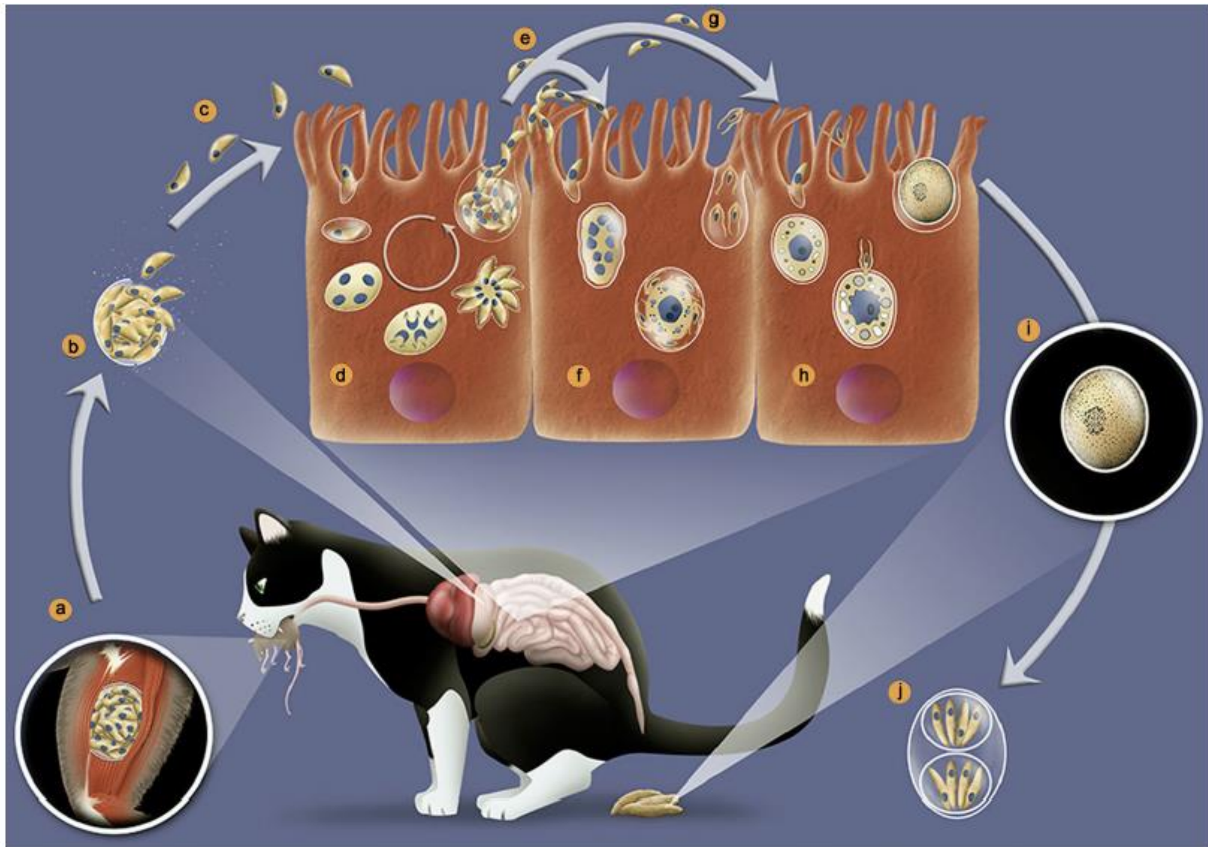

Fig. 1.7 *Life cycle of Toxoplasma gondii in the definitive host (felids).*

Note: a, DH ingest prey with tissue cysts. b, Bradyzoites are liberated in the intestines. c, Bradyzoites invade epithelial cells of the intestine of the DH. d, Schizogony starts with bradyzoites differentiating to merozoites. e-f, Gametogony starts by differentiation of merozoites to gamonts. g-h, Formation of the oocysts starts after fertilization. i, Oocyst is released in the feces of the DH and sporulates in the environment. From *"The life cycle of Toxoplasma gondii reviewed using animations" by Attias, M., Teixeira, D.E., Benchimol, M., Vommaro, R.C., Crepaldi, P.H. and De Souza, W. 2020. Parasites & Vectors, 13(1), p. 588. Copyright 2020 by BioMed Central Ltd.*

1.2.3 *Toxoplasma gondii*

1.2.3.1 Life cycle and transmission

Toxoplasma gondii is a zoonotic parasite with a global distribution. Its definitive hosts are domestic and wild felids (Hill, Chirukandoth and Dubey, 2005). Intermediate hosts can become infected by ingesting water or food containing the oocysts in environments contaminated with feces of felids. Another route of infection for the IH unique to *Toxoplasma* among the Sarcocystidae is through carnivory, where IH ingest tissues of other IH containing tissue cysts (Hill, Chirukandoth and Dubey, 2005; Dubey, 2010).

The dissemination of oocysts to human settlements and coasts has been observed through fresh runoff water (Schuster *et al.*, 2005; VanWormer *et al.*, 2014). Moreover, filter feeding marine bivalves and fish can pick up and concentrate oocysts. Experimental trials have shown that mussels and oysters can accumulate oocysts from water and remain infectious for bioassay in mice (Arkush *et al.*, 2003; Lindsay *et al.*, 2004). Fish have previously been reported to filter the parasite oocyst, and retain infectiousness; as well, DNA has been detected in different species of fish (Massie *et al.*, 2010; Marino *et al.*, 2019).

Congenital transmission holds significant importance, particularly in humans, sheep, goats, and pigs. When a pregnant woman or animal acquires a primary infection, tachyzoites can colonize placental tissues through parasitemia, leading to placentitis, embryonic death and resorption, fetal death, abortion, stillbirth, or neonatal death (Hill, Chirukandoth and Dubey, 2005; Dubey, 2010; Robert-Gangneux and Dardé, 2012). Among these species, sheep and goats are particularly susceptible to *T. gondii*, making the parasite infection a major cause of reproductive losses worldwide (Stelzer *et al.*, 2019).

Furthermore, humans can also contract the infection through organ transplantation and blood transfusion (Robert-Gangneux and Dardé, 2012). Toxoplasmosis is most commonly associated with transplants involving the heart and heart-lung from a previously infected donor. However, due to the immunosuppressive therapies required for these procedures, toxoplasmosis may also result from a reactivation of a latent infection in the patient (Dard *et al.*, 2018).

1.2.3.2 Public health significance.

It is estimated that approximately 25-30% of the human population is infected with *T. gondii* (Dubey, 2010; Robert-Gangneux and Dardé, 2012). Human toxoplasmosis is considered one of the top leading causes of hospitalizations and deaths among all foodborne illnesses in the USA (Scallan *et al.*, 2011). Several factors have been associated with prevalence in humans, with dietary, economic, social, and cultural habits, as well as quality of water and sanitation coverage the most important (Dubey, 2010). While this infection is often asymptomatic in individuals with a healthy immune system, certain groups, such as pregnant women and immunocompromised patients, are at higher risk. Congenital toxoplasmosis can lead to different outcomes depending on the trimester of gestation. In severe cases, it can result in fetal death, stillbirth, or developmental abnormalities (McLeod *et al.*, 2014). The risk for immunocompromised patients is influenced by the duration and degree of immunosuppression. The most common manifestation of the disease in these individuals is toxoplasmic encephalitis, although it can also affect other vital organs, such as heart (Robert-Gangneux and Dardé, 2012).

1.2.3.3 *Toxoplasma gondii* in the Canadian North

In the Canadian North, *T. gondii* is recognized as the most prevalent parasitic infection among Inuit communities (Goyette *et al.*, 2014). Specifically, Nunavik, located in northern Québec, reports the highest seroprevalence: 43% in Inuit individuals associated with the consumption of bivalves, marine mammals and geese (Ducrocq *et al.*, 2021). Numerous studies have investigated the seroprevalence of *T. gondii* in a wide diversity of animal species, including those harvested as country foods such as geese, seals, and caribou (Jenkins *et al.*, 2013; Bachand *et al.*, 2019; Hernández-Ortiz *et al.*, 2023); Additionally, researchers have monitored animals considered as sentinels in the parasite cycle, such as foxes, wolverines, polar bear, and lynx (Sharma *et al.*, 2019; Pilfold *et al.*, 2021; Bouchard *et al.*, 2022, 2023). Given the zoonotic nature of *T. gondii* and the concerning prevalence in Inuit population, this thesis places a primary emphasis on the investigation into the presence of *T. gondii* in harvested wildlife from the Canadian North.

1.2.4 *Neospora caninum*

1.2.4.1 Life cycle and transmission

This parasite is only relatively recently recognized, and the life cycle has not been fully elucidated. It is thought that canids, primarily domestic dogs, serve as the DH for the parasite. Additionally, oocysts have been also identified in various wild canids, such as coyotes, grey wolves, and dingos (Basso *et al.*, 2001; Dubey, Schares and Ortega-Mora, 2007; Dubey *et al.*, 2011; Almería, 2013). While foxes have been considered potential DH, conclusive evidence is yet to be established (Almería, 2013; Elmore *et al.*, 2013). Cattle serve as the most significant domestic IH for the parasite (Dubey, Schares and Ortega-Mora, 2007), although it has also been identified in various wild ruminants all over the world (Almería, 2013). Transmission to IH is thought to occur through the ingestion of sporulated oocysts excreted in canid feces. Additionally, the parasite can be transmitted transplacentally in both definitive and intermediate hosts (Dubey, Schares and Ortega-Mora, 2007; Marugan-Hernandez, 2017). Unlike *T. gondii*, this parasite is not thought to transmit via carnivory among IH.

1.2.4.2 Animal health significance

There is currently no concrete evidence indicating a potential zoonotic risk associated with *N. caninum*. As a result, no specific measures are recommended from a public health perspective. However, this parasite significantly impacts beef and dairy production due to the economic losses resulting from reproductive failures. *Neospora caninum* is recognized as the leading cause of abortion in cattle worldwide (Dubey, Schares and Ortega-Mora, 2007; Marugan-Hernandez, 2017; Lindsay and Dubey, 2020b). *Neospora* has been detected in a wide range of host species through serological and molecular techniques; however, clinical manifestations are infrequent. In some ruminants, neosporosis has been reported to cause emaciation, pulmonary disorders, and even death (Donahoe *et al.*, 2015). Congenital neosporosis and reproductive losses have also been observed in farmed red deer (*Cervus elaphus*) (Soler *et al.*, 2022).

1.2.4.3 *Neospora* spp. in the Canadian North

In the Canadian North, there is evidence of antibodies to *Neospora* (exposure) in caribou (Carlsson *et al.*, 2019; Hernández-Ortiz *et al.*, 2023), seals (Reiling *et al.*, 2019) and polar bears (Pilfold *et*

al., 2021). Additionally, DNA of *Neospora* has been detected in clams in Nunavut (Fung *et al.*, 2021).

1.2.5 *Sarcocystis* spp.

1.2.5.1 Life cycle and transmission

The genus *Sarcocystis* exhibits a wide diversity of animal definitive and intermediate hosts, with over 100 species described in mammals, birds, and reptiles. Some species of *Sarcocystis* can infect numerous hosts belonging to different orders. Generally, *Sarcocystis* spp. are more specific to their intermediate hosts compared to their definitive hosts (Dubey *et al.*, 2016; Lindsay and Dubey, 2020b). Infection of DH occurs when they ingest muscular or neural tissue containing tissue cysts, known as sarcocysts. A distinctive feature of *Sarcocystis*, unlike *Toxoplasma* and *Neospora*, is that sporulation takes place in the lamina propria of the DH's intestine (versus in the environment) following fertilization and oocyst formation. As sporulation is asynchronous, both unsporulated and sporulated oocysts can be found simultaneously. The oocyst wall is thin and often ruptures, releasing the sporocysts into the intestinal lumen, where they are eventually excreted in the feces of the DH. Occasionally, unsporulated and partially sporulated oocysts are also excreted in feces (Dubey *et al.*, 2016).

1.2.5.2 Public and animal health significance

Three known species of *Sarcocystis* utilize humans as the definitive hosts: *S. hominis* and *S. heydorni* with cattle serving as IH, and *S. suihominis*, with pigs as IH (Rosenthal, 2021). Humans can also become dead-end IH through accidental or aberrant infections with several unidentified species of *Sarcocystis* (Dubey *et al.*, 2016). From an animal health perspective, the manifestation of disease is uncommon. However, in a wide array of animals such as livestock, lamas, horses, bears, sea otters, and lynx, reported clinical signs include eosinophilic myositis, muscular damage, reduce in milk production, abortion and even encephalitis. (Forest *et al.*, 2000; Dubey *et al.*, 2016; Saeed *et al.*, 2018; Miller *et al.*, 2020; Greenfield *et al.*, 2022; Dubey and Rosenthal, 2023).

1.2.5.3 *Sarcocystis* spp. in the Canadian North

As a non-zoonotic parasite, and with minimal understanding of its significance for animal health, there are not many studies of *Sarcocystis* in the Canadian North. Sarcocysts are frequently

considered incidental findings on examination of cardiac and skeletal muscle from wildlife intermediate hosts. DNA of the parasite has been reported in in seals (Reiling *et al.*, 2019). Additionally, the parasite has been identified in wolverine from Nunavut (Dubey *et al.*, 2010), and Canadian lynx (Forest *et al.*, 2000).

1.2.6 Methods of detection of tissue-cyst coccidian parasites in wildlife

The significance of diagnostic testing for infectious diseases in wildlife is increasing, driven by growing concerns regarding pathogens affecting wildlife populations, biodiversity, and the potential implications for human health. Diagnostic testing in wildlife presents challenges surpassing those encountered in domestic animals. These challenges include obstacles related to animal and samples accessibility, compromised sample quality, and limited understanding of pathogenesis and epidemiology of diseases in wildlife context (Ryser-Degiorgis, 2013). Nevertheless, several studies have successfully adapted and applied existing techniques to assess wildlife populations.

1.2.6.1 Bioassays

The gold standard for detection of *T. gondii* infection involves isolating the parasite using laboratory animals, such as mice and cats (Dubey, 2010). This method has also proven effective for *N. caninum* and *Sarcocystis* spp. (Dubey *et al.*, 2000; Gondim *et al.*, 2017; Costa *et al.*, 2018). The procedure consists in inoculating laboratory animals with infected tissues or body fluids, allowing the parasites to establish infection in the new host. Following a period of 6 to 8 weeks, the experimental animals are euthanized to examine their tissues and fluids (Dubey, 2010). However, the bioassay is a resource-intensive and time-consuming procedure, requiring the ongoing care of laboratory animals and the duration of the infection (Liu *et al.*, 2015).

1.2.6.2 Microscopic examination

Conventional techniques involve the detection of oocysts in the feces of the definitive hosts through fecal flotation. Additionally, tissue cysts can be identified by histology examination and immunohistochemistry in various tissues of infected animals, including aborted fetus (Dubey, 2010; Lindsay and Dubey, 2020b). The equipment for microscopy ranges from basic to advance technologies, including tools like the electron microscope.

1.2.6.3 Serological techniques

Numerous techniques have been developed for the detection of antibodies against *T. gondii* as it is a parasite of public concern. In this section, I aboard into some of the most relevant techniques. Among the early methods employed in humans is the Sabin-Feldman dye test, which was considered the gold standard; however, it has disadvantages due to its reliance on live tachyzoites, posing a biohazard risk, and necessitating serum from a healthy human.

The modified agglutination test (MAT) stands out as a widely used technique for diagnosing *T. gondii* in both humans and animals. Its advantages include the absence of a need for a conjugate or secondary antibody and the lack of special equipment requirements. The test consists of mixing the serum sample with formalin fixed tachyzoites and Evans blue dye solution. In the case of a negative sample, the tachyzoites precipitate at the bottom of the well, forming a distinctive blue dot. Conversely, if the sample is positive, antibodies bind with the tachyzoite, resulting in agglutination and a clear appearance in the well (Dubey, 2010). Despite its simplicity, result interpretation can be challenging, and the test may produce false positive or negative results (Liu *et al.*, 2015).

The indirect fluorescence antibody test (IFA) has been a widely used diagnostic tool for *T. gondii* in both humans and animals. Additionally, it is the standard serological technique for the diagnosis of bovine neosporosis (Sinnott *et al.*, 2017). The procedure involves the use of tachyzoites (for *T. gondii* or *N. caninum*) or merozoites (for *Sarcocystsis* spp.) (Miller *et al.*, 2010; Lindsay and Dubey, 2020; Silva *et al.*, 2023). Formalin-fixed parasites are incubated with the serum sample in serial dilution over slides with wells. Subsequently, a conjugate or secondary antibody labeled with a fluorochrome is added to the reaction, and the results can be observed under a fluorescence microscope. However, disadvantages include the necessity for a species-specific antibody and the challenges in determining a cut-point value due to the serum dilution factor.

The enzyme-linked immunosorbent assay (ELISA) stands out as the leading detection method in clinical laboratories worldwide for the detection of diseases-associated biomarkers (Crowther, 2010). In traditional ELISA, the process unfolds on solid plastic supports (plates), where antibodies capture antigens, followed by a substrate that generates a measurable signal with the assistance of an enzyme. The use of ELISA plates offers a significant advantage, allowing the

simultaneously testing of a large number of samples and the potential utilization of automated systems (Liu *et al.*, 2015). Moreover, ELISA can not only determine the presence of an antigen but also quantify its concentration. For *T. gondii* and *N. caninum*, different protocols have been established using a diverse range of antigens, including full proteins from tachyzoite lysates to single recombinant proteins, that have been employed in commercial kits too (Dubey, 2010; Liu *et al.*, 2015; Lindsay and Dubey, 2020). Conversely, ELISAs for *Sarcocystis* spp. are less efficient due to the diversity of the parasite, posing challenges in identifying the specific species (Lindsay and Dubey, 2020). Similar to IFA, a notable disadvantage is that ELISA requires a species-specific conjugate, adding complexity when working with wildlife samples (Crowther, 2010).

1.2.6.4 Molecular techniques

The Polymerase chain reaction (PCR) is considered an important tool for detecting DNA of *T. gondii*, *N. caninum* and *Sarcocystis* spp. This method has the advantages of being quick, and have high sensitivity and specificity, making it versatile for various applications such as diagnosis, DNA quantification of parasites, sequencing, and species identification. To enhance sensitivity, different genes have been targeted including B1 gene and the 529 bp repeat element for *T. gondii*; the Nc-5 gene for *N. caninum*, and the ITS1 and 18S rDNA for coccidia (Dubey, 2010; Liu *et al.*, 2015; Sinnott *et al.*, 2017; Lindsay and Dubey, 2020b).

1.3 THESIS OBJECTIVES

Overarching goals:

I. To determine which wildlife species, and which tissues within individual hosts, harbor *Toxoplasma gondii* and other *Sarcocystidae*

- **i.** To detect and identify *T. gondii* and other *Sarcocystidae* in samples collected from wildlife harvested in the Canadian Arctic

II. To develop and evaluate methods of detection of *Toxoplasma* and related parasites in wildlife.

- **i.** To standardize an indirect ELISA with purified Antigen p30 for detection of IgG vs *T. gondii.*
- **ii.** To evaluate and compare serological techniques in wildlife for detection of IgG vs *T. gondii*.

  Hypothesis: Indirect p30 ELISA is likely to have higher analytical and clinical sensitivity and specificity than Indirect crude extract ELISA, and commercial ELISA.
- **iii.** To compare molecular techniques for detection of DNA of *T. gondii* in wildlife tissue.

  Hypothesis: T. gondii DNA is likely to be detected at higher sensitivity by the magnetic capture PCR than conventional PCR method.
- **iv.** To compare molecular techniques for detection of DNA of *Sarcocystidae* in wildlife tissue.

1.4 References

Almería, S. (2013) ‘*Neospora caninum* and Wildlife’, *ISRN Parasitology*, 2013, pp. 1–23. Available at: https://doi.org/10.5402/2013/947347.

Amstrup, S.C., Marcot, B.G. and Douglas, D.C. (2013) ‘A Bayesian network modeling approach to forecasting the 21st century worldwide status of polar bears’, in E.T. DeWeaver, C.M. Bitz, and L.-B. Tremblay (eds) *Geophysical Monograph Series*. Washington, D.C.: American Geophysical Union, pp. 213–268. Available at: https://doi.org/10.1029/180GM14.

Arkush, K.D., Miller, M.A., Leutenegger, C.M., Gardner, I.A., Packham, A.E., Heckeroth, A.R., Tenter, A.M., Barr, B.C. and Conrad, P.A. (2003) ‘Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*)’, *International Journal for Parasitology*, 33(10), pp. 1087–1097. Available at: https://doi.org/10.1016/S0020-7519(03)00181-4.

Attias, M., Teixeira, D.E., Benchimol, M., Vommaro, R.C., Crepaldi, P.H. and De Souza, W. (2020) ‘The life-cycle of *Toxoplasma gondii* reviewed using animations’, *Parasites & Vectors*, 13(1), p. 588. Available at: https://doi.org/10.1186/s13071-020-04445-z.

Atwood, T.C., Peacock, E., McKinney, M.A., Lillie, K., Wilson, R., Douglas, D.C., Miller, S. and Terletzky, P. (2016) ‘Rapid environmental change drives increased land use by an Arctic marine predator’, *PLOS ONE*. Edited by S. Li, 11(6), p. e0155932. Available at: https://doi.org/10.1371/journal.pone.0155932.

Atwood, T.C., Simac, K., Breck, S.W., York, G. and Wilder, J. (2017) ‘Human–polar bear interactions in a changing Arctic: existing and emerging concerns’, in A. Butterworth (ed.) *Marine Mammal Welfare*. Cham: Springer International Publishing (Animal Welfare), pp. 397–418. Available at: https://doi.org/10.1007/978-3-319-46994-2_22.

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E. and Jenkins, E. (2019) ‘Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada’, *Parasites & Vectors*, 12(1), p. 155. Available at: https://doi.org/10.1186/s13071-019-3408-9.

Basso, W., Venturini, L., Venturini, M.C., Hill, D.E., Kwok, O.C.H., Shen, S.K. and Dubey, J.P. (2001) 'First Isolation of *Neospora caninum* from the feces of a naturally infected dog', *Journal of Parasitology*, 87(3), pp. 612–618. Available at: https://doi.org/10.1645/0022-3395(2001)087[0612:FIONCF]2.0.CO;2.

Berta, A., Sumich, J.L. and Kovacs, K.M. (2006) *Marine mammals: evolutionary biology*. 2. ed. Amsterdam: Elsevier.

Binnington, M.J., Lei, Y.D., Pokiak, L., Pokiak, J., Ostertag, S.K., Loseto, L.L., Chan, H.M., Yeung, L.W.Y., Huang, H. and Wania, F. (2017) 'Effects of preparation on nutrient and environmental contaminant levels in Arctic beluga whale (*Delphinapterus leucas*) traditional foods', *Environmental Science: Processes & Impacts*, 19(8), pp. 1000–1015. Available at: https://doi.org/10.1039/C7EM00167C.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Buhler, K., Al-Adhami, B., Su, C., Fenton, H., G.-Gouin, G., Roth, J.D., Rodrigues, C.W., Pamak, C., Simon, A., Bachand, N., Leighton, P. and Jenkins, E. (2022) 'Are foxes (*Vulpes* spp.) good sentinel species for *Toxoplasma gondii* in northern Canada?', *Parasites & Vectors*, 15(1), p. 115. Available at: https://doi.org/10.1186/s13071-022-05229-3.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Jung, T.S., Harms, N.J., Willier, C.N., Boonstra, R., Majchrzak, Y.N., Peers, M.J.L., Gouin, G.-G., Al-Adhami, B., Simon, A., Leighton, P. and Jenkins, E.J. (2023) 'Canada Lynx (*Lynx canadensis*) as potential reservoirs and sentinels of *Toxoplasma gondii* in Northern Canada', *Zoonotic Diseases*, 3(1), pp. 6–17. Available at: https://doi.org/10.3390/zoonoticdis3010002.

Carlsson, A.M., Curry, P., Elkin, B., Russell, D., Veitch, A., Branigan, M., Campbell, M., Croft, B., Cuyler, C., Côté, S.D., Leclerc, L.-M., Tryland, M., Nymo, I.H. and Kutz, S.J. (2019) 'Multi-pathogen serological survey of migratory caribou herds: A snapshot in time', *PLOS ONE*. Edited by J.P. Stewart, 14(7), p. e0219838. Available at: https://doi.org/10.1371/journal.pone.0219838.

Chan, H.M., Fediuk, K., Hamilton, S., Rostas, L., Caughey, A., Kuhnlein, H., Egeland, G. and Loring, E. (2006) 'Food security in Nunavut, Canada: barriers and recommendations.',

International Journal of Circumpolar Health, 65(5), pp. 416–431. Available at: https://doi.org/10.3402/ijch.v65i5.18132.

Chester, S.R. (2016) *The arctic guide: wildlife of the far north*. 1st edition. Princeton, NJ: Princeton University Press.

COSEWIC (2018) *COSEWIC assessment and status report on the polar bear, Ursus maritimus, in Canada*. Ottawa, ON: Committee on the Status of Endangered Wildlife in Canada = Comité sur la situation des espèces en péril au Canada. Available at: https://wildlife-species.canada.ca/species-risk-registry/virtual_sara/files/cosewic/OursBlancPolarBear-2019-Eng.pdf.

Costa, R.C., Mesquita, L.P., Nunes, M.V.L., Oliveira, I.M., Oliveira, L.F.S., Souza, A.R., Maiorka, P.C. and Varaschin, M.S. (2018) '*Neospora caninum* bioassay in gerbils using placental tissues from naturally infected goats', *Veterinary Parasitology*, 249, pp. 70–73. Available at: https://doi.org/10.1016/j.vetpar.2017.11.009.

Cottee-Jones, H.E.W. and Whittaker, R.J. (2012) 'perspective: The keystone species concept: a critical appraisal', *Frontiers of Biogeography*, 4(3). Available at: https://doi.org/10.21425/F5FBG12533.

Crowther, J.R. (2010) *The ELISA guidebook*. New York: Humana Press.

Dard, C., Marty, P., Brenier-Pinchart, M.-P., Garnaud, C., Fricker-Hidalgo, H., Pelloux, H. and Pomares, C. (2018) 'Management of toxoplasmosis in transplant recipients: an update', *Expert Review of Anti-infective Therapy*, 16(6), pp. 447–460. Available at: https://doi.org/10.1080/14787210.2018.1483721.

Donahoe, S.L., Lindsay, S.A., Krockenberger, M., Phalen, D. and Šlapeta, J. (2015) 'A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife', *International Journal for Parasitology: Parasites and Wildlife*, 4(2), pp. 216–238. Available at: https://doi.org/10.1016/j.ijppaw.2015.04.002.

Dowsley, M. (2009) ‘Inuit-organised polar bear sport hunting in Nunavut territory, Canada’, *Journal of Ecotourism*, 8(2), pp. 161–175. Available at: https://doi.org/10.1080/14724040802696049.

Dubey, J.P. (2010) *Toxoplasmosis of animals and humans*. 2nd ed. Boca Raton: CRC Press.

Dubey, J.P., Calero-Bernal, R., Rosenthal, B., Speer, C.A. and Fayer, R. (2016) *Sarcocystosis of animals and humans*. Second edition. Boca Raton: CRC Press, Taylor & Francis Group.

Dubey, J.P., Jenkins, M.C., Rajendran, C., Miska, K., Ferreira, L.R., Martins, J., Kwok, O.C.H. and Choudhary, S. (2011) ‘Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*’, *Veterinary Parasitology*, 181(2–4), pp. 382–387. Available at: https://doi.org/10.1016/j.vetpar.2011.05.018.

Dubey, J.P., Reichard, M.V., Torretti, L., Garvon, J.M., Sundar, N. and Grigg, M.E. (2010) ‘Two new species of Sarcocystis (Apicomplexa: Sarcocystidae) infecting the wolverine (*Gulo gulo*) from Nunavut, Canada’, *Journal of Parasitology*, 96(5), pp. 972–976. Available at: https://doi.org/10.1645/GE-2412.1.

Dubey, J.P. and Rosenthal, B.M. (2023) ‘Bovine sarcocystosis: *Sarcocystis* species, diagnosis, prevalence, economic and public health considerations, and association of *Sarcocystis* species with eosinophilic myositis in cattle’, *International Journal for Parasitology*, 53(9), pp. 463–475. Available at: https://doi.org/10.1016/j.ijpara.2022.09.009.

Dubey, J.P., Saville, W.J.A., Lindsay, D.S., Stich, R.W., Stanek, J.F., Speer, C.A., Rosenthal, B.M., Njoku, C.J., Kwok, O.C.H., Shen, S.K. and Reed, S.M. (2000) ‘Completion of the life cycle of *Sarcocystis neurona’*, *The Journal of Parasitology*, 86(6), p. 1276. Available at: https://doi.org/10.2307/3285013.

Dubey, J.P., Schares, G. and Ortega-Mora, L.M. (2007) ‘Epidemiology and control of neosporosis and *Neospora caninum*’, *Clinical Microbiology Reviews*, 20(2), pp. 323–367. Available at: https://doi.org/10.1128/CMR.00031-06.

Ducrocq, J., Ndao, M., Yansouni, C.P., Proulx, J., Mondor, M., Hamel, D., Lévesque, B., De Serres, G. and Talbot, D. (2021) ‘Epidemiology associated with the exposure to *Toxoplasma*

gondii in Nunavik's Inuit population using the 2017 *Qanuilirpitaa* cross-sectional health survey', *Zoonoses and Public Health*, 68(7), pp. 803–814. Available at: https://doi.org/10.1111/zph.12870.

Elmore, S.A., Lalonde, L.F., Samelius, G., Alisauskas, R.T., Gajadhar, A.A. and Jenkins, E.J. (2013) 'Endoparasites in the feces of arctic foxes in a terrestrial ecosystem in Canada', *International Journal for Parasitology: Parasites and Wildlife*, 2, pp. 90–96. Available at: https://doi.org/10.1016/j.ijppaw.2013.02.005.

Feldhamer, G.A., Thompson, B.C. and Chapman, J.A. (eds) (2003) *Wild mammals of North America: biology, management, and conservation*. 2nd ed. Baltimore, Md: Johns Hopkins University Press.

Festa-Bianchet, M., Ray, J.C., Boutin, S., Côté, S.D. and Gunn, A. (2011) 'Conservation of caribou (*Rangifer tarandus*) in Canada: an uncertain future.', *Canadian Journal of Zoology*, 89(5), pp. 419–434. Available at: https://doi.org/10.1139/z11-025.

Forest, T.W., Abou-Madi, N., Summers, B.A., Tornquist, S.J. and Cooper, B.J. (2000) '*Sarcocystis neurona*-like encephalitis in a Canada lynx (*Felis lynx canadiensis*)', *Journal of Zoo and Wildlife Medicine*, 31(3), pp. 383–387. Available at: https://doi.org/10.1638/1042-7260(2000)031[0383:SNLEIA]2.0.CO;2.

Friesen, T.M. and Arnold, C.D. (1995) 'Zooarchaeology of a focal resource: dietary importance of beluga whales to the precontact Mackenzie Inuit', *ARCTIC*, 48(1), pp. 22–30. Available at: https://doi.org/10.14430/arctic1221.

Fung, R., Manore, A.J.W., Harper, S.L., Sargeant, J.M., Shirley, J., Caughey, A. and Shapiro, K. (2021) 'Clams and potential foodborne *Toxoplasma gondii* in Nunavut, Canada', *Zoonoses and Public Health*, 68(3), pp. 277–283. Available at: https://doi.org/10.1111/zph.12822.

Garibaldi, A. and Turner, N. (2004) 'Cultural keystone species: implications for ecological conservation and restoration', *Ecology and Society*, 9(3). Available at: https://doi.org/10.5751/ES-00669-090301.

Gondim, L.S.Q., Jesus, R.F., Ribeiro-Andrade, M., Silva, J.C.R., Siqueira, D.B., Marvulo, M.F.V., Aléssio, F.M., Mauffrey, J.-F., Julião, F.S., Savani, E.S.M.M., Soares, R.M. and Gondim, L.F.P. (2017) '*Sarcocystis neurona* and *Neospora caninum* in Brazilian opossums (Didelphis spp.): Molecular investigation and in vitro isolation of *Sarcocystis* spp.', *Veterinary Parasitology*, 243, pp. 192–198. Available at: https://doi.org/10.1016/j.vetpar.2017.07.002.

Goyette, S., Cao, Z., Libman, M., Ndao, M. and Ward, B.J. (2014) 'Seroprevalence of parasitic zoonoses and their relationship with social factors among the Canadian Inuit in Arctic regions', *Diagnostic Microbiology and Infectious Disease*, 78(4), pp. 404–410. Available at: https://doi.org/10.1016/j.diagmicrobio.2013.08.026.

Greenfield, J.B., Anderson, M.V., Dorey, E.A., Redman, E., Gilleard, J.S., Nemeth, N.M. and Rothenburger, J.L. (2022) 'Molecular characterization of *Sarcocystis* spp. as a cause of protozoal encephalitis in a free-ranging black bear', *Journal of Veterinary Diagnostic Investigation*, 34(1), pp. 146–152. Available at: https://doi.org/10.1177/10406387211038389.

Harwood, L.A., Iacozza, J., Auld, J.C., Norton, P. and Loseto, L. (2014) 'Belugas in the Mackenzie River estuary, NT, Canada: Habitat use and hot spots in the Tarium Niryutait Marine Protected Area', *Ocean & Coastal Management*, 100, pp. 128–138. Available at: https://doi.org/10.1016/j.ocecoaman.2014.08.004.

Harwood, L.A., Innes, S., Norton, P. and Kingsley, M. (1996) 'Distribution and abundance of beluga whales in the Mackenzie estuary, southeast Beaufort Sea, and west Amundsen Gulf during late July 1992', *Canadian Journal of Fisheries and Aquatic Sciences*, 53(10), pp. 2262–2273. Available at: https://doi.org/10.1139/f96-180.

Hill, D.E., Chirukandoth, S. and Dubey, J.P. (2005) 'Biology and epidemiology of *Toxoplasma gondii* in man and animals', *Animal Health Research Reviews*, 6(01), pp. 41–61. Available at: https://doi.org/10.1079/AHR2005100.

Hummel, M. and Ray, J.C. (2014) *Caribou and the north: a shared future*. Toronto: Dundurn.

Inuit Tapiriit Kanatami (2017) ‘An Inuit-specific approach for the Canadian food policy’, *Inuit Tapiriit Kanatami*, 20 November. Available at: https://www.itk.ca/inuit-specific-approach-for-canadian-food-policy/ (Accessed: 5 July 2023).

Jenkins, E.J., Castrodale, L.J., de Rosemond, S.J.C., Dixon, B.R., Elmore, S.A., Gesy, K.M., Hoberg, E.P., Polley, L., Schurer, J.M., Simard, M. and Thompson, R.C.A. (2013) ‘Tradition and Transition: parasitic zoonoses of people and animals in Alaska, Northen Canada, and Greenland’, in *Advances in Parasitology*. Elsevier, pp. 33–204. Available at: https://doi.org/10.1016/B978-0-12-407706-5.00002-2.

Kenny, T.-A. and Chan, H.M. (2017) ‘Estimating wildlife harvest based on reported consumption by Inuit in the Canadian Arctic’, *ARCTIC*, 70(1). Available at: https://doi.org/10.14430/arctic4625.

Kenny, T.-A., Fillion, M., Simpkin, S., Wesche, S.D. and Chan, H.M. (2018) ‘Caribou (*Rangifer tarandus*) and Inuit nutrition security in Canada’, *EcoHealth*, 15(3), pp. 590–607. Available at: https://doi.org/10.1007/s10393-018-1348-z.

Kenny, T.-A., Hu, X.F., Kuhnlein, H.V., Wesche, S.D. and Chan, H.M. (2018) ‘Dietary sources of energy and nutrients in the contemporary diet of Inuit adults: results from the 2007–08 Inuit Health Survey’, *Public Health Nutrition*, 21(7), pp. 1319–1331. Available at: https://doi.org/10.1017/S1368980017003810.

Kuhnlein, H.V. and Receveur, O. (1996) ‘Dietary change and traditional food systems of indigenous peoples’, *Annual Review of Nutrition*, 16(1), pp. 417–442. Available at: https://doi.org/10.1146/annurev.nu.16.070196.002221.

Kuhnlein, H.V. and Receveur, O. (2007) ‘Local cultural animal food contributes high levels of nutrients for Arctic Canadian indigenous adults and children1’, *The Journal of Nutrition*, 137(4), pp. 1110–1114. Available at: https://doi.org/10.1093/jn/137.4.1110.

Lindsay, D.S., Collins, M.V., Mitchell, S.M., Wetch, C.N., Rosypal, A.C., Flick, G.J., Zajac, A.M., Lindquist, A. and Dubey, J.P. (2004) ‘Survival of *Toxoplasma gondii* oocysts in eastern

oysters (*Crassostrea virginica*)', *Journal of Parasitology*, 90(5), pp. 1054–1057. Available at: https://doi.org/10.1645/GE-296R.

Lindsay, D.S. and Dubey, J.P. (2020) 'Neosporosis, toxoplasmosis, and sarcocystosis in ruminants', *Veterinary Clinics of North America: Food Animal Practice*, 36(1), pp. 205–222. Available at: https://doi.org/10.1016/j.cvfa.2019.11.004.

Liu, Q., Wang, Z.-D., Huang, S.-Y. and Zhu, X.-Q. (2015) 'Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii'*, *Parasites & Vectors*, 8(1), p. 292. Available at: https://doi.org/10.1186/s13071-015-0902-6.

Lunn, N.J., Servanty, S., Regehr, E.V., Converse, S.J., Richardson, E. and Stirling, I. (2016) 'Demography of an apex predator at the edge of its range: impacts of changing sea ice on polar bears in Hudson Bay', *Ecological Applications*, 26(5), pp. 1302–1320. Available at: https://doi.org/10.1890/15-1256.

Marino, A.M.F., Giunta, R.P., Salvaggio, A., Castello, A., Alfonzetti, T., Barbagallo, A., Aparo, A., Scalzo, F., Reale, S., Buffolano, W. and Percipalle, M. (2019) '*Toxoplasma gondii* in edible fishes captured in the Mediterranean basin', *Zoonoses and Public Health*, 66(7), pp. 826–834. Available at: https://doi.org/10.1111/zph.12630.

Marugan-Hernandez, V. (2017) '*Neospora caninum* and Bovine Neosporosis: Current Vaccine Research', *Journal of Comparative Pathology*, 157(2–3), pp. 193–200. Available at: https://doi.org/10.1016/j.jcpa.2017.08.001.

Massie, G.N., Ware, M.W., Villegas, E.N. and Black, M.W. (2010) 'Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish', *Veterinary Parasitology*, 169(3–4), pp. 296–303. Available at: https://doi.org/10.1016/j.vetpar.2010.01.002.

McCall, A.G., Derocher, A.E. and Lunn, N.J. (2015) 'Home range distribution of polar bears in western Hudson Bay', *Polar Biology*, 38(3), pp. 343–355. Available at: https://doi.org/10.1007/s00300-014-1590-y.

McLeod, R., Lykins, J., Gwendolyn Noble, A., Rabiah, P., Swisher, C.N., Heydemann, P.T., McLone, D., Frim, D., Withers, S., Clouser, F. and Boyer, K. (2014) 'Management of congenital

toxoplasmosis', *Current Pediatrics Reports*, 2(3), pp. 166–194. Available at: https://doi.org/10.1007/s40124-014-0055-7.

Miller, M.A., Conrad, P.A., Harris, M., Hatfield, B., Langlois, G., Jessup, D.A., Magargal, S.L., Packham, A.E., Toy-Choutka, S., Melli, A.C., Murray, M.A., Gulland, F.M. and Grigg, M.E. (2010) 'A protozoal-associated epizootic impacting marine wildlife: Mass-mortality of southern sea otters (*Enhydra lutris nereis*) due to *Sarcocystis neurona* infection', *Veterinary Parasitology*, 172(3–4), pp. 183–194. Available at: https://doi.org/10.1016/j.vetpar.2010.05.019.

Miller, M.A., Moriarty, M.E., Henkel, L., Tinker, M.T., Burgess, T.L., Batac, F.I., Dodd, E., Young, C., Harris, M.D., Jessup, D.A., Ames, J., Conrad, P.A., Packham, A.E. and Johnson, C.K. (2020) 'Predators, disease, and environmental change in the nearshore ecosystem: Mortality in southern sea otters (*Enhydra lutris nereis*) from 1998–2012', *Frontiers in Marine Science*, 7, p. 582. Available at: https://doi.org/10.3389/fmars.2020.00582.

Musiani, M., Leonard, J.A., Cluff, H.D., Gates, C.C., Mariani, S., Paquet, P.C., Vilà, C. and Wayne, R.K. (2007) 'Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat color and association with migratory caribou: WOLF GENETIC AND ECOLOGICAL DIFFERENTIATION', *Molecular Ecology*, 16(19), pp. 4149–4170. Available at: https://doi.org/10.1111/j.1365-294X.2007.03458.x.

Pilfold, N.W., Richardson, E.S., Ellis, J., Jenkins, E., Scandrett, W.B., Hernández-Ortiz, A., Buhler, K., McGeachy, D., Al-Adhami, B., Konecsni, K., Lobanov, V.A., Owen, M.A., Rideout, B. and Lunn, N.J. (2021) 'Long-term increases in pathogen seroprevalence in polar bears (*Ursus maritimus*) influenced by climate change', *Global Change Biology*, 27(19), pp. 4481–4497. Available at: https://doi.org/10.1111/gcb.15537.

Reiling, S.J., Measures, L., Feng, S., Boone, R., Merks, H. and Dixon, B.R. (2019) '*Toxoplasma gondii*, *Sarcocystis* sp. and *Neospora caninum*-like parasites in seals from northern and eastern Canada: potential risk to consumers', *Food and Waterborne Parasitology*, 17, p. e00067. Available at: https://doi.org/10.1016/j.fawpar.2019.e00067.

Robert-Gangneux, F. and Dardé, M.-L. (2012) 'Epidemiology of and diagnostic strategies for toxoplasmosis', *Clinical Microbiology Reviews*, 25(2), pp. 264–296. Available at: https://doi.org/10.1128/CMR.05013-11.

Roberts, L.S., Janovy, J., Nadler, S. and Roberts, L.S. (2013) *Gerald D. Schmidt & Larry S. Roberts' foundations of parasitology*. Ninth edition. New York: McGraw Hill.

Rosenthal, B.M. (2021) 'Zoonotic *Sarcocystis*', *Research in Veterinary Science*, 136, pp. 151–157. Available at: https://doi.org/10.1016/j.rvsc.2021.02.008.

Ryser-Degiorgis, M.-P. (2013) 'Wildlife health investigations: needs, challenges and recommendations', *BMC Veterinary Research*, 9(1), p. 223. Available at: https://doi.org/10.1186/1746-6148-9-223.

Saeed, M.A., Rashid, M.H., Vaughan, J. and Jabbar, A. (2018) 'Sarcocystosis in South American camelids: The state of play revisited', *Parasites & Vectors*, 11(1), p. 146. Available at: https://doi.org/10.1186/s13071-018-2748-1.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011) 'Foodborne Illness Acquired in the United States—Major Pathogens', *Emerging Infectious Diseases*, 17(1), pp. 7–15. Available at: https://doi.org/10.3201/eid1701.P11101.

Schuster, C.J., Aramini, J.J., Ellis, A.G., Marshall, B.J., Robertson, W.J., Medeiros, D.T. and Charron, D.F. (2005) 'Infectious disease outbreaks related to drinking water in Canada, 1974–2001', *Canadian Journal of Public Health*, 96(4), pp. 254–258. Available at: https://doi.org/10.1007/BF03405157.

Searles, E. (2002) 'Food and the making of modern inuit identities', *Food and Foodways*, 10(1–2), pp. 55–78. Available at: https://doi.org/10.1080/07409710212485.

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N. and Jenkins, E. (2019) 'Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of *Toxoplasma gondii* in naturally infected wolverines (*Gulo gulo*) from the Yukon, Canada', *Food*

and Waterborne Parasitology, 15, p. e00046. Available at: https://doi.org/10.1016/j.fawpar.2019.e00046.

Sheikh, N., Egeland, G.M., Johnson-Down, L. and Kuhnlein, H.V. (2011) 'Changing dietary patterns and body mass index over time in Canadian Inuit communities', *International Journal of Circumpolar Health*, 70(5), pp. 511–519. Available at: https://doi.org/10.3402/ijch.v70i5.17863.

Silva, T.M.V.D., De Santi, M., Gonçalves, L.R., Merino, M.M.J., André, M.R. and Machado, R.Z. (2023) 'Reactivity against *Sarcocystis neurona* and *Sarcocystis falcatula*-like in horses from Southeastern and Midwestern Brazil', *Revista Brasileira de Parasitologia Veterinária*, 32(3), p. e007623. Available at: https://doi.org/10.1590/s1984-29612023031.

Sinnott, F.A., Monte, L.G., Collares, T.F., Silveira, R.M. and Borsuk, S. (2017) 'Review on the immunological and molecular diagnosis of neosporosis (years 2011–2016)', *Veterinary Parasitology*, 239, pp. 19–25. Available at: https://doi.org/10.1016/j.vetpar.2017.04.008.

Soler, J.P., Moré, G., Urtizbiría, F., Hecker, Y.P., Cirone, K.M., Scioli, M.V., Paolicchi, F.A., Fiorentino, M.A., Uriarte, E.L.L., Cantón, G.J., Verna, A.E., Morrell, E.L. and Moore, D.P. (2022) 'Epidemic abortions due to *Neospora caninum* infection in farmed red deer (*Cervus elaphus*)', *Parasitology Research*, 121(5), pp. 1475–1485. Available at: https://doi.org/10.1007/s00436-022-07488-6.

Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L.M., Maksimov, P., Gethmann, J., Conraths, F.J. and Schares, G. (2019) '*Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact', *Food and Waterborne Parasitology*, 15, p. e00037. Available at: https://doi.org/10.1016/j.fawpar.2019.e00037.

Storrie, L., Hussey, N.E., MacPhee, S.A., O'Corry-Crowe, G., Iacozza, J., Barber, D.G., Nunes, A. and Loseto, L.L. (2022) 'Year-round dive characteristics of male beluga whales from the Eastern Beaufort Sea population indicate seasonal shifts in foraging strategies', *Frontiers in Marine Science*, 8, p. 715412. Available at: https://doi.org/10.3389/fmars.2021.715412.

Taillon, J., Brodeur, V. and Rivard, S. (2016) *Biological status report of migratory caribou, Leaf River herd*. Québec: Ministère des forêts, de la faune et des parcs. Available at: mffp.gouv.qc.ca.

Thiemann, G.W., Iverson, S.J. and Stirling, I. (2008) 'Polar bear diets and arctic marine food webs: insights from fatty acid analysis', *Ecological Monographs*, 78(4), pp. 591–613. Available at: https://doi.org/10.1890/07-1050.1.

Tyrrell, M. (2008) 'Nunavik Inuit perspectives on beluga whale management in the Canadian Arctic', *Human Organization*, 67(3), pp. 322–334. Available at: https://doi.org/10.17730/humo.67.3.47826252k0623352.

VanWormer, E., Miller, M.A., Conrad, P.A., Grigg, M.E., Rejmanek, D., Carpenter, T.E. and Mazet, J.A.K. (2014) 'Using molecular epidemiology to track *Toxoplasma gondii* from terrestrial carnivores to marine hosts: implications for public health and conservation', *PLoS Neglected Tropical Diseases*. Edited by E. Ghedin, 8(5), p. e2852. Available at: https://doi.org/10.1371/journal.pntd.0002852.

Vaudry, S. (2016) 'Conflicting understandings in polar bear co-management in the Inuit Nunangat: Enacting Inuit knowledge and identity', in T.M. Herrmann and T. Martin (eds) *Indigenous Peoples' Governance of Land and Protected Territories in the Arctic*. Cham: Springer International Publishing, pp. 145–163. Available at: https://doi.org/10.1007/978-3-319-25035-9_8.

Waugh, D., Pearce, T., Ostertag, S.K., Pokiak, V., Collings, P. and Loseto, L.L. (2018) 'Inuvialuit traditional ecological knowledge of beluga whale (*Delphinapterus leucas*) under changing climatic conditions in Tuktoyaktuk, NT', *Arctic Science*, pp. 1–17. Available at: https://doi.org/10.1139/as-2017-0034.

Würsig, B.G., Thewissen, J.G.M. and Kovacs, K.M. (eds) (2018) *Encyclopedia of marine mammals*. Third edition. London, United Kingdom; San Diego, CA, United States: Academic Press, an imprint of Elsevier.

TRANSITION STATEMENT CHAPTER 2

Caribou rank in the top five country foods consumed by Inuit. Of particular concern is the elevated human seroprevalence of *Toxoplasma gondii* in Nunavik, which has been linked to consumption of harvested wildlife in previous epidemiological studies. Previous work in our lab explored prevalence of this parasite in caribou in Nunavik sampled by wildlife biologists, but not those intended for human consumption. Consequently, I focused on hunter-harvested caribou from the Nunavik region, aiming to discern their potential role as sources of the parasite for human populations. In collaboration with local hunters and the Inuit owned Makivvik Corporation, I obtained samples from caribou harvested from the Leaf River herd. Our objective was to explore the seroprevalence of *Toxoplasma gondii* and *Neospora caninum* (a related parasite that could have significance for caribou health and reproduction, although it is not zoonotic). as well as the tissue prevalence for these parasites and *Sarcocystis* spp. in caribou. While it was not the primary objective, this work explored the diversity of *Sarcocystis* spp. in caribou, including detection of the more pathogenic genus, *Besnoitia*, and provided insights into tissue predilection and dynamics between caribou and terrestrial carnivore definitive hosts for *Sarcocystis* spp. in both the Palearctic and Nearctic. This work was published in the International Journal for Parasitology: Parasites and Wildlife, on which I served as first author, conducted the laboratory and data analysis, and ensured that results were communicated to stakeholders, including the Nunavik Regional Board of Health and Social Services.

CHAPTER 2: *TOXOPLASMA GONDII* AND RELATED SARCOCYSTIDAE PARASITES IN HARVESTED CARIBOU FROM NUNAVIK, CANADA

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2.1 Abstract

Caribou are keystone species important for human harvest and of conservation concern; even so, much is unknown about the impact of parasites on caribou health and ecology. The aim of this study was to determine the seroprevalence, tissue prevalence, and diversity of tissue-dwelling coccidian parasites (including *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp.) in 88 migratory caribou (*Rangifer tarandus*) harvested for human consumption in two communities in Nunavik, Québec, Canada. Both *T. gondii and N. caninum* have potential to cause abortions and neurological disease in caribou. Seroprevalence for antibodies to *T. gondii* using ELISA on fluid from thawed hearts was 18% overall, and no DNA of *T. gondii* was detected in tissues, which has positive implications for food safety since this parasite is zoonotic. Seroprevalence for antibodies to *N. caninum* using competitive ELISA was 5%, and DNA of *N. caninum* was detected in only one heart sample. DNA of *Sarcocystis*, a non-zoonotic, related coccidian, was detected in tissue samples from 85% of caribou, with higher prevalence in heart (82%) than skeletal muscle (47%). This is the first time that *Sarcocystis* spp. from caribou in Canada have been identified to species level, many of which have been described in reindeer from Fennoscandia. The high prevalence and diversity of *Sarcocystis* spp. suggests intact trophic relationships between canids and caribou in Nunavik. *Besnoitia* spp. was serendipitously detected in three muscle samples, a parasite previously associated with skin lesions in caribou in Nunavik. Community-level differences in *T. gondii* exposure and prevalence of *Sarcocystis* spp. in skeletal muscle tissues may reflect differences in hunter selection of individual animals and muscles, or possibly regional differences in the ecology of carnivore definitive hosts for these parasites. Further work is needed to explore effects of tissue coccidians in caribou, their taxonomic classifications, and community level differences in parasite prevalence and diversity.

Keywords.

Caribou, *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis*

2.2 Introduction

Caribou are keystone species in the tundra and taiga ecosystems of the arctic and subarctic regions (Gunn *et al.*, 2011). However, many caribou populations are declining due to indirect and direct anthropogenic pressures on caribou and their habitat (Festa-Bianchet *et al.*, 2011; Kenny, Fillion, *et al.*, 2018). Apart from their ecological role, caribou are considered culturally significant for Inuit communities and are among the most frequently consumed "country food" in different regions of Inuit Nunangat. Caribou contributes 19% to the total country food consumption in Nunavik, and caribou were consumed by 95% of the Inuit population in 2017 (Kenny and Chan, 2017; Kenny, Hu, *et al.*, 2018; Johnson-Down *et al.*, 2021).

North American free-ranging caribou (*Rangifer tarandus*) have been classified into four ecotypes: Peary caribou, adapted to the High Arctic deserts; mountain caribou, considered sedentary in their alpine environments; woodland caribou, also sedentary in the boreal forests; and migratory caribou, with herds of hundreds or thousands of individuals, migrating seasonally between the boreal forest and the tundra (Festa-Bianchet *et al.*, 2011; Taillon, Brodeur and Rivard, 2016). The Leaf River herd in Nunavik is classified as a migratory ecotype and exhibits long-term fluctuations in population numbers. Between 1975-2001, the population size increased, but had decreased when counted in 2011, a decade later. This, along with Indigenous knowledge, suggests a continued decrease at the last population estimate in 2015 (Taillon, Brodeur and Rivard, 2016). Caribou populations are facing mounting threats, and parasites are believed to influence health of the herds (Gunn *et al.*, 2011).

Coccidian parasites from the family Sarcocystidae usually have predator-prey heteroxenous life cycle with both intestinal and tissue stages. Sexual reproduction occurs during the intestinal phase within the definitive hosts, mainly mammalian carnivores. Asexual reproduction, in turn, occurs in vasculature and tissues of their vertebrate intermediate hosts (Roberts *et al.*, 2013). Apart from *Sarcocystis* spp., Sarcocystidae oocysts are generally shed in the feces of the definitive hosts and sporulate in the environment prior to becoming infective for intermediate hosts (Fig. 1). On ingestion by a naïve intermediate host, sporozoites are released and develop to tachyzoites disseminating to somatic tissues, where they establish as tissue cysts containing numerous bradyzoites. A carnivore definitive host becomes infected by ingesting tissue cysts containing bradyzoites in prey. The bradyzoites invade the intestinal cells, undergo schizogony (merogony),

gametogenesis, sexual reproduction, and are finally released in feces as oocysts (Roberts *et al.*, 2013).

As opposed to the general Sarcocystidae life cycle, where sporulation occurs in the environment and the sporulated oocyst is the infective stage, sporulation of *Sarcocystis* spp. occurs within the intestine of the definitive host and sporocysts are the infective stage for the intermediate host (Lindsay and Dubey, 2020). The same cycle has been proposed for *Besnoitia tarandi*; however, transmission between hosts is not fully understood and experimental infections to induce oocyst shedding in potential definitive hosts have thus far been unsuccessful (Florin-Christensen and Schnittger, 2018; Schares *et al.*, 2019). Additionally, *T. gondii* has a unique ability for tissue cysts to transmit between intermediate hosts through carnivory, maintaining the life cycle in ecosystems with few definitive hosts (Lindsay and Dubey, 2020). Finally, for *T. gondii, N. caninum*, and some species of *Sarcocystis*, tachyzoites can be also transmitted transplacentally, with detrimental effects for the fetus.

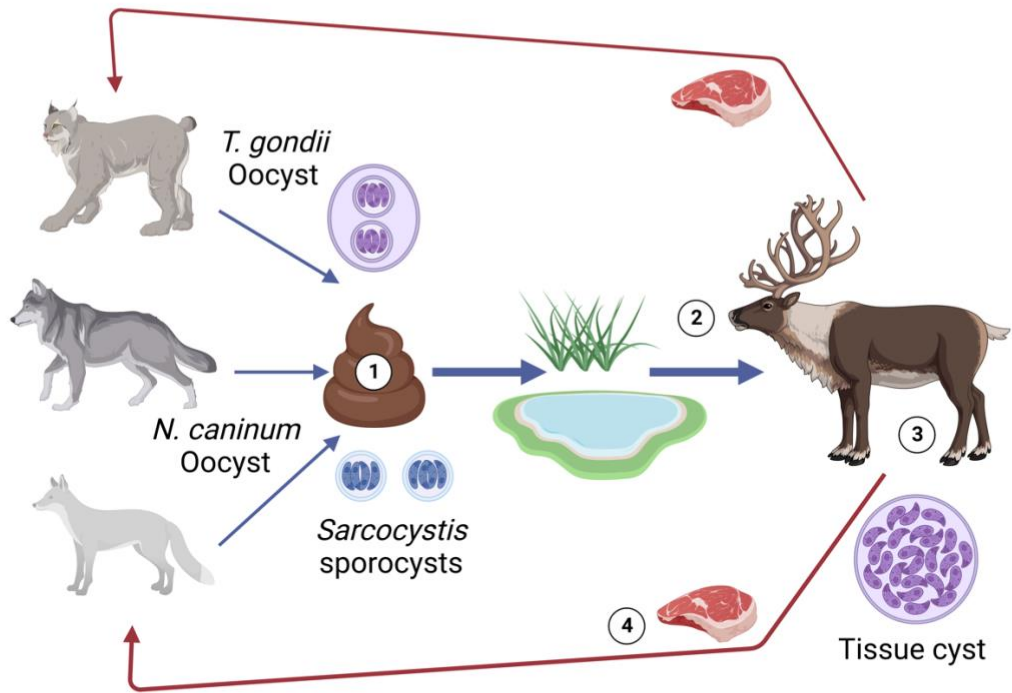

Fig. 2.1 General life cycle of Sarcocystidae parasites.

1, For *Toxoplasma gondii* and *Neospora caninum*, oocysts are shed in feces by definitive hosts (DH, felids and canids, respectively), sporulate and become infective in the environment, whereas for *Sarcocystis* spp., sporulation occurs in the intestine of the definitive hosts and sporocysts are immediately infective for intermediate hosts. 2, Intermediate hosts (IH) ingest sporulated oocysts or sporocysts in food, water or soil. 3, Sporozoites are released, divide rapidly, and tachyzoites disseminate to somatic tissues of the IH, and form tissue cysts. 4, DH becomes infected by ingesting prey species with bradyzoites within tissue cysts. Created with BioRender.com

Toxoplasma gondii causes disease and spontaneous abortion in livestock and could affect caribou reproduction (Carlsson *et al.*, 2019). Additionally, *T. gondii* is zoonotic (the only coccidian parasite in this study that is zoonotic) (Dubey, 2010) and is the most common parasite in Inuit communities based on human serosurveys (Goyette *et al.*, 2014), with a seroprevalence of 43% in Nunavik (Ducrocq *et al.*, 2021).*Toxoplasma* in humans can be acquired through ingestion of oocysts in contaminated food or water, consumption of tissue cysts in raw or undercooked meat, congenital transmission from acutely infected mothers to the fetus during pregnancy, as well as through transfusion or organ transplantation from an infected individual (Robert-Gangneux and Dardé, 2012). While often asymptomatic in individuals with a healthy immune system, congenital toxoplasmosis can lead to fetal death, stillbirth, or developmental abnormalities (McLeod *et al.*, 2014), while immunosuppressed individuals may develop toxoplasmic encephalitis, along with other complications (Robert-Gangneux and Dardé, 2012).

Neospora caninum is one of the major causes of abortion in cattle (Barry *et al.*, 2019) and causes disease and abortion in cervids (Soler et al. 2022). Pathogenic species of *Sarcocystis* can cause abortion in livestock; however, effects on fetal health are not well understood (Florin-Christensen and Schnittger, 2018). Six *Sarcocystis* spp. have been reported in *Rangifer* from Europe with unknown health consequences; findings of sarcocysts in cardiac and skeletal muscle on histology are often considered incidental (Dahlgren and Gjerde, 2007). Caribou infected with *Besnoitia tarandi* show alopecia and ulceration in skin, and in severe cases, may become emaciated (Ducrocq *et al.*, 2012; Schares *et al.*, 2019).

In order to determine how likely caribou are to be a source of human exposure to *T. gondii*, to set a baseline for prevalence of coccidian parasites circulating in caribou, and to gain insights into parasite transmission through predator-prey relationships in a northern ecosystem, we determined the seroprevalence, tissue prevalence, and diversity of tissue-dwelling coccidian parasites in caribou harvested in Nunavik.

2.3 Material and Methods

2.3.1 Study design and area

The project was an observational and cross-sectional study using serological and molecular techniques to detect antibodies from heart fluid and DNA of coccidian parasites from tissues of harvested caribou (*Rangifer tarandus*) from the Leaf River herd in Nunavik (northern Québec, Canada). Samples were collected by hunters from the communities of Tasiujaq and Umiujaq, in collaboration with the local Hunting Fishing Trapping Associations and Makivvik Corporation. The known definitive hosts for Sarcocystidae native to this region include gray wolf (*Canis lupus*), red fox (*Vulpes vulpes*), Arctic fox (*Vulpes lagopus,* previously *Alopex lagopus*), Canadian lynx (*Lynx canadensis*) and black bear (*Ursus americanus*) (Chester, 2016).

2.3.2 Sampling design and collection

Sample kits containing re-sealable plastic bags were labeled with the tissue type and an animal identification number and sent to the local Hunting Fishing Trapping Associations. Approximately 100 grams each of heart, brain and skeletal muscle were collected by local hunters between 2018 to 2022, and stored at -20ºC until processed at the Zoonotic Parasite Research Unit, University of Saskatchewan. Fluid from thawed hearts was used in lieu of sera as validated by (Sharma *et al.*, 2019).

2.3.3 Serological tests

Heart fluid was collected from the storage bags containing the heart while thawing and centrifuged at 1200 x g for 5 minutes; the supernatant was subsequently transferred to a 1.5 mL Eppendorf tube and stored at -20ºC until further testing (Sharma *et al.*, 2019). Commercial serological tests were available for *T. gondii* and *N. caninum* that do not rely on host species-specific antibodies; however, there is no commercially available serological test for either *Besnoitia tarandi* or generic level *Sarcocystis*. Furthermore, serological cross reactivity among *Sarcocystis* spp. is inconsistent (Dubey *et al.*, 2015). Therefore, serological tests for only *T. gondii* and *N. caninum* were performed.

2.3.3.1 Indirect enzyme-linked immunosorbent assay (ELISA) for *Toxoplasma gondii*

The commercially available indirect ELISA IDVet kit (ID Screen® Toxoplasmosis Indirect Multi-species, IDVet Innovative Diagnostics. Grabels, France) targeting the *T. gondii* p30 protein from tachyzoites surface was performed according to manufacturer's instructions. Heart fluid samples were diluted 1:2 in dilution buffer and loaded in duplicate on the plates. Sera from experimentally infected reindeer pre- and post-infection were added to each plate as internal negative and positive controls at 1:10 dilution. Optical densities (OD) from kit controls were used to calculate the sample/positive percentage (S/P%) using the formula S/P%= $[(OD_{sample} - OD_{negative\ control} / OD_{positive\ control} - OD_{negative\ control})] \times 100$. Samples were considered negative if S/P% was less than 40%, positive if S/P% was higher than 50%, and samples with S/P% between 40 and 50% were considered suspect.

2.3.3.2 Competitive ELISA for *Neospora caninum*

The commercially available competitive ELISA kit (*Neospora caninum* antibody test kit, cELISA, VMRD, WA, USA) was performed following the manufacturer's protocol. Fifty µL/well per sample, including kit controls, were added directly to the plate in duplicate. Results were interpreted by calculating the percentage of inhibition (%I) using the kit controls. Results were classified as negative when the samples were <30 %I, and positive if the samples were >30 %I.

2.3.4 Molecular tests.

2.3.4.1 Magnetic capture (MC) DNA extraction and quantitative PCR (qPCR) for *T. gondii*

Heart and brain tissues from the same animal were pooled if collectively less than 100g, or analyzed separately if the weight of each tissue was at least 100 g; afterwards, DNA was extracted as described by (Opsteegh *et al.*, 2010). Each qPCR run included one beef negative control without spiking and two beef positive controls spiked with 2.5 x10^5 /mL and 2.5 x10^4 mL cell-cultured *T. gondii* tachyzoites (VEG type III). Heart tissue from experimentally infected reindeer (Bouchard *et al.*, 2017) was used as an internal positive control. DNA was amplified by a qPCR targeting the 188 bp *T. gondii* sequence within the 529 repeat-element using the Tox 9F (5'-AGGAGAGATA TCAGGACTGTAG-3') and Tox 11R (5'-GCGTCGTCTC GTCTAGATCG-3') primers, and

performed using a BIO-RAD CFX96 DNA thermal cycler (Bio-Rad, Hercules, CA, USA) as previously described (Bachand *et al.*, 2019). The reaction was considered positive if the Ct-value was less than or equal to 35, negative if the Ct-value exceeded 35.

2.3.4.2 DNA extraction and conventional PCR for tissue dwelling coccidians

A second DNA extraction was performed from 25 mg of heart, brain, and muscle tissue using a DNeasy® Blood & Tissue Kit (QIAGEN Group, Germany) following manufacturer's instructions. DNA was quantified using a Nanodrop 2000c spectrophotometer (Thermo Scientific USA) and extractions were stored at -20ºC until testing. To compare with the MC RT-PCR, DNA was extracted separately from heart and brain tissues (known predilection sites for *T. gondii*) and assayed with primers targeting the *T. gondii* 529 bp repeat element as per (Homan *et al.*, 2000), with forward primer TOX4 (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and reverse primer TOX5 (5'-CGCTGCAGACACAGTGCATCTGGATT-3'). Reactions took place in a BIO-RAD Touch C1000 thermocycler (Bio-Rad, CA, USA), with initial denaturation of 94ºC for 7 min, 35 cycles of 94ºC for 1 min, 61ºC for 1 min and 72ºC for 1 min, and a final extension of 72ºC for 10 min.

DNA extracted separately from heart and skeletal muscle samples from each animal was assayed with primers for a 257 bp region of the Nc5 genomic region of *N. caninum* using forward primer Np4 (5´-CCTCCCAATGCGAACGAAA-3´) and reverse primer Np7 (5´-GGGTGAACCGAGGGAGTTG-3´) (Barry *et al.*, 2019). Reactions followed an initial denaturation of 94ºC for 4 min, 40 cycles of 95ºC for 45 s, 61ºC for 1 min and 72ºC for 1 min, and a final extension of 72ºC for 10 min.

Finally, DNA extracted separately from heart and skeletal muscle samples were assayed with genus level primers for a ~700 bp fragment from the 18S rRNA gene of *Sarcocystis* using SarcoForward (5'-CGCAAATTACCCAATCCTGA-3') and SarcoReverse (5'-ATTTCTCATAAGGTGCAGGAG-3') (Moré *et al.*, 2011). These primers were designed to be genus-specific for *Sarcocystis*, but also annealed to *Besnoitia* 18S rRNA, amplifying the region. Reactions involved initial denaturation of 95ºC for 4 min, 40 cycles of 94ºC for 40 s, 59ºC for 30 s and 72ºC for 1min, and a final extension of 72ºC for 6 min. Purified PCR products at band positions consistent with *Sarcocystis* were sent to the National Research Council in Saskatoon,

Saskatchewan, Canada for Sanger sequencing using the same primers as PCR. An additional nested PCR was performed using pan-apicomplexan primers targeting the first internal transcribed spacer (ITS-1) (Michaels *et al.*, 2016) to confirm *Besnoitia* positive samples. Sequences were assembled using QIAGEN CLC Main Workbench (QIAGEN Aarhus, Denmark). Assembled sequences were compared with GenBank sequences using the BLAST tool from the National Center for Biotechnology Information (NCBI, MD, USA). Assembled 18S rDNA sequences were aligned with selected Sarcocystidae reference sequences from Genbank using the online version of MAFFT (Katoh, Rozewicki and Yamada, 2019) with standard parameters (Table 1). A sequence of *Eimeria adeneodei* was included as an outgroup (Table 1). The aligned matrix was manually viewed, edited, and truncated in MEGA7 (Kumar, Stecher and Tamura, 2016) and exported for analysis. For a distance approach, a data-display network was constructed from uncorrected p-distances using all characters in Splitstree4 (Huson and Bryant, 2006). As a measure of statistical support, bootstraps were calculated from 1000 replicates. RAxML8 (Stamatakis, 2014) was used for a maximum likelihood approach using the GTRCAT approximation and calculating bootstraps by invoking the autoMRE bootstopping function. The topology was viewed in FigTree4 (http://tree.bio.ed.ac.uk/software/figtree/) and exported to Corel PaintshopPro X8 for finalization.

Table 2.1 Reference sequences (18S rRNA) with designated *Sarcocystis* and related coccidian species names, hosts from which the organism was recovered (if provided) and accession numbers from GenBank used in the phylogenetic analysis.

NA = not applicable.

Group	Designated name	Host	GenBank accession number
I	*S. grueneri*	*Rangifer t. tarandus*	EF056010
II	*S. alces*	*Alces alces*	EU282018
II	*S. alces*	*Alces alces*	KF831273
II	*S. capracanis*	Not provided	L76472
II	*S. tarandivulpes*	*Rangifer t. tarandus*	EF467657
III	*S. alceslatrans*	*Alces alces*	KF831276
III	*S. rangi*	*Rangifer t. tarandus*	EF056011
III	*S. rangi*	*Rangifer t. tarandus*	EF467655
IV	*S. cf tarandi*	*Cervus nippon*	LC349468
IV	*S. cf tarandi*	*Cervus nippon centralis*	LC481020
IV	*S. cf tarandi*	*Cervus nippon centralis*	LC481021
IV	*S. elongata*	*Cervus elaphus*	GQ251020
IV	*S. elongata*	*Cervus elaphus*	GQ251019
IV	*S. tarandi*	*Rangifer t. tarandus*	EF056017
IV	*S. tarandi*	*Rangifer t. tarandus*	GQ250976
IV	*S. tarandi*	*Rangifer t. tarandus*	EF056018
V	*S. silva*	*Alces alces*	EU282016
V	*S. silva*	*Capreolus capreolus*	JN226122
VI	*S. rangiferi*	*Rangifer t. tarandus*	GQ250981
VI	*S. rangiferi*	*Rangifer t. tarandus*	EF056015
VII	*S. scandinavica*	*Alces alces*	EU282032
VII	*S. scandinavica*	*Alces alces*	EU282027
VIII	*Besnoitia besnoiti*	Not provided	AF109678

VIII	*Besnoitia jellisoni*	*Culture-derived zoites*	AF291426
VIII	*Hammondia hammondi*	Not provided	AF096498
VIII	*Cytoisospora belli*	Not provided	DQ060683
VIII	*Neospora caninum*	*Canis familiaris*	U16159
VIII	*Toxoplasma gondii*	Not provided	EF472967
Outgroup	*Eimeria adeneodei*	Not provided	AF324212
NA	*S. cervicanis*	*Cervus elaphus*	KY973354
NA	*S. cervicanis*	*Cervus elaphus*	KY973333
NA	*S. cruzi*	*Bos taurus*	KT901173
NA	*S. cruzi*	*Bos taurus*	JX679467
NA	*S. hirsuta*	*Bos taurus*	AH006015
NA	*S. hirsuta*	*Bos taurus*	KT901163
NA	*S. neurona*	*Phoca vitulina richardsii*	AF252406
NA	*S. neurona*	Cultured tachyzoites	U07812

2.3.5 Data analysis.

Seroprevalence, tissue prevalence and their 95% confidence intervals (CI) were calculated using the Ausvet Epitools calculator (Sergeant, 2018).

Samples were grouped based on their respective communities of harvest, and the two groups were compared using Fisher's exact Chi-square test (IBM SPSS Statistics). Serological and molecular tests were compared between ELISA and MC qPCR for *T. gondii*, and between cELISA and PCR for *N. caninum* using McNemar's chi-square test for related samples. If not significantly different, the kappa coefficient (κ) was used to determine the level of agreement between two tests.

2.4 Results.

2.4.1 Detection of antibodies against *T. gondii* and *N. caninum*

Heart fluid from 16 of 88 (18%; 95% CI: 11.5, 28. Fig. 2.2) caribou were positive for antibodies to *T. gondii*. Seroprevalence was significantly higher in samples submitted from Tasiujaq (27%; 95% CI: 17, 40) than those from Umiujaq (3%; 95% CI: 0.5, 15; p=0.004). For *N. caninum*, only 4 samples tested positive (5%; 95% CI: 0.6, 8); there was no statistical difference between communities.

2.4.2 Detection of parasite DNA.

DNA of *T. gondii* was not detected in any sample using either magnetic capture qPCR or conventional PCR. DNA of *N. caninum* was detected in one heart sample (prevalence: 1.5%, 95% CI: 0.3, 7.9) from a caribou that was negative for antibodies to *N. caninum* by cELISA. DNA of *Sarcocystis* spp. was detected significantly more often in heart tissue (82%; 95% CI: 72, 88) than muscle tissue (47%; 95% CI: 36, 58. Fig. 2.3) (p=0.002). Prevalence of *Sarcocystis* spp. in skeletal muscle samples, but not heart samples, was significantly higher in Tasiujaq (64%; 95% CI: 50, 76) than Umiujaq (19%; 95% CI: 9, 36; p<0.001). A total of 34 caribou (39%) had DNA of *Sarcocystis* spp. in both heart and skeletal muscle.

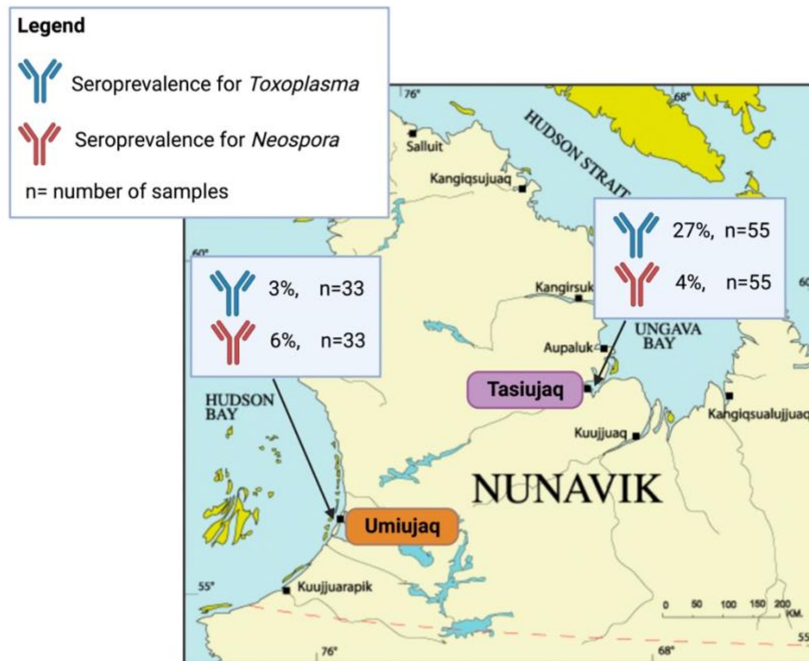

Fig. 2.2 Observed seroprevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in caribou harvested from 2 communities in Nunavik. Source: Nunavik Research Centre, Makivik Corporation.

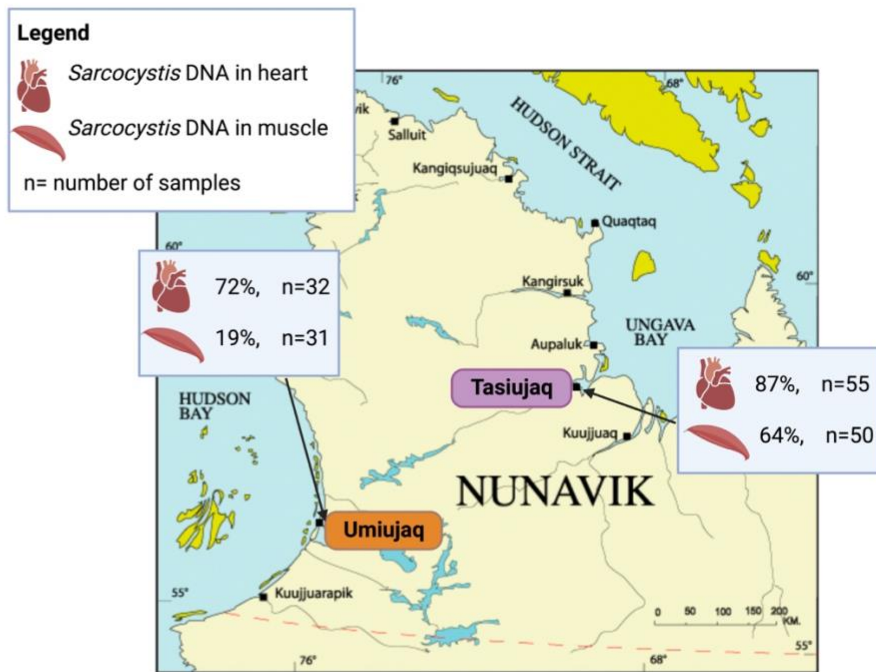

Fig. 2.3 *Sarcocystis* DNA prevalence in heart and muscle of caribou harvested by 2 communities in Nunavik, Québec, Canada.

2.4.3 Comparison between serology and molecular tests.

Sixteen caribou were positive for *T. gondii* by serology and negative for molecular. McNemar chi-square showed statistical difference between the tests (χ^2 =14, df=1, p <0.001, n=88). There was no statistical difference for *N. caninum* between serology and molecular (χ^2 = 1.3, df= 1, p=0.248, n=88) and only a fair agreement (κ=0.39).

2.4.4 Sequencing results and phylogeny for *Sarcocystis* spp.

In total, 32 sequences were generated as a subsample of the 71 positive heart samples. On average, the sequences shared a 99.4 (±1.24) percentage of identity (%ID) with *S. grueneri* using BLAST. Sixteen sequences were successfully generated from 38 positive skeletal muscle samples. Thirteen of these sequences represent 5 different *Sarcocystis* spp. as follows: five sequences had an average of 99 %ID (±1.11) with *S. tarandi*, three had 99.6 %ID (±0.44) with *S. tarandivulpes*, two had 99.8 %ID with *S. rangi*, two had 100 %ID to *S. rangiferi*, and one had 99.8 %ID with *S. scandinavica*. Additionally, 3 samples from the 18S rRNA PCR generated sequences identical to *Besnoitia* spp; these samples were tested with ITS-1 nested PCR and the sequences had 100%ID with *Besnoitia* spp.

The aligned matrix consisted of 74 ingroup and one outgroup sequence with a final length of 658 characters. The assignment of 8 groups was intended for discussion puposes rather than for taxonomic designation. The subfamily Toxoplasmatinae (Group VIII) was supported as a monophyletic clade across both analyses (DDN bs: 100, ML bs: 95) including *Isospora belli* in the grouping (Fig. 4 and 5). Sequences of *Besnoitia* spp. obtained from GenBank, as well as those generated in this study were included within the Group VIII. It was not possible to differentiate between *B. besnoiti*, *B. tarandi* and *B. jellisoni* using 18S sequences or ITS-1 sequences. The subfamily Sarcocystinae was recovered as monophyletic in the ML analysis, although it lacked support with the inclusion of *S. neurona* (ML bs: 67) (Fig. 4). However, when *S. neurona* is excluded, the clade enjoys bootstrap support of 100. All 32 sequences recovered from heart samples grouped with a *S. grueneri* reference sequence in a well-supported monophyly (DDN bs: 100, ML bs: 96) (Group I). Sequences generated from muscle tissue were more diverse and represented members of groups II through VII (red dots in Fig. 2.4 and 2.5). Group II comprised a well-supported *S. tarandivulpes* grouping inclusive of sequences generated here (DDN bs: 95, ML

bs: 97), sister to a *S. alces* and *S. capracanis* clade. Two more samples grouped alongside *S. rangi* forming a well-supported monophyly in Group III sister to *S. alcestrans,* completing Group III. Despite forming a monophyletic clade, Group IV lacked support in the ML analysis and was rendered paraphyletic by Group V in the network analysis. The sister grouping of Group V to Group IV was also not supported in the ML analysis. Group IV consisted of a polyphyletic grouping of reference sequences designated as *S. tarandi, S. cf. tarandi* and *S. elongata* as well as sample sequences generated here. Inferring identity of these samples beyond members of Group IV is thus not possible. Group V, consisting of *S. silva* sequences, was confidently supported as a monophyletic group in both methods (DDN bs: 90, ML bs: 89). Group VI comprised two sample sequences from this study alongside *S. rangiferi* reference sequences in a monophyletic group in both the network (bs 94) and ML phylogeny (bs 99). A single sample sequence grouped alongside two reference sequences of *S. scandinavica* forming Group VII. We could therefore confidently infer species level identification through phylogenetic and network reconstruction for most sample sequences and group level identification to the remaining sequences (see supplementary table).

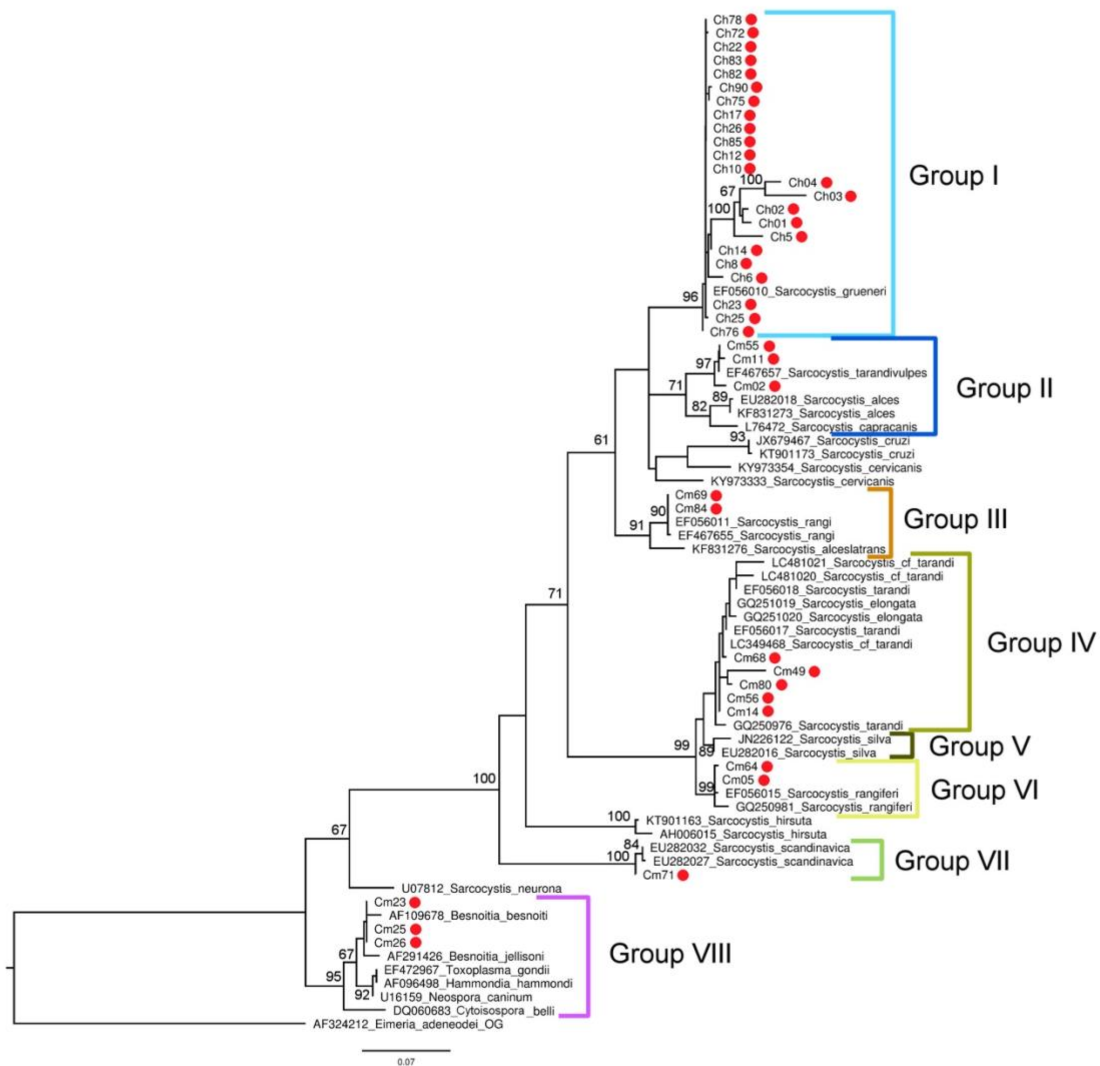

Fig. 2.4 Maximum likelihood topology for tissue dwelling coccidians (mostly *Sarcocystis* spp.) generated from 18S rDNA sequence data analyzed in RAxML 8 under the GTRCAT approximation.

Group names bear no taxonomic designation but merely assigned for discussion purposes. Bootstrap values > 60 are displayed above branches as branch/node support. Red dots indicate sequences generated in this study.

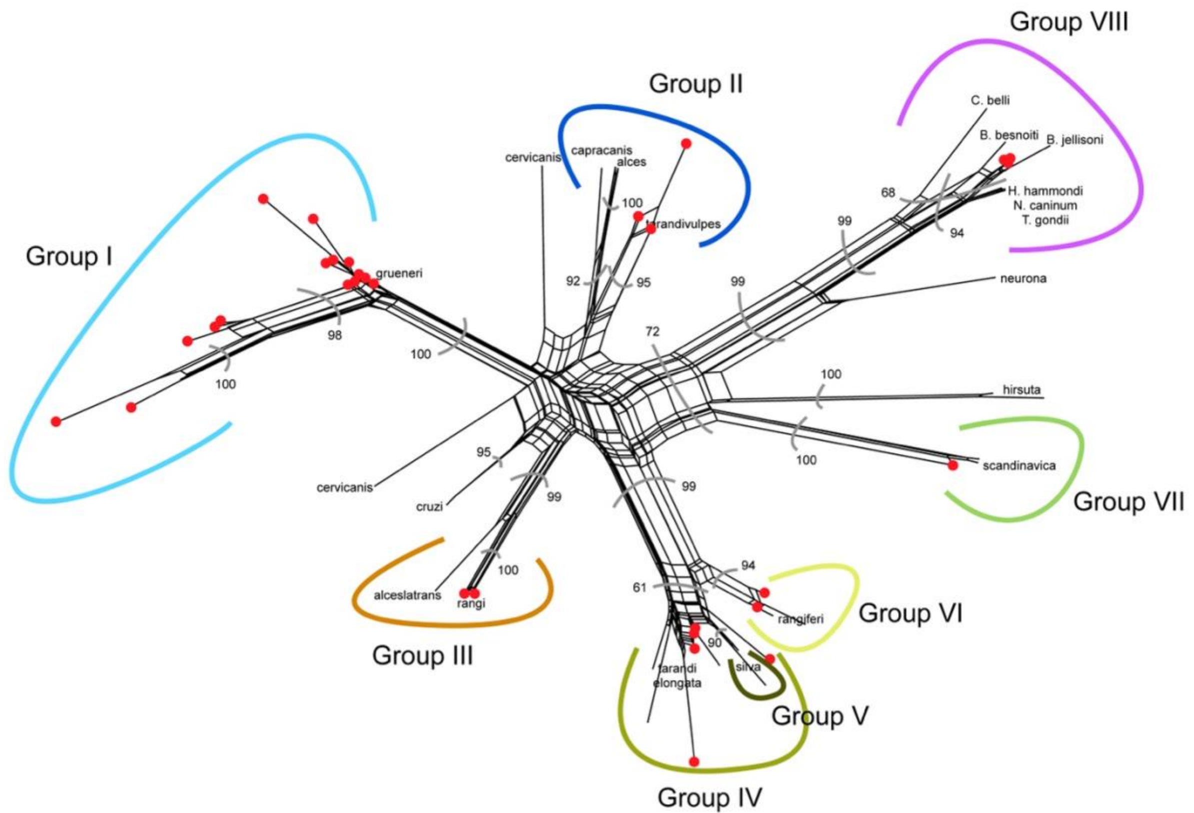

Fig. 2.5 A data-display network constructed from uncorrected 18S rDNA p-distances, using all characters, for tissue dwelling coccidians (mostly *Sarcocystis* spp.).

Group names bear no taxonomic designation but merely assigned for discussion purposes. Bootstrap supports are displayed by the grey curves and associated values imposed on the network. Red dots indicate sequences generated in this study.

2.5 Discussion

The main impetus for this study was to determine tissue and seroprevalence of *T. gondii* in harvested caribou from Nunavik, due to food safety concerns from northern communities, and the potential impact of this and related coccidian parasites on caribou reproduction and health. In this study, antibodies for *T. gondii* were detected in heart fluid of 18% of harvested caribou using ELISA, lower than the seroprevalence of 26% reported by Bachand (Bachand *et al.*, 2019) when screening sera of live captured caribou/calf pairs from the same region using a modified agglutination test (MAT). Both estimates are higher than the overall seroprevalence for *T. gondii* in migratory caribou across Canada (2%), of which 1% was found for Leaf River (Carlsson *et al.*, 2019). Various sample types (whole blood, blood on filter papers, sera, and frozen hemolyzed blood) and tests (MAT and ELISA) were used in this study (Carlsson *et al.*, 2019). Therefore, differences in seroprevalence could well be related to differences in tests and sample type. In the current study, fluid from thawed heart tissue was used for serology, based on previous work that demonstrated that heart fluid performed better than filter paper eluate, and we used a commercial ELISA that had higher sensitivity, specificity, and reproducibility than MAT (Sharma *et al.*, 2019). Other studies have shown that heart fluid can have higher antibody titers than sera or meat fluid from skeletal muscles when using the IDVET kit (Wallander *et al.*, 2015). Seroprevalence results should be interpreted carefully as the presence of antibodies indicates previous exposure to the parasite, and for *T. gondii*, potentially chronic infection with tissue cysts (Merks *et al.*, 2023). There may well be spatial, temporal and population-level differences in prevalence including sample selection by biologists versus those selected by hunters for harvesting (Kutz *et al.*, 2013); for example, the low prevalence of *T. gondii* that we observed may in part be due to hunter selection of caribou that appeared healthiest, as their primary purpose is for consumption.

DNA of *T. gondii* was not detected in large volumes (100 g) of tissues known to be predilection sites for *T. gondii*, using two different methods. This could be due to the limitations of molecular techniques when detecting DNA of tissue dwelling parasites. For instance, tissue cysts may have been missed in the sections analyzed, leading to false-negative results. Additionally, the tissue prevalence may be underestimated due to the possibility that the parasite burden in naturally infected wildlife (especially herbivores) may be below the detection limit of the test (Wyrosdick and Schaefer, 2015).

Regardless, the low tissue burden of *T. gondii* in caribou in Nunavik, as reported in this study, suggests that the risk of food-borne transmission from caribou to humans is low. This is consistent with recent research indicating that *T. gondii* seroprevalence in Inuit is associated with consumption of other sources, potentially marine animals and geese, rather than caribou and other terrestrial wildlife (Ducrocq *et al.*, 2021). Discrepancies between serology and molecular results for *T. gondii* in wildlife samples, including caribou, have been reported before (Bachand *et al.*, 2019; Bouchard *et al.*, 2022). Differences observed may be linked to animals that contracted the infection at a young age, combined with long-lasting antibody presence and a relatively low parasite burden in herbivores (compared to carnivores). Carnivores, with higher overall tissue burdens, showed a stronger correlation between serology and tissue burden (Sharma *et al.*, 2019; Bouchard *et al.*, 2022).

The only potential sylvatic definitive host for *T. gondii* in Nunavik is the Canadian lynx. Seroprevalence in Canadian lynx in Québec (QC) ranges between 14% and 36% when using MAT (Simon *et al.*, 2013). More recent data using the ELISA IDVET kit reported a seroprevalence of 36% (n = 18/50) in southern QC and 86% (n = 6/7) in Nunavik, and a tissue prevalence of 24% and 86%, respectively (Bouchard *et al.*, 2023). In the same study, only one of 62 lynx was positive for DNA consistent with *T. gondii* in feces. Canadian lynx are, therefore, a potential local source of *T. gondii* exposure for caribou. Further studies are needed, including increased sample size, to better understand the role of the lynx in the *T. gondii* cycle including the transmission from lynx to caribou in Nunavik.

In the current study, seroprevalence for *T. gondii* was significantly higher in caribou harvested in Tasiujaq (27%, Ungava Bay) than Umiujaq (3%, Hudson Bay). This could be due to differences in sample size, demographics, hunter preferences between the two communities, or reflect the true differences in exposure of caribou in the two locations. Our findings differ from previous studies which indicated that the seroprevalence of *T. gondii* was higher in foxes in Hudson Bay (65%) compared to those in Ungava bay (29%) (Bouchard *et al.*, 2022). Differences in diet could account for these findings, with foxes consuming migratory geese or marine foods more likely to be exposed to *T. gondii* than foxes consuming rodents (Bouchard, unpublished).

In the present study, seroprevalence for *N. caninum* in caribou was 5%, lower than overall seroprevalence reported in migratory caribou across Canada (27%), and higher than the 0% reported for the Leaf River herd (Carlsson *et al.*, 2019). Other studies reported 2% from boreal caribou in Canada (Bondo *et al.*, 2019) and 11.5% in Alaska (Stieve *et al.*, 2010). This is the first report of *N. caninum* DNA detected in heart tissue, from a caribou that was sero-negative. Similar to our *T. gondii* findings, this study highlights inconsistencies between serological and molecular testing for *Neospora*. It is possible that these discrepancies are due to biological factors; however, comparing the results of this study with others is challenging, primarily because serology is typically conducted on adults, while molecular tests are performed on aborted fetuses and placenta (Sinnott *et al.*, 2017; Basso *et al.*, 2022). *Neospora caninum* is the major cause of abortion in cattle and can result in ataxia and muscle weakness in calves and farmed red deer (*Cervus elaphus*), but its effects on fertility and health of free ranging cervids, including caribou, have not been described (Florin-Christensen and Schnittger, 2018; Soler *et al.*, 2022). The known definitive hosts for *N. caninum* in North America are domestic dogs (Basso *et al.*, 2001), gray wolves (Dubey *et al.*, 2011) and coyotes (Almería, 2013). In the Nunavik region, it is likely that wolves are involved, as well as sled dogs living in the communities (Salb *et al.*, 2008). Further work is needed to determine definitive hosts, transmission, and wildlife health significance of *N. caninum* in Nunavik and elsewhere in the Canadian North.

The high tissue prevalence of *Sarcocystis* DNA observed in this study was not entirely surprising. While it is possible that there may have been some cross contamination at the sampling level by hunters, high prevalence of *Sarcocystis* in muscle tissue has been reported previously in woodland and barren-ground caribou from Newfoundland and Labrador (Khan and Evans, 2006). While there was no significant difference in prevalence of *Neospora* in heart tissue between the two communities in this study, prevalence of *Sarcocystis* DNA in muscle samples was significantly higher in Tasiujaq (64%) compared to Umiujaq (19%). This might; however, be attributed to the differences in muscle sample selection by the two communities. Hunters from Umiujaq typically provided more flexor and extensor muscles, whereas those from Tasiujaq tended to submit larger leg muscle samples from the quadriceps or biceps. It is possible that *Sarcocystis* has a preference for larger leg muscles over flexor and extensor muscles.

This study represents the first report of species-level identification of *Sarcocystis* spp. in caribou in Canada. In heart, we found only *Sarcocystis grueneri,* which has been reported in heart tissue from *Rangifer* species in Norway by microscopic and molecular methods (Gjerde, 1984; Dahlgren and Gjerde, 2007). Three of the five species that we identified in muscle tissue (*S. rangi, S. tarandivulpes and S. rangiferi*) had also been previously reported in *Rangifer* species from Norway and Iceland (Dahlgren and Gjerde, 2007; Dahlgren *et al.*, 2007) as well as *S. tarandi*, one of the Group IV species in the present study. The ML analysis did not showed support for the *Sarcocystis* spp. present in group IV; consequently, the classification at species level of these sequences is not clear. *Sarcocystis scandinavica,* previously reported in moose (*Alces alces*) from Norway (Dahlgren and Gjerde, 2008), was identified in one sample. The presence of similar *Sarcocystis* spp. in caribou in Canada and Fennoscandia, as reported in this study, could be attributed to the introduction of animals from Europe. For example, the similarity between the species of *Sarcocystis* found in reindeer in Norway and Iceland suggest a likely introduction of these parasites through the importation of semi-domestic *Rangifer* species from Norway to Iceland (Dahlgren *et al.*, 2007). It is possible that a similar situation ocurred in Eastern Canada with the introduction of Scandinavian *Rangifer* in 1908 (Khan and Evans, 2006). The definitive hosts for the *Sarcocystis* species identified in the present study are most likely canids such as wolves, red foxes, arctic foxes and domestic dogs (Gjerde, 1985; Salb *et al.*, 2008; Lesniak *et al.*, 2018). According to Indigenous and local knowledge, wolf and fox populations have increased in Nunavik during the last decade, possibly increasing the risk of transmission of *Sarcocystis* to caribou. Migration of definitive hosts between Europe and Canada could also explain the presence of similar species of *Sarcocystis* in *Rangifer* in Canada and Fennoscandia. Arctic foxes move long distances on sea ice; for example, from Svalbard to Canada, representing an additional possible route of introduction (Fuglei and Tarroux, 2019).

Finding *Besnoitia* in skeletal muscle samples was somewhat unexpected as the primers used targeted *Sarcocystis*. However, *Besnoitia* spp. are not uncommon in caribou populations from Canada and Alaska and have been recorded in caribou from Québec and Labrador (Ducrocq *et al.*, 2013; Schares *et al.*, 2019). While *Besnoitia* infection is more commonly associated with dermal lesions, the parasite does undergo a muscle dissemination stage. It is also possible that hunters cut through the skin, possibly contaminating the muscle sample submitted for screening. Hunters did not report signs of besnoitiosis in the caribou harvested for this study; however, the disease might

have a significant impact in caribou health, therefore, monitoring these populations is recommended.

The phylogenetic tree had a congruent topology with previous studies comparing parasites from the Sarcocystidae family infecting ruminants, including members of the genus *Rangifer* (Dahlgren, Gouveia-Oliveira and Gjerde, 2008; Gjerde, 2013). The reference sequence of *Cystoisospora belli* (DQ060683) was not surprisingly recovered in the subfamily Toxoplasmatinae, as *Isospora* is known to be polyphyletic (Dahlgren, Gouveia-Oliveira and Gjerde, 2008). Groups I, II and III showed well-supported and close relationships, suggesting that the species in these groups share an evolutionary history and might have similar definitive hosts. In the same way, groups IV, V, VI and VII may share definitive hosts that differ from those of Groups I-III (Dahlgren, Gouveia-Oliveira and Gjerde, 2008). Further investigation into *Sarcocystis* spp. is needed to explain the wide diversity of this group of parasites, which may reflect differences at the genus, rather than species, level.

2.6 Conclusions

This study suggests that the risk of human exposure and transmission of *T. gondii,* a food-borne parasite, through consumption and handling of harvested caribou is relatively low, but not zero. The relatively low prevalence of *T. gondii* and *N. caninum* in caribou in Nunavik reported in this study also suggests a low impact on caribou health and reproduction, although it is likely that hunters harvest the healthiest animals in a herd. The high prevalence and diversity of *Sarcocystis* *s*pp. found in this study suggests intact trophic relationships between caribou and their likely definitive hosts, such as wolves and foxes, in Nunavik. As well, the close relationship of *Sarcocystis* species in caribou in Nunavik to those found in *Rangife*r from Norway and Iceland provides insight into connectivity and phylogeography of both hosts and parasites. Further research is needed to understand effects of tissue coccidians on caribou health and their implications for Inuit food safety and security. Investigating the cycles of the parasites and their transmission to caribou through their predators, as well as the history of *Rangifer* and the parasites across the Arctic, are important areas for future research.

2.7 Acknowledgements

We thank all hunters and community coordinators that contributed to this study by submitting samples, in particular Elena Berthe and Eddie Kumarluk. We are thankful to the personnel at the Nunavik Research Centre (Makivvik Corp.), regional and local Hunting Fishing Trapping Associations for storage of the samples, logistic help, and transport, and for sharing their knowledge. We thank to the Nunavik Regional Board of Health and Social Services for their valuable feedback. We thank Brent Wagner, Yunxiu Dai, Champika Fernando, Michelle Sniatynski and Marìa Jarque for helping in laboratory analyses. This study was supported by the Natural Science and Engineering Research Council of Canada, ArcticNet Networks of Centres of Excellence, Polar Knowledge, and Consejo Nacional de Ciencia y Tecnología (CONACyT,

Mexico).

2.8 Supplementary material

Supplementary Table. Sequences (18S rRNA) generated in the present study with designated *Sarcocystis* and related coccidian species names, tissue from which the organism was recovered, %ID with Blast, bootstrap values obtained from the data-display network (DDN) and maximum likelihood (ML) topology, accession numbers from GenBank and for BOLDSystem.

Sample	Host	Tissue source	Inferred identity	BLAST (% ID)	DDN bs	ML bs	GenBank accession number	BOLD Systems number
Ch01	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (97.73)*	100	96	OQ740639	HOZ001-23
Ch02	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (97.91)*	100	96	OQ740647	HOZ002-23
Ch03	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (94.88)*	100	96	OQ740646	HOZ003-23
Ch04	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (95.89)*	100	96	OQ740645	HOZ004-23
Ch05	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (97.31)*	100	96	OQ740644	HOZ005-23
Ch06	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.5)*	100	96	OQ740643	HOZ006-23
Ch08	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (94.84)*	100	96	OQ740642	HOZ007-23
Ch10	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740641	HOZ008-23
Ch12	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740640	HOZ009-23
Ch14	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.67)*	100	96	OQ740661	HOZ010-23
Ch17	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740660	HOZ011-23
Ch22	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740659	HOZ012-23
Ch23	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740658	HOZ013-23
Ch25	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.84)*	100	96	OQ740657	HOZ014-23
Ch26	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740656	HOZ015-23
Ch72	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.83)*	100	96	OQ740655	HOZ016-23
Ch75	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.82)*	100	96	OQ740654	HOZ017-23
Ch76	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.77)*	100	96	OQ740653	HOZ018-23
Ch78	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740652	HOZ019-23
Ch82	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740651	HOZ020-23
Ch83	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740650	HOZ021-23
Ch85	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (100)*	100	96	OQ740649	HOZ022-23

Ch90	*Rangifer tarandus*	Heart	*S. grueneri*	*S. grueneri (99.63)*	100	96	OQ740648	HOZ023-23
Cm02	*Rangifer tarandus*	Muscle	*S. tarandivulpes*	*S. tarandivulpes (99.13)*	95	97	OQ740669	HOZ024-23
Cm05	*Rangifer tarandus*	Muscle	*S. rangiferi*	*S. ranginferi (100)*	94	99	OQ740664	HOZ025-23
Cm11	*Rangifer tarandus*	Muscle	*S. tarandivulpes*	*S. tarandivulpes (99.67)*	95	97	OQ740667	HOZ026-23
Cm14	*Rangifer tarandus*	Muscle	*Group IV member*	*S. tarandi (100)*	61	-	OQ740638	HOZ027-23
Cm23	*Rangifer tarandus*	Muscle	*Besnoitia sp*	*Besnoitia sp (100)*	46	54	OQ740632	HOZ028-23
Cm25	*Rangifer tarandus*	Muscle	*Besnoitia sp*	*Besnoitia sp (100)*	46	54	OQ740631	HOZ029-23
Cm26	*Rangifer tarandus*	Muscle	*Besnoitia sp*	*Besnoitia sp (100)*	46	54	OQ740633	HOZ030-23
Cm49	*Rangifer tarandus*	Muscle	*Group IV member*	*S. tarandi (97.58)*	61	-	OQ740634	HOZ031-23
Cm55	*Rangifer tarandus*	Muscle	*S. tarandivulpes*	*S. tarandivulpes (100)*	95	97	OQ740668	HOZ032-23
Cm56	*Rangifer tarandus*	Muscle	*Group IV member*	*S. tarandi (99.82)*	61	-	OQ740635	HOZ033-23
Cm64	*Rangifer tarandus*	Muscle	*S. rangiferi*	*S. ranginferi (100)*	94	99	OQ740665	HOZ034-23
Cm68	*Rangifer tarandus*	Muscle	*Group IV member*	*S. tarandi (99.83)*	61	-	OQ740636	HOZ035-23
Cm69	*Rangifer tarandus*	Muscle	*S. rangi*	*S. rangi (99.84)*	100	90	OQ740662	HOZ036-23
Cm71	*Rangifer tarandus*	Muscle	*S. scandinavica*	*S. scandinavica (99.83)*	100	100	OQ740666	HOZ037-23
Cm80	*Rangifer tarandus*	Muscle	*Group IV member*	*S. tarandi (98.85)*	61	-	OQ740637	HOZ038-23
Cm84	*Rangifer tarandus*	Muscle	*S. rangi*	*S. rangi (99.83)*	100	90	OQ740663	HOZ039-23

2.9 References

Almería, S. (2013) ‘*Neospora caninum* and Wildlife’, *ISRN Parasitol*, 2013, pp. 1–23. Available at: https://doi.org/10.5402/2013/947347.

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E. and Jenkins, E. (2019) ‘Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada’, *Parasit Vectors*, 12(1), p. 155. Available at: https://doi.org/10.1186/s13071-019-3408-9.

Barry, R., Nissly, R.H., Feria, W., Thirumalapura, N., Tewari, D., Jayarao, B.M. and Kuchipudi, S.V. (2019) ‘A probe-based real-time PCR assay for the detection of *Neospora caninum* in clinical samples from cattle’, *Vet Parasitol*, 269, pp. 2–6. Available at: https://doi.org/10.1016/j.vetpar.2019.04.002.

Basso, W., Holenweger, F., Schares, G., Müller, N., Campero, L.M., Ardüser, F., Moore-Jones, G., Frey, C.F. and Zanolari, P. (2022) ‘*Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted foetuses’, *Food Waterborne Parasitol*, 28, p. e00176. Available at: https://doi.org/10.1016/j.fawpar.2022.e00176.

Basso, W., Venturini, L., Venturini, M.C., Hill, D.E., Kwok, O.C.H., Shen, S.K. and Dubey, J.P. (2001) ‘First isolation of *Neospora caninum* from the feces of a naturally infected dog’, *J Parasitol*, 87(3), pp. 612–618. Available at: https://doi.org/10.1645/0022-3395(2001)087[0612:FIONCF]2.0.CO;2.

Bondo, K.J., Macbeth, B., Schwantje, H., Orsel, K., Culling, D., Culling, B., Tryland, M., Nymo, I.H. and Kutz, S. (2019) ‘Health survey of boreal caribou (*Rangifer tarandus caribou*) in Northeastern British Columbia, Canada’, *J Wildl Dis*, 55(3), p. 544. Available at: https://doi.org/10.7589/2018-01-018.

Bouchard, É., Sharma, R., Bachand, N., Gajadhar, A.A. and Jenkins, E.J. (2017) ‘Pathology, clinical signs, and tissue distribution of *Toxoplasma gondii* in experimentally infected reindeer

(*Rangifer tarandus*)', *Int J Parasitol Parasites Wildl*, 6(3), pp. 234–240. Available at: https://doi.org/10.1016/j.ijppaw.2017.08.004.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Buhler, K., Al-Adhami, B., Su, C., Fenton, H., G.-Gouin, G., Roth, J.D., Rodrigues, C.W., Pamak, C., Simon, A., Bachand, N., Leighton, P. and Jenkins, E. (2022) 'Are foxes (*Vulpes* spp.) good sentinel species for *Toxoplasma gondii* in northern Canada?', *Parasit Vectors*, 15(1), p. 115. Available at: https://doi.org/10.1186/s13071-022-05229-3.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Jung, T.S., Harms, N.J., Willier, C.N., Boonstra, R., Majchrzak, Y.N., Peers, M.J.L., Gouin, G.-G., Al-Adhami, B., Simon, A., Leighton, P. and Jenkins, E.J. (2023) 'Canada Lynx (*Lynx canadensis*) as potential reservoirs and sentinels of *Toxoplasma gondii* in Northern Canada', *Zoonotic Dis*, 3(1), pp. 6–17. Available at: https://doi.org/10.3390/zoonoticdis3010002.

Carlsson, A.M., Curry, P., Elkin, B., Russell, D., Veitch, A., Branigan, M., Campbell, M., Croft, B., Cuyler, C., Côté, S.D., Leclerc, L.-M., Tryland, M., Nymo, I.H. and Kutz, S.J. (2019) 'Multi-pathogen serological survey of migratory caribou herds: A snapshot in time', *PLoS One*. Edited by J.P. Stewart, 14(7), p. e0219838. Available at: https://doi.org/10.1371/journal.pone.0219838.

Chester, S.R. (2016) *The arctic guide: wildlife of the far north*. 1st edition. Princeton, NJ: Princeton University Press.

Dahlgren, S.S. and Gjerde, B. (2007a) 'Genetic characterisation of six *Sarcocystis* species from reindeer (*Rangifer tarandus tarandus*) in Norway based on the small subunit rRNA gene', *Vet Parasitol*, 146(3–4), pp. 204–213. Available at: https://doi.org/10.1016/j.vetpar.2007.02.023.

Dahlgren, S.S. and Gjerde, B. (2008) '*Sarcocystis* in moose (*Alces alces*): molecular identification and phylogeny of six *Sarcocystis* species in moose, and a morphological description of three new species', *Parasitol Res*, 103(1), pp. 93–110. Available at: https://doi.org/10.1007/s00436-008-0936-1.

Dahlgren, S.S., Gjerde, B., Skirnisson, K. and Gudmundsdottir, B. (2007) 'Morphological and molecular identification of three species of *Sarcocystis* in reindeer (*Rangifer tarandus tarandus*)

in Iceland', *Vet Parasitol*, 149(3–4), pp. 191–198. Available at: https://doi.org/10.1016/j.vetpar.2007.08.015.

Dahlgren, S.S., Gouveia-Oliveira, R. and Gjerde, B. (2008) 'Phylogenetic relationships between *Sarcocystis* species from reindeer and other Sarcocystidae deduced from ssu rRNA gene sequences', *Vet Parasitol*, 151(1), pp. 27–35. Available at: https://doi.org/10.1016/j.vetpar.2007.09.029.

Dubey, J.P. (2010) *Toxoplasmosis of animals and humans*. 2nd ed. Boca Raton: CRC Press.

Dubey, J.P., Calero-Bernal, R., Rosenthal, B.M., Speer, C.A. and Fayer, R. (2015) *Sarcocystosis of Animals and Humans, Second Edition*. Boca Raton: CRC Press.

Dubey, J.P., Jenkins, M.C., Rajendran, C., Miska, K., Ferreira, L.R., Martins, J., Kwok, O.C.H. and Choudhary, S. (2011) 'Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*', *Vet Parasitol*, 181(2–4), pp. 382–387. Available at: https://doi.org/10.1016/j.vetpar.2011.05.018.

Ducrocq, J., Beauchamp, G., Kutz, S., Simard, M., Elkin, B., Croft, B., Taillon, J., Côté, S.D., Brodeur, V., Campbell, M., Cooley, D., Cuyler, C. and Lair, S. (2012) 'Comparison of gross visual and microscopic assessment of four anatomic sites to monitor *Besnoitia tarandi* in barren-ground caribou (*Rangifer tarandus*)', *J Wildl Dis*, 48(3), pp. 732–738. Available at: https://doi.org/10.7589/0090-3558-48.3.732.

Ducrocq, J., Beauchamp, G., Kutz, S., Simard, M., Taillon, J., Côté, S.D., Brodeur, V. and Lair, S. (2013) 'Variables associated with *Besnoitia tarandi* prevalence and cyst density in barren-ground caribou (*Rangifer tarandus*) populations', *J Wildl Dis*, 49(1), pp. 29–38. Available at: https://doi.org/10.7589/2012-05-125.

Ducrocq, J., Ndao, M., Yansouni, C.P., Proulx, J., Mondor, M., Hamel, D., Lévesque, B., De Serres, G. and Talbot, D. (2021) 'Epidemiology associated with the exposure to *Toxoplasma gondii* in Nunavik's Inuit population using the 2017 *Qanuilirpitaa* cross-sectional health survey', *Zoonoses Public Health*, 68(7), pp. 803–814. Available at: https://doi.org/10.1111/zph.12870.

Festa-Bianchet, M., Ray, J.C., Boutin, S., Côté, S.D. and Gunn, A. (2011) ‘Conservation of caribou (*Rangifer tarandus*) in Canada: an uncertain future’, *Can J Zool*, 89(5), pp. 419–434. Available at: https://doi.org/10.1139/z11-025.

Florin-Christensen, M. and Schnittger, L. (eds) (2018) *Parasitic Protozoa of Farm Animals and Pets*. Cham: Springer International Publishing. Available at: https://doi.org/10.1007/978-3-319-70132-5.

Fuglei, E. and Tarroux, A. (2019) ‘Arctic fox dispersal from Svalbard to Canada: one female’s long run across sea ice’, *Polar Res*, 38(0). Available at: https://doi.org/10.33265/polar.v38.3512.

Gjerde, B. (1984) ‘A light microscopic comparison of the cysts of four species of *Sarcocysti*s infecting the domestic reindeer (*Rangifer tarandus*) in Northern Norway’, *Acta Vet Scand*, 25(2), pp. 195–204. Available at: https://doi.org/10.1186/BF03547264.

Gjerde, B. (1985) ‘the fox as a definitive host for *Sarcocystis rangi* from reindeer (*Rangifer tarandus tarandus*)’, *Acta Vet Scand*, 26(1), pp. 140–142. Available at: https://doi.org/10.1186/BF03546572.

Gjerde, B. (2013) ‘Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene’, *Int J Parasitol*, 43(7), pp. 579–591. Available at: https://doi.org/10.1016/j.ijpara.2013.02.004.

Goyette, S., Cao, Z., Libman, M., Ndao, M. and Ward, B.J. (2014) ‘Seroprevalence of parasitic zoonoses and their relationship with social factors among the Canadian Inuit in Arctic regions’, *Diagn Microbiol Infect Dis*, 78(4), pp. 404–410. Available at: https://doi.org/10.1016/j.diagmicrobio.2013.08.026.

Gunn, A., Canada, Environment Canada, and Canadian Councils of Resource Ministers (2011) *Northern caribou population trends in Canada*. Ottawa: Canadian Councils of Resource Ministers.

Homan, W.L., Vercammen, M., De Braekeleer, J. and Verschueren, H. (2000) ‘Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for

diagnostic and quantitative', *Int J Parasitol*, 30(1), pp. 69–75. Available at: https://doi.org/10.1016/S0020-7519(99)00170-8.

Huson, D.H. and Bryant, D. (2006) 'Application of phylogenetic networks in evolutionary studies.', *Mol Biol Evol*, 23(2), pp. 254–67. Available at: https://doi.org/10.1093/molbev/msj030.

Johnson-Down, L., Allaire, J., Little, M. and Ayotte, P. (2021) *Country and Market Food Consumption and Nutritional Status. Nunavik Inuit Health Survey 2017 Qanuilirpitaa? How are we now?* Available at: https://nrbhss.ca/sites/default/files/health_surveys/Country_Food_and_Market_Food_Consumption_and_Nutritional_Status_fullreport_en.pdf (Accessed: 4 July 2022).

Katoh, K., Rozewicki, J. and Yamada, K.D. (2019) 'MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization', *Brief Bioinform*, 20(4), pp. 1160–1166. Available at: https://doi.org/10.1093/bib/bbx108.

Kenny, T.-A. and Chan, H.M. (2017) 'Estimating wildlife harvest based on reported consumption by Inuit in the Canadian Arctic', *Arctic*, 70(1). Available at: https://doi.org/10.14430/arctic4625.

Kenny, T.-A., Fillion, M., Simpkin, S., Wesche, S.D. and Chan, H.M. (2018) 'Caribou (*Rangifer tarandus*) and Inuit nutrition security in Canada', *EcoHealth*, 15(3), pp. 590–607. Available at: https://doi.org/10.1007/s10393-018-1348-z.

Kenny, T.-A., Hu, X.F., Kuhnlein, H.V., Wesche, S.D. and Chan, H.M. (2018) 'Dietary sources of energy and nutrients in the contemporary diet of Inuit adults: results from the 2007–08 Inuit Health Survey', *Public Health Nutr*, 21(7), pp. 1319–1331. Available at: https://doi.org/10.1017/S1368980017003810.

Khan, R.A. and Evans, L. (2006) 'Prevalence of *Sarcocystis* spp. in two subspecies of caribou (*Rangifer tarandus*) in Newfoundland and Labrador, and foxes (*Vulpes vulpes*), wolves (*Canis lupus*), and Husky dogs (*Canis familiaris*) as potential definitive hosts', *J Parasitol*, 92(3), pp. 662–663. Available at: https://doi.org/10.1645/GE-753R1.1.

Kumar, S., Stecher, G. and Tamura, K. (2016) 'MEGA7: Molecular Evolutionary Genetics Analysis version 7.0.', *Molecular Biology and Evolution* [Preprint].

Kutz, S., Ducrocq, J., Cuyler, C., Elkin, B., Gunn, A., Kolpashikov, L., Russell, D. and White, R.G. (2013) 'Standardized monitoring of Rangifer health during International Polar Year', *Rangifer*, pp. 91–114. Available at: https://doi.org/10.7557/2.33.2.2532.

Lesniak, I., Heckmann, I., Franz, M., Greenwood, A.D., Heitlinger, E., Hofer, H. and Krone, O. (2018) 'Recolonizing gray wolves increase parasite infection risk in their prey', *Ecol Evol*, 8(4), pp. 2160–2170. Available at: https://doi.org/10.1002/ece3.3839.

Lindsay, D.S. and Dubey, J.P. (2020) 'Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants', *Vet Clin North Am Food Anim Pract*, 36(1), pp. 205–222. Available at: https://doi.org/10.1016/j.cvfa.2019.11.004.

McLeod, R., Lykins, J., Gwendolyn Noble, A., Rabiah, P., Swisher, C.N., Heydemann, P.T., McLone, D., Frim, D., Withers, S., Clouser, F. and Boyer, K. (2014) 'Management of congenital Toxoplasmosis', *Curr Pediatr Rep*, 2(3), pp. 166–194. Available at: https://doi.org/10.1007/s40124-014-0055-7.

Merks, H., Boone, R., Janecko, N., Viswanathan, M. and Dixon, B.R. (2023) 'Foodborne protozoan parasites in fresh mussels and oysters purchased at retail in Canada', *Int J Food Microbiol*, 399, p. 110248. Available at: https://doi.org/10.1016/j.ijfoodmicro.2023.110248.

Michaels, L., Rejmanek, D., Aguilar, B., Conrad, P. and Shapiro, K. (2016) 'California mussels (*Mytilus californianus*) as sentinels for marine contamination with *Sarcocystis neurona*', *Parasitol*, 143(6), pp. 762–769. Available at: https://doi.org/10.1017/S0031182016000354.

Moré, G., Abrahamovich, P., Jurado, S., Bacigalupe, D., Marin, J.C., Rambeaud, M., Venturini, L. and Venturini, M.C. (2011) 'Prevalence of *Sarcocystis* spp. in Argentinean cattle', *Vet Parasitol*, 177(1–2), pp. 162–165. Available at: https://doi.org/10.1016/j.vetpar.2010.11.036.

Opsteegh, M., Langelaar, M., Sprong, H., den Hartog, L., De Craeye, S., Bokken, G., Ajzenberg, D., Kijlstra, A. and der Giessen, J. van (2010) 'Direct detection and genotyping of *Toxoplasma*

gondii in meat samples using magnetic capture and PCR’, *Int J Food Microbiol*, 139(3), pp. 193–201. Available at: https://doi.org/10.1016/j.ijfoodmicro.2010.02.027.

Robert-Gangneux, F. and Dardé, M.-L. (2012) ‘Epidemiology of and diagnostic strategies for Toxoplasmosis’, *Clin Microbiol Rev*, 25(2), pp. 264–296. Available at: https://doi.org/10.1128/CMR.05013-11.

Roberts, L.S., Janovy, J., Nadler, S. and Roberts, L.S. (2013) *Gerald D. Schmidt & Larry S. Roberts’ foundations of parasitology*. Ninth edition. New York: McGraw Hill.

Salb, A.L., Barkema, H.W., Elkin, B.T., Thompson, R.C.A., Whiteside, D.P., Black, S.R., Dubey, J.P. and Kutz, S.J. (2008) ‘Dogs as sources and sentinels of parasites in humans and wildlife, Northern Canada’, *Emerg Infect Dis*, 14(1), pp. 60–63. Available at: https://doi.org/10.3201/eid1401.071113.

Schares, G., Jutras, C., Bärwald, A., Basso, W., Maksimov, A., Schares, S., Tuschy, M., Conraths, F.J. and Brodeur, V. (2019) ‘*Besnoitia tarandi* in Canadian woodland caribou – Isolation, characterization and suitability for serological tests’, *Int J Parasitol Parasites Wildl*, 8, pp. 1–9. Available at: https://doi.org/10.1016/j.ijppaw.2018.11.005.

Sergeant, E. (2018) *Epitools Epidemiological Calculators. Ausvet.* Available at: https://epitools.ausvet.com.au/ (Accessed: 2 April 2022).

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N. and Jenkins, E. (2019) ‘Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of *Toxoplasma gondii* in naturally infected wolverines (*Gulo gulo*) from the Yukon, Canada’, *Food Waterborne Parasitol*, 15, p. e00046. Available at: https://doi.org/10.1016/j.fawpar.2019.e00046.

Simon, A., Poulin, M.B., Rousseau, A.N., Dubey, J.P. and Ogden, N.H. (2013) ‘Spatiotemporal dynamics of *Toxoplasma gondii* infection in canadian lynx (*Lynx canadensis*) in Western Québec, Canada’, *J Wildl Dis*, 49(1), pp. 39–48. Available at: https://doi.org/10.7589/2012-02-048.

Sinnott, F.A., Monte, L.G., Collares, T.F., Silveira, R.M. and Borsuk, S. (2017) 'Review on the immunological and molecular diagnosis of Neosporosis (years 2011–2016)', *Vet Parasitol*, 239, pp. 19–25. Available at: https://doi.org/10.1016/j.vetpar.2017.04.008.

Soler, J.P., Moré, G., Urtizbiría, F., Hecker, Y.P., Cirone, K.M., Scioli, M.V., Paolicchi, F.A., Fiorentino, M.A., Uriarte, E.L.L., Cantón, G.J., Verna, A.E., Morrell, E.L. and Moore, D.P. (2022) 'Epidemic abortions due to *Neospora caninum* infection in farmed red deer (*Cervus elaphus*)', *Parasitol Res*, 121(5), pp. 1475–1485. Available at: https://doi.org/10.1007/s00436-022-07488-6.

Stamatakis, A. (2014) 'RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies', *Bioinform*, 30(9), pp. 1312–1313. Available at: https://doi.org/10.1093/bioinformatics/btu033.

Stieve, E., Beckmen, K., Kania, S.A., Widner, A. and Patton, S. (2010) '*Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Alaska wildlife', *J Wildl Dis*, 46(2), pp. 348–355. Available at: https://doi.org/10.7589/0090-3558-46.2.348.

Taillon, J., Brodeur, V. and Rivard, S. (2016) *Biological status report of migratory caribou, Leaf River herd*. Québec: Ministère des forêts, de la faune et des parcs. Available at: mffp.gouv.qc.ca.

Wallander, C., Frössling, J., Vågsholm, I., Burrells, A. and Lundén, A. (2015) '"Meat Juice" Is Not a Homogeneous Serological Matrix', *Foodborne Pathog Dis*, 12(4), pp. 280–288. Available at: https://doi.org/10.1089/fpd.2014.1863.

Wyrosdick, H.M. and Schaefer, J.J. (2015) '*Toxoplasma gondii* : history and diagnostic test development', *Anim Health Res Rev*, 16(2), pp. 150–162. Available at: https://doi.org/10.1017/S1466252315000183.

TRANSITION STATEMENT CHAPTER 3

Continuing my investigation into potential sources of *T. gondii* for Inuit communities, this chapter focused on hunter-harvested beluga whales from the Eastern Beaufort Sea in the Northwest Territories. The harvest of this beluga population by Inuvialuit has been happening for more than 500 years and remains integral to their cultural heritage and diet. In 2014, previous researchers detected *T. gondii* in this beluga population, raising concerns that this parasite posed a new threat to human and beluga health. Therefore, in collaboration with local hunters and the Department of Fisheries and Oceans Canada, I tested serum and tissue samples collected from harvested beluga population in this region starting in 2015. My primary objective was to examine serum samples for antibodies against *T. gondii*, employing different commercial kits and protocols to ensure the consistency and reliability of our results. This chapter not only delves into the challenges of serology in wildlife samples, but also used molecular techniques for detecting *T. gondii* DNA, including the magnetic capture qPCR. Although the focus was on *T. gondii*, samples were also tested for another food borne parasite, *Trichinella* spp. (for which human infections have been recorded from other marine mammals, such as polar bear and walrus), which was fortunately not detected. Overall, these findings support that food borne parasites are not highly prevalent in belugas harvested for human consumption in this population at the current time. Finally, I detected DNA of a related coccidian parasite, *Sarcocystis* spp., which was genetically distinct from terrestrial species of this parasite, raising questions about the life cycle of the parasite and suggesting a potential marine cycle involving beluga whales and their predators. This work is in review by the Journal “Diseases of Aquatic Organisms”, on which I serve as first author. I conducted the laboratory analyses since 2017, all the data analyses and statistics, and ensured results were reported to the relevant Hunter Trapper Organizations, the Fisheries Joint Management Council, and the government of the Northwest Territories.

CHAPTER 3. LOW PREVALENCE OF ZOONOTIC FOOD-BORNE PARASITES *TOXOPLASMA GONDII* AND *TRICHINELLA* SPP., BUT HIGH PREVALENCE OF *SARCOCYSTIS* SPP., IN EASTERN BEAUFORT SEA BELUGA WHALES (*DELPHINAPTERUS LEUCAS*) HARVESTED IN THE CANADIAN ARCTIC

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3.1 Abstract

Beluga whales (*Delphinapterus leucas*) play a significant role as a traditional food source for Inuit communities in the Canadian Arctic, raising concerns about zoonotic parasites. The aim of this study was for harvesters, government, and veterinary researchers to jointly determine prevalence of selected parasites in beluga whales harvested from the Eastern Beaufort Sea population between 2014 and 2021 using serological assays, histology and PCR. Multiple serological assays were conducted on blood samples of 120 belugas. Among these assays, a commercial ELISA and IFAT were assessed to be the most reproducible, and detected antibodies to *Toxoplasma gondii* in less than 1% of belugas, before and after lipid removal. The overall tissue prevalence for DNA of *T. gondii* was 1% (n=1/97) based on magnetic capture DNA extraction using primers targeting a 529 bp repeat element unique to *T. gondii*. *Trichinella* larvae were not detected in diaphragms (n=42) or tongues (n=108) by the pepsin/HCl artificial digestion method. Sarcocysts were observed on histology in 6/26 animals examined in 2015-16 and DNA of an unidentified species of *Sarcocystis* was detected in the skeletal muscle of 59% (n=46/78) of belugas using a nested PCR with pan-apicomplexan primers. Phylogenetic analyses indicate a close relationship between the *Sarcocystis* found in belugas and in other marine, but not terrestrial, mammals, suggesting a unique marine life cycle requiring further investigation to determine definitive hosts and transmission routes. Overall, due to low prevalence of *T. gondii* and *Trichinella* spp., our findings suggest that the risk of human transmission through the consumption of belugas is low.

3.2 Introduction

Beluga whales (*Delphinapterus leucas*) are toothed cetaceans (suborder Odontoceti) widely distributed across the circumpolar Arctic (Würsig, Thewissen and Kovacs, 2018). These whales hold great importance for northern Indigenous communities. Beluga whales rank among the top five most frequently consumed country foods by Inuit, and between 9% to 60% of the Inuit population relies on them for nutrition and food security (Sheikh *et al.*, 2011; Kenny *et al.*, 2018; Johnson-Down *et al.*, 2021) In the western Canadian Arctic, beluga serve as a crucial food source for Inuvialuit, and the harvesting, preparation and sharing of qilalugaq (beluga in the Inuvialuit language) is an integral part of Inuvialuit culture (Waugh *et al.*, 2018; Ovitz et al., 2024).

For more than 500 years, Inuvialuit have been harvesting belugas from the Eastern Beaufort Sea (EBS) population in the Mackenzie Estuary (Fig. 3.1) (McGhee, 1988; Friesen and Arnold, 1995). In the 1970's, an oil and gas industry established the first harvest monitoring, and in the 1980's the monitoring was standardized by the Department of Fisheries and Oceans (DFO) in the 1980s (Harwood *et al.*, 2002). Since 1989, the Canada/Inuvialuit Fisheries Joint Management Committee (FJMC), which includes delegates of the Inuvialuit Game Council and members appointed by the Minister of DFO, manages the harvest of EBS belugas (FJMC, 2013).

Assessment of beluga health is a key component of the management strategy, and is monitored from both western science and Inuvialuit knowledge perspectives (Loseto *et al.*, 2018; Ostertag *et al.*, 2018). The EBS beluga population is considered to be stable and currently not of conservation concern (COSEWIC, 2004).

Fig. 3.1 Map of the Inuvialuit Settlement Region (ISR).

Showing the Mackenzie Estuary (orange circle), the approximately location of Hendrickson Island (yellow pin), East Whitefish station (red pin) and Tuktoyaktuk (blue pin). Map obtained from DFO website (dfo-mpo.gc.ca/oceans) and edited by BioRender.com

Harvesting and consumption of wild animals could pose a potential risk of transmission of zoonotic parasites such as the apicomplexan *Toxoplasma gondii* and the nematode *Trichinella* spp. to humans. Toxoplasmosis is considered to be the most prevalent parasitic infection in people in northern communities in Canada (Goyette *et al.*, 2014), and, in some communities, exposure has been associated with the consumption of marine food sources, such as bivalves, belugas and walrus (Ducrocq *et al.*, 2021). Marine transmission of *T. gondii* is of concern for both human health (Jenkins *et al.*, 2015; Murray, 2023) and beluga health, as the parasite has been isolated from stranded belugas from eastern Canada (Mikaelian *et al.*, 2000; Iqbal *et al.*, 2018). An unpublished report of *T. gondii* and a related parasite (*Sarcocystis* sp.) in the EBS beluga population (Haman *et al.*, 2013) raised community concerns (Murray, 2023) as none of the preparation methods (fresh, dried, boiled or smoked) (Binnington *et al.*, 2017; Waugh *et al.*, 2018) of beluga products in the Inuvialuit Settlement Region (ISR) would not necessarily inactivate *T. gondii* (Jones and Dubey, 2012).

Trichinella spp. are nematodes that causes trichinellosis, a foodborne zoonotic disease with worldwide distribution (Pozio, 2007; Gajadhar, 2015). In the Canadian Arctic, *Trichinella* spp. rank as the second most prevalent parasitic infection in the human population (Goyette *et al.*, 2014). The way in which primarily fish- and invertebrate-eating marine mammals become infected with *Trichinella* spp. remains somewhat puzzling, as the parasite typically requires the ingestion of meat from terrestrial carnivores. Outbreaks in humans have been associated with the consumption of raw, dried or fermented meat from carnivorous wildlife hosts, including bears and walruses in the North American Arctic (Gottstein *et al.*, 2009; Jenkins *et al.*, 2013; Springer *et al.*, 2017). Infections observed in walruses (Gajadhar and Forbes, 2010) have raised concerns about the potential infection of belugas and other marine mammals with this parasite. To our knowledge, there are not reports or findings of *Trichinella* in the EBS beluga population.

Only a limited number of *Sarcocystis* spp. are known to be zoonotic (*S. hominis*, *S. heydorni*, *S. suihominis*), and their transmission to humans is associated with consumption of beef and pork (Dubey *et al.*, 2016; Rosenthal, 2021). Currently, there is no known risk of transmission of *Sarcocystis* spp. from marine mammals to humans. Microscopic observations of *Sarcocystis* spp. in beluga whales have not shown any associated significant pathology (De Guise *et al.*, 1993). Nevertheless, reports have linked one species, *Sarcocystis neurona*, to cases of encephalitis in a

broad diversity of marine mammals (Dubey *et al.*, 2003; Gibson *et al.*, 2011; Barbosa *et al.*, 2015), in belugas, however, *S. neurona* has not been reported. The lack of information about *Sarcocystis* spp. in marine mammals raises a need for further investigation on the parasites diversity and transmission in beluga whales through molecular and phylogenetic analyses.

Both *T. gondii* and *Sarcocystis* spp. have a predator-prey life cycle that relies on a definitive host (predator) releasing infectious stages of the parasites to the environment. Intermediate hosts acquire the parasite from the environment (or for *T. gondii*, also from consuming another intermediate host), and the cycle continues when a new definitive host preys upon the intermediate host (Dubey *et al.*, 2016; Florin-Christensen and Schnittger, 2018). The mode of transmission of these parasites to marine mammals, such as belugas, is not well understood; however various potential routes have been described, including coastal run-off water and ingestion of filter-feeding fishes and invertebrates harboring the infectious stages of the parasites (Fig. 3.2) (Michaels *et al.*, 2016; Shapiro *et al.*, 2019; Fung *et al.*, 2021).

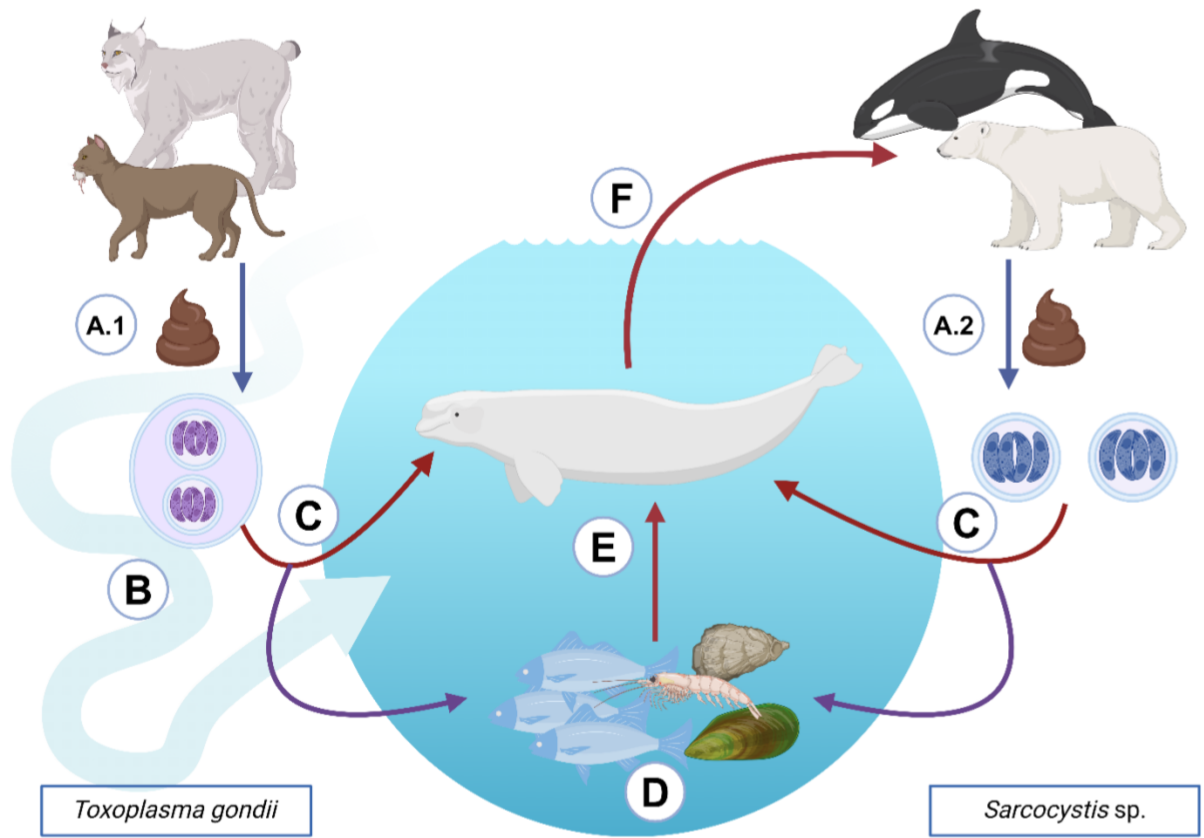

Fig. 3.2 Potential route of transmission of the coccidian parasites, *Toxoplasma gondii* and *Sarcocystis* spp. to beluga whales serving as intermediate hosts.

A.1, oocysts of *T. gondii* are excreted in the feces of felids, the definitive hosts (DH), and **A.2**, sporocysts of *Sarcocystis* spp. are passed in the feces of potential marine DH such as polar bears and killer whales. **B**, Oocysts of *T. gondii* can be transported in terrestrial runoff and rivers into marine environments. **C**, belugas may directly ingest oocysts and/or sporocysts from contaminated seawater. **D**, filter-feeding fish, and invertebrates could concentrate infectious oocysts. **E**, belugas may acquire *T. gondii* and/or *Sarcocystis* spp. by ingesting filter feeding fish and invertebrates. **F**, Life cycle of *Sarcocystis* spp. continues when the DH ingest sarcocysts in tissues of infected beluga; life cycle for *T. gondii* can also continue from beluga to their predators as this parasite can transmit among intermediate hosts through carnivory. Created with Biorender.com.

Therefore, the goal of our study was for veterinary researchers to work with harvesters, government personnel, and the FJMC to determine the status of *T. gondii* and *Trichinella* spp., two zoonotic food-borne parasites, as well as the non-zoonotic parasite *Sarcocystis* spp., in beluga whales harvested from the Eastern Beaufort Sea in Canada over a 7-year period (2014-2021). This study employs serological, molecular, and artificial digestion methodologies to address community concerns about safe consumption of harvested belugas, while also providing insight on the diversity and transmission of food-borne tissue parasites transmitted through carnivory.

3.3 Materials and Methods

3.3.1 Project permits

This study is exempt from University of Saskatchewan and University of Manitoba Animal Research ethics because animals were harvested for purposes other than research. Samples were collected under the Department of Fisheries and Oceans (DFO) permits for three of seven study years: 2014 (S-14/15-3020-YK), 2020 (S-20/21-3012-YK) and 2021 (S-21/22-3016-YK). From 2015-2020, samples were collected under Northwest Territories (NT) Scientific Research Licence "Beluga health and food borne parasites in the Inuvialuit Settlement region" held by E. Jenkins as follows: 15708 (2015), 15813 (2016), 15999 (2017), 16298 (2018), 16453 (2019), and 16650 (2020).

In 2020 and 2021, due to COVID-19 travel restrictions, no researchers traveled into the study sites and samples were collected solely by community whale monitors. Samples in all years were collected under a DFO Licence to Fish for Scientific Purposes with letters of support from the FJMC and the Tuktoyaktuk Hunters and Trappers Committee. Beluga samples were shipped out of the Northwest Territories with a DFO Marine Mammal Transportation License, to the DFO in Winnipeg; afterwards, to the University of Saskatchewan.

3.3.2 Study population and sample collection

The eastern Beaufort Sea (EBS) beluga population migrates during the spring from the Bering Sea (Storrie *et al.*, 2022) to their summer range in the Canadian Beaufort Sea, where they form a dense congregation in the Mackenzie Estuary during late June and early July (Harwood *et al.*, 1996). The FJMC Beluga Monitoring Program employs community-based beluga monitors who are stationed

at core harvest locations (Harwood *et al.*, 2002). In July of each year from 2014 – 2021, samples were obtained by harvested hunted whales and collected by whale monitors and researchers using a standardized protocol at annual harvest at Hendrickson Island and East Whitefish, NT, Canada (Fig. 1). A total of 146 whales were sampled, further details of which blood samples were collected from neck or heart transections, centrifuged at 1200 x for 15 min, and serum was recovered and stored at -80ºC until further analyses. Approximately 100 g of heart, brain, skeletal muscle, tongue and diaphragm from each beluga were collected, placed in labeled Whirl-Pak® bags, and stored at -20ºC until further analyses.

3.3.3 Serological tests for *T. gondii*

Serum samples were tested for antibodies to *T. gondii* using two different commercial indirect enzyme linked immunosorbent assays (ELISA) employing mammalian specific conjugates, a commercial and an in-house modified agglutination test (MAT), and an in-house indirect immunofluorescence assay (IFA) using commercially coated slides. As internal controls, we used serum samples from grey seals (*Halichoerus grypus*) experimentally infected with *T. gondii* (Gajadhar *et al.*, 2004) obtained from the Centre for Foodborne and Animal Parasitology (Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada). Results were interpreted as positive, negative, or inconclusive, with the last regarded as negative in this study. In order to eliminate lipid interference reported in other studies of marine mammals, lipids were extracted from each serum sample (n=96) using chloroform (Blanchet *et al.*, 2014), as long as there was sufficient volume available. Treated samples were re-tested, and the results were compared before and after lipid extraction.

3.3.3.1 New Life Diagnostics commercial ELISA and MAT

A total of 53 serum samples from 2014-2016 were tested using the commercial ELISA and MAT from New Life Diagnostics (NLD; New Life Diagnostics LLC, Carlsbad, CA, USA) according to manufacturer instructions. Briefly, for the MAT NLD, samples were serially diluted from 1:25 to 1:200 in buffer; afterwards, 25 μL of the diluted sera and 25 μL of antigen working solution were added to each of 96 round-bottom wells, mixed through pipetting, and incubated at room temperature (RT) overnight. Positive and negative controls provided in the kit were added to each plate. The plate was read 24 h later by the analyst and a second reader. A blue “button” at the bottom of the well indicated a negative result; a clear bottom indicated a positive result. Serum samples and kit controls were diluted 1:100 and tested in duplicate by ELISA. Samples were

considered negative if the optical density (OD) value was equal to or less than 0.2 OD, and positive if the OD value was higher than 0.2 OD.

3.3.3.2 IDVet commercial ELISA

The indirect ELISA IDVet kit (ID Screen Toxoplasmosis Indirect Multi-species, IDVet Innovative Diagnostics. Grabels, France) was performed on all samples (2014-2021, n=120) according to manufacturer instructions. As in-house negative and positive controls, serum samples from captive grey seals pre- and post- experimental infection with *T. gondii* were added to each plate. Sample/positive percentage (S/P%) was calculated using the formula S/P%= $[(OD_{sample} - OD_{negative\ control} / OD_{positive\ control} - OD_{negative\ control})] \times 100$. Samples were considered negative if S/P% was less than 40%, positive if S/P% was higher than 50%, and samples with S/P% between 40 and 50% were considered inconclusive.

3.3.3.3 In-House MAT

The test was performed as per Al-Adhami *et al.*, (2016) on all samples (2014-2021). Briefly, serum samples were titrated at serial two-fold dilutions from 1:25 to 1:200. The antigen mixture for each plate was prepared by mixing 200 µL of antigen stock solution (*T. gondii* whole-cell tachyzoites treated with formalin, University of Tennessee Research Foundation, USA), 2.5 mL of bovine serum albumine/borate buffer, 35 µL of 2-β-mercaptoethanol and 50 µL of Evans blue dye solution. Equal volumes (25 µL) of antigen mixture and serum dilutions were added into each of 96 round-bottom wells on a plate. The plate was covered and incubated at room temperature for 24 h. A blue button at the bottom of the well indicated a negative result, while a clear well bottom indicated a positive result.

3.3.3.4 Immunofluorescence assay

The assay was performed on all samples (2014-2021) using *T. gondii* fixed microscope slides (FA Substrate slide, VMRD, WA, USA) according to manufacturer instructions. Samples were added to slide wells at two-fold serial dilutions from 1:10 to 1:40 and incubated in a humid chamber at 37ºC for 30 min; afterwards, protein A/G FITC conjugate (BioVision, Milpitas, CA, USA) was added at 1:300 dilution and incubated as before. Slides were read on a fluorescent microscope at 20-40x by the performer of the test and a second observer. Samples were considered positive when

the wells showed a clear peripheral staining of tachyzoites. Wells with only apical or no staining of tachyzoites were considered negative.

3.3.4 Histopathology

Samples of epaxial muscle, heart, and brain, were collected from 26 belugas harvested on Hendrickson Island in 2015 and 2016 as part of an in-depth health assessment involving Canadian Wildlife Health Cooperative veterinarians. The tissues were fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax, and stained with hematoxylin, phloxine, and saffron (HPS). Slides were observed under a light microscope for histopathological analysis, which involved effort specifically to detect coccidian tissue cysts.

3.3.5 *Toxoplasma gondii* DNA extraction and amplification

The magnetic capture quantitative PCR (MC qPCR) was selected as an alternative to bioassays, with increased sensitivity over other direct detection methods by using a homogenized large sample (100 g of tissue vs 25 mg), and improved specificity through the use of a sequence-specific DNA extraction and qPCR using primers for the 529 bp repeat element unique to *T. gondii* (Opsteegh *et al.*, 2010). This MC qPCR has been used to detect *T. gondii* DNA from wildlife (Bachand *et al.*, 2019; Sharma *et al.*, 2019; Engel *et al.*, 2023). DNA was extracted from heart and brain, considered predilection sites for *T. gondii* in experimentally infected animals (Opsteegh *et al.*, 2010; Juránková *et al.*, 2014). Briefly, between 30 to 100 g of tissue from each beluga were incubated overnight on 2.5 mL of cell lysis buffer per gram of tissue. Heart tissue from an experimentally infected reindeer (Bouchard *et al.*, 2017) was used as a positive reference control. Additionally, one beef sample was used as negative control, and two beef samples spiked with 2.5 x 10^5 and 2.5 x 10^6 tachyzoites/mL as positive controls were included in each run.

Real-time PCR amplification was performed in a Bio-Rad CFX 96 DNA thermal cycler (BioRad, Hercules, California, USA) using the primers Tox-9F (5'-AGGAGAGATA TCAGGACTGTAG-3') and Tox-11R (5'-GCGTCGTCTC GTCTAGATCG-3'). Each reaction of 25 µL was run in duplicate, and positive, negative, and 'no template' controls were added to each run. As a standard curve for quantification of positive samples we used dilution series of *T. gondii* plasmid DNA was used as a standard curve for quantification of positive samples. The reaction was considered

positive if Ct-value was less or equal to 35; reaction was considered negative if Ct-value was equal to zero or above 35 (Bachand *et al*, 2019).

In order to compare sensitivity of conventional vs MC qPCR, and when only small amounts of skeletal muscle were available (vs 100 g needed for MC qPCR), a second DNA extraction was performed from 25 mg of heart, brain, and skeletal muscle using a DNeasy® Blood & Tissue Kit (QIAGEN Group, Germany) following manufacturer instructions on samples from beluga harvested in 2017-2021. DNA was quantified using a Nanodrop 2000c spectrophotometer (Thermo Scientific USA) and extractions were stored at -20ºC until assay with primers targeting the *T. gondii* 529 bp repeat element as per (Homan *et al.*, 2000) with forward primer TOX4 5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and reverse TOX5 (5'-CGCTGCAGACACAGTGCATCTGGATT-3'). PCR was performed using a BIO-RAD Touch C1000 thermocycler (Bio-Rad, CA, USA), with initial denaturation of 94ºC for 7 min, 35 cycles of 94ºC for 1 min, 61ºC for 1 min and 72ºC for 1 min, and a final extension of 72ºC 10 min.

3.3.6 Amplification of ITS-1 region of apicomplexan parasites and sequence analysis

DNA extractions from skeletal muscle were assayed by a nested PCR with pan-apicomplexan primers (External Forward DF 5'-TACCGATTGAGTGTTCCGGTG-3'; Internal Forward diF 5'-CGTAACAAGGTTTCCGTAGG-3'; External Reverse DR 5'-GCAATTCACATTGCGTTTCGC-3'; Internal Reverse diR 5'-TTCATCGTTGCGCGAGCCAAG-3') targeting the Internal Transcribed Spacer 1 (ITS-1) region as per (Michaels *et al.*, 2016). Reactions for both external and internal primers involved initial denaturation of 94ºC for 3 min, 35 cycles of 95ºC for 40s, 59.5ºC for 40 s, 72ºC for 90 s, and a final extension of 72ºC for 4 min. Purified PCR products were only observed at band positions of ~1000 bp, and these were sent for Sanger sequencing using the internal PCR primers by the National Research Council in Saskatoon, Saskatchewan, Canada.

Sequences were assembled using QIAGEN CLC Main Workbench (QIAGEN Aarhus, Denmark) and compared with GenBank sequences using the BLAST tool from the National Center for Biotechnology Information (NCBI, MD, USA). Sarcocystidae sequences from GenBank representing common parasite species, identified from muscle tissue of the animal serving as the

IH for the parasite, were selected and used as references. The assembled ITS-1 sequences generated from the present study were aligned with selected reference sequences, using the online version of MAFFT (https://mafft.cbrc.jp/alignment/server/) with standard parameters (Table 3.1). The aligned matrix was manually viewed, edited, and truncated in MEGA7 (www.megasoftware.net) and exported for analysis. For a distance approach, a data-display network was constructed from uncorrected p-distances using all characters in Splitstree4 (University of Tübingen, Germany). As a measure of statistical support, bootstraps were calculated from 1000 replicates. RAxML8 (Stamatakis, 2014) was used for a maximum likelihood approach using the GTRCAT approximation and calculating bootstraps by invoking the autoMRE bootstopping function. The topology was viewed in FigTree4 (http://tree.bio.ed.ac.uk/software/figtree/) and exported to Corel PaintshopPro X8 for finalization.

Table 3.1 Reference sequences for the ITS-1 region of Sarcocystidae from GenBank used for phylogenetic analysis.

Organism	Host	GenBank accession number
S. arctica	*Vulpes lagopus*	KF601306
		KF601309
S. buffalonis	*Bubalus bubalis*	KU247925
		KU247926
S. canis	*Ursus maritimus*	DQ176645
S. cruzi	*Bos taurus*	EF622148
		EF622176
		EF622149
S. felis	*Felis catus*	AY190081
		AY190082
S. fusiformis	*Bubalus bubalis*	KR186139
		KR186140
S. levinei	Buffalo*	MH793427
		MH793428
S. lutrae	*Lutra lutra*	KM657777
		KM657794
S. neurona	*Phoca vitulina*	AF252407
	Mephitis mephitis	AY082648
	Felis catus	MN172273
S. rangiferi	*Rangifer tarandus*	N/P
***Sarcocystis* sp.**	*Arctocephalus tropicalis*	MW264422
		MH918015
	Physeter macrocephalus	HQ184185
	Phoca vitulina	MT460246
	Ursus americanus	MW960105
		MW960104
Toxoplasma gondii	*Phoca vitulina*	AF252408
Neospora caninum	*Canis familiaris*	AF038861
Eimeria tenella	Not provided	AF026388

3.3.7 Recovery of larvae of *Trichinella* spp. in muscle samples

Samples from 2014-2019 were tested by the internationally recognized gold standard method to recover larvae of *Trichinella* spp. (Forbes and Gajadhar, 1999). Briefly, 5 g each of diaphragm and tongues were pooled from five belugas for a total of 25 g per pool. Each pool was homogenized in a blender and digested in 1% pepsin/HCl solution at 37ºC on a magnetic stir plate for 90 minutes, followed by pouring through a 180-micron mesh sieve and sequential sedimentation through two separatory funnels. Then, around 25 mL of the sediment was drained twice and transferred to two Petri dishes to examine for the presence of *Trichinella* larvae by experienced personnel under a stereomicroscope.

3.3.8 Data analysis.

Seroprevalence, tissue prevalence and their 95% confidence intervals (CI) were calculated using the Ausvet Epitools epidemiological calculators (Sergeant, 2018). Links between status of infection and sex or age of the whales (when known) were analysed using Fisher's exact Chi square test (IBM SPSS Statistics). Serological assays (IDVET with NLD ELISA, NLD MAT, IFA and in-house MAT), both before and after chloroform clean-up, were compared using McNemar's chi-square test for related samples (IBM SPSS Statistics). The agreement between the serological tests was assessed using the Kappa coefficient (k). Kappa values of 0.81, 0.61-0.80, 0.41-0.60, and 0.40 were considered to represent excellent, substantial, moderate to good, and slight to poor agreement, respectively (Viera and Garrett, 2005).

3.4 Results

3.4.1 Beluga whale samples collected.

A total of 146 beluga whales were sampled between 2014 and 2021: 115 whales from Hendrickson Island and 31 from East Whitefish (Table 3.2). The sex of all but 7 animals was documented, with an overall female-to-male ratio of 17:122. The age of all but 10 belugas was determined and categorized into three groups: there were 2 Juveniles (≤9 years old), 112 Adults (10-39 years old), and 22 Elders (≥40 years old).

Table 3.2 Number of sampled beluga whales by year and location (EW:HI), along with sex (F:M:U), mean body length in cm (min, max), and mean age in years (min, max).

Year	Location (EW:HI)	Total	Sex (F:M:U)	Mean lenght (min,max)	Mean age (min,max)
2014	13:15	28	4:24:0	411.7 (335.3-48.7.7)	30.6 (14-57)
2015	7:17	24	6:17:1	403.8 (345.4-454.7)	30 (17-55)
2016	6:10	16	0:13:3	414 (340.4-444.5)	17.8 (8.7-33.3)
2017	5:15	20	1:19:0	420.2 (360.7-454.7)	27.3 (10-69)
2018	0:9	9	0:9:0	435.6 (411.5-454.7)	37 (25.4-55.3)
2019	0:15	15	1:13:1	404.7 (165.1-454.7)	36 (21.6-58.2)
2020	0:16	16	4:11:1	410.2 (315-449.6)	26.6 (9.1-51.9)
2021	0:18	18	1:16:1	392.6 (302.3-457.2)	25.9 (13-42)
Total	31:115	146	17:122:7	410 (165.1-487.7)	28.7 (8.7-69)

EW:HI, East Whitefish-to-Hendrickson Island

F:M:U, Female-to-Male-to-Unidentified

Min,max, Minimum and maximum

3.4.2 Serological assays for *Toxoplasma gondii*

Antibodies were detected on MAT NLD in 96% (n=51/53, 95% CI: 87.3-98.9%) of the 2014-16 samples before the lipid removal chloroform clean-up, and 91% (n=48/53, 95% CI: 79.8-95.9%) after the clean-up, with no statistically significant difference (p=0.250) and moderate agreement (k=0.547). Seroprevalence with ELISA NLD on the same samples was 49% (n=26/53, 95% CI: 36.1-62.1%) and 11% (n=6/53, 95% CI: 5.3-22.6%) before and after the lipid removal, respectively, with a statistically significant difference (p<0.001) and poor agreement (k=0.234) (Table 3.3).

Due to these unexpectedly high seroprevalences, inconsistency with kit controls from NLD, and difficulties in procurement, we re-tested these 53 samples from 2014-16 and switched to the IDVET ELISA kit for remaining serum samples collected in 2017-2021 (n=120). Poor agreement was observed between IDVET ELISA with the NLD ELISA (p<0.001, k=0.039) and MAT (p<0.001, k=0.004) methods. Antibodies were detected in only one sample (AREW-15-06), and this sample was also positive on IFA (titer 1:20), both before and after lipid removal (n=1/120, 95% CI: 0.2-4.6%).

All 120 samples were considered negative on in-House MAT. Due to insufficient serum volume, chloroform clean-up was feasible for only 96 samples (2014-2020) for re-testing by IDVET ELISA and IFA. Table 3.3 shows no significant differences between chloroform treated and untreated samples for both IDVET and IFA, and no significant difference between results from IDVET ELISA and IFA, demonstrating an excellent level of agreement (p=1, K=1).

Table 3.3 Proportion of samples positive to *Toxoplasma gondii* in serum collected from harvested beluga whales (n=96) using different serological tests before and after chloroform clean-up to remove lipids.

Serological test	Seroprevalence (n +ve/total tested, 95% CI)		Kappa	p value McNemar test
	Before chloroform clean-up	After chloroform clean-up		
MAT NLD	96% (51/53, 87-99%)	91% (48/53, 80-96%)	0.547	0.250
ELISA NLD	49% (26/53, 36-62%)	11% (6/53, 5-23%)	0.234	<0.001
ELISA IDVet	1% (1/96, 0.2-5.7%)	1% (1/96, 0.2-5.7%)	1.0	1
IFA	1% (1/96, 0.2-5.7%)	1% (1/96, 0.2-5.7%)	1.0	1
In-house MAT	0% (0/120, 0-3.1%)	np	np	np

n +ve, number of positives.

np, not performed; CI, confidence interval.

3.4.3 Histopathology findings

Cyst-like structures, containing elongated basophil cells corresponding to *Sarcocystis* spp., were found in skeletal muscle tissue from 6/16 belugas for which a necropsy was performed in 2015, and 0/10 in 2016. These parasitic cysts were not associated with inflammatory changes (Fig. 3.3). No coccidian cysts were observed in the heart or brain.

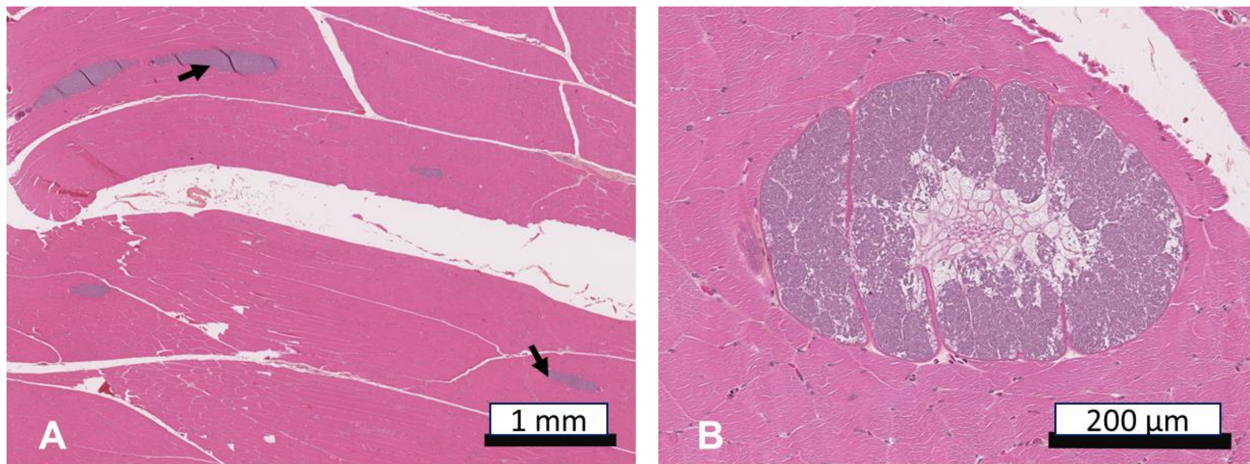

Fig. 3.3 A, Tissue cysts of *Sarcocystis* sp. (sarcocysts, black arrows) in muscle sections of beluga, stained with HPS. B, Sarcocyst containing numerous bradyzoites.

3.4.4 DNA of *Toxoplasma gondii* using MCqPCR and conventional PCR

From 131 heart and 102 brain tissues analyzed by MCqPCR, *T. gondii* DNA was detected in only one heart sample from 2015, identified as ARHI-15-06 (0.8%, 95%CI: 0.1-4.2%). The average weight of tissue tested was 71 g (range 31-100g) for heart and 79 g (range 30-100g) for brain. By conventional PCR, no DNA of *T. gondii* was not detected in any of 53 heart or 55 brain samples.

3.4.5 Internal Transcribed Spacer-1 PCR and sequence analysis.

DNA of *Sarcocystis* sp., but not *T. gondii*, was amplified from 59% (n=46/78, 95% CI: 47.9.-69.2%) of skeletal muscle samples. There were no statistically significant differences in infection status for *Sarcocystis* and *T. gondii* between male and female belugas, nor among the different age groups. A total of 32 sequences were generated from belugas in the present study (supplementary material), all with an average of 99.2% identity (%ID) on BLAST with *Sarcocystis* sp. reported previously in a sperm whale and fur seals (Fig. 3.4 and 3.4; DDN bs: 100, ML bs:100). The aligned matrix consisted of 33 ingroup sequences and one outgroup (AF026388). A well-supported monophyletic *Sarcocystis* clade (ML bs: 95) could be separated into two large clades. One well-supported clade grouping *Sarcocystis* spp. from ruminants as intermediate hosts (IH) (Fig. 3.3 ML bs: 100) and the second consisting of *Sarcocystis* spp. isolated from carnivores as IH, including the sequences generated in this study (Fig. 3.3 and 3.5; DDN bs:100, ML bs: 98). *Sarcocystis neurona* was dubiously recovered as a sister to either the carnivore clade (ML bs: 98) or, sister to *T. gondii* and *Neospora caninum* (DDN bs: 76).

3.4.6 *Trichinella* testing.

No Trichinella spp. larvae were recovered from any of the 108 whales' tongues collected between 2014 to 2019, or 42 diaphragms collected from 2017 and 2019.

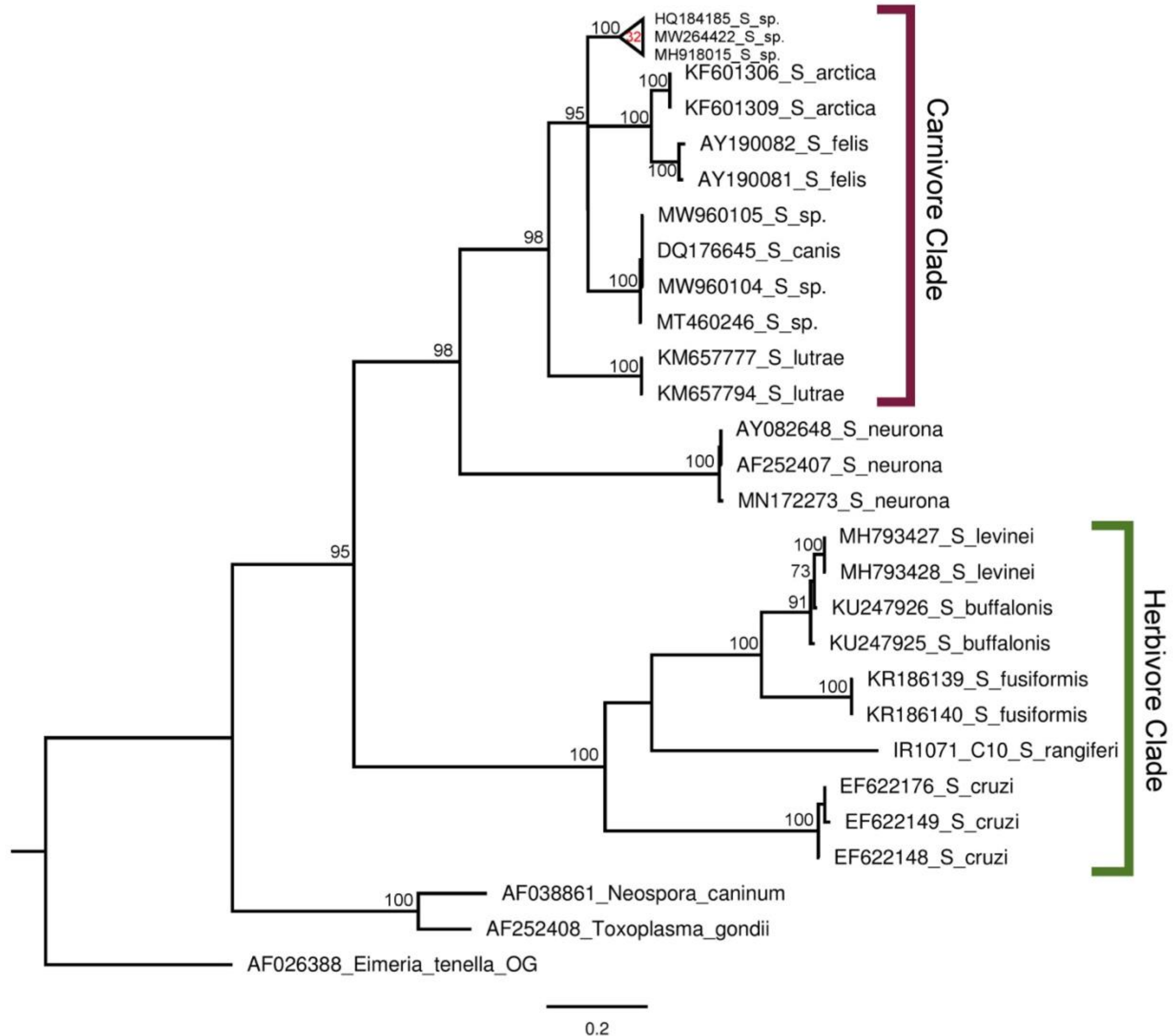

Fig. 3.4 Maximum likelihood topology generated from ITS-1 region sequence data of Sarcocystidae analyzed in RAxML 8 under the GTRCAT approximation.

Bootstrap values > 60 are displayed above branches as branch/node support. The 32 sequences from beluga generated in this study are grouped with those from fur seal (MW264422, MH918015) and sperm whale (HQ184185).

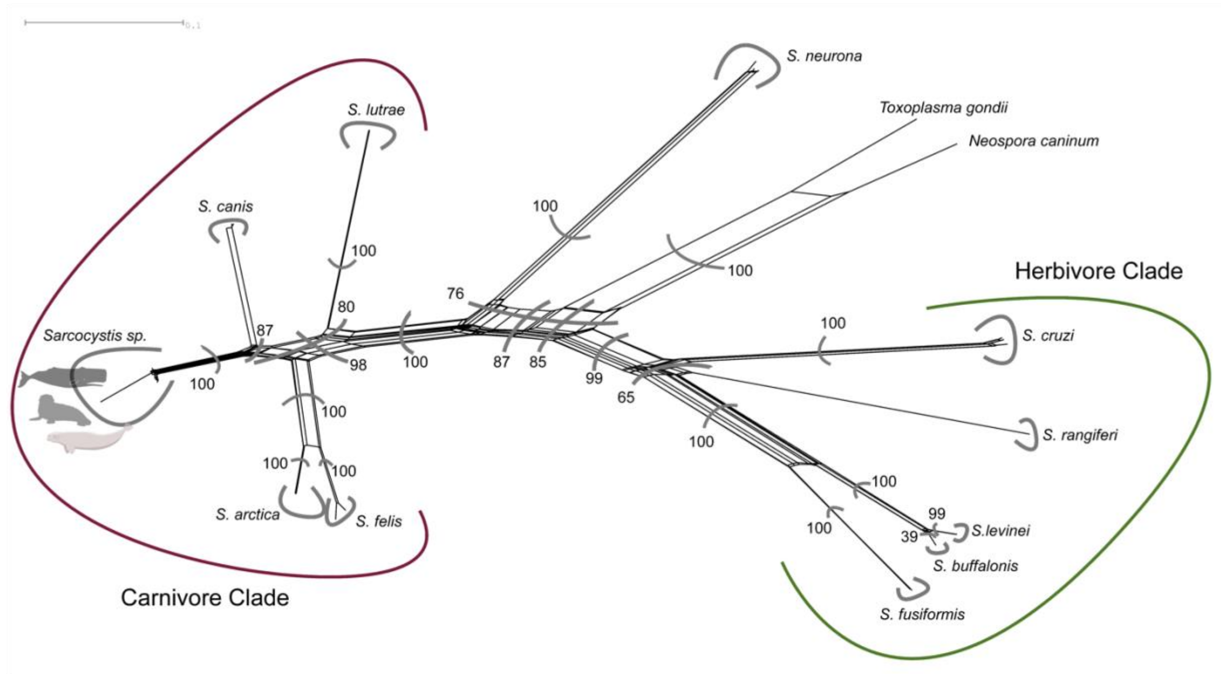

Fig. 3.5 A data-display network constructed from uncorrected ITS-1 region of Sarcocystidae p-distances, using all characters.

Bootstrap supports are displayed by the grey curves and associated values imposed on the network. The 32 Sequences from beluga generated in this study are grouped with those from fur seal and sperm whale. Edited with BioRender.com

3.5 Discussion

The main purpose of this study was to detect and characterize zoonotic food-borne parasites of concern in beluga whales from the EBS population harvested for human consumption. Although we used multiple, state-of-the-art serological and molecular methods, we detected low prevalence of *T. gondii,* and no evidence of *Trichinella* spp., in the EBS beluga population, which is reassuring from a food safety perspective. Tissue prevalence of *T. gondii* reported in the present study was low (1%) compared to a previous unpublished report (13%) in the EBS population sampled over 3 years prior to 2014 (Haman *et al.*, 2013), which may reflect methodological differences and/or interannual variation in transmission. Tissue prevalence in the present study is also lower than the 44% reported from stranded St. Lawrence Estuary (SLE) belugas (Iqbal *et al.*, 2018). Our findings are consistent with observations of lower exposure of fox and humans to *T. gondii* in the ISR (Western Canadian Arctic) compared with the Eastern Canadian Arctic (Goyette *et al.*, 2014; Ducrocq *et al.*, 2021; Bouchard *et al.*, 2022). The low tissue prevalence observed in the present study may be also be attributed to selection bias, as hunters from the ISR tend to select belugas based on physical characteristics indicative of good health (Ostertag *et al.*, 2018); in contrast to the SLE belugas, which were found stranded with clinical conditions or deceased. Therefore, the results from the present study results may not be representative of the true prevalence in the total beluga population (Nusser *et al.*, 2008).

Differences in exposure to *T. gondii* and immunocompetence between EBS and SLE beluga populations could be related to geographic distribution and proximity to human settlements and domestic cat populations. The SLE, which is supplied with water from the highly populated Laurentian Great Lakes basin, has a range of pathogens and pollutants that can have implications for beluga health (Lair *et al*., 2016). Polychlorinated biphenyls (PCBs) in liver and blubber may have immunosuppressive effects on the whales (Mikaelian *et al.*, 2000; Hobbs *et al.*, 2003; Raach *et al.*, 2011; Noël *et al.*, 2014). Both immunosuppression and the presence of significant environmental contamination with oocysts of *T. gondii* could jointly contribute to the high prevalence of *T. gondii* in the SLE beluga population (Mikaelian *et al.*, 2000). In contrast, the ISR is a remote area with small human population (~6200), and therefore a small domestic cat population (Government of Canada, 2022). This combination of minimal anthropogenic activities

and low environmental circulation of *T. gondii* in the Western Canadian Arctic may account for the low prevalence in the ISR beluga population.

The Canadian lynx (*Lynx canadensis*) is the only wild felid found in the Canadian Arctic and therefore the only potential wild definitive host for *T. gondii*. Studies conducted in lynx in eastern Canada have reported seroprevalences between 14% to 41% (Simon *et al.*, 2013; Bouchard *et al.*, 2023). Bouchard (2023) also reported tissue prevalence rates of 17% and 32% from lynx in Yukon and eastern Canada, respectively. However, the normal geographical distribution of Canadian lynx is limited to the tree line (Chester, 2016), which may reduce marine transmission of *T. gondii* oocysts through coastal run-off water (Miller *et al.*, 2002; Conrad *et al.*, 2005).

Serology is an indirect diagnostic method that serves as an alternative to the direct detection of a pathogen, and is useful for monitoring previous exposure to a pathogen in wildlife populations (Gilbert *et al.*, 2013). However, serology has certain limitations in wildlife, including the need for test validation for each animal species, the lack of established control standards, and the risk of cross-reactivity with closely related pathogens (Gilbert *et al.*, 2013). For these reasons, we compared different serological tests to detect antibodies against *T. gondii* in serum samples obtained from belugas, and used serum samples from experimentally infected grey seals as internal controls which worked as expected (Gajadhar *et al.*, 2004). Seroprevalence using MAT NLD was much higher (91 to 96%) than the rest of the techniques used in the present study. Similar findings were reported by van de Velde *et al.* (2016) comparing a commercial MAT with IDVET ELISA and commercial slides for IFA for diverse marine mammals. ELISA and IFA utilize a protein A/G conjugate (or a secondary antibody species specific) that binds with IgG antibodies across multiple mammalian species. In contrast, agglutination tests bind sample antibodies with tachyzoites; however, non-specific binding with other substances in the sample may lead to false positive results (Blanchet *et al.*, 2014).

To minimize nonspecific binding in the present study, serum samples were subjected to lipid removal by chloroform clean-up followed by re-testing. Seroprevalence for MAT NLD was not significantly different after chloroform treatment. For ELISA NLD, seroprevalence in beluga samples was significantly decreased after chloroform treatment ($p<0.001$), but not to the extent observed in a study on harbour porpoises (*Phocoena phoceona*), where seroprevalence decreased from 45% to 0% (Blanchet *et al.*, 2014). In our study, only one beluga sample was positive on both

ELISA IDVET and IFA, and there was no difference before and after the chloroform clean-up. Additionally, all samples tested negative in an in-house MAT. One potential explanation for this finding lies in the methodology, as ELISA and IFA use an enzyme-conjugated protein A/G, facilitating the selective binding with host antibodies (IgG) specific to *T. gondii*, consequently, increasing test sensitivity. In contrast, in MAT, the fixed tachyzoites are exposed to the total antibodies present in the samples, contributing to the observed differences in results. Results of both molecular and serological analyses in the present study suggest that IDVET ELISA, VMRD IFAT, and in-house MAT produced reasonably strong and reliable results for beluga samples, and that lipid removal did not change our findings.

In order to identify the species of *Sarcocystis* observed on histopathology, and to ensure that the lack of detection of DNA of *T. gondii* in the present study was not attributed to mutations at the target site of magnetic capture PCR (the 529 bp repeated element of *T. gondii*), we performed an additional PCR using pan-coccidian primers targeting the highly conserved ITS-1 region. This region has been previously utilized for the detection of *T. gondii* and *Sarcocystis* spp. in marine mammals, as well as bivalves (Gibson *et al.*, 2011; Michaels *et al.*, 2016; O'Byrne *et al.*, 2021). *Sarcocystis* spp. DNA, but not *T. gondii*, amplified from skeletal muscle of beluga at a high prevalence. This high prevalence was not unexpected based on previous observations in beluga from this population (Haman *et al.*, 2013), beluga in the SLE region (De Guise *et al.*, 1993), and marine mammals such as sea otters, pinnipeds, and cetaceans as well as other wildlife and livestock (Khan and Evans, 2006; Haman *et al.*, 2013; Dubey *et al.*, 2016; Hernández-Ortiz *et al.*, 2023). However, it is important to note that the tissue prevalence of *Sarcocystis* spp. observed in this study may not represent the true prevalence in the EBS beluga population, primarily due to hunter selection bias for adult male belugas. While there was no statistical difference in *Sarcocystis* infection between sexes (with a female-to-male ration of 17:122) and among age groups (with juveniles-to-adults-to-elders ration of 2:112:22), the sample sizes for females, juveniles and older belugas were relatively low.

Evidence of DNA of *Sarcocystis* in skeletal muscle from 59% of EBS belugas demonstrates that they commonly serve as intermediate hosts for this parasite. Belugas become infected by ingesting the infectious stage of *Sarcocystis* (sporocysts) released in the feces of carnivore definitive hosts. Consequently, definitive hosts would be infected through consumption of sarcocysts in belugas by

predation and scavenging (De Guise *et al.*, 1993). In nature, the predators of beluga include polar bears (e.g., Smith and Sjare, 1990; Thiemann *et al.*, 2008) and killer whales (Lefort *et al.*, 2020). In beluga, we did not detect species of *Sarcocystis* commonly shed by terrestrial carnivores, such as *S. canis* or *S. neurona*; the latter is potentially more pathogenic and therefore this finding was reassuring for beluga health. Our phylogenetic evidence suggests that the *Sarcocystis* sp. present in beluga is closely related to that found in other marine species. It is possible that the definitive host is a previously undescribed terrestrial carnivore, through marine contamination with sporocysts shed by the definitive hosts on land as proposed for *T. gondii* and *S. neurona* (Miller *et al.*, 2002; VanWormer *et al.*, 2014; Shapiro *et al.*, 2019; O'Byrne *et al.*, 2021). Transmission of terrestrial species of *Sarcocystis* to marine mammals may thus occur through the ingestion of sporocysts in fish and other invertebrates that filter water and concentrate the infectious stages of the parasites (Arkush *et al.*, 2003; Massie *et al.*, 2010; Michaels *et al.*, 2016; Reiling *et al.*, 2019).

The phylogenetic analysis for the present study based on the ITS-1 region revealed two distinct clades in the genus *Sarcocystis* excluding *S. neurona*. Each of these clades showed well-supported relationships, suggesting that the *Sarcocystis* spp. within each clade share an evolutionary history. Sequences generated in this study have a well-supported relationship to previous *Sarcocystis* sequences found in fur seals (MW264422, MH918015) and a sperm whale (HQ184185) (Gibson *et al.*, 2011). These geographically separated findings (EBS in Canada, Iguape in Brazil, and Washington in USA) strongly suggest the possibility of a marine cycle for this particular *Sarcocystis* species, and it is supported by the low similarity of the sequence generated in this study to any sequences from terrestrial animals. Therefore, the transmission via runoff water from land to sea is less likely to be the primary source of transmission of this *Sarcocystis* sp. to belugas. Further investigation is necessary to better understand the transmission dynamics and host-parasite relationships of *Sarcocystis* in beluga whales, to identify the potential definitive hosts such as polar bears or killer whales, and to gain insights into the ecological interactions and potential risks for beluga population. Furthermore, it is important to conduct studies using primers targeting different regions of the genome of Sarcocystidae, to generate a more comprehensive understanding of the genetic diversity and relationships among *Sarcocystis* and related parasites.

We did not detect larvae of *Trichinella* spp. in beluga in the present study. Although *Trichinella* is common in more carnivorous marine mammals, such as polar bears and walruses (Forbes, 2000;

Springer *et al.*, 2017), there are relatively few confirmed cases in piscivorous seals and whales. Infection of only one beluga from Alaska has been confirmed through the artificial digestion method (Forbes, 2000; Jenkins *et al.*, 2013). Transmission of *Trichinella* in animals occurs through predation, cannibalism, or scavenging of infected muscle tissue (Gottstein *et al.*, 2009). Belugas, however, are generalist predators that feed on a variety of fishes and also invertebrates (Quakenbush *et al.*, 2015; Choy *et al.*, 2020) which are not part of the parasite's life cycle. The lack of detection of *Trichinella* spp. in diaphragm and tongue samples from belugas in the present study may well present a truly low prevalence in beluga population.

3.6 Conclusion

This study provides compelling evidence that the EBS beluga population has a low occurrence of food-borne zoonotic parasites (*T. gondii* and *Trichinella* spp.), and high prevalence of a previously undescribed species of *Sarcocystis*. Similar to most *Sarcocystis* spp., this is unlikely to be zoonotic. Therefore, the risk of human transmission of *T. gondii* and *Trichinella* spp. through consumption and harvesting belugas is low at the current time. The validation of serological tests for marine mammals is critical to prevent false positive or negative results, but is difficult in the absence of a 'gold standard'. We therefore suggest multiple serological tests and multiple lines of evidence (such as molecular testing, histopathology, followed by immunohistochemistry) for surveillance for *T. gondii* in wildlife populations in the future, especially if trying to detect changes over time. The findings regarding the prevalence and diversity of *Sarcocystis* suggest the existence of a marine life cycle of this parasite in beluga whales, potentially involving beluga predators as definitive hosts. However, further research is required to understand marine transmission of *Sarcocystis* and the evolutionary relationships of the Sarcocystidae. Finally, continued surveillance for pathogens and contaminants in beluga as risk factors for beluga health as well as food security and safety among Inuit harvesters, is needed through continued community-based harvest monitoring and enhanced local capacity for testing.

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3.8 Supplementary Material.

Sequences (ITS1) generated in the present study with designated *Sarcocystis* species names, tissue from which the organism was recovered, %ID with Blast, bootstrap values obtained from the data-display network (DDN) and maximum likelihood (ML) topology, accession numbers from GenBank and maximum likelihood (ML) topology, accession numbers from GenBank and for BOLD Systems.

Sample	**Host**	**Tissue source**	**Inferred identity**	**BLAST (% ID)**	**DDN bs**	**ML bs**	**GenBank accession number**	**BOLD Systems number**
ARHI17_13	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524192	DLSA001-23
ARHI15_02	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524223	DLSA002-23
ARHI20_01	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524222	DLSA003-23
ARHI20_02	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524221	DLSA004-23
ARHI20_03	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524220	DLSA005-23
ARHI20_15	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524219	DLSA006-23
ARHI21_02	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524218	DLSA007-23
ARHI21_04	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524217	DLSA008-23
ARHI21_06	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524216	DLSA009-23
ARHI21_07	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524215	DLSA010-23
ARHI21_14	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524214	DLSA011-23
ARHI20_07	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.24	100	100	OR524213	DLSA015-23
ARHI20_13	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524212	DLSA016-23
ARHI20_04	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524211	DLSA017-23

ARHI21_08	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.27	100	100	OR524210	DLSA018-23
ARHI17_16	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524209	DLSA019-23
ARHI21_03	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524208	DLSA020-23
ARHI21_19	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.28	100	100	OR524207	DLSA021-23
ARHI21_15	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524206	DLSA023-23
ARHIb17_13	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.14	100	100	OR524205	DLSA024-23
ARHI21_16	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524204	DLSA025-23
ARHI17_08	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524203	DLSA026-23
ARHI17_14	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524202	DLSA027-23
ARHIb17_08	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524201	DLSA028-23
ARHI17_18	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.16	100	100	OR524200	DLSA029-23
ARHI19_05	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	99.03	100	100	OR524199	DLSA030-23
ARHI15_20	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	98.67	100	100	OR524198	DLSA031-23
ARHI20_14	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	98.31	100	100	OR524197	DLSA032-23
ARHI21_05	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	98.79	100	100	OR524196	DLSA033-23
ARHI20_08	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	98.41	100	100	OR524195	DLSA035-23
ARHI17_15	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	98.19	100	100	OR524194	DLSA036-23
ARHI19_03	*Delphinapterus leucas*	Skeletal muscle	*Sarcocystis* sp.	95.43	100	100	OR524193	DLSA037-23

3.9 References

Al-Adhami, B.H., Simard, M., Hernández-Ortiz, A., Boireau, C. and Gajadhar, A.A. (2016) ‘Development and evaluation of a modified agglutination test for diagnosis of *Toxoplasma* infection using tachyzoites cultivated in cell culture’, *Food and Waterborne Parasitology*, 2, pp. 15–21. Available at: https://doi.org/10.1016/j.fawpar.2015.12.001.

Arkush, K.D., Miller, M.A., Leutenegger, C.M., Gardner, I.A., Packham, A.E., Heckeroth, A.R., Tenter, A.M., Barr, B.C. and Conrad, P.A. (2003) ‘Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*)’, *International Journal for Parasitology*, 33(10), pp. 1087–1097. Available at: https://doi.org/10.1016/S0020-7519(03)00181-4.

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E. and Jenkins, E. (2019) ‘Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada’, *Parasites & Vectors*, 12(1), p. 155. Available at: https://doi.org/10.1186/s13071-019-3408-9.

Barbosa, L., Johnson, C.K., Lambourn, D.M., Gibson, A.K., Haman, K.H., Huggins, J.L., Sweeny, A.R., Sundar, N., Raverty, S.A. and Grigg, M.E. (2015) ‘A novel *Sarcocystis neurona* genotype XIII is associated with severe encephalitis in an unexpectedly broad range of marine mammals from the northeastern Pacific Ocean’, *International Journal for Parasitology*, 45(9–10), pp. 595–603. Available at: https://doi.org/10.1016/j.ijpara.2015.02.013.

Binnington, M.J., Lei, Y.D., Pokiak, L., Pokiak, J., Ostertag, S.K., Loseto, L.L., Chan, H.M., Yeung, L.W.Y., Huang, H. and Wania, F. (2017) ‘Effects of preparation on nutrient and environmental contaminant levels in Arctic beluga whale (*Delphinapterus leucas*) traditional foods’, *Environmental Science: Processes & Impacts*, 19(8), pp. 1000–1015. Available at: https://doi.org/10.1039/C7EM00167C.

Blanchet, M., Godfroid, J., Breines, E., Heide-Jørgensen, M., Nielsen, N., Hasselmeier, I., Iversen, M., Jensen, S. and Åsbakk, K. (2014) ‘West Greenland harbour porpoises assayed for antibodies against *Toxoplasma gondii*: false positives with the direct agglutination method’,

Diseases of Aquatic Organisms, 108(3), pp. 181–186. Available at: https://doi.org/10.3354/dao02715.

Bouchard, É., Sharma, R., Bachand, N., Gajadhar, A.A. and Jenkins, E.J. (2017) 'Pathology, clinical signs, and tissue distribution of *Toxoplasma gondii* in experimentally infected reindeer (*Rangifer tarandus*)', *International Journal for Parasitology: Parasites and Wildlife*, 6(3), pp. 234–240. Available at: https://doi.org/10.1016/j.ijppaw.2017.08.004.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Buhler, K., Al-Adhami, B., Su, C., Fenton, H., G.-Gouin, G., Roth, J.D., Rodrigues, C.W., Pamak, C., Simon, A., Bachand, N., Leighton, P. and Jenkins, E. (2022) 'Are foxes (*Vulpes* spp.) good sentinel species for *Toxoplasma gondii* in northern Canada?', *Parasites & Vectors*, 15(1), p. 115. Available at: https://doi.org/10.1186/s13071-022-05229-3.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Jung, T.S., Harms, N.J., Willier, C.N., Boonstra, R., Majchrzak, Y.N., Peers, M.J.L., Gouin, G.-G., Al-Adhami, B., Simon, A., Leighton, P. and Jenkins, E.J. (2023) 'Canada Lynx (*Lynx canadensis*) as potential reservoirs and sentinels of *Toxoplasma gondii* in Northern Canada', *Zoonotic Diseases*, 3(1), pp. 6–17. Available at: https://doi.org/10.3390/zoonoticdis3010002

Chester, S.R. (2016) *The arctic guide: wildlife of the far north*. 1st edition. Princeton, NJ: Princeton University Press.

Choy, E., Giraldo, C., Rosenberg, B., Roth, J., Ehrman, A., Majewski, A., Swanson, H., Power, M., Reist, J. and Loseto, L. (2020) 'Variation in the diet of beluga whales in response to changes in prey availability: insights on changes in the Beaufort Sea ecosystem', *Marine Ecology Progress Series*, 647, pp. 195–210. Available at: https://doi.org/10.3354/meps13413.

Conrad, P.A., Miller, M.A., Kreuder, C., James, E.R., Mazet, J., Dabritz, H., Jessup, D.A., Gulland, F. and Grigg, M.E. (2005) 'Transmission of Toxoplasma: Clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment', *International Journal for Parasitology*, 35(11–12), pp. 1155–1168. Available at: https://doi.org/10.1016/j.ijpara.2005.07.002.

COSEWIC (2004) *COSEWIC assessment and update status report on the beluga whale Delphinapterus leucas in Canada.* Available at: https://publications.gc.ca/site/archivee-archived.html?url=https://publications.gc.ca/collections/Collection/CW69-14-170-2004E.pdf (Accessed: 23 March 2023).

De Guise, S., Lagacé, A., Girard, C. and Béland, P. (1993) 'Intramuscular *Sarcocystis* in two beluga whales and an Atlantic White-Sided dolphin from the St. Lawrence Estuary, Quebec, Canada', *Journal of Veterinary Diagnostic Investigation*, 5(2), pp. 296–300. Available at: https://doi.org/10.1177/104063879300500231.

Dubey, J.P., Calero-Bernal, R., Rosenthal, B., Speer, C.A. and Fayer, R. (2016) *Sarcocystosis of animals and humans*. Second edition. Boca Raton: CRC Press, Taylor & Francis Group.

Dubey, J.P., Zarnke, R., Thomas, N.J., Wong, S.K., Bonn, W.V., Briggs, M., Davis, J.W., Ewing, R., Mense, M., Kwok, O.C.H., Romand, S. and Thulliez, P. (2003) '*Toxoplasma gondii*, N*eospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals', *Veterinary Parasitology*, 116(4), pp. 275–296. Available at: https://doi.org/10.1016/S0304-4017(03)00263-2.

Ducrocq, J., Ndao, M., Yansouni, C.P., Proulx, J., Mondor, M., Hamel, D., Lévesque, B., De Serres, G. and Talbot, D. (2021) 'Epidemiology associated with the exposure to *Toxoplasma gondii* in Nunavik's Inuit population using the 2017 *Qanuilirpitaa* cross-sectional health survey', *Zoonoses and Public Health*, 68(7), pp. 803–814. Available at: https://doi.org/10.1111/zph.12870.

Engel, L., Hamedy, A. and Koethe, M. (2023) 'Direct detection and quantification of *Toxoplasma gondii* in meat samples from feral raccoons (*Procyon lotor*) in Germany by magnetic-capture real-time PCR', *Parasitology Research*, 122(1), pp. 307–313. Available at: https://doi.org/10.1007/s00436-022-07730-1.

FJMC. 2013. Fisheries Joint Management Committee: Beaufort Sea Beluga Management Plan. 4th Amended Printing Inuvik, Northwest Territories.

Florin-Christensen, M. and Schnittger, L. (eds) (2018) *Parasitic Protozoa of Farm Animals and Pets*. Cham: Springer International Publishing. Available at: https://doi.org/10.1007/978-3-319-70132-5.

Forbes, L.B. (2000) 'The occurrence and ecology of *Trichinella* in marine mammals', *Veterinary Parasitology*, 93(3–4), pp. 321–334. Available at: https://doi.org/10.1016/S0304-4017(00)00349-6.

Forbes, L.B. and Gajadhar, A.A. (1999) 'A validated *Trichinella* digestion assay and an associated sampling and quality assurance system for use in testing pork and horse meat', *Journal of Food Protection*, 62(11), pp. 1308–1313. Available at: https://doi.org/10.4315/0362-028X-62.11.1308.

Friesen, T.M. and Arnold, C.D. (1995) 'Zooarchaeology of a focal resource: dietary importance of beluga whales to the precontact Mackenzie Inuit', *ARCTIC*, 48(1), pp. 22–30. Available at: https://doi.org/10.14430/arctic1221.

Fung, R., Manore, A.J.W., Harper, S.L., Sargeant, J.M., Shirley, J., Caughey, A. and Shapiro, K. (2021) 'Clams and potential foodborne *Toxoplasma gondii* in Nunavut, Canada', *Zoonoses and Public Health*, 68(3), pp. 277–283. Available at: https://doi.org/10.1111/zph.12822.

Gajadhar, A. (2015) *Foodborne parasites in the food supply web: occurrence and control*. Boston, MA: Elsevier.

Gajadhar, A.A. and Forbes, L.B. (2010) 'A 10-year wildlife survey of 15 species of Canadian carnivores identifies new hosts or geographic locations for *Trichinella* genotypes T2, T4, T5, and T6', *Veterinary Parasitology*, 168(1–2), pp. 78–83. Available at: https://doi.org/10.1016/j.vetpar.2009.10.012.

Gajadhar, A.A., Measures, L., Forbes, L.B., Kapel, C. and Dubey, J.P. (2004) 'Experimental *Toxoplasma gondii* infection in grey seals (*Halichoerus grypus*)', *Journal of Parasitology*, 90(2), pp. 255–259. Available at: https://doi.org/10.1645/GE-144R.

Gibson, A.K., Raverty, S., Lambourn, D.M., Huggins, J., Magargal, S.L. and Grigg, M.E. (2011) 'Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected

marine sentinel species', *PLoS Neglected Tropical Diseases*. Edited by J.M. Vinetz, 5(5), p. e1142. Available at: https://doi.org/10.1371/journal.pntd.0001142.

Gilbert, A.T., Fooks, A.R., Hayman, D.T.S., Horton, D.L., Müller, T., Plowright, R., Peel, A.J., Bowen, R., Wood, J.L.N., Mills, J., Cunningham, A.A. and Rupprecht, C.E. (2013) 'Deciphering serology to understand the ecology of infectious diseases in wildlife', *EcoHealth*, 10(3), pp. 298–313. Available at: https://doi.org/10.1007/s10393-013-0856-0.

Gottstein, B., Pozio, E. and Nockler, K. (2009) 'Epidemiology, diagnosis, treatment, and control of trichinellosis', *Clinical Microbiology Reviews*, 22(1), pp. 127–145. Available at: https://doi.org/10.1128/CMR.00026-08.

Government of Canada, S.C. (2022) *Census Profile, 2021 Census of Population*. Available at: https://www12.statcan.gc.ca/census-recensement/2021/dp-pd/prof/index.cfm?Lang=E (Accessed: 10 May 2023).

Goyette, S., Cao, Z., Libman, M., Ndao, M. and Ward, B.J. (2014) 'Seroprevalence of parasitic zoonoses and their relationship with social factors among the Canadian Inuit in Arctic regions', *Diagnostic Microbiology and Infectious Disease*, 78(4), pp. 404–410. Available at: https://doi.org/10.1016/j.diagmicrobio.2013.08.026.

Haman, K.H., Raverty, S., Wendte, J.M., Loseto, L., Ferguson, S.H. and Grigg, M.E. (2013) '*Toxoplasma gondii* infected tissues from hunter harvested beluga (*Delphinapterus leucas*) in the Western Canadian Arctic'. *44th Conference of the International Association for Aquatic Animal Medicine (IAAAM)*, San Francisco, California, 21 April. Available at: https://www.vin.com/apputil/project/defaultadv1.aspx?pid=11375&catid=&id=5768539&meta=generic&authorid=.

Harwood, L.A., Innes, S., Norton, P. and Kingsley, M. (1996) 'Distribution and abundance of beluga whales in the Mackenzie estuary, southeast Beaufort Sea, and west Amundsen Gulf during late July 1992', *Canadian Journal of Fisheries and Aquatic Sciences*, 53(10), pp. 2262–2273. Available at: https://doi.org/10.1139/f96-180.

Harwood, L.A., Norton, P., Day, B. and Hall, P.A. (2002) 'The harvest of beluga whales in Canada's Western Arctic: Hunter-based monitoring of the size and composition of the catch', *ARCTIC*, 55(1), pp. 10–20. Available at: https://doi.org/10.14430/arctic687.

Hernández-Ortiz, A., Bouchard, É., Snyman, L.P., Al-Adhami, B.H., Gouin, G.-G., Neelin, M. and Jenkins, E.J. (2023) '*Toxoplasma gondii* and related Sarcocystidae parasites in harvested caribou from Nunavik, Canada', *International Journal for Parasitology: Parasites and Wildlife*, 21, pp. 246–254. Available at: https://doi.org/10.1016/j.ijppaw.2023.06.008.

Hobbs, K.E., Muir, D.C.G., Michaud, R., Béland, P., Letcher, R.J. and Norstrom, R.J. (2003) 'PCBs and organochlorine pesticides in blubber biopsies from free-ranging St. Lawrence River Estuary beluga whales (*Delphinapterus leucas*), 1994–1998', *Environmental Pollution*, 122(2), pp. 291–302. Available at: https://doi.org/10.1016/S0269-7491(02)00288-9.

Homan, W.L., Vercammen, M., De Braekeleer, J. and Verschueren, H. (2000) 'Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative', *Int J Parasitol*, 30(1), pp. 69–75. Available at: https://doi.org/10.1016/S0020-7519(99)00170-8

Iqbal, A., Measures, L., Lair, S. and Dixon, B. (2018) '*Toxoplasma gondii* infection in stranded St. Lawrence Estuary beluga *Delphinapterus leucas* in Quebec, Canada', *Diseases of Aquatic Organisms*, 130(3), pp. 165–175. Available at: https://doi.org/10.3354/dao03262.

Jenkins, E.J., Castrodale, L.J., de Rosemond, S.J.C., Dixon, B.R., Elmore, S.A., Gesy, K.M., Hoberg, E.P., Polley, L., Schurer, J.M., Simard, M. and Thompson, R.C.A. (2013) 'Tradition and Transition: parasitic zoonoses of people and animals in Alaska, Northen Canada, and Greenland', in *Advances in Parasitology*. Elsevier, pp. 33–204. Available at: https://doi.org/10.1016/B978-0-12-407706-5.00002-2.

Jenkins, E.J., Simon, A., Bachand, N. and Stephen, C. (2015) 'Wildlife parasites in a One Health world', *Trends in Parasitology*, 31(5), pp. 174–180. Available at: https://doi.org/10.1016/j.pt.2015.01.002.

Johnson-Down, L., Allaire, J., Little, M. and Ayotte, P. (2021) *Country and Market Food Consumption and Nutritional Status. Nunavik Inuit Health Survey 2017 Qanuilirpitaa? How are we now?* Available at: https://nrbhss.ca/sites/default/files/health_surveys/Country_Food_and_Market_Food_Consumption_and_Nutritional_Status_fullreport_en.pdf (Accessed: 4 July 2022).

Jones, J.L. and Dubey, J.P. (2012) 'Foodborne Toxoplasmosis', *Clinical Infectious Diseases*, 55(6), pp. 845–851. Available at: https://doi.org/10.1093/cid/cis508.

Juránková, J., Basso, W., Neumayerová, H., Baláž, V., Jánová, E., Sidler, X., Deplazes, P. and Koudela, B. (2014) 'Brain is the predilection site of *Toxoplasma gondii* in experimentally inoculated pigs as revealed by magnetic capture and real-time PCR', *Food Microbiology*, 38, pp. 167–170. Available at: https://doi.org/10.1016/j.fm.2013.08.011.

Kenny, T.-A., Hu, X.F., Kuhnlein, H.V., Wesche, S.D. and Chan, H.M. (2018) 'Dietary sources of energy and nutrients in the contemporary diet of Inuit adults: results from the 2007–08 Inuit Health Survey', *Public Health Nutrition*, 21(7), pp. 1319–1331. Available at: https://doi.org/10.1017/S1368980017003810.

Khan, R.A. and Evans, L. (2006) 'Prevalence of *Sarcocystis* spp. in Two subspecies of caribou (*Rangifer tarandus*) in Newfoundland and Labrador, and foxes (*Vulpes vulpes*), wolves (*Canis lupus*), and husky dogs (*Canis familiaris*) as potential definitive hosts', *Journal of Parasitology*, 92(3), pp. 662–663. Available at: https://doi.org/10.1645/GE-753R1.1.

Lair, S., Measures, L.N. and Martineau, D. (2016) 'Pathologic findings and trends in mortality in the beluga (*Delphinapterus leucas*) population of the St Lawrence Estuary, Quebec, Canada, From 1983 to 2012', *Veterinary Pathology*, 53(1), pp. 22–36. Available at: https://doi.org/10.1177/0300985815604726.

Lefort, K.J., Matthews, C.J.D., Higdon, J.W., Petersen, S.D., Westdal, K.H., Garroway, C.J. and Ferguson, S.H. (2020) 'A review of Canadian Arctic killer whale (*Orcinus orca*) ecology', *Canadian Journal of Zoology*, 98(4), pp. 245–253. Available at: https://doi.org/10.1139/cjz-2019-0207.

Loseto, L., Lam, J. and Iacozza, J. (2018) ‘Beluga Summit: knowledge sharing of the eastern Beaufort Sea beluga whale’, *Arctic Science*, 4(3), pp. i–iv. Available at: https://doi.org/10.1139/as-2018-0011.

Massie, G.N., Ware, M.W., Villegas, E.N. and Black, M.W. (2010) ‘Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish’, *Veterinary Parasitology*, 169(3–4), pp. 296–303. Available at: https://doi.org/10.1016/j.vetpar.2010.01.002.

McGhee, R. (1988) *Beluga hunters: an archaeological reconstruction of the history and culture of the Mackenzie Delta Kittegaryumiut*. Rejacketed edition. Ottawa: University of Ottawa Press.

Michaels, L., Rejmanek, D., Aguilar, B., Conrad, P. and Shapiro, K. (2016) ‘California mussels (*Mytilus californianus*) as sentinels for marine contamination with *Sarcocystis neurona*’, *Parasitology*, 143(6), pp. 762–769. Available at: https://doi.org/10.1017/S0031182016000354.

Mikaelian, I., Boisclair, J., Dubey, J., Kennedy, S. and Martineau, D. (2000) ‘Toxoplasmosis in beluga whales (*Delphinapterus leucas*) from the St Lawrence Estuary: two case reports and a serological survey’, *Journal of Comparative Pathology*, 122(1), pp. 73–76. Available at: https://doi.org/10.1053/jcpa.1999.0341.

Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E. and Conrad, P.A. (2002) ‘Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*)’, *International Journal for Parasitology*, 32(8), pp. 997–1006. Available at: https://doi.org/10.1016/S0020-7519(02)00069-3.

Murray, L. (2023) *The 2016 Beluga Summit: planning and proceedings*. Winnipeg, Manitoba: Fisheries and Oceans Canada, Arctic and Aquatic Research Division, Freshwater Institute, Central and Arctic Region.

Noël, M., Loseto, L.L., Helbing, C.C., Veldhoen, N., Dangerfield, N.J. and Ross, P.S. (2014) ‘PCBs are associated with altered gene transcript profiles in Arctic beluga whales (*Delphinapterus leucas*)’, *Environmental Science & Technology*, 48(5), pp. 2942–2951. Available at: https://doi.org/10.1021/es403217r.

Nusser, S.M., Clark, W.R., Otis, D.L. and Huang, L. (2008) 'Sampling considerations for disease surveillance in wildlife populations', *Journal of Wildlife Management*, 72(1), pp. 52–60. Available at: https://doi.org/10.2193/2007-317.

O'Byrne, A.M., Lambourn, D.M., Rejmanek, D., Haman, K., O'Byrne, M., VanWormer, E. and Shapiro, K. (2021) '*Sarcocystis neurona* transmission from opossums to marine mammals in the Pacific Northwest', *EcoHealth*, 18(1), pp. 84–94. Available at: https://doi.org/10.1007/s10393-021-01536-w.

Opsteegh, M., Langelaar, M., Sprong, H., den Hartog, L., De Craeye, S., Bokken, G., Ajzenberg, D., Kijlstra, A. and der Giessen, J. van (2010) 'Direct detection and genotyping of *Toxoplasma gondii* in meat samples using magnetic capture and PCR', *International Journal of Food Microbiology*, 139(3), pp. 193–201. Available at: https://doi.org/10.1016/j.ijfoodmicro.2010.02.027.

Ostertag, S.K., Loseto, L.L., Snow, K., Lam, J., Hynes, K. and Gillman, D.V. (2018) '"That's how we know they're healthy": the inclusion of traditional ecological knowledge in beluga health monitoring in the Inuvialuit Settlement Region', *Arctic Science*, pp. 1–29. Available at: https://doi.org/10.1139/as-2017-0050.

Ovitz, K.L., Matari, K.G.A., O'Hara, S., Esagok, D., Inuvik Hunters and Trappers Committee (IHTC) and Loseto, L.L. (2024). Observations of social and environmental change on Kendall Island *(Ukiivik)*, a traditional whaling camp in the Inuvialuit Settlement Region., *Arctic Science*, 10(1), pp. 140–168. Available at: https://doi.org/10.1139/as-2022-0016

Pozio, E. (2007) 'World distribution of *Trichinella* spp. infections in animals and humans', *Veterinary Parasitology*, 149(1–2), pp. 3–21. Available at: https://doi.org/10.1016/j.vetpar.2007.07.002.

Quakenbush, L.T., Suydam, R.S., Brown, A.L., Lowry, L.F., Frost, K.J. and Mahoney, B.A. (2015) 'Diet of beluga whales, *Delphinapterus leucas*, in Alaska from stomach contents, March-November', *Marine Fisheries Review*, 77(1), pp. 70–84. Available at: https://doi.org/10.7755/MFR.77.1.7.

Raach, M., Lebeuf, M. and Pelletier, É. (2011) 'PBDEs and PCBs in the liver of the St Lawrence Estuary beluga (*Delphinapterus leucas*): a comparison of levels and temporal trends with the blubber', *Journal of Environmental Monitoring*, 13(3), p. 649. Available at: https://doi.org/10.1039/c0em00310g.

Reiling, S.J., Measures, L., Feng, S., Boone, R., Merks, H. and Dixon, B.R. (2019) '*Toxoplasma gondii*, *Sarcocystis* sp. and *Neospora caninum*-like parasites in seals from northern and eastern Canada: potential risk to consumers', *Food and Waterborne Parasitology*, 17, p. e00067. Available at: https://doi.org/10.1016/j.fawpar.2019.e00067.

Rosenthal, B.M. (2021) 'Zoonotic *Sarcocysti*s', *Research in Veterinary Science*, 136, pp. 151–157. Available at: https://doi.org/10.1016/j.rvsc.2021.02.008.

Sergeant, E. (2018) *Epitools Epidemiological Calculators. Ausvet.* Available at: https://epitools.ausvet.com.au/ (Accessed: 2 April 2022).

Shapiro, K., VanWormer, E., Packham, A., Dodd, E., Conrad, P.A. and Miller, M. (2019) 'Type X strains of *Toxoplasma gondii* are virulent for southern sea otters (*Enhydra lutris nereis)* and present in felids from nearby watersheds', *Proceedings of the Royal Society B: Biological Sciences*, 286(1909), p. 20191334. Available at: https://doi.org/10.1098/rspb.2019.1334.

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N. and Jenkins, E. (2019) 'Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of *Toxoplasma gondii* in naturally infected wolverines (*Gulo gulo*) from the Yukon, Canada', *Food and Waterborne Parasitology*, 15, p. e00046. Available at: https://doi.org/10.1016/j.fawpar.2019.e00046.

Sheikh, N., Egeland, G.M., Johnson-Down, L. and Kuhnlein, H.V. (2011) 'Changing dietary patterns and body mass index over time in Canadian Inuit communities', *International Journal of Circumpolar Health*, 70(5), pp. 511–519. Available at: https://doi.org/10.3402/ijch.v70i5.17863.

Simon, A., Poulin, M.B., Rousseau, A.N., Dubey, J.P. and Ogden, N.H. (2013) 'Spatiotemporal dynamics of *Toxoplasma gondii* infection in Canadian lynx (*Lynx canadensis*) in Western

Québec, Canada', *Journal of Wildlife Diseases*, 49(1), pp. 39–48. Available at: https://doi.org/10.7589/2012-02-048.

Smith, T.G. and Sjare, B. (1990) 'Predation of belugas and narwhals by polar bears in nearshore areas of the Canadian high Arctic', *ARCTIC*, 43(2), pp. 99–102. Available at: https://doi.org/10.14430/arctic1597.

Springer, Y.P., Casillas, S., Helfrich, K., Mocan, D., Smith, M., Arriaga, G., Mixson, L., Castrodale, L. and McLaughlin, J. (2017) 'Two outbreaks of trichinellosis linked to consumption of walrus meat — Alaska, 2016–2017', *MMWR. Morbidity and Mortality Weekly Report*, 66(26), pp. 692–696. Available at: https://doi.org/10.15585/mmwr.mm6626a3.

Stamatakis, A. (2014) 'RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies', *Bioinformatics*, 30(9), pp. 1312–1313. Available at: https://doi.org/10.1093/bioinformatics/btu033.

Storrie, L., Hussey, N.E., MacPhee, S.A., O'Corry-Crowe, G., Iacozza, J., Barber, D.G., Nunes, A. and Loseto, L.L. (2022) 'Year-round dive characteristics of male beluga whales from the Eastern Beaufort Sea population indicate seasonal shifts in foraging strategies', *Frontiers in Marine Science*, 8, p. 715412. Available at: https://doi.org/10.3389/fmars.2021.715412.

Thiemann, G.W., Iverson, S.J. and Stirling, I. (2008) 'Polar bear diets and Arctic marine food webs: insights from fatty acid analysis', *Ecological Monographs*, 78(4), pp. 591–613. Available at: https://doi.org/10.1890/07-1050.1.

VanWormer, E., Miller, M.A., Conrad, P.A., Grigg, M.E., Rejmanek, D., Carpenter, T.E. and Mazet, J.A.K. (2014) 'Using molecular epidemiology to track *Toxoplasma gondii* from terrestrial carnivores to marine hosts: implications for public health and conservation', *PLoS Neglected Tropical Diseases*. Edited by E. Ghedin, 8(5), p. e2852. Available at: https://doi.org/10.1371/journal.pntd.0002852.

van de Velde, N. *et al.* (2016) '*Toxoplasma gondii* in stranded marine mammals from the North Sea and Eastern Atlantic Ocean: Findings and diagnostic difficulties', *Veterinary Parasitology*, 230, pp. 25–32. Available at: https://doi.org/10.1016/j.vetpar.2016.10.021.

Viera, A.J., Garett, J.M., 2005. Understanding interobserver agreement: the kappa statistic. *Fam. Med*. 37, 360-363

Waugh, D., Pearce, T., Ostertag, S.K., Pokiak, V., Collings, P. and Loseto, L.L. (2018) ‘Inuvialuit traditional ecological knowledge of beluga whale (*Delphinapterus leucas*) under changing climatic conditions in Tuktoyaktuk, NT’, *Arctic Science*, pp. 1–17. Available at: https://doi.org/10.1139/as-2017-0034.

Würsig, B.G., Thewissen, J.G.M. and Kovacs, K.M. (eds) (2018) *Encyclopedia of Marine Mammals*. Third edition. London, United Kingdom; San Diego, CA, United States: Academic Press, an imprint of Elsevier.

TRANSITION STATEMENT CHAPTER 4

I encountered many challenges in Chapter 2 and 3 regarding serology techniques for the detection of *T. gondii* in wildlife samples using commercially available kits. For example, one kit yielded almost 100% seroprevalence in belugas, which was initially thought to be due to interference due to high lipids common in the blood of arctic marine mammals. Therefore, Chapter 4 focused on pilot studies needed to develop in-House ELISAs, including comparison of a specific antigen target (p30) compared to crude extract from tachyzoites of *T. gondii*. This allowed more control and transparency over the various steps involved, concurrently mitigating costs as well as challenges associated with commercial kits primarily designed for livestock. In this chapter, we focus on the optimization of in-house ELISAs using polar bear serum previously tested for *T. gondii* using a commercial kit that has behaved as expected in a range of mammalian species, and is itself becoming a gold standard for veterinary diagnostics. While polar bears might seem an unusual choice for this work, I had access to archived sera from known individual animals as part of a large collaborative study exploring the effects of climate change on exposure of polar bears to terrestrial pathogens (see Appendix; for this study I performed all the laboratory analyses for *T. gondii* and provided input into the publication in Global Change Biology, a high impact journal). As well, polar bears have significance as country foods for certain Inuit communities and play a role as sentinels for pathogen transmission in the Arctic environments. I plan to submit this chapter as a manuscript to Food and Waterborne Parasitology, on which I will serve as first author, having worked closely with the Canadian Food Inspection Agency to develop, and validate these in house assays.

CHAPTER 4. EVALUATION OF THE IN-HOUSE DEVELOPED ELISA FOR DETECTION OF ANTIBODIES TO *TOXOPLASMA GONDII* IN SERUM SAMPLES FROM POLAR BEAR

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4.1 Abstract

Toxoplasma gondii is a zoonotic protozoan parasite that can cause significant disease in most warm-blooded animals, including humans. Surveillance testing of human and animal populations is essential to estimate disease prevalence, assess food safety risks and establish control programs. Serological tests are the most practical methods by which to detect the prevalence of *Toxoplasma* infection in a broad range of hosts. An in-house indirect ELISA was developed using crude extract (CE) antigen (CE IELISA) and a commercial purified p30 antigen (P30 IELISA) have been developed and evaluated for the detection of antibodies to *Toxoplasma gondii* in serum samples of polar bears. Performance of the two in-house developed assays was evaluated using a commercially available kit. Results indicated that the in-house CE IELISA had relative sensitivity and specificity of 0.744 and 0.865 respectively. Additionally, and moderate to good agreement between CE IELISA and the commercial kit; therefore, we considered these tests suitable for testing polar bear samples. P30 IELISA was considered as a not reliable test for testing polar bear samples, due data showed low dispersion (standard deviation= 0.101) and overlapping between positive and negative samples.

4.2 Introduction

The protozoan *Toxoplasma gondii* is a worldwide-distributed zoonotic obligate intracellular parasite and it is considered one of the most successful parasites globally (Dubey, 2010). Its complex life cycle involves the developmental stages of oocysts, tachyzoites and bradyzoites, all of which are infective for susceptible hosts. Members of the family Felidae are the only known definitive hosts, whereas a wide range of warm-blooded species including humans, livestock, wild mammals and birds serve as intermediate hosts for this parasite (Robert-Gangneux and Dardé, 2012). While parasite infection is usually subclinical or asymptomatic, it holds significant importance in public and animal health due to its potential severe consequences during pregnancy (fetal loss or congenital toxoplasmosis) and encephalitis in immunosuppressed patients (Dubey and Jones, 2008; Stelzer *et al.*, 2019).

The gold standard diagnostic test to detect *T. gondii* in humans and animals is the isolation of the parasite by bioassay in laboratory animals (Liu *et al.*, 2015). This requires fresh, unfrozen samples and does not align with responsible animal use. However, isolating DNA of the parasite, as well

as other pathogens, from wildlife can be challenging with low parasite burdens and also requires invasive tissue sampling (Gilbert *et al.*, 2013). Therefore, serological techniques for detection of antibodies in serum/blood samples are important tools for surveillance studies that do not require invasive sampling.

Among the diverse range of serological tests, the enzyme-linked immunosorbent assay (ELISA) is the most amenable to large scale surveillance testing of sera (Tabatabaei and Ahmed, 2022). This assay has the potential to be sensitive, can be semi-automated for large scale testing, and the reading of results is less subjective than in other serological assays (Liu *et* al., 2015, Hill *et al.*, 2006; Al-Adhami and Gajadhar, 2014). The conventional indirect ELISA usually involves the preparation of lysates or crude extracts (CE) of whole tachyzoites grown in mice or tissue cultures, which vary between laboratories and are difficult to standardize. In recent years, the use of purified recombinant proteins has developed as an alternative to increase sensitivity and specificity for ELISAs, with the advantage of precise antigens and easy standardization (Liu *et al.*, 2015).

Many of these recombinant proteins play a role in host cell invasion, immune modulation and/or virulence attenuation of *T. gondii*, and are highly immunogenic (Boothroyd *et al.*, 1998; Holec-Gąsior, 2013). Among the *T. gondii* antigens, surface antigen 1 (SAG1), also known as p30, stands out as the most immunodominant and stage-specific protein in tachyzoites (Kasper, Bradley and Pfefferkorn, 1984). It represents approximately 3 to 5 % of the total protein content of *Toxoplasma*. This protein's high immunogenicity makes it a valuable choice as a diagnostic reagent (Bonhomme *et al.*, 1994). As a result, it is widely utilized in various commercial ELISA kits (Dard *et al.*, 2016), including indirect multi-species assays which use a horseradish peroxidase (HRP) A/G as a conjugate and can therefore be used in multiple mammalian species, ideal for rare species for which species-specific conjugates are seldom available, such as polar bears.

The commercial ELISA test kit IDVET ELISA (ID Screen® Toxoplasmosis Indirect Multi-species, IDVet Innovative Diagnostics, Grabels, France) is widely accepted as a reliable test for screening animals for *Toxoplasma* infection regardless of host species. (Liyanage *et al.*, 2021). It has been also used as a screening tool for wildlife animals (Bachand *et al.*, 2019; Calvo-Mac *et al.*, 2020; Engel *et al.*, 2022). A substantial to excellent agreement has been reported between the IDVET ELISA and the magnetic capture qPCR for detection of DNA of *T. gondii*, serving as a reference test in carnivore samples (Sharma *et al.*, 2019; Bouchard *et al.*, 2022).

Polar bears (*Ursus maritimus*) are listed as Special Concern by COSEWIC and Threatened by the USA. These bears are considered sentinel species for environmental issues in the arctic regions, such as climate change and pollution. Additionally, as polar bears are apex predators, they are indicators of changes in lower trophic levels and in the Arctic marine ecosystems (COSEWIC, 2018). Furthermore, polar bears are considered sentinels of disease dynamics and pathogens, such as *T. gondii* (Pilfold *et al.*, 2021).

In this study, serum samples from polar bears collected in Western Hudson Bay, Canada under a long-term serological surveillance of several pathogens including *Toxoplasma*, and provided by (Pilford et *al.*, 2021) were used to evaluate the performance of the developed in-house indirect ELISAs. These same samples were previously tested by the commercial IDVET ELISA in our laboratory.

4.3 Materials and Methods

4.3.1 Samples collection

A subset of polar bear serum samples (n=297) was selected from a larger pre-existing collection (bank) previously tested for *T. gondii* (Pilfold *et al.*, 2021). To ensure a representative and diverse sample set, repetitive samples from individuals recaptured during the sampling phase (Lunn *et al.*, 2016) were excluded. To preserve homogeneity within the dataset, a balanced distribution of positive and negative samples, as well as female (n=145) and male (n=152) individuals, was maintained (65 negative females: 68 negative males, 80 positive females:84 positive males).

4.3.2 Antigen preparation

4.3.2.1 Tachyzoites crude extract

The crude extract (CE) was prepared and obtained from the Centre for Food-borne and Animal Parasitology (Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada). Briefly, tachyzoites from *T. gondii* RH strain were lysed and prepared as previously described (Al-Adhami and Gajadhar, 2014). Stocks of zoites (previously harvested from cell culture, washed, and filtered) were re-suspended in PBS, immersed in liquid nitrogen for 1 min and then thawed immediately in a 65°C water bath for 1 min. The cycle was repeated 3 more times. Tachyzoites were disrupted by

sonication for 20 s at 4°C at an amplitude of 60%. The homogenate was centrifuged at 10,000 x g for 30 min at 4°C, and the supernatant was collected and stored at -20°C.

The protein concentration was measured using the Pierce® Rapid Gold BCA Protein Assay kit (Thermo Scientific). The standard curve was performed using BSA starting at 2 mg/mL. The plate was read and measured in a Varioskan LUX multiomode microplate reader (Thermo Fisher Scientfic) at 480 nm of wavelength.

4.3.2.2 Purification of P30

Protein p30 was purified from the CE by immunoprecipitation using Pierce® Crosslink Immunoprecipitation Kit (Thermo Scientific, USA). Ten µg of mouse monoclonal antibodies (Mab clone P30/3, Bio-Rad) were bound to a protein A/G agarose spin column and immobilized using Dimethyl sulfoxide (DMSO) as a crosslinker. A volume of 450 µL of the CE containing 133.7 µg of protein, was added to the spin column and incubated overnight at 4ºC. The next day, the column was washed, and the antigen was eluted from the Agarose-Protein A/G-Mab complex. Afterwards, the eluate was detected on SDS gel (Fig. 4.1). This process required large number of tachyzoites and yielded small volume of the antigen (95 µL containing <125 µg/ml protein) which was inadequate to proceed with coating ELISA plates. Therefore, a commercially available purified p30 (*Toxoplasma gondii* p30 protein, Fitzgerald Industries International, Inc. MA, USA) was utilized to continue further testing. The lyophilized product was reconstituted in Glycine buffer saline pH 9.0 at a final concentration of 1 mg/ mL following the manufacturer instructions.

4.3.3 Preparation of standard reagents and optimization of the in-house developed IELISA

4.3.3.1 Protein profiles of the antigens

To identify and confirm the presence of antigen proteins, as well as to estimate their molecular weight, SDS-PAGE was performed, comparing the CE antigen, an in-house p30 purified protein and the commercial p30 protein. Band profiles for all fractions on SDS-PAGE were detected and the molecular weights analyzed using Image Lab software V.5.2.1 (BioRad, USA) (Fig. 4.1).

4.3.3.2 Western Blot

To confirm the reactivity of the antigens with anti-*Toxoplasma* antibodies, serum samples collected from pigs (*Sus scrofa*), gray seal (*Halichoerus grypus*), and reindeer (*Rangifer tarandus*) experimentally infected with *T. gondii* were used in a WB analysis. Additionally, polar bears sera previously tested by IDVET (Pilfold *et al.*, 2021) were added. Positive and negative control serum samples from each species were included on each blot. The crude extract was loaded into a Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 0.75 µg/mL per well except for the wells used for polar bear sera (2.7 µg/mL). Proteins were transferred to nitrocellulose membranes, and the blots were washed and blocked in 5% skim milk in TBS. Sera were diluted at 1:250 in a 1% skim milk Tris Buffered Saline with 0.05% Tween-20 (TBST) solution. Protein A/G conjugate diluted at 1:10,000 was used as a secondary antibody. Bands were developed with a commercial substrate (Clarity® Western ECL Substrate, Bio-Rad, USA) dissolved in Tris Buffered Saline (TBS) and visualized in a gel documentation system (Fig. 4.2, 4.3).

4.3.3.3 Optimization of in-house indirect ELISA (IELISA)

Sera from pigs and polar bears confirmed as *T. gondii* antibody-positive or -negative by commercially available ELISA-IgG test kits were used as reference sera (Forbes, et al.2012, Pilfold et al. 2021). Additionally, the plate included bovine samples with known positive serostatus for *Besnoitia*, *Neospora*, one pig sample for *Trichinella* T6, and one rabbit sample for *Cryptosporidium*, respectively. The sera were tested in duplicates with a 2-fold dilution at 1:25, and the non-species-specific protein A/G conjugated to horseradish peroxidase was diluted at 1:10K and 1:40K.

4.3.4 Protocol for in-house IELISA using polar bear serum samples

A panel of 297 polar bear samples were tested using both the CE and p30 protein antigens. An indirect ELISA was performed as previously described (Al-Adhami & Gajadhar, 2014) with modifications following the optimized conditions (4.3.3.3). Microplates (Costar, Corning Inc., USA) were coated with 100 µL of the antigens in carbonate buffer (pH 9.6) and divided into two, with p30 on one side and crude extract on the other. The plates were incubated overnight at 4ºC. Post-incubation, the plates were thrice washed with TBS, subsequently blocked with 200 µL of TBST containing 2% BSA for 1 h at 37 ºC, succeeded by another three wash cycles. The serum

samples, diluted 1:25 with 5% skim milk TBST 1% BSA, were added in duplicates, 100 µL/well, to both halves of the plate. A one-hour incubation at 37ºC was followed by three washing rounds. Following this, 100 µL of HRP-protein A/G conjugate, diluted 1:10K in TBST 1% BSA, were added to each well and incubated 1 h at 37 ºC. Subsequently, the plate was washed thrice, and 100 µL of 3,3',5,5' – tetramethylbenzidine (TMB) substrate solution (R&D Inc, USA) was added and incubated in the dark for 20 min at RT. The reaction was stopped by adding 50 µL of 2N H_2SO_4. The absorbance was read at a wavelength of 450 nm using a microplate spectrophotometer (Varioskan LUX multimode microplate reader, Thermo Scientific). Afterwards, the Optical Density (OD) values were obtained for subsequent analysis.

4.3.5 Commercial ELISA kit

The commercially available indirect ELISA IDVET kit (ID Screen® Toxoplasmosis Indirect Multi-species, IDVet Innovative Diagnostics, Grabels, France) was used to perform the testing following the manufacturer instructions as previously reported (Pilfold *et al.*, 2021). The sample/positive percentage (S/P%) was calculated using the formula S/P%= $[(OD_{sample} - OD_{negative\ control} / OD_{positive\ control} - OD_{negative\ control})]$ x 100. Samples were considered negative if S/P% was less than 40%, positive if S/P% was higher than 50%, and samples with S/P% between 40 and 50% were considered suspicious. Polar bear samples were tested at 1:10 dilution following the manufacturer instructions.

4.3.6 Data analysis

Considering the ELISA IDVet kit as a gold standard, data from the three ELISAs was analyzed using Diagnostic Test Evaluation and Comparison from Epitools (www.epitools.ausvet.com). For this test, positive samples by IDVET were labeled as Infected, and negative samples as Uninfected. The Youden's J value obtained from Epitools (0.920) was used as cut-point value for the CE IELISA. The developed in-house IELISAs were compared with the IDVET ELISA using a cross table, and the test parameters were calculated (relative sensitivity, relative specificity, positive predictive value, negative predictive value). IDVET kit and In-house IELISAs were compared using the McNemar's test of homogeneity (IBM SPSS Statistics). Agreement between p30 in-house IELISA, in-house CE IELISA, and IDVET kit was estimated by calculating Kappa (k) value. Kappa values of ≥0.81, 0.61–0.80, 0.40–0.60, and ≤0.40 were considered to represent excellent,

substantial, moderate to good, and slight to poor agreement, respectively (Viera and Garret, 2005). The relative sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence intervals were calculated as previously described (WOAH, 2023).

4.4 Results

4.4.1 Performance of prepared antigens and WB

The SDS-PAGE revealed the protein banding pattern for the CE (Fig. 4.1), along with the in-house purified p30 protein and the commercial p30. As mentioned earlier (4.3.2.2) the in-house purified p30 was not used in further testing due to the minimal protein yield. In the WB, antibodies from experimentally infected pig recognized seven major antigens from the CE, with molecular weight of 79, 75, 65, 55, 45, 35 and 30 kDa, respectively (Fig. 4.2). Positive controls from experimentally infected seal, reindeer and polar bear recognized similar antigens to those found in the pig sample, and all positive controls identified p30 protein. For the p30 WB, all positive controls recognized the commercially purified protein (Fig. 4.3). Additionally, negative controls from pig and seal recognized p30 in both WBs.

Using check*er*board titration, the previously established test conditions for the mammalian in-house indirect ELISA (ELISA-A/G) (Al-Adhami and Gajadhar, 2014) were optimized as follows: 20 ng and 28 ng of coating antigen per well for p30 and CE antigens, respectively. Data was plotted to compare the serum dilutions from the eight internal reference controls (Fig. 4.4).

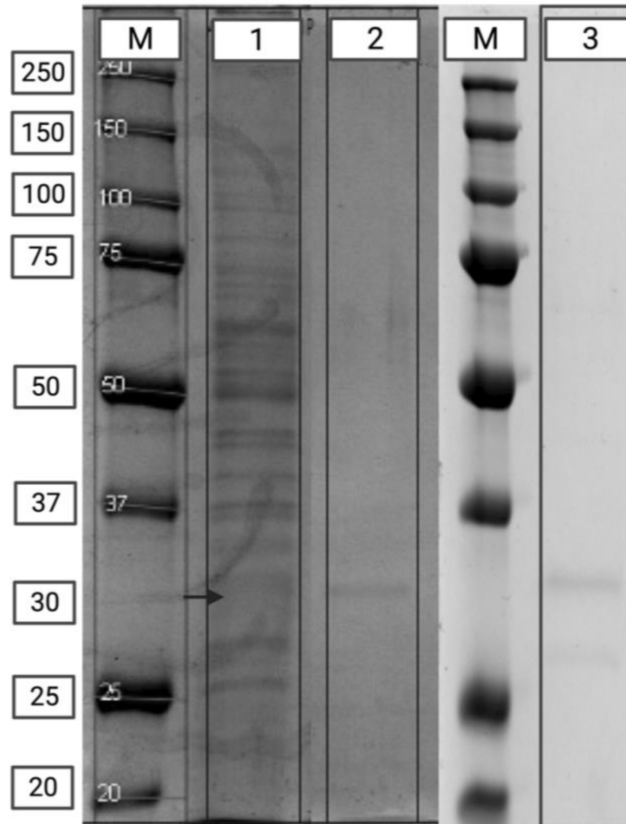

Fig. 4.1 SDS-PAGE comparing antigens of *Toxoplasma gondii* used for IELISAs.

M, marker showing molecular weight in kDa. Lane 1, protein profile from crude extract antigen. Lane 2, in-house purified p30 protein. Lane 3, commercial p30 protein. Arrow shows the 30 kDa protein.

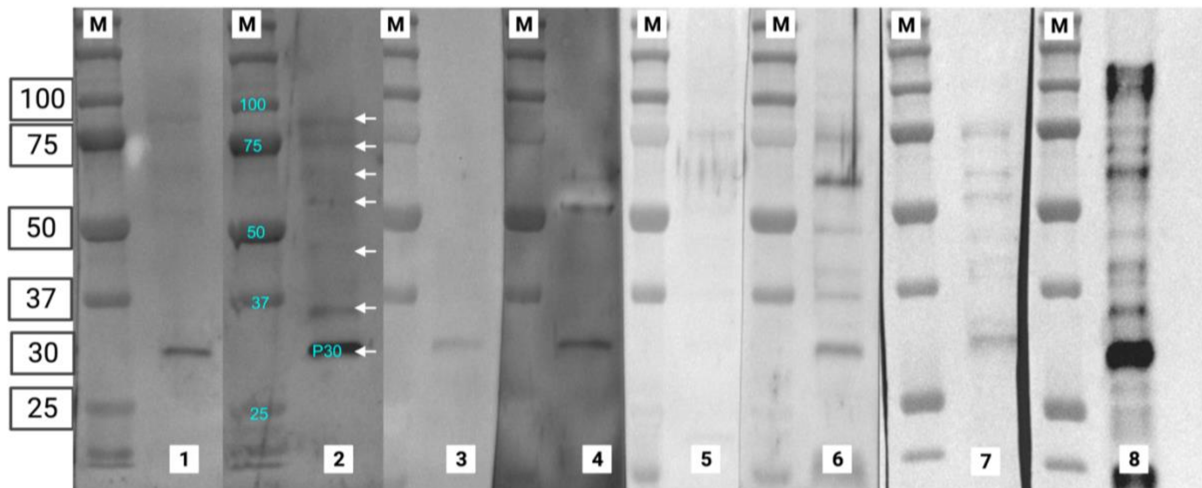

Fig. 4.2 Western Blot protein profiles of crude extract of *Toxoplasma gondii* CE.

Sera from pre-bleed pig (1), experimentally infected pig (2), pre-bleed seal (3), experimentally infected seal (4), pre-bleed reindeer (5), experimentally infected reindeer (6), polar bear tested negative by IDVET (7), and polar bear tested positive by IDVET (8). M, marker showing molecular weights in kDa, repeated for each sample. Arrows indicate the 7 major proteins (79, 75, 65, 55, 45, 35 and 30 kDa) recognized by antibodies from infected pig, seal, reindeer, and polar bear. Note that protein concentration loaded in each of well 7 & 8 was 3.6X the amount loaded in each of other wells.

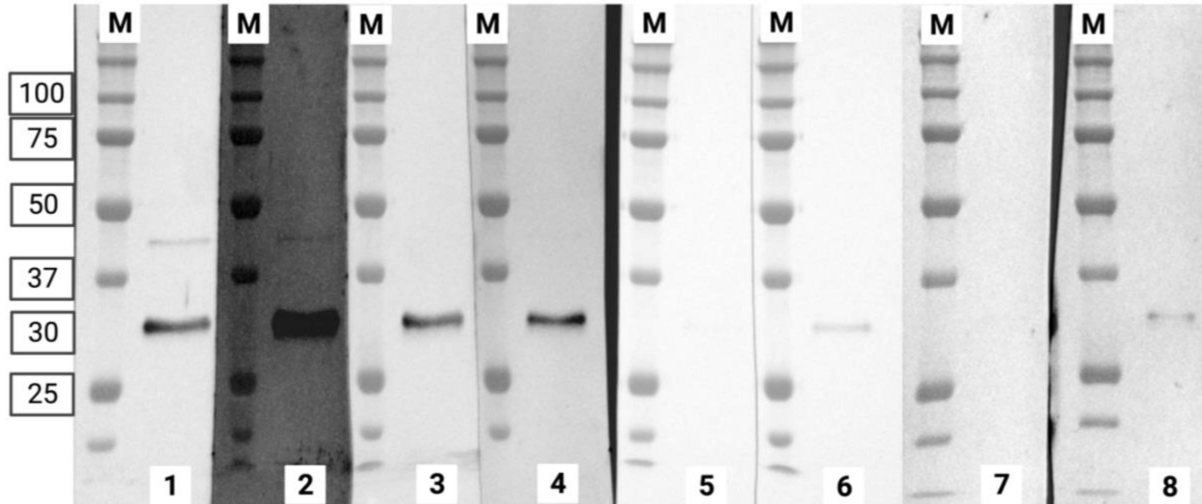

Fig. 4.3 Western Blot protein profiles of the commercial p30 antigen.

Sera from pre-bleed pig (1), experimentally infected pig (2), pre-bleed seal (3), experimentally infected seal (4), pre-bleed reindeer (5), experimentally infected reindeer (6), polar bear tested negative by IDVET (7), and polar bear tested positive by IDVET (8). M, marker showing molecular weights in kDa, repeated for each sample.

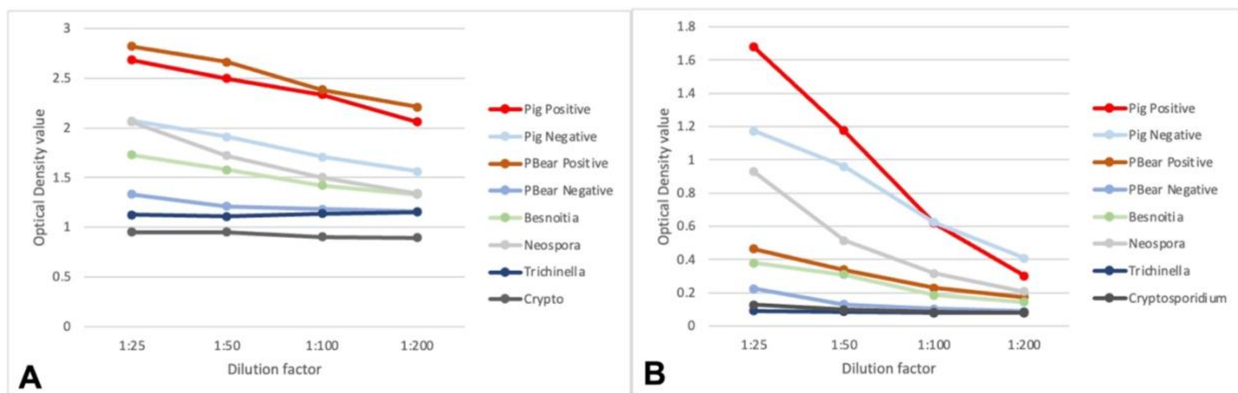

Fig. 4.4 Checkerboard titration comparing the two antigens.

A, crude extract at 28 ng /well; B, p30 protein at 20 ng/well. Sera from pigs and polar bears confirmed as *T. gondii* antibody-positive or –negative were included on the plate. Serum samples of known positive status to *Besnoitia*, *Neospora caninum*, *Trichinella* T6 and *Cryptosporidium*, respectively were added to the plate.

4.4.2 Panel of tested serum samples from polar bear

A total of 297 serum samples previously tested by IDVET for *T. gondii* were used for the in-house IELISAs. From these samples, 145 were from female and 152 were from male animals. Sex of the animals and status by IDVET are shown in Table 4.1. These samples were simultaneously re-tested on the developed P30 and CE IELISA.

4.4.3 Distribution of raw data for in-house CE and P30 IELISAs

Boxplots of test results by "disease" (IDVET test) status and a strip chart of test results were obtained from the analysis in Epitools. The IDVET ELISA showed a good separation between negative and positive samples (St. deviation= 1.246), with almost no overlapping of the data (Fig 5). For the in-house CE IELISA, data points were more dispersed (St. deviation= 0.793, Table 1) with some overlap between the negatives and positive samples with low OD (Fig. 4.5). In contrast, data from the P30 IELISA showed low dispersion (St. deviation= 0.101, Table 4.1) and almost complete overlap between positive and negative samples (Fig. 4.5). Consequently, P30 IELISA was not included in further analysis.

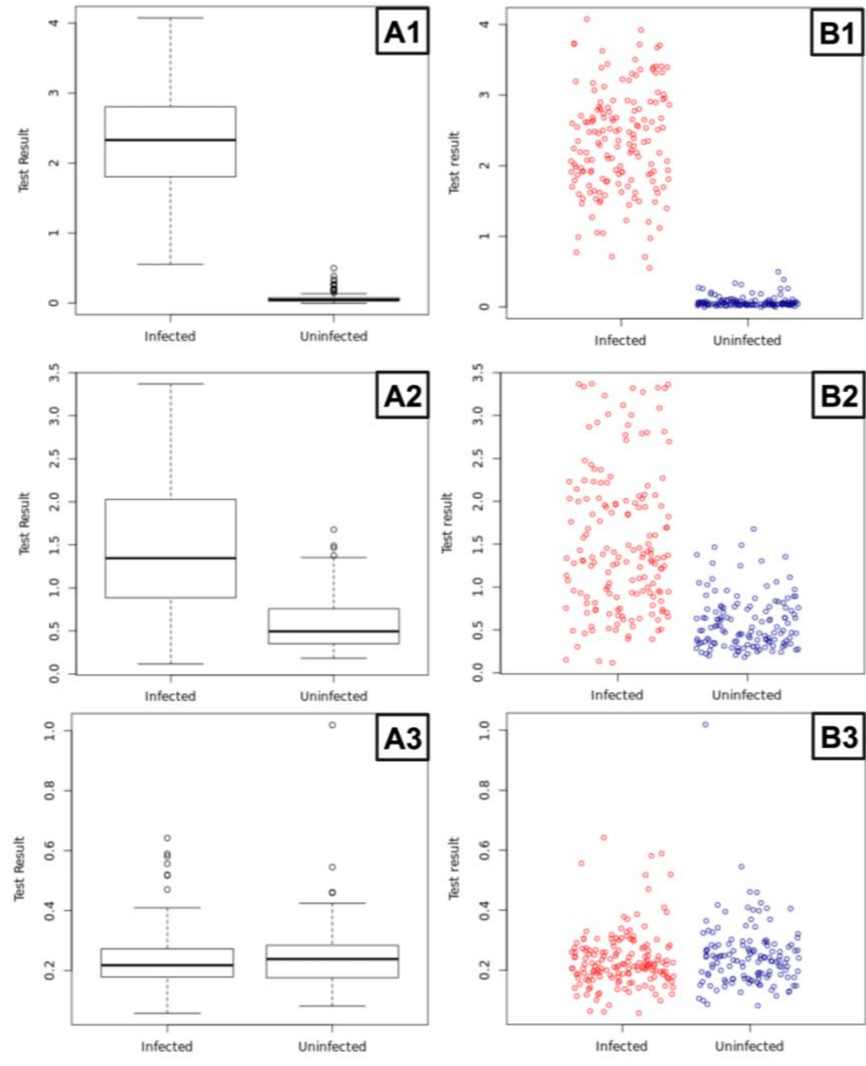

Fig. 4.5 Box plots (A) and Strip charts (B) showing the distribution of the optical density values from the IDVET (1), crude extract (2) and p30 protein (3) ELISAs using 297 polar bear samples.

Table 4.1 Comparison of optical density values from three ELISAs for detection of *Toxoplasma gondii* antibodies from naturally infected polar bears.

	P30 protein		**Crude extract**		**IDVET**	
	Negative	Positive	Negative	Positive	Negative	Positive
Mean	0.247	0.234	0.534	1.516	0.07	2.326
	0.240		1.099		1.316	
Median	0.238	0.217	0.495	1.345	0.046	2.328
	0.255		0.840		1.478	
Standard deviation	0.108	0.095	0.309	0.821	0.077	0.522
	0.101		0.793		1.246	
Minimum	0.081	0.057	0.182	0.117	-0.004	0.550
Maximum	1.019	0.642	1.677	3.370	0.497	4.074
Yourden's J cut-point	0.470		0.920		0.550	

4.4.4 Evaluation of the performance of in-house CE IELISA

The proportion of positive samples was 55.2% (95% confidence intervals [CI]: 49.5-60.8%) for IDVET ELISA and 47.1% (95% CI: 41.5-52.8%) for the CE IELISA. Agreement between tests was considered moderate to good (k=0.592).

Positives by IDVET ELISA (n=122) were classified in four groups using the 25, 50 and 75 percentiles as follow: low positive 0.5-1.797 (19 samples), medium positive 1.798-2.326 (26 samples), high positive 2.327-2.802 (37 samples), and very highly positive ≥2.803 (40 samples). The proportion of samples that disagreed between tests was 20.2% (95% CI: 16-25.1%): 37/42 samples that tested negative by CE IELISA (false negatives) were considered low-medium positives by IDVET, and 6 samples were considered high and very high. Eighteen samples that tested positive by CE IELISA were considered negative by IDVET (false positives) (Table 4.2).

Table 4.2 Two by two table comparing the in-house CE IELISA against the IDVET ELISA to detect *T. gondii* antibodies in naturally infected polar bears.

	IDVET ELISA		
CE ELISA 0.92	Positive	Negative	Total
Positive	122	18	140
Negative	42	115	157
Total	164	133	297
Relative sensitivity	0.744		
Relative specificity	0.865		
PPV	0.871		
NPV	0.728		
Kappa	0.598		
McNemar test (p value)	0.003		

CE- Crude extract

PPV- Positive predictive value

NPV- Negative predictive value

4.5 Discussion

In the present study, a crude extract antigen (CE) prepared from our cell-cultured tachyzoites, and a p30 antigen obtained from commercial sources have been utilized to develop ELISAs to diagnose *T. gondii* infection in polar bears. Previous studies evaluated the performance of the P30 ELISA using field samples and /or samples of known positive or negative status for *T. gondii* infection. Results of the P30 ELISA were compared with those generated by traditional ELISAs using native *Toxoplasma* proteins, WB, and IFAT. It was concluded that the P30 ELISA is a useful tool for the specific and sensitive detection of antibodies to *T. gondii* in serum samples collected from naturally exposed and experimentally infected animals. (Pardini *et al.*, 2012, Kimbita *et al.*, 2001; Maksimov *et al.*, 2011; Sudan, Tewari and Singh, 2015). In our study, the in-house –developed CE IELISA showed promising results with serum samples from polar bears but the P30 IELISA failed to discriminate between positive and negative samples. The SDS-PAGE and WB analyses conducted on the commercial p30 antigen showed its reactivity with serum samples of known positive status to *T. gondii* (Fig 4.3). However, this antigen failed the testing on ELISA plates, and we have no clear explanation for this result. Further work is needed to evaluate different batches of commercially available p30 proteins on ELISA plate. Also, it would have been useful to purify recombinant p30 from the tachyzoites crude extract in the laboratory. However, our trial yielded minimal amount of p30 that was not adequate to be used to develop the ELISA. Preparation of p30 in the laboratory would be achieved by cloning and expression of the amino acid sequence in plasmids (Qi *et al.*, 2022) or by affinity chromatography (Hosseininejad *et al.*, 2009). However, these procedures are time and cost consuming as they require the maintenance of either *T. gondii* tachyzoites *in vitro* or bacteria, such as *E. coli*, for the expression of plasmids with the sequence of interest. This was considered to be performed but disrupted by the COVID-19 pandemic closure as well as the by occupational health concerns of maintaining tachyzoites *in vitro*.

Expanding the panel of serum controls could improve the overall optimization of the IELISA. Some authors suggest using between four to five samples as controls, to assess the range of antibodies against the specific antigen (Crowther, 2010). While this approach is ideal for any ELISA, the challenges presented by wildlife samples remain a constant factor in optimizing serological tests.

In the current study, we demonstrated the suitability of protein A/G conjugate as a reagent for detection of *T. gondii* IgG antibodies in polar bear samples. The effectiveness of this reagent has been previously reported in both domestic and wildlife species (Al-Adhami and Gajadhar, 2014) including black bears (*Ursus americanus*) and grizzly bears (*Ursus arctos*) (Godfroid, Beckmen and Helena Nymo, 2016; Dorion *et al.*, 2021), demonstrating that the combined utilization of both proteins A and G leads to favorable performance for multiple species. The affinity of protein A and protein G varies among host species antibodies (Merck KGaA, 2023); therefore, when dealing with single host species, the employment of a single protein is adequate for conducting the test. In the case of polar bears and other carnivores, antibodies exhibit a higher affinity with protein A than for G (Nymo *et al.*, 2013).

We found moderate to good agreement between the in-house CE IELISA and the commercially available ELISA kit (k=0.592). The relative sensitivity and specificity of the in-house CE IELISA were 0.744 and 0.865 respectively, which was considered a good performance of the test despite both false negatives and false positives were detected, and would be suitable for broad epidemiological studies where cost is a concern. We acknowledge that the commercial kit is not a perfect classification but was the best option in the absence of reference standards for polar bears and tissues from these animals to determine presence of the parasite. An alternative approach for comparing these 3 tests in the absence of a true gold standard involves the utilization of latent-class models, which obviate the need for designating a gold standard and provide flexible means of estimating test accuracy (Enøe, Georgiadis and Johnson, 2000). These methodologies enable the estimation of the relative sensitivity and specificity for two tests when the true infection status of the samples is unknown, as there is no necessity for the test to be designated as perfect for analytical purposes (International Office of Epizootics and Biological Standards Commission, 2018). Additionally, these methods are well-suited for comparing antigen and antibody detection tests for *T. gondii* since antibodies are potentially detectable for most of an infected animal's lifespan (Gardner, Greiner and Dubey, 2010).

4.6 Conclusion

This study involved a comparison between a crude extract in-house IELISA, a commercial p30 protein in-house IELISA, and a commercially available ELISA kit for the detection of *T. gondii* antibodies in polar bear serum samples. Inexplicably, the commercially obtained p30 antigen was unable to differentiate between positive and negative samples, and performed poorly as compared to the crude extract. The evaluation revealed a good performance of the CE IELISA, and a moderate to good agreement with the commercial ELISA. Our findings support the employment of protein A/G as a conjugate for detection of antibodies against *T. gondii* in polar bear sera. Additionally, this data underscores the importance of considering approaches to test evaluation test accuracy, especially where gold standards and controls are lacking, which is often the case with samples from wildlife of conservation significance. Future work is recommended to continue exploring the options of purified or recombinant p30 protein as an antigen for diagnostic tests for *T. gondii.*

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4.8 References

Al-Adhami, B.H. and Gajadhar, A.A. (2014) 'A new multi-host species indirect ELISA using protein A/G conjugate for detection of anti-Toxoplasma gondii IgG antibodies with comparison to ELISA-IgG, agglutination assay and Western blot', *Veterinary Parasitology*, 200(1–2), pp. 66–73. Available at: https://doi.org/10.1016/j.vetpar.2013.11.004.

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E. and Jenkins, E. (2019) 'Serological and molecular detection of Toxoplasma gondii in terrestrial and marine wildlife harvested for food in Nunavik, Canada', *Parasites & Vectors*, 12(1), p. 155. Available at: https://doi.org/10.1186/s13071-019-3408-9.

Bonhomme, A., Thirion, C., Boulanger, F., Charton, F., Burlet, H., Pinon, J.M. and Alix, A.J.P. (1994) '*Toxoplasma gondii* -structure variations of the antigen P30', *Parasitology*, 108(3), pp. 281–287. Available at: https://doi.org/10.1017/S0031182000076125.

Boothroyd, J.C., Hehl, A., Knoll, L.J. and Manger, I.D. (1998) 'The surface of Toxoplasma: more and less', *International Journal for Parasitology*, 28(1), pp. 3–9. Available at: https://doi.org/10.1016/S0020-7519(97)00182-3.

Bouchard, É., Sharma, R., Hernández-Ortiz, A., Buhler, K., Al-Adhami, B., Su, C., Fenton, H., G.-Gouin, G., Roth, J.D., Rodrigues, C.W., Pamak, C., Simon, A., Bachand, N., Leighton, P. and Jenkins, E. (2022) 'Are foxes (Vulpes spp.) good sentinel species for Toxoplasma gondii in northern Canada?', *Parasites & Vectors*, 15(1), p. 115. Available at: https://doi.org/10.1186/s13071-022-05229-3.

Calvo-Mac, C., Gutleb, A.C., Contal, S., Ilukewitsch, V., Muñoz-Zanzi, C. and Medina-Vogel, G. (2020) 'Exposure to Toxoplasma gondii in Marine Otters (Lontra felina) and Domestic Cats (Felis catus) in an Arid Environment in Chile', *Journal of Wildlife Diseases*, 56(4). Available at: https://doi.org/10.7589/2019-10-269.

COSEWIC (2018) *COSEWIC assessment and status report on the polar bear, Ursus maritimus, in Canada*. Ottawa, ON: Committee on the Status of Endangered Wildlife in Canada = Comité sur la situation des espèces en péril au Canada. Available at: https://wildlife-

species.canada.ca/species-risk-registry/virtual_sara/files/cosewic/OursBlancPolarBear-2019-Eng.pdf.

Crowther, J.R. (2010) *The ELISA guidebook*. New York: Humana Press.

Dard, C., Fricker-Hidalgo, H., Brenier-Pinchart, M.-P. and Pelloux, H. (2016) 'Relevance of and New Developments in Serology for Toxoplasmosis', *Trends in Parasitology*, 32(6), pp. 492–506. Available at: https://doi.org/10.1016/j.pt.2016.04.001.

Dorion, B., Black, W., Wolff, P., Murray, L., Nomi, K. and Bildfell, R. (2021) 'Seroprevalence of Toxoplasma gondii in American Black Bears (Ursus americanus) in Nevada, USA, using an Enzyme-linked Immunosorbent Assay', *Journal of Wildlife Diseases*, 57(2). Available at: https://doi.org/10.7589/JWD-D-20-00066.

Dubey, J. and Jones, J. (2008) '*Toxoplasma gondii* infection in humans and animals in the United States', *International Journal for Parasitology*, 38(11), pp. 1257–1278. Available at: https://doi.org/10.1016/j.ijpara.2008.03.007.

Dubey, J.P. (2010) *Toxoplasmosis of animals and humans*. 2nd ed. Boca Raton: CRC Press.

Engel, L., Hamedy, A., Kornacka-Stackonis, A., Langner, T., Birka, S. and Koethe, M. (2022) 'Toxoplasma gondii in raccoons (Procyon lotor) in Germany: a serosurvey based on meat juice', *Parasitology Research*, 121(12), pp. 3417–3425. Available at: https://doi.org/10.1007/s00436-022-07646-w.

Enøe, C., Georgiadis, M.P. and Johnson, W.O. (2000) 'Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown', *Preventive Veterinary Medicine*, 45(1–2), pp. 61–81. Available at: https://doi.org/10.1016/S0167-5877(00)00117-3.

Gardner, I.A., Greiner, M. and Dubey, J.P. (2010) 'Statistical Evaluation of Test Accuracy Studies for *Toxoplasma gondii* in Food Animal Intermediate Hosts', *Zoonoses and Public Health*, 57(1), pp. 82–94. Available at: https://doi.org/10.1111/j.1863-2378.2009.01281.x.

Gilbert, A.T., Fooks, A.R., Hayman, D.T.S., Horton, D.L., Müller, T., Plowright, R., Peel, A.J., Bowen, R., Wood, J.L.N., Mills, J., Cunningham, A.A. and Rupprecht, C.E. (2013) 'Deciphering Serology to Understand the Ecology of Infectious Diseases in Wildlife', *EcoHealth*, 10(3), pp. 298–313. Available at: https://doi.org/10.1007/s10393-013-0856-0.

Godfroid, J., Beckmen, K. and Helena Nymo, I. (2016) 'Removal of Lipid from Serum Increases Coherence between Brucellosis Rapid Agglutination Test and Enzyme-linked Immunosorbent Assay in Bears in Alaska, USA', *Journal of Wildlife Diseases*, 52(4), pp. 912–915. Available at: https://doi.org/10.7589/2015-11-298.

Hill, D.E., Chirukandoth, S., Dubey, J.P., Lunney, J.K. and Gamble, H.R. (2006) 'Comparison of detection methods for Toxoplasma gondii in naturally and experimentally infected swine', *Veterinary Parasitology*, 141(1–2), pp. 9–17. Available at: https://doi.org/10.1016/j.vetpar.2006.05.008.

Holec-Gąsior, L. (2013) 'Toxoplasma gondii Recombinant Antigens as Tools for Serodiagnosis of Human Toxoplasmosis: Current Status of Studies', *Clinical and Vaccine Immunology*, 20(9), pp. 1343–1351. Available at: https://doi.org/10.1128/CVI.00117-13.

Hosseininejad, M., Azizi, H.R., Hosseini, F. and Schares, G. (2009) 'Development of an indirect ELISA test using a purified tachyzoite surface antigen SAG1 for sero-diagnosis of canine *Toxoplasma gondii* infection', *Veterinary Parasitology*, 164(2–4), pp. 315–319. Available at: https://doi.org/10.1016/j.vetpar.2009.05.029.

International Office of Epizootics and Biological Standards Commission (2018) *Manual of diagnostic tests and vaccines for terrestrial animals: (mammals, birds and bees)*.

Kasper, L.H., Bradley, M.S. and Pfefferkorn, E.R. (1984) 'Identification of stage-specific sporozoite antigens of Toxoplasma gondii by monoclonal antibodies.', *The Journal of Immunology*, 132(1), pp. 443–449. Available at: https://doi.org/10.4049/jimmunol.132.1.443.

Kimbita, E.N., Xuan, X., Huang, X., Miyazawa, T., Fukumoto, S., Mishima, M., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Suzuki, N., Mikami, T. and Igarashi, I. (2001) 'Serodiagnosis of Toxoplasma gondii infection in cats by enzyme-linked immunosorbent assay

using recombinant SAG1', *Veterinary Parasitology*, 102(1–2), pp. 35–44. Available at: https://doi.org/10.1016/S0304-4017(01)00522-2.

Liu, Q., Wang, Z.-D., Huang, S.-Y. and Zhu, X.-Q. (2015) 'Diagnosis of toxoplasmosis and typing of Toxoplasma gondii', *Parasites & Vectors*, 8(1), p. 292. Available at: https://doi.org/10.1186/s13071-015-0902-6.

Liyanage, K.L.D.T.D., Wiethoelter, A., Hufschmid, J. and Jabbar, A. (2021) 'Descriptive Comparison of ELISAs for the Detection of Toxoplasma gondii Antibodies in Animals: A Systematic Review', *Pathogens*, 10(5), p. 605. Available at: https://doi.org/10.3390/pathogens10050605.

Lunn, N.J., Servanty, S., Regehr, E.V., Converse, S.J., Richardson, E. and Stirling, I. (2016) 'Demography of an apex predator at the edge of its range: impacts of changing sea ice on polar bears in Hudson Bay', *Ecological Applications*, 26(5), pp. 1302–1320. Available at: https://doi.org/10.1890/15-1256.

Maksimov, P., Buschtöns, S., Herrmann, D.C., Conraths, F.J., Görlich, K., Tenter, A.M., Dubey, J.P., Nagel-Kohl, U., Thoms, B., Bötcher, L., Kühne, M. and Schares, G. (2011) 'Serological survey and risk factors for Toxoplasma gondii in domestic ducks and geese in Lower Saxony, Germany', *Veterinary Parasitology*, 182(2–4), pp. 140–149. Available at: https://doi.org/10.1016/j.vetpar.2011.05.049.

Merck KGaA (2023) *Protein G and Protein A Bind to Different IgG*. Available at: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/protein-biology/protein-pulldown/protein-a-g-binding?gclid=EAIaIQobChMI77njlZnsgQMV-h-tBh1oyg-FEAAYAyAAEgL37_D_BwE (Accessed: 10 October 2023).

Nymo, I.H., Godfroid, J., Åsbakk, K., Larsen, A.K., Das Neves, C.G., Rødven, R. and Tryland, M. (2013) 'A protein A/G indirect enzyme-linked immunosorbent assay for the detection of anti-*Brucella* antibodies in Arctic wildlife', *Journal of Veterinary Diagnostic Investigation*, 25(3), pp. 369–375. Available at: https://doi.org/10.1177/1040638713485073.

Pilfold, N.W., Richardson, E.S., Ellis, J., Jenkins, E., Scandrett, W.B., Hernández-Ortiz, A., Buhler, K., McGeachy, D., Al-Adhami, B., Konecsni, K., Lobanov, V.A., Owen, M.A., Rideout, B. and Lunn, N.J. (2021) 'Long-term increases in pathogen seroprevalence in polar bears (*Ursus maritimus*) influenced by climate change', *Global Change Biology*, 27(19), pp. 4481–4497. Available at: https://doi.org/10.1111/gcb.15537.

Qi, T., Ai, J., Sun, Y., Ma, H., Kang, M., You, X. and Li, J. (2022) 'Application of Toxoplasma gondii-specific SAG1, GRA7 and BAG1 proteins in serodiagnosis of animal toxoplasmosis', *Frontiers in Cellular and Infection Microbiology*, 12, p. 1029768. Available at: https://doi.org/10.3389/fcimb.2022.1029768.

Robert-Gangneux, F. and Dardé, M.-L. (2012) 'Epidemiology of and Diagnostic Strategies for Toxoplasmosis', *Clinical Microbiology Reviews*, 25(2), pp. 264–296. Available at: https://doi.org/10.1128/CMR.05013-11.

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N. and Jenkins, E. (2019) 'Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of Toxoplasma gondii in naturally infected wolverines (Gulo gulo) from the Yukon, Canada', *Food and Waterborne Parasitology*, 15, p. e00046. Available at: https://doi.org/10.1016/j.fawpar.2019.e00046.

Stelzer, S., Basso, W., Benavides Silván, J., Ortega-Mora, L.M., Maksimov, P., Gethmann, J., Conraths, F.J. and Schares, G. (2019) 'Toxoplasma gondii infection and toxoplasmosis in farm animals: Risk factors and economic impact', *Food and Waterborne Parasitology*, 15, p. e00037. Available at: https://doi.org/10.1016/j.fawpar.2019.e00037.

Sudan, V., Tewari, A.K. and Singh, H. (2015) 'Serodiagnosis of Toxoplasma gondii infection in bovines from Kerala, India using a recombinant surface antigen 1 ELISA', *Biologicals*, 43(4), pp. 250–255. Available at: https://doi.org/10.1016/j.biologicals.2015.04.002.

Tabatabaei, M.S. and Ahmed, M. (2022) 'Enzyme-Linked Immunosorbent Assay (ELISA)', in S.L. Christian (ed.) *Cancer Cell Biology*. New York, NY: Springer US, pp. 115–134. Available at: https://doi.org/10.1007/978-1-0716-2376-3_10.

World Organization for Animal Health (WOAH). 2023. Principles and methods of validation of diagnostic assays for infectious diseases. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 1.1.6 PP 1-27. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.06_VALIDATION.pdf

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

5.1 The risk of exposure to *Toxoplasma gondii* from harvesting and consuming wildlife in communities in the Canadian North

Toxoplasma gondii is a globally distributed zoonotic food-borne parasite, with significant implications for public health, particularly among vulnerable populations. It is estimated that one-third of the global population has been exposed to the parasite, and in the Canadian Arctic, it has been reported as the most prevalent parasitic infection within Inuit communities (Goyette *et al.*, 2014). Given this backdrop, the main purpose of this research was to address community concerns about food safety and investigate the presence of *T. gondii* in wildlife species traditionally harvested and consumed in the Canadian North, that could potentially contribute to the high seroprevalence for *T. gondii* within certain communities.

Chapters 2 and 3 of this research centered around two keystone species, caribou and beluga whales, both of which rank among the top five most frequently consumed country foods in the Canadian North (Sheikh *et al.*, 2011; Kenny *et al.*, 2018). To comprehensively investigate this objective, multiple methodologies, including serological and molecular approaches, were employed.

In Chapter 2, we reported our findings in caribou harvested for human consumption from the Leaf River herd in Nunavik, Québec. Our initial approach involved utilizing a commercially available ELISA kit as a screening tool to explore the level of exposure to *T. gondii* within this caribou population. In addition, we used the best available "food quality" direct detection method of magnetic capture DNA extraction followed by a qPCR (MC qPCR), that has a higher sensitivity compared with conventional DNA extractions, due the use of 4000 times more tissue. We found an overall seroprevalence of 18%, which indicates exposure, but low tissue prevalence, as we did not detect amplified DNA of the parasite in heart and brain tissues.

For Chapter 3, our focus turned to the Beaufort Sea beluga population in the Inuvialuit Settlement Region of the Northwest Territories, in which *T. gondii* had recently been described as a novel threat to northern health due to climate change. At the invitation of the community and federal government collaborators, we employed a variety of serological tests on serum samples obtained from beluga harvested for human consumption over 5 years. Commercial IDVET ELISA and the IFA had higher accuracy and efficiency as screening tools. These assessments revealed one positive serum sample out of 96. Likewise, multiple molecular techniques were employed testing

heart, brain and muscle tissues, finding only one positive from 131 heart tissue samples on MC qPCR.

Our findings suggest that while the risk of transmission from harvested caribou and beluga to the human population is very low, it is not entirely absent. It is important to emphasize that our findings are derived from selectively harvested wildlife, reflecting selection bias due to the preferences and knowledge of the hunters. Consequently, the results of this research may not be representative of the actual prevalence within these animal populations. However, they are reassuring that hunter-selected animals in these 2 populations have low tissue prevalence of an important zoonotic food-borne parasite for the time period of this research.

While this research addresses the potential risks of caribou in Nunavik and belugas in the ISR, it leaves unanswered questions about how communities may still be exposed to the parasite. Modeling findings have suggested the existence of both oocyst- and tissue cyst-borne exposure routes in Nunavik (Ducrocq *et al.*, 2021). Food-borne transmission was associated with consumption of migratory birds and marine mammals, such as walrus, and seal livers. Previous studies have demonstrated exposure to *T. gondii* in migratory birds in the Canadian Arctic, such as geese (Elmore *et al.*, 2014; Bachand *et al.*, 2019), supporting the hypothesis that migratory birds are infected with oocysts from soil and water in southern areas during their overwinter nesting, and return to their northern nesting areas carrying the tissue stages of the parasite.

The consumption of harvested pinnipeds, such as seals and walrus, has been identified as a potential route of *T. gondii* transmission to human populations. Numerous studies on pinnipeds have reported a wide range of prevalence rates, varying from 0% to 83% by serological testing and 0% to100% by molecular techniques (van de Velde *et al.*, 2016; Dubey *et al.*, 2020). A comprehensive study between 1999 and 2006, covering seven Inuit communities and involving harvested pinnipeds, employed both serological and molecular methods, reporting a seropositive rate of 10.4% (86/828), but no DNA was detected (Simon *et al.*, 2011), similar to the findings in this thesis. In more recent studies, seroprevalence in ringed seals in the Arctic has ranged from 10 to 30% (Bachand *et al.*, 2019, Sudlovenick, in press). DNA of *T. gondii* was detected in 26% of seals from Nunavik (Reiling *et al.*, 2019). In contrast, seals from subarctic regions exhibit higher prevalence to *T. gondii*: 53% seroprevalence and 37% tissue prevalence in gray seals in the St. Lawrence gulf (Sauvé *et al.*, 2020). Additionally, Reilling reported tissue prevalence ranging from

40% to 63% in gray, hooded, and harp seals from the Gulf of St. Lawrence and islands of Nova Scotia.

Pinnipeds spend considerable amounts of time both in water and on land or ice. Activities such as copulation, birth and nursing take place out of the water, rendering these animals potentially more exposed to oocysts, especially in southern areas where felid definitive hosts are frequently present. Furthermore, pinnipeds primarily subsist on fish and aquatic invertebrates, although some species occasionally include birds and other pinnipeds in their diet (Berta, Sumich and Kovacs, 2006), heightening the risk of trasmission of *T. gondii* through oocysts in filter feeders and tissue cysts in birds and marine mammals.

Further research is strongly recommended to ascertain the sources of *T. gondii* transmission to communities in the Canadian Arctic. While existing research has predominantly focused on harvested wildlife, it would be prudent to expand investigations into market foods, drinking water sources, and enhanced health surveys. Employing innovative serological and molecular techniques for determining potential sources in humans, such as detecting antibodies against oocyst/sporozoite antigens, or exploring methods to trace whether the source originated from outside these regions, would provide a more comprehensive understanding of the transmission pathways.

5.2 Risk of Sarcocystidae transmission in marine mammals

Chapter 3 of this thesis presents research findings regarding *T. gondii* and *Sarcocystis* spp. in beluga whales from the Eastern Beaufort Sea. This investigation contributes to the broader global inquiry into the mechanisms of transmission of apicomplexan parasites to marine mammals. While land to sea transmission has been recognized as an important (and perhaps penultimate) source of marine toxoplasmosis, less attention has been given to non-zoonotic *Sarcocystis* spp. This thesis provides genetic evidence of a potential marine cycle of *Sarcocystis* spp., or at the least, an unrecognized terrestrial source of a novel *Sarcocystis* spp. Numerous studies have focused on southern sea otters as sentinels and models for understanding parasite transmission within marine ecosystems.

- **Fresh runoff water**. The most likely source of infection is believed to be environment exposure to *T. gondii* oocyst (Conrad *et al.*, 2005) and *Sarcocystis neurona* sporocysts

(Rejmanek *et al.*, 2009), particularly through coastal fresh runoff water (Miller *et al.*, 2002). Spatial analysis has documented a strong association between *T. gondii* seropositive otters and location of maximal freshwater outflow along California coast. Otters sampled at these maximal flow sites were 3 times more likely to be seropositive than those sampled at low flow sites (Miller *et al.*, 2002). Arctic systems are strongly interrelated making this arbitrary compartmentalization a difficult base upon which to produce a holistic discussion of why contaminants end up where they do, and how the environment conspires to put certain ecosystem components at risk far from any contaminating sources. Northward flowing rivers that drain 107 km2 may be major conduits to the Arctic Ocean of contaminants originating from point sources and/or atmospheric deposition to the terrestrial ecosystems. Approximately 78% of the total annual discharge to the Arctic Ocean is via 10 large rivers, one of those the Mackenzie River in Canada, draining 184x10'4 km2 (Macdonald 2000)

- **Terrestrial carnivores as sources and sentinels of Sarcocystidae infections.** Researchers have examined the potential role of terrestrial carnivores as both reservoirs and sentinels for Sarcocystidae infections in sea otters. The detection of *T. gondii* Genotype X in terrestrial carnivores, such as feral cats, bobcats, mountain lions, and foxes, alongside the high prevalence of this genotype in California sea otters, has provided compelling evidence of the influence of carnivores inhabiting areas in close proximity to sea otter habitats on the transmission of *T. gondii* within coastal environments (VanWormer *et al.*, 2014).

Furthermore, reports from Canada have provided evidence of *T. gondii* in a diverse range of terrestrial carnivores such as foxes, wolverines, and wolves, as well as polar bears which could be involved in both terrestrial and marine cycles (Bouchard *et al.*, 2019; Sharma *et al.*, 2019; Brandell *et al.*, 2021; Pilfold *et al.*, 2021). Although felids, which serve as the only definitive hosts for *T. gondii*, are largely absent in the Canadian Arctic, the documented presence of the parasite in carnivore species serving as intermediate hosts reinforces that *T. gondii* is indeed circulating throughout the Arctic region.

Polar bears are the best sentinel species in the monitoring of Sarcocystidae parasites, due to their status as top predators in the Arctic ecosystems and their ability to sample both

marine and terrestrial food webs. Their exposure to these parasites is likely increased through their carnivorous feeding habits. Ringed seals represent the primary prey for polar bears, although their diet also include other pinnipeds, cetaceans, seabirds, and, during periods on land, they may consume seaweed, eggs, chicks, reindeer calves and carrion whenever it becomes available (Berta, Sumich and Kovacs, 2006). As part of a collaborative effort during my PhD program, I served as coauthor on paper reporting an overall seroprevalence of 55.9% for *T. gondii* in a long-term surveillance project involving polar bears from the western Hudson Bay population, which is becoming increasing landlocked as summer ice continues to shrink (Pilfold *et al.*, 2021). Moreover, this paper demonstrate a consistent increase in seroprevalence of *T. gondii* in this population over 3 study periods (1980s, 1990s, and 2010s) and a link to higher seroprevalence in wetter summers, possibly due to increased terrestrial run-off containing oocysts, These findings underscore the potential of polar bears as valuable indicators for assessing the prevalence and impact of Sarcocystidae parasites in the Arctic environments.

- **Oocyst concentration by filter-feeding marine invertebrates.** The inevitable dilution of oocysts of *T. gondii* and sporocysts of *Sarcocystis* spp. shed by terrestrial carnivores into freshwater reduces the risk of direct consumption by marine mammals, because the environmental density of the parasite in the oceans may be low to infectied animals (Arkush *et al.*, 2003). However, parasites in the marine environment can be concentrated by filter-feeding marine bivalves, which are a major prey species for marine mammals such as sea otters, pinnipeds, and cetaceans. Bivalve accumulation of *T. gondii* oocysts under laboratory conditions has been described in mussels and oysters, demonstrating that oocysts concentrated from water remain infectious for bioassay in mice (Arkush *et al.*, 2003; Lindsay *et al.*, 2004). DNA of *T. gondii* and *Sarcocystis neurona* have been reported from a wild mussel collected from a known high-risk area for infection in sea otters in California (Miller *et al.*, 2008; Michaels *et al.*, 2016).

- **Filter-feeding fish.** Accumulation of oocysts by bivalves does not provide a plausible explanation for the transmission of these parasites to piscivorous mammals, such as belugas, which primarily subsist on fish as a significant part of their diet. Fish have previously been recognized as biotic vectors for organic material transfer between salt

marshes and coastal environments (Massie *et al.*, 2010). Both anchovies and Pacific sardines were capable of filtering *T. gondii* oocysts and recovering from intestines under laboratory conditions, which remained infectious to mice. Furthermore, investigations into fish at wholesale markets in Sicily have reported the presence of *T. gondii* DNA in gills and intestines of 32 from 147 samples across 12 different fish species (Marino *et al.*, 2019). Moreover, the DNA of *T. gondii* has been successfully amplified from various Arctic fish species, including Atlantic salmon and Arctic char from Nunavik (Reiling and Dixon, 2019). Future work is needed to determine if fish merely retain oocysts in their gills, or if they harbour tissue cysts containing bradyzoites of *T. gondii*.

- **Unique marine cycle of the parasite.** Our findings for *Sarcocystis* spp. from Chapter 3 strongly suggest a possibility of a unique marine pathway for infection in belugas. This is primarily attributed to the differences between the *Sarcocystis* DNA sequences generated during this research and those associated in terrestrial hosts. These findings indicate a potential marine life cycle for the parasites, with belugas acting as intermediate hosts; beluga predators, such as polar bears or killer whales, might serve as the definitive hosts, releasing sporocysts into arctic marine ecosystems.

While the specific marine sources of exposure to *T. gondii* and other Sarcocystidae in marine mammals remain unclear, further work is needed to better understand risk factors, sources of infection, the marine life cycle of the parasite, and the importance in marine mammal health.

5.3 A traveling parasite: *Sarcocystis* spp.

In Chapter 2, we reported our findings of multiple *Sarcocystis* spp. in caribou from Nunavik, revealing species-specific tissue predilection sites. For example, the DNA of *S. grueneri* was exclusively detected in cardiac tissue of the caribou, while *S. rangi*, *S. rangiferi*, *S. tarandivulpes*, *S. tarandi* were solely identified in skeletal muscle. To the best of our knowledge, this report represents the first identification of *Sarcocystis* species in Canada. Of particular interest is that all these parasite species have been previously documented in both Norway and Iceland. This observation raises a compelling question: how are identical parasite species distributed across both Europe and North America?

- **What we know about *Rangifer* in the past**. The natural history of *Rangifer* in North America has been hypothesized to be a dynamic evolutionary process during the last glacial period. At this time, *Rangifer* populations were dispersed across Eurasia, North America, and the Beringia region. These populations migrated and colonized contemporary circumpolar habitats as the glacial ice gradually receded. Investigations suggest that three primary *Rangifer* groups emerged in correspondence with their respective geographic distribution (Flagstad and Røed, 2003). One group includes nearly all *Rangifer* ecotypes in North America, and is believed to have its origin in Beringia, with potential connections to Siberia, Central and Western Europe. A second group, exclusively Eurasian, appears to have emerged from isolated populations that thrived in insular environments from Western Europe. The third group was woodland (migratory) caribou in Quėbec, which seem to have originated in habitats located further south, at a time when the continental ice sheet extended over substantial portion of the United States (Flagstad and Røed, 2003).

  It is plausible that *Rangifer* populations and *Sarcocystis* spp. have maintained a long-term coexistence since the last glacial period, with limited genetic variation, as corroborated by successful recovery of parasite DNA from ancient samples, of species which continue to persist in contemporary times. For instance, there have been reports of the extraction of *Toxascaris leonina* DNA from cougar coprolites originating from the Pleistocene epoch (Petrigh *et al.*, 2019). Additionally, DNA of *Trypanosoma cruzi* has been detected from mummies in South America dating back to approximately 9000 years BP (Araújo *et al.*, 2009). This evidence suggests the enduring presence and genetic stability of certain parasites over extended temporal scales.

- **Introduction of European fauna to North America.** The translocation of semi-domesticated *Rangifer* from Eurasia, commonly known as reindeer, to North America has occurred on multiple occasions, primarily driven by husbandry purposes and the intention to enhance economic conditions for the indigenous communities inhabiting the respective regions targeted for those introductions. The first recorded instance occurred between 1821 and 1902 when reindeer from Siberia were brought to Alaska. Subsequently, in 1908, reindeer from Norway were introduced to Labrador. Some of these introduced animals were further relocated to the Northwest Territories in 1911, and to Quebec in 1918, which

may be the most likely route from the European *Sarcocystis* to stablish in the caribou population from this thesis. Another importation of reindeer from Norway took place in 1921, this time to Baffin Island in Nunavut. Lastly, from 1929 to 1935, reindeer from Alaska were transported to the Mackenzie Delta in the Northwest Territories (Scotter, 1970).

These attempts were marked by considerable losses of individual animals, and although the introductions often failed to establish, the introduction of reindeer from Eurasia raises the potential for the introduction of *Sarcocystis* parasites from Europe into North America. Definitive hosts of these parasites in these regions, such as wolves and foxes, could well have consumed the sarcocysts from the introduced reindeer through predation or scavenging, subsequently perpetuating the parasitic life cycle and thereby disseminating sporozoites into the environment where native caribou populations reside.

- **Carnivore long-distance movements.** Sea ice and the nomadic behaviour of Arctic foxes is another connector of parasites between the Palearctic and Nearctic. Factors motivating these animals' departure from breeding home ranges include the scarcity of food resources and competition within densely populated fox territories. Arctic foxes embark on extensive journeys, utilizing sea ice as a platform for exploration and dispersal. In a recent study, the movements of a female Arctic fox were tracked over a four-month period, revealing a total cumulative distance traversed of 4415 km, encompassing an inter-continental journey from Svalbard in Europe to Ellesmere Island in Nunavut, Canada (Fuglei and Tarroux, 2019). This stands as the longest-distance migration recorded for an Arctic fox.

  Arctic fox may serve as potential carriers of *Sarcocystis* spp., either originating from their breeding home or acquired through predation and scavenging activities during their extensive travels. Given that foxes are recognized as definitive hosts for *Sarcocystis* spp., the dispersal of the parasite may occur via excretion of the sporocysts in the feces of these animals, disseminated from their point of origin to their ultimate destination. Such behaviour may also be pertinent to other carnivores, including wolves, particularly where their ranges overlap with migratory wildlife habitats, thus contributing to the transmission and life cycle of *Sarcocystis* spp.

5.4 Limitations and Future Directions

The findings presented in this thesis have substantially contributed to our understanding of the ecology, epidemiology, and diversity of Sarcocystidae parasites in harvested wildlife across the Canadian North. However, it is important to mention the limitations of our research. One of the foremost challenges we encountered is the limitation imposed by sample size and bias in wildlife research. Our access to specimens is contingent on agreements with communities and hunters, and the availability of animals. Furthermore, we recognize the potential influence of selection bias on our research outcomes, which may not reflect the true prevalence of these parasites within the animal populations under examination. Indeed, anecdotally many hunters deliberately avoid harvesting animals that behave strangely or appear thin or unwell. An additional constraint was the absence of known positive and negative controls, which could enhance the precision and robustness of our methodologies.

Chapter 2 reported our findings related to serology for *T. gondii* and *N. caninum*, as well as the molecular findings for these two parasites and *Sarcocystis* spp. in caribou from Nunavik during a constrained time period, setting a baseline. However, to enhance our comprehension of the dynamics of these parasites within caribou populations, further research is highly recommended. This could be achieved through longitudinal studies involving examination of samples obtained from live-trapped and collared animals, providing insight into the dynamic patterns of parasitic infections. Additionally, investigating animals that hunters suspect may be diseased could yield valuable data as well. Moreover, extending the study to encompass caribou populations from various ecotypes and geographic regions will offer a more comprehensive view of the situation across Canada and North America.

Chapter 3 explored *T. gondii* and *Sarcocystis* spp. in beluga whales from Eastern Beaufort Sea. The study of Sarcocystidae parasites within marine ecosystems needs deeper exploration. Extending research to encompass belugas from the Eastern Canadian region holds the potential to provide insights into one of the most pressing inquiries concerning the transmission of these parasites in the marine environment. Beluga whales in in Eastern Canada are more likely to be exposed to contaminants, due to their proximity to larger human settlements. This presents unique opportunity to detect any correlations between immunosuppressive effects of contaminants and

parasitic exposure, and to identify potential risks for the health of beluga populations as well as human harvesters.

The methodological challenges encountered across all chapters of this thesis underscore the need for continued optimization of these techniques, especially in wildlife samples which are often difficult to collect and store optimally and can harbour mixed infections with non-target organisms. In this case, it was a fortunate accident that the primers we used for *Sarcocystis* spp. also detected the more pathogenic *Besnoitia* sp. in hunter harvested caribou. Exploiting statistical approaches, such as latent class models, can significantly enhance ability to compare tests in the absence of known controls. In particular, for Chapter 4, due to the surprising failure of commercially derived p30 antigen to discriminate among sera positive and negative for antibodies to *T. gondii*, we recommend the development of in-house antigen for *T. gondii*, potentially through gene cloning and the synthesis of recombinant proteins. This approach could afford greater quality control compared to relying on commercial suppliers of proteins.

While our research into *Sarcocystis* spp. in both caribou and beluga was somewhat serendipitous (our primary focus was on *T. gondii* as a zoonosis), these findings incentivized more in-depth investigations. We successfully amplified *Sarcocystis* DNA from caribou samples, targeting the 18S rDNA region, and from beluga samples, targeting the ITS1 region. The sequences generated enabled species-level identification and comparative analyses with reference sequences available in GenBank. Phylogenetic analysis has not only facilitated the identification of parasite species but also elucidated the diversity of the genus *Sarcocystis*. Future work could target different genes within the parasite, such as mitochondrial DNA (COX), with the aim of generating more comprehensive sequences. This should be conducted not only in caribou and beluga but also in predators and other animals coexisting within the same habitats higher up the food chain and with higher prevalence and intensity of tissue cysts. Such investigations hold the promise of advancing our comprehension of the diversity of the parasite and the complex host-parasite interactions.

Obtaining wildlife samples is inherently challenging, especially when dealing with harvested animals that hold significant nutritional and cultural value for indigenous communities. Therefore, it is crucial to promote collaborative research partnerships with other institutions and, notably, to actively engage with indigenous communities. This thesis would not have been possible without the dedication and effort invested by hunters and community coordinators throughout the sampling

process. To the extent possible (especially during COVID), our research team consistently communicated our findings back to the communities through summaries, bulletins, and presentations, all facilitated by our collaborators. This includes an international and multiagency team that provided archived polar bear sera that represents an immense effort by many biologists, pilots, and conservation personnel. Information developed in this thesis has been reported to communities, hunter trapper organizations, the Fisheries Joint Management Committee in Inuvik, and the Nunavik Regional Board of Health and Social Services, along with national and international scientific peers. Furthermore, during my doctoral program, I was fortunate to collaborate with the University of Waterloo in a side project that involved deeper interactions with the communities and an exploration of country food in the Inuvialuit Settlement Region. Additionally, I played a role in establishing a molecular technique for detection of *T. gondii* in wildlife harvested for food consumption at the Nunavik Research Centre, operated by the Makivvik Corporation in Kuujjuaq, Québec. Ultimately this thesis was conducted with the fundamental purpose of addressing community concerns related to food safety, and, as such, the results are intended for the benefit and well-being of these communities.

5.5 Conclusion

This thesis has made a substantial contribution to our understanding of Sarcocystidae parasites in harvested wildlife from the Canadian North. Additionally, the data derived from this study has addressed concerns within communities regarding food safety. Our findings indicate that the consumption of harvested caribou from Nunavik and beluga whales from the Eastern Beaufort Sea have low, but not zero, risk of transmitting *T. gondii* to the human population. It is important to carefully tailor public health messages to promote safe practices when handling and preparing country foods to protect high risk groups (like pregnant and immunocompromised people), while at the same time balancing the many nutritional and cultural benefits of harvesting and consuming traditional foods.

Furthermore, this research has expanded our knowledge of *Sarcocystis* spp. in caribou and beluga populations, emphasizing the need for further investigations to gain a deeper understanding of the diversity of the parasite and its interactions among both intermediate and definitive hosts. My thesis has also underscored the importance and challenges of developing serological techniques for wildlife samples, focusing on the challenges and tools required to improve these

methodologies. Finally, this thesis exemplifies the collaborative efforts aimed at benefiting communities in the Canadian Arctic. It aligns with the One Health concept, addressing the interrelation of human, animal, and environmental health. The research contributes insights into the risk of zoonotic diseases, emphasizing community concerns and food safety.

5.6 References

Araújo, A., Jansen, A.M., Reinhard, K. and Ferreira, L.F. (2009) 'Paleoparasitology of Chagas disease: a review', *Memórias do Instituto Oswaldo Cruz*, 104(suppl 1), pp. 9–16. Available at: https://doi.org/10.1590/S0074-02762009000900004.

Arkush, K.D., Miller, M.A., Leutenegger, C.M., Gardner, I.A., Packham, A.E., Heckeroth, A.R., Tenter, A.M., Barr, B.C. and Conrad, P.A. (2003) 'Molecular and bioassay-based detection of *Toxoplasma gondii* oocyst uptake by mussels (*Mytilus galloprovincialis*)', *International Journal for Parasitology*, 33(10), pp. 1087–1097. Available at: https://doi.org/10.1016/S0020-7519(03)00181-4.

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E. and Jenkins, E. (2019) 'Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada', *Parasites & Vectors*, 12(1), p. 155. Available at: https://doi.org/10.1186/s13071-019-3408-9.

Berta, A., Sumich, J.L. and Kovacs, K.M. (2006) *Marine mammals: evolutionary biology*. 2. ed. Amsterdam: Elsevier.

Bouchard, É., Elmore, S.A., Alisauskas, R.T., Samelius, G., Gajadhar, A.A., Schmidt, K., Ross, S. and Jenkins, E.J. (2019) 'Transmission dynamics of *Toxoplasma gondii* in arctic foxes (*Vulpes lagopus*): a long-term mark-recapture serologic study at Karrak Lake, Nunavut, Canada', *Journal of Wildlife Diseases*, 55(3), p. 619. Available at: https://doi.org/10.7589/2018-06-144.

Brandell, E.E., Cross, P.C., Craft, M.E., Smith, D.W., Dubovi, E.J., Gilbertson, M.L.J., Wheeldon, T., Stephenson, J.A., Barber-Meyer, S., Borg, B.L., Sorum, M., Stahler, D.R., Kelly, A., Anderson, M., Cluff, H.D., MacNulty, D.R., Watts, D.E., Roffler, G.H., Schwantje, H., Hebblewhite, M., Beckmen, K., Fenton, H. and Hudson, P.J. (2021) 'Patterns and processes of pathogen exposure in gray wolves across North America', *Scientific Reports*, 11(1), p. 3722. Available at: https://doi.org/10.1038/s41598-021-81192-w.

Conrad, P.A., Miller, M.A., Kreuder, C., James, E.R., Mazet, J., Dabritz, H., Jessup, D.A., Gulland, F. and Grigg, M.E. (2005) 'Transmission of *Toxoplasma*: Clues from the study of sea

otters as sentinels of *Toxoplasma gondii* flow into the marine environment', *International Journal for Parasitology*, 35(11–12), pp. 1155–1168. Available at: https://doi.org/10.1016/j.ijpara.2005.07.002.

Dubey, J.P., Murata, F.H.A., Cerqueira-Cézar, C.K., Kwok, O.C.H. and Grigg, M.E. (2020) 'Recent epidemiologic and clinical importance of *Toxoplasma gondii* infections in marine mammals: 2009–2020', *Veterinary Parasitology*, 288, p. 109296. Available at: https://doi.org/10.1016/j.vetpar.2020.109296.

Ducrocq, J., Ndao, M., Yansouni, C.P., Proulx, J., Mondor, M., Hamel, D., Lévesque, B., De Serres, G. and Talbot, D. (2021) 'Epidemiology associated with the exposure to *Toxoplasma gondii* in Nunavik's Inuit population using the 2017 *Qanuilirpitaa* cross-sectional health survey', *Zoonoses and Public Health*, 68(7), pp. 803–814. Available at: https://doi.org/10.1111/zph.12870.

Elmore, S.A., Huyvaert, K.P., Bailey, L.L., Milhous, J., Alisauskas, R.T., Gajadhar, A.A. and Jenkins, E.J. (2014) 'Toxoplasma gondii exposure in arctic-nesting geese: A multi-state occupancy framework and comparison of serological assays', *International Journal for Parasitology: Parasites and Wildlife*, 3(2), pp. 147–153. Available at: https://doi.org/10.1016/j.ijppaw.2014.05.005.

Flagstad, Ø. and Røed, K.H. (2003) 'Refugial origins of reindeer (*Rangifer tarandus l.*) Inferred from mitochondrial DNA sequences', *Evolution*, 57(3), pp. 658–670. Available at: https://doi.org/10.1111/j.0014-3820.2003.tb01557.x.

Fuglei, E. and Tarroux, A. (2019) 'Arctic fox dispersal from Svalbard to Canada: one female's long run across sea ice', *Polar Research*, 38(0). Available at: https://doi.org/10.33265/polar.v38.3512.

Goyette, S., Cao, Z., Libman, M., Ndao, M. and Ward, B.J. (2014) 'Seroprevalence of parasitic zoonoses and their relationship with social factors among the Canadian Inuit in Arctic regions', *Diagnostic Microbiology and Infectious Disease*, 78(4), pp. 404–410. Available at: https://doi.org/10.1016/j.diagmicrobio.2013.08.026.

Kenny, T.-A., Hu, X.F., Kuhnlein, H.V., Wesche, S.D. and Chan, H.M. (2018) 'Dietary sources of energy and nutrients in the contemporary diet of Inuit adults: results from the 2007–08 Inuit Health Survey', *Public Health Nutrition*, 21(7), pp. 1319–1331. Available at: https://doi.org/10.1017/S1368980017003810.

Lindsay, D.S., Collins, M.V., Mitchell, S.M., Wetch, C.N., Rosypal, A.C., Flick, G.J., Zajac, A.M., Lindquist, A. and Dubey, J.P. (2004) 'Survival of *Toxoplasma gondii* oocysts in eastern oysters (*Crassostrea virginica*)', *Journal of Parasitology*, 90(5), pp. 1054–1057. Available at: https://doi.org/10.1645/GE-296R.

Marino, A.M.F., Giunta, R.P., Salvaggio, A., Castello, A., Alfonzetti, T., Barbagallo, A., Aparo, A., Scalzo, F., Reale, S., Buffolano, W. and Percipalle, M. (2019) '*Toxoplasma gondii* in edible fishes captured in the Mediterranean basin', *Zoonoses and Public Health*, 66(7), pp. 826–834. Available at: https://doi.org/10.1111/zph.12630.

Massie, G.N., Ware, M.W., Villegas, E.N. and Black, M.W. (2010) 'Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish', *Veterinary Parasitology*, 169(3–4), pp. 296–303. Available at: https://doi.org/10.1016/j.vetpar.2010.01.002.

Michaels, L., Rejmanek, D., Aguilar, B., Conrad, P. and Shapiro, K. (2016) 'California mussels (*Mytilus californianus*) as sentinels for marine contamination with *Sarcocystis neurona*', *Parasitology*, 143(6), pp. 762–769. Available at: https://doi.org/10.1017/S0031182016000354.

Miller, M., Miller, W., Conrad, P., James, E., Melli, A., Leutenegger, C., Dabritz, H., Packham, A., Paradies, D. and Harris, M. (2008) 'Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, runoff and toxoplasmosis of sea otters', *International Journal for Parasitology*, 38(11), pp. 1319–1328. Available at: https://doi.org/10.1016/j.ijpara.2008.02.005.

Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd, E., Harris, M.D., Ames, J.A., Packham, A.E. and Conrad, P.A. (2002) 'Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*)', *International Journal for Parasitology*, 32(8), pp. 997–1006. Available at: https://doi.org/10.1016/S0020-7519(02)00069-3.

Petrigh, R.S., Martínez, J.G., Mondini, M. and Fugassa, M.H. (2019) ‘Ancient parasitic DNA reveals *Toxascaris leonina* presence in Final Pleistocene of South America’, *Parasitology*, 146(10), pp. 1284–1288. Available at: https://doi.org/10.1017/S0031182019000787.

Pilfold, N.W., Richardson, E.S., Ellis, J., Jenkins, E., Scandrett, W.B., Hernández-Ortiz, A., Buhler, K., McGeachy, D., Al-Adhami, B., Konecsni, K., Lobanov, V.A., Owen, M.A., Rideout, B. and Lunn, N.J. (2021) ‘Long-term increases in pathogen seroprevalence in polar bears (*Ursus maritimus*) influenced by climate change’, *Global Change Biology*, 27(19), pp. 4481–4497. Available at: https://doi.org/10.1111/gcb.15537.

Reiling, S. and Dixon, B. (2019) ‘*Toxoplasma gondii*: How an Amazonian parasite became an Inuit health issue’, *Canada Communicable Disease Report*, 45(7/8), pp. 183–190. Available at: https://doi.org/10.14745/ccdr.v45i78a03.

Reiling, S.J., Measures, L., Feng, S., Boone, R., Merks, H. and Dixon, B.R. (2019) ‘*Toxoplasma gondii*, *Sarcocystis* sp. and *Neospora caninum*-like parasites in seals from northern and eastern Canada: potential risk to consumers’, *Food and Waterborne Parasitology*, 17, p. e00067. Available at: https://doi.org/10.1016/j.fawpar.2019.e00067.

Rejmanek, D., Vanwormer, E., Miller, M.A., Mazet, J.A.K., Nichelason, A.E., Melli, A.C., Packham, A.E., Jessup, D.A. and Conrad, P.A. (2009) ‘Prevalence and risk factors associated with *Sarcocystis neurona* infections in opossums (*Didelphis virginiana*) from central California’, *Veterinary Parasitology*, 166(1–2), pp. 8–14. Available at: https://doi.org/10.1016/j.vetpar.2009.08.013.

Sauvé, C., Hernández-Ortiz, A., Jenkins, E., Mavrot, F., Schneider, A., Kutz, S., Saliki, J. and Daoust, P. (2020) ‘Exposure of the Gulf of St. Lawrence grey seal Halichoerus grypus population to potentially zoonotic infectious agents’, *Diseases of Aquatic Organisms*, 142, pp. 105–118. Available at: https://doi.org/10.3354/dao03536.

Scotter, G.W. (1970) ‘Reindeer husbandry as a land use in Northern Canada’, in International Union for Conservation of Nature and Natural Resources. Productivity and conservation in northern circumpolar lands. IUCN Publications new series, pp 159-168.

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N. and Jenkins, E. (2019) 'Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of *Toxoplasma gondii* in naturally infected wolverines (*Gulo gulo*) from the Yukon, Canada', *Food and Waterborne Parasitology*, 15, p. e00046. Available at: https://doi.org/10.1016/j.fawpar.2019.e00046.

Sheikh, N., Egeland, G.M., Johnson-Down, L. and Kuhnlein, H.V. (2011) 'Changing dietary patterns and body mass index over time in Canadian Inuit communities', *International Journal of Circumpolar Health*, 70(5), pp. 511–519. Available at: https://doi.org/10.3402/ijch.v70i5.17863.

Simon, A., Chambellant, M., Ward, B.J., Simard, M., Proulx, J.F., Levesque, B., Bigras-Poulin, M., Rousseau, A.N. and Ogden, N.H. (2011) 'Spatio-temporal variations and age effect on *Toxoplasma gondii* seroprevalence in seals from the Canadian Arctic', *Parasitology*, 138(11), pp. 1362–1368. Available at: https://doi.org/10.1017/S0031182011001260.

VanWormer, E., Miller, M.A., Conrad, P.A., Grigg, M.E., Rejmanek, D., Carpenter, T.E. and Mazet, J.A.K. (2014) 'Using molecular epidemiology to track *Toxoplasma gondii* from terrestrial carnivores to marine hosts: implications for public health and conservation', *PLoS Neglected Tropical Diseases*. Edited by E. Ghedin, 8(5), p. e2852. Available at: https://doi.org/10.1371/journal.pntd.0002852.

van de Velde, N., Devleesschauwer, B., Leopold, M., Begeman, L., IJsseldijk, L., Hiemstra, S., IJzer, J., Brownlow, A., Davison, N., Haelters, J., Jauniaux, T., Siebert, U., Dorny, P. and De Craeye, S. (2016) '*Toxoplasma gondii* in stranded marine mammals from the North Sea and Eastern Atlantic Ocean: Findings and diagnostic difficulties', *Veterinary Parasitology*, 230, pp. 25–32. Available at: https://doi.org/10.1016/j.vetpar.2016.10.021.

APPENDIX A

This appendix included a manuscript published in Global Change Biology in 2021, as a part of a multidisciplinary collaboration between San Diego Zoo Global, Environment and Climate Change Canada, University of Saskatchewan, and the Canadian Food Inspection Agency. Polar bear sera were examined by different serological tests to detect antibodies against seven pathogens: *Toxoplasma gondii*, *Neospora caninum*, *Trichinella* spp., *Francisella tularensis*, *Bordetella bronchiseptica*, canine morbillivirus (CDV) and canine parvovirus (CPV). My contribution for this publication included material preparation, performance of analysis and interpretation of the results for *T. gondii* and *N. caninum.* As well, as editing the corresponding sections in the manuscript. Because polar bears are apex predators in the Arctic environments, they serve as sentinels for pathogens, including those mentioned in this thesis.

LONG-TERM INCREASES IN PATHOGEN SEROPREVALENCE IN POLAR BEARS (*URSUS MARITIMUS*) INFLUENCED BY CLIMATE CHANGE

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Abstract

The influence of climate change on wildlife disease dynamics is a burgeoning conservation and human health issue, but few long-term studies empirically link climate to pathogen prevalence. Polar bears (*Ursus maritimus*) are vulnerable to the negative impacts of sea ice loss as a result of accelerated Arctic warming. While studies have associated changes in polar bear body condition, reproduction, survival, and abundance to reductions in sea ice, no long-term studies have documented the impact of climate change on pathogen exposure. We examined 425 serum samples from 381 adult polar bears, collected in western Hudson Bay, Canada, for antibodies to selected pathogens across three time periods: 1986-1989 (n = 157), 1995-1998 (n = 159), and 2015-2017 (n = 109). We ran serological assays for antibodies to seven pathogens: *Toxoplasma gondii*, *Neospora caninum*, *Trichinella* spp., *Francisella tularensis*, *Bordetella bronchiseptica*, canine morbillivirus (CDV), and canine parvovirus (CPV). Seroprevalence of zoonotic parasites (*T. gondii*, *Trichinella* spp.) and bacterial pathogens (*F. tularensis*, *B. bronchiseptica*) increased significantly between 1986-1989 and 1995-1998, ranging from +6.2% to +20.8%, with *T. gondii* continuing to increase into 2015-2017 (+25.8% overall). Seroprevalence of viral pathogens (CDV, CPV) and *N. caninum* did not change with time. *Toxoplasma gondii* seroprevalence was higher following wetter summers, while seroprevalences of *Trichinella* spp. and *B. bronchiseptica* were positively correlated with hotter summers. Seroprevalence of antibodies to *F. tularensis* increased following years polar bears spent more days on land, and polar bears previously captured in human settlements were more likely to be seropositive for *Trichinella* spp. As the Arctic has warmed due to climate change, zoonotic pathogen exposure in WH polar bears has increased, driven by numerous altered ecosystem pathways.

Introduction

Pathogens can impact the health of wildlife in a range of ways, from sublethal effects to eruptive outbreaks causing significant population declines (Alexander & Appel, 1994; Rijks et al., 2005; Sasan et al., 2019). Concomitant exposure and multi-pathogen infections can also alter host response, potentially compounding the burden on the immune system (Cox, 2001; Pedersen & Fenton, 2007). Therefore, factors that influence pathogen persistence are important considerations for conservation management. This is imperative with respect to zoonotic pathogens, which can cause significant health challenges in humans (Shereen et al., 2020), with cascading impacts on the conservation of wildlife (Hockings et al., 2020; Lindsey et al., 2020).

Climate change is expected to amplify some pathogens in wildlife, with warming temperatures and changing precipitation regimes potentially causing an increase in pathogen persistence, prevalence, emergence, and transmission (Altizer et al., 2013). However, host-pathogen ecology, including density-dependent processes and vectors, needs careful examination for the role of climate (Harvell et al., 2009; McDonald et al., 2016; Ogden & Lindsay, 2016). Despite the recent rise in climate-disease interaction studies (Altizer et al., 2013), and the formation of a crisis discipline ('Conservation Medicine'; Aguirre et al., 2002), there remains a paucity of long-term empirical studies associating the prevalence of wildlife disease with climatic factors. Because not all pathogens respond to changes in climate in the same way (Karvonen et al., 2010; Lafferty, 2009; Menge et al., 2016), further empirical study is necessary to improve forecasting models.

The Arctic is an important ecosystem for monitoring climate-disease interactions because it is warming twice as fast as the rest of the planet (Cohen et al., 2014), is relatively simple with low species diversity (Post et al., 2013), and many northern people depend on the traditional harvest of wildlife which can lead to zoonotic exposure risk (McDonald et al., 1990; Møller et al., 2010). Recent Arctic studies have shown linkages between climate change and the life cycles of endoparasites (Hoar et al., 2012; Kutz et al., 2005), ectoparasites (Descamps, 2013; Larsson et al., 2007), and biting insects (Culler et al., 2015). Although there has been a long history of investigations into disease prevalence in Arctic wildlife (Connell, 1949; Elton, 1931), the majority have been cross-sectional rather than longitudinal (Carlsson et al., 2019; Clausen & Hjort, 1986; Dick & Belosevic, 1978; Elmore et al., 2016). While calls have been made for Arctic climate-

disease interaction studies (Bradley et al., 2005; Burek et al., 2008), logistical challenges have been a major barrier to long-term, repeat sample collection.

Polar bears (*Ursus maritimus*) are a widely-dispersed apex carnivore, with a species range covering the circumpolar Arctic and extending across more than 35 degrees in latitude (PBSG, 2018). Given their range and trophic position, polar bears may be a sentinel species for changing disease dynamics in the Arctic (Stirling & Derocher, 2012). Jensen et al. (2010) reported an increase in the seroprevalence of *Toxoplasma gondii* in polar bears from Svalbard, Norway from 24.3% in the 1990s (Oksanen et al., 2009), to 47.6% in 2006-08. Jensen et al. (2010) speculated that the increase was linked to enhanced survival of oocysts in warmer water from the North Atlantic Current. Atwood et al. (2017) documented an increase over time in the seroprevalence of *T. gondii* and *Coxiella burnetii* in polar bears from the southern Beaufort Sea, 2007-14. Polar bears that spent summer and fall on land were found to be seven times more likely to be seropositive for *T. gondii* than those that stayed on the sea ice (Atwood et al., 2017), and had a heightened immune system response (Whiteman et al., 2019). As increased use of land during summer and fall months is related to sea ice loss as a consequence of climate change (Atwood et al., 2016b; Gleason & Rode, 2009), Atwood et al. (2017) was the first polar bear study to link an ecosystem pathway for increased pathogen prevalence to climate change.

Polar bears of the western Hudson Bay (WH) migrate to and from land annually, following the melt and refreeze patterns of sea ice in the Bay (Derocher & Stirling, 1990a). Between 1979-2014, the number of ice-free days in WH increased 8.6 days/decade (Stern & Laidre, 2016), altering polar bear migratory phenology (Castro de la Guardia et al., 2017; Cherry et al., 2013). While on land, polar bears forage opportunistically on a variety of terrestrial foods (Derocher et al., 1993; Russell, 1975), but lose approximately 1 kg of mass per day (Pilfold et al., 2016). As a result, longer ice-free seasons in Hudson Bay are correlated with decreases in body condition, reproduction, and survival of polar bears (Derocher & Stirling, 1995; Lunn et al., 2016; Regehr et al., 2007; Sciullo et al., 2016). Additionally, increased time on land is positively correlated with human-polar bear interactions in communities (Towns et al., 2009). However, the consequences of climate change and longer ice-free seasons on pathogen exposure in WH polar bears are unknown.

The objectives of this study were to examine whether the seroprevalence of seven pathogens in WH polar bears has changed over a 32-year period (1986-2017), and assess any temporal trends in seropositivity against biotic and abiotic variables for potential influential drivers of changes in pathogen exposure. It has been shown that sex and age can influence seroprevalence to some pathogens in polar bears (Jensen et al., 2010; Oksanen et al., 2009; Rah et al., 2005). Additionally, while sea ice has been the primary focus for climate interaction studies in polar bears (Stirling & Derocher, 2012), the Hudson Bay ecosystem is also undergoing changes in air temperature and precipitation regimes (Macrae et al., 2014; Figure 1). We aimed to resolve which factors are most influential to changing pathogen exposure in polar bears through a serological analysis from the longest continuously-studied polar bear population in the world.

Materials & Methods

Study Area

Hudson Bay is a shallow inland sea (Jones and Anderson 1994), with a seasonal sea ice regime that influences the climate dynamics of the ecosystem (Rouse, 1998). Between 1986-2017, annual mean (± SE) air temperature as recorded at Churchill Airport, Manitoba (Churchill A, Station ID 5060600, 58°44'24.0" N, 94°04'12.0" W, Environment Canada, 2020) was –6.30 ± 0.07 °C, with a mean air temperature of 9.90 ± 0.06 °C in summer (June-September) and –22.7 ± 0.1 °C in winter (December-March). Mean annual precipitation between 1986-2017 was 412.2 ± 3.1 mm. The proportion of precipitation falling during summer increased over the study period, along with air temperature (Figure 1).

During the winter, Hudson Bay is ice-covered. WH polar bears occupy and travel over much of the Bay, with mean annual home ranges for adult females of approximately 230,000 - 380,000 km^2 (McCall *et al.*, 2014; Figure 1). Sea ice breakup begins in May and the Bay is ice-free by August, which forces WH bears to spend the summer and fall onshore. WH polar bears congregate during the ice-free months along the western coastline of Hudson Bay in the lowlands (Derocher & Stirling, 1990a; Stirling et al., 2004). The lowlands are dominated by poorly drained peat bog plateaus and channel fens, with a mixture of open-canopy spruce-lichen woodlands

(Macrae et al., 2014). During the ice-free period, WH polar bears can also be found near human settlements, in particular Churchill, MB, which employs the Polar Bear Alert Program to minimize human–polar bear conflict by intercepting bears that come close to town (Kearney, 1989; Towns et al., 2009).

Sample Collection

Samples were collected in 1986-1989, 1995-1998 and 2015-2017 as part of ongoing, long-term studies of WH polar bears (Lunn et al., 2016). Bears were located by helicopter and chemically immobilized via remote injection following standard protocols (Stirling et al., 1989). A vestigial premolar was extracted for age estimation (Calvert & Ramsay, 1988) from previously unmarked bears older than 1 year. Blood was drawn from a femoral vein using a 30cc or 60cc syringe within half an hour of capture, immediately transferred into red/grey SST tubes, and kept in a cooler until it was spun in a centrifuge to separate the serum at the end of each field day. All capture and handling methods were reviewed and approved annually by the Environment and Climate Change Canada Western and Northern Animal Care Committee.

Sera were stored at –70 °C until needed. Freezers were monitored with alarm systems to ensure serum samples did not undergo freeze-thaw cycles. While we did not analyse for the possible influence of sample degradation over time, we are confident that storage at –70 °C prevented proteolysis and had negligible impact on our ability to detect antibodies (Cecchini et al., 1992; Dard et al., 2017). We focused the analysis on sera from adult polar bears (≥ 5 years) and when possible, balanced samples between males and females, within and over years (Table 1). Negative control sera were obtained in 2019 from two adult male polar bears (aged 5 and 7 years) born and raised in captivity at the Toronto Zoo.

Serological Analyses

We ran serological assays to detect exposure to seven pathogens (Table 2): *Toxoplasma gondii*, *Neospora caninum*, *Trichinella* spp., *Francisella tularensis*, *Bordetella bronchiseptica*, canine morbillivirus (CDV) and canine parvovirus (CPV). We selected these pathogens to have representation from parasitic, bacterial and viral diseases, as well as a range of documentation history in polar bears. We selected pathogens well-documented in polar bears for comparison across to other parts of their range (*T. gondii, Trichinella* spp., CDV), pathogens with minimal

documentation for follow up (*N. caninum, F. tularensis*), and pathogens that have not been surveyed before (*B. bronchiseptica*, CPV), to provide a baseline for future monitoring.

Toxoplasma gondii

IgG antibodies against *T. gondii* were detected using a commercial indirect-ELISA (iELISA) kit (ID Screen® Toxoplasmosis Indirect Multi-species, IDvet Innovative Diagnostics, Grabels, France) that has shown high specificity for *T. gondii* and non-cross reactivity with *N. caninum* (ID Vet, 2020). In addition to kit controls and negative controls from captive polar bears, serum samples previously collected from wolverines and known to be positive and/or negative by molecular methods (Sharma et al., 2019) were used as internal controls. Results were obtained as sample/positive percentage (S/P %), calculated using the optical density (OD) of the positive and negative kit controls, and the OD of test samples using the following formula: S/P%= [(OD sample – OD negative control) / (OD positive control – OD negative control)] x 100. S/P% values of $\geq$ 50 were considered positive.

Neospora caninum

Serum samples were tested for the presence of antibodies to *N. caninum* using a commercially available competitive-ELISA (cELISA) kit (*Neospora Caninum* Antibody Test Kit cELISA, Veterinary Medical Research & Development. WA, USA), in which both the antigen and monoclonal antibody conjugate have shown high specificity to *N. caninum* and non-cross reactivity with *T. gondii* or *Sarcocystis* spp. (Baszler et al., 1996). The test was performed following the manufacturer's instructions. Results were calculated and expressed as % Inhibition (% I) using the formula % I = 100 × [1 - (Sample OD / negative control OD)]. Samples were considered positive if the test sample produced $\geq$ 30% inhibition and negative if the test sample produced < 30% inhibition.

Trichinella spp.

Antibodies to *Trichinella* spp. were detected using an in-house cELISA and iELISA at the Canadian Food Inspection Agency's Centre for Food-borne and Animal Parasitology. Negative control sera came from captive polar bears. Both assays utilized Nunc MaxiSorp 96-well plates (Thermo Fisher Scientific, Rochester, NY) coated overnight at 2-8℃ with excretory-secretory (E-

S) antigen of *T. spiralis* first-stage larvae diluted in carbonate/bicarbonate buffer (pH 9.6). Washing of ELISA plates was performed with Tris-buffered saline containing 0.05% Tween-20 (TBST). After coating with antigen, the plates were washed, then blocked for 2 h at room temperature with 2% Bovine Serum Albumin (BSA; Sigma-Aldrich, St. Louis, MO) in Tris-buffered saline.

All polar bear sera used in this study were initially tested by cELISA using plates coated with 50 ng/well of the E-S antigen. Immune serum from a pig experimentally infected with *T. spiralis* served as a positive repeatability control. Sera were diluted 1/50 in TBST containing 1% BSA and the working dilution of an in-house produced mouse monoclonal antibody (M3963, IgG1 isotype) specific to TSL-1 (immunodominant carbohydrate epitope containing β-tyvelose) was determined by checkerboard titration. Positive control and test sera were loaded in duplicate, whereas the negative controls were tested in triplicate. Since the two negative control sera produced very similar test values in every assay run, absorbance values for only one of them were used to calculate % I. The plates were incubated for 1 h at room temperature, with shaking (450 rpm). After three washes with TBST, the plates were incubated for 30 min as above with cross-adsorbed anti-mouse IgG (Fc):HRP (Thermo Fisher Scientific) diluted 1/5,000 in TBST-1% BSA. The plates were washed six times and incubated for 15 min at room temperature with 3,3',5,5'–Tetramethylbenzidine (TMB; Seracare, Milford, MA). The reaction was stopped using TMB BlueSTOP solution (Seracare) and the plates were read at 650 nm using a SpectraMax Plus 384 microplate spectrophotometer and SoftMax Pro 6.2 software (Molecular Devices, Sunnyvale, CA). The results were expressed as % I using the same equation as that described above for *Neospora caninum* cELISA. Testing by cELISA enabled selection of a subset of positive polar bear sera with high % I values that were instrumental in developing the iELISA. To produce bear-specific conjugate for iELISA, goat anti-bear serum (Forensic; MP Biomedicals, Solon, OH, USA) was purified using the NAb Protein A/G Spin Kit (Thermo Scientific). The eluted antibodies were transferred into 20 mM HEPES, 300 mM NaCl using Sephadex G-25 Desalting Columns (GE Healthcare, Buckinghamshire, UK). Three hundred µl of this preparation containing 4 mg of purified IgG were conjugated to HRP using a Lightning-Link HRP Labelling Kit (Novus Biologicals, Littleton, CO, USA). The conjugate was diluted twofold in an equal volume of sterile glycerol and stored at -20ºC. To perform the assay, ELISA plates were coated with 100 ng/well of the E-S antigen. A polar bear serum sample that exhibited 92.2% inhibition on preliminary testing

by cELISA (above) was used as a positive control. Test and control sera were diluted 1/200 in TBST containing 1% BSA and 5% skim milk and incubated in duplicate for 1 h at room temperature, with shaking (450 rpm). This was followed by incubation for 1 h as above with the anti-bear HRP conjugate diluted 1/5,000 in TBST-1% BSA. After the final wash, incubation with the TMB substrate was carried out for 10 min. The subsequent steps were as those for cELISA. The absorbance values in iELISA were normalized as percent of positive control (PP). The cut-off for iELISA was calculated as four times the mean of PP values of the two negative control sera. There was a strong correlation between these *Trichinella* assays (Pearson correlation coefficient: r = 0.93).

Franciscella tularensis

A microagglutination assay (MAT) was used to detect IgM and IgG antibodies for *F. tularensis* (Sato et al., 1990). Serum samples from Arctic foxes (*Vulpes lagopus*) that were previously tested with a MAT were used as positive and negative controls. A high positive control (1:1024), low positive control (1:128), and negative control were used for each run. Titers ≥ 1:128 were considered positive.

Bordetella bronchiseptica

An ELISA for *B. bronchiseptica* - reactive IgG antibodies was performed as previously described using anti-canine IgG as the conjugate (Ellis et al., 2011; Ellis et al., Submitted). A previously determined cut-off value of 15 OD units, which was based on the testing of positive and negative dog sera, was used as an indicator of biologically significant antibody response after exposure to *B. bronchiseptica* antigens (Ellis et al., 2011; Ellis et al., Submitted).

Canine parvovirus

Serum antibodies reactive with CPV antigens were measured using an ELISA that was developed using standard procedures. Briefly, single component, high titer (>1/8000 hemagglutination titer), low passage canine parvovirus 2a vaccine (Vanguard Plus CPV, Zoetis, kindly provided by Dr. J. Wu) was used as antigen to coat plates. Optimal dilution of this antigen and secondary sheep anti-canine IgG was determined in a checkboard titration using known CPV positive and negative canine sera. A 1/500 dilution of antigen was used and sera were tested at a

1/100 final dilution. Units of activity were determined by comparison with the optical density values of the canine positive control serum.

Canine morbillivirus

Serum antibodies reactive with CDV were detected in an immunoperoxidase plaque staining assay that was conducted according to previously described methods (Soma et al., 2001), with minor modifications. Briefly, Vero cells were infected in suspension with approximately 0.1 m.o.i. of the Onderstepoort strain of CDV. Approximately 10^5 cells were plated into 96-well tissue culture plates. Monolayers were fixed in acetone seventy-two hours later, and reacted with a 1/50 dilution of polar bear sera or CDV- positive or negative canine sera, followed by peroxidase-conjugated sheep anti-canine IgG antiserum. Individual wells were scored as positive or negative.

Statistical Analyses

Seroprevalence of each pathogen between 1986-1989 (n = 157), 1995-1998 (n = 159), and 2015-2017 (n = 109), and co-occurrence between pathogens, were compared using a Pearson Chi-square. Polar bears were sampled a maximum of once per year, but some individuals were sampled multiple times with a time period (1986-1989, 1995-1998, 2015-2017). To minimize overestimates resulting from enduring antibodies, individuals that were sampled multiple times (27/41 total repeats) were only counted on their first positive result, not subsequent positive results within the time period. This resulted in only 3.7% (14/381) of the seroprevalence dataset from the same bears repeatedly sampled across time periods. Any individual seroconversions within a time period were recorded as independent observations. Because the number of repeat positives removed varied by pathogen, sample sizes for seroprevalence estimates are unequal. We assessed mean age of bears in each time period with a one-way ANOVA, to exclude the explanation of an age-based bias influencing seroprevalence changes over time.

To examine which factors were influential to changes in pathogen exposure in polar bears, we considered potentially influential sets of biological and climatic variables on an annual time scale (Table 3 & 4). Biological variables included age, sex, weight, body condition and conflict history of the bear. Briefly, the variable ‘Age’ was estimated from the cementum layers of an extracted vestigial premolar (Calvert & Ramsay, 1988), while ‘Sex’ was determined in the field. Polar bears were given a subjective fatness rating of 1-5 at the time of capture (Stirling et al.,

2008), and bears rated 1-2 were considered in 'Poor' condition, bears rated 3 were 'Average', and bears rated 4-5 were considered in 'Good' condition. Prior to modelling, categories of body condition were dummy coded with 'Average' forming the reference category. The variable 'Weight' was estimated from measurements of straight-line length and axillary girth during handling, following the calculations in Thiemann et al. (2011). Lastly, polar bears with any history of capture as part of the Polar Bear Alert Program in the town of Churchill prior to sampling, were categorized as 'Conflict' bears.

Climatic variables included number of ice-free days, summer temperature, winter minimum temperature, annual temperature, summer precipitation, and annual precipitation. Summer was defined as June – September, while winter was defined as December – March. All climate variables, with exception of sea ice, were measured at the weather station at Churchill Airport, Manitoba (Churchill A, Station ID 5060600, 58°44'24.0" N, 94°04'12.0" W, Environment Canada, 2020). The variable 'IceFree' was the number of days in a year of less than 15% sea ice concentration as determined by SSM/I with a spatial grain of 25 km^2 (Cavalieri et al., 1996), within the home range of WH polar bears (McCall et al., 2014). All WH polar bears migrate to land during the ice-free period, and migration phenology correlates with thresholds of sea ice concentration (Cherry et al., 2013; Stirling et al., 1999). We used a threshold of 15% sea ice concentration as it aligns with the climatic definition of "ice-free" (Parkinson et al., 1999), and is a conservative measure of the total number of days polar bears likely spent on land. Lastly, all climatic measurements were taken from the year prior to the year of the serum sample. Temporal lags were tested out to 3-years from year of sample, and a 1-year lag resulted in the best fit (Table S1).

We related the seroprevalence (1/0) of pathogens that showed significant change over time to variables using binomial (logit link) generalized linear mixed models (GLMM), and the same constrained set of *a priori* models for each pathogen, balanced for equal representation of all variables (Table S2, S3). We included Bear ID as a random effect to control for repeat samples from the same individuals. Prior to modelling, we mean-centered continuous covariates about zero, and examined Pearson correlation coefficients for any pairs of factors that had a coefficient > 0.7. To assess the relative influence of biological and climatic factors, we used Akaike's information criterion for small samples (AIC_C; Burnham & Anderson, 2002). We evaluated sets of biological and climatic factors separately, and identified top factors for each as having an AIC_C $w_i \geq 0.60$. To

assess the comparative strength of biological and climatic factors on their influence on disease exposure, we combined top biological and climatic factors into one model, and used log-likelihood ratio tests and conditional-R^2 (Nakagawa & Schielzeth, 2013) to examine model improvement. The analyses were conducted in R (Version 3.3.3; R Development Core Team 2017), all reported variances are 95% confidence intervals, and the alpha cut-off value was set to 0.05 for all significance tests.

Results

Seroprevalence

The mean age of adult females and males sampled was 14.1 ± 0.8 (n = 211) and 12.4 ± 0.7 (n = 214), respectively, and there was no significant difference in mean age between the time periods ($F_{2,422}$ = 0.60, P = 0.55). Mean seroprevalence was 55.9% (228/408) for *T. gondii*, 9.7% (41/424) for *N. caninum*, 66.0% (270/409) for *Trichinella* spp., 52.3% (216/413) for *F. tularensis*, 68.4% (281/411) for *B. bronchiseptica*, 24.0% (100/417) for CDV, and 7.0% (28/400) for CPV. Co-occurrence between pathogens did not significantly vary from random (Table 5), with the exception of *T. gondii* and *N. caninum* (χ^2 = 13.5, df = 1, P < 0.001), in which all seropositive cases of *N. caninum* were observed in bears also seropositive for *T. gondii*.

Seroprevalence Over Time

Seroprevalence varied significantly between time periods for *T. gondii* (χ^2 = 17.4, df = 2, P < 0.001), *Trichinella* spp. (χ^2 = 13.4, df = 2, P = 0.001), *F. tularensis* (χ^2 = 9.9, df = 2, P = 0.007) and *B. bronchiseptica* (χ^2 = 23.1, df = 2, P < 0.001). The seroprevalence of all four pathogens increased between 1986-89 and 1995-98 (Figure 2). Only *T. gondii* seroprevalence continued to significantly increase between 1995-98 and 2015-17, increasing by an overall total of 25.8% (Figure 2a). Seroprevalence of *N. caninum* (χ^2 = 4.4, df = 2, P = 0.11), and viral pathogens CDV (χ^2 = 0.4, df = 2, P = 0.83) and CPV (χ^2 = 2.7, df = 2, P = 0.26) did not significantly vary between time periods. Of the 381 individual polar bears sampled, 38 individuals were sampled twice and 3 individuals were sampled three times. The number of observed seroconversions varied by

pathogen, with only one observation for *T. gondii* and CPV, and 15 observations for *B. bronchiseptica*, with a range of 1-21 years between observations (Table 6).

Biological Factors

Sex was a top factor in the seroprevalence of both *Trichinella* spp. ($AIC_C\ w_i = 0.83$) and *B. bronchiseptica* ($AIC_C\ w_i = 1.00$). Males were 1.82 ($CI_{95} = 1.03 – 3.23$) times more likely to be seropositive for *Trichinella* spp. than females, while females were 4.95 ($CI_{95} = 2.53 – 9.71$) times more likely to be seropositive for *B. bronchiseptica*. Age was a top factor in the seroprevalences of *Trichinella* spp. ($AIC_C\ w_i = 0.92$) and *B. bronchiseptica* ($AIC_C\ w_i = 0.98$). The likelihood of being seropositive for both *Trichinella* spp. ($\beta = 0.06$, $CI_{95} \pm 0.05$) and *B. bronchiseptica* ($\beta = 0.09$, $CI_{95} \pm 0.06$) increased with age. Polar bears previously captured in human settlements ('Conflict') was a top factor for *Trichinella* spp. ($AIC_C\ w_i = 0.81$) and *F. tularensis* ($AIC_C\ w_i = 0.74$). Conflict polar bears were 2.24 ($CI_{95} = 1.11 – 4.54$) times more likely to be seropositive for *Trichinella* spp., and 1.97 ($CI_{95} = 1.16 – 3.32$) times less likely for *F. tularensis*. Body condition was a top factor for seroprevalence of *F. tularensis* ($AIC_C\ w_i = 0.70$), with bears rated in good condition 1.84 ($CI_{95} = 1.07 – 3.19$) times more likely to be seropositive for *F. tularensis*. No biological factors had a ranking $AIC_C\ w_i \geq 0.60$ for *T. gondii.* In all cases, the addition of top biological factors significantly improved overall model fit for all pathogens modelled (Table 7).

Climate Factors

Mean summer temperature was a top factor in seroprevalence of *Trichinella* spp. ($AIC_C\ w_i = 0.72$) and *B. bronchiseptica* ($AIC_C\ w_i = 0.76$). The likelihood of being seropositive for *Trichinella* spp. ($\beta = 0.51$, $CI_{95} \pm 0.35$) and *B. bronchiseptica* ($\beta = 0.78$, $CI_{95} \pm 0.35$) increased with warmer summers. In addition, mean minimum winter temperature was a top factor for *Trichinella* spp. ($AIC_C\ w_i = 0.73$), with bears more likely to be seropositive following warmer winters ($\beta = 0.20$, $CI_{95} \pm 0.18$). Total precipitation in the summer months ($AIC_C\ w_i = 0.85$), and annually ($AIC_C\ w_i = 0.85$), were top factors in the seroprevalence of *T. gondii*. Wetter summers ($\beta = 0.12$, $CI_{95} \pm 0.08$) within dryer years overall ($\beta = -0.13$, $CI_{95} \pm 0.07$) increased the likelihood of polar bears seropositive for *T. gondii*. The number of ice-free days was a top factor in seroprevalence of *F. tularensis* ($AIC_C\ w_i = 0.77$), with increased likelihood of being seropositive

following years with more ice-free days ($\beta = 0.03$, $CI_{95} \pm 0.02$). In all cases, the addition of top climatic factors significantly improved overall model fit for all diseases modelled (Table 7).

Discussion

Climate change is expected to increase the severity and scope of outbreaks of some diseases in wildlife, as warmer and wetter conditions are hypothesized to facilitate the transmission and survival of pathogens (Altizer et al., 2013), including expanding pathogen range into latitudes not previously observed (Baker-Austin et al., 2013; Dudley et al., 2015). However, empirical evidence of climate-mediated change in disease prevalence, even in Arctic systems with accelerated warming, has been lacking. We detected increases in the seroprevalence of four of the seven pathogens we surveyed in WH polar bears across at least one time period. Seroprevalences increased for zoonotic parasites (*T. gondii*, *Trichinella* spp.) and bacterial pathogens (*F. tularensis* and *B. bronchiseptica*), but remained unchanged for viruses (CDV, CPV) and *N. caninum*. We also found support for climate-mediated changes in the prevalence of each of the four zoonotic pathogens we surveyed.

The protozoan parasite *Toxoplasma gondii* increased in seroprevalence between all time periods, peaking at 69.6% in 2015-17. Our findings are higher than any previous report for adult polar bears (Atwood et al., 2017; Jensen et al., 2010; Kirk et al., 2010; Naidenko et al., 2013; Rah et al., 2005). Polar bears in Hudson Bay have the longest on-land period relative to other subpopulations (PBSG, 2018), suggesting that terrestrial exposure may be an important factor in *T. gondii* seroprevalence (Atwood et al. 2017). *Toxoplasma gondii* seroprevalence did not vary with sex or across age, but rather, was associated to changing precipitation patterns in Hudson Bay during the period in which polar bears are on land.

Wetter summers, within dryer years overall, were correlated with a higher seroprevalence of *T. gondii* for WH polar bears. The positive correlation between precipitation and *T. gondii* seroprevalence reflects patterns documented in other regions with definitive felid hosts (Afonso et al., 2010), intermediate wildlife host species (*e.g.* crows *Covus* spp. Salant et al., 2013; roe deer *Capreolus capreolus* Gamarra et al., 2008; wild rabbits *Oryctolagus cunniculus* Almeria et al., 2004), and is consistent with the expectations of *T. gondii* transmission under future climate

scenarios (Meerburg & Kijlstra, 2009). Potential terrestrial *T. gondii* exposure includes polar bears ingesting surface water contaminated with oocysts shed by domestic or wild felid hosts, with wetter conditions during summer months favouring sporulation and spread (Dubey, 2010). In Churchill MB, domestic cats are kept as pets, but bears that were previously captured there were not more likely to have *T. gondii* antibodies. Canada lynx (*Lynx canadensis*) are suspected to serve as definitive hosts of *T. gondii,* and seropositive lynx have been documented in the surrounding Hudson Bay watershed (Labelle et al., 2001; Simon et al., 2013a). However, while Canada lynx range includes the Hudson Bay lowlands (Vashon, 2016), the current status of lynx in the WH study area is unknown. Observations by trappers have been rare (L. Fishback – pers. comm.), and lynx are the only endemic carnivore not detected by trail cameras that have been operating along the coast of Wapusk National Park since 2011 (D. Clark – pers. comm.; Clark et al., 2019).

Like other Arctic carnivores, polar bears may also be exposed to *T. gondii* through the consumption of tissue cysts in intermediate host species, and precipitation may modulate exposure. On the sea ice, ringed seals (*Pusa hispida*) are the primary prey for WH polar bears (Thiemann et al., 2008), and ringed seals in Canada have been documented with *T. gondii* seroprevalences ranging from 10-26% (Bachand et al., 2019; Reiling et al., 2019; Simon et al., 2011). Surface runoff contaminated with *T. gondii* oocysts into coastal marine environments is a suspected source of infection for marine mammals in the Arctic (Simon et al., 2013b). Higher precipitation during the summer months coincides with the hyperphagic feeding period for ringed seals (Young & Ferguson, 2013), which may increase exposure to *T. gondii* oocysts lodged in the alimentary canal of filter feeding fish (Massie et al., 2010). Alternatively, *T. gondii* exposure for polar bears could involve the consumption of terrestrial intermediate hosts such as Arctic fox (*Vulpes lagopus;* Prestrud et al., 2007) or Arctic-nesting migratory lesser snow geese (*Chen caerulescens*; Bachand et al., 2019; Elmore et al., 2015). While observations of Arctic fox consumption are very rare in Hudson Bay (but see: Richardson & Brook, 2004), snow geese are preyed upon in the summer months by some polar bears in WH (Gormezano & Rockwell, 2013). In years with higher summer precipitation, plant growth is enhanced and nesting survival of snow geese improves (Lecomte et al., 2009), which may bolster goose populations and encounter rates for polar bears.

Neospora caninum is a tissue dwelling coccidian parasite closely related to *T. gondii*, but neosporosis has only been recognized for about 30 years (Dubey et al., 2007). Atwood et al. (2017)

was the first to report *N. caninum* antibodies in polar bears, recorded in 3.7% of southern Beaufort Sea polar bears, 2007-14. Comparatively, mean *N. caninum* seroprevalence in WH was more than double the southern Beaufort Sea subpopulation. Additionally, all WH polar bears with *N. caninum* antibodies, also had antibodies to *T. gondii*, potentially reflecting a common route of exposure. Gray wolves (*Canis lupus*) are the only definitive hosts for *N. caninum* (Dubey et al., 2011) to overlap with polar bears in WH. As intermediate hosts, polar bears are most likely infected by consuming oocysts shed by canids into the environment.

Transmission by carnivory among intermediate hosts, common for terrestrial mammalian transmission of *T. gondii* (Dubey, 2010), to our knowledge has not been demonstrated for *N. caninum*. If this was a potential route of exposure for bears, caribou (*Rangifer tarandus*) and ringed seals may contribute to exposure. Caribou were found in 10% of WH polar bear scats collected on land (Gormezano & Rockwell, 2013), and ringed seals are the primary prey on the sea ice. *Neospora caninum*-like DNA was reported in the tissue of 26% ringed seals in eastern Hudson Bay (Reiling et al., 2019), while more than 80% of the Quaminuriaq caribou herd of Hudson Bay were reported as *N. caninum* seropositive (Carlsson et al., 2019). However, if feeding on intermediate hosts exposed polar bears to *N. caninum*, we would expect a seroprevalence comparable to *T. gondii*. The *N. caninum* seroprevalence we observed aligns with the generally lower oocyst shedding pattern observed in canids (Dubey & Schares, 2011), and likely reflects the overall level circulating in the Hudson Bay ecosystem.

Trichinella spp. have a long history of documentation in polar bears, and previous studies found prevalences comparable to our study (Asbakk et al., 2010; Larsen & Kjos-Hanssen, 1983; Naidenko et al., 2013; Rah et al., 2005). Our results are consistent with previous findings that *Trichinella* spp. seroprevalence increases with age in polar bears, although we did not find uniformity between sexes (Asbakk et al., 2010; Rah et al., 2005); rather, males had higher seroprevalence than females. Because of the low *Trichinella* spp. prevalence in arctic marine mammals (Jenkins et al., 2013), transmission of *Trichinella* spp. in polar bears likely relies on hunting or scavenging terrestrial carnivores (Richardson & Brook, 2004), or cannibalism (Forbes, 2000; Larsen & Kjos-Hanssen, 1983). The finding that older males are more likely to be seropositive supports cannibalism as a mode of transmission, as cannibalism is more frequently observed in adult males (Taylor et al., 1985). Dietary studies of WH polar bears also supports

cannibalism, as polar bears have been identified in scat remains, but not other terrestrial carnivores (Derocher et al., 1993; Gormezano & Rockwell, 2013; Russell, 1975).

Trichinella spp. seroprevalence in WH polar bears was positively correlated to both warmer summer and winter temperatures across all time periods, but was not correlated with annual temperatures, suggesting a season-specific response. Although serology cannot differentiate amongst *Trichinella* spp., it is most probable that the species in this study are *Trichinella nativa* and/or *Trichinella* -T6, which are freeze-tolerant and the most common sylvatic taxa found in wildlife in Canada (Gajadhar & Forbes, 2010). Mean annual minimum winter temperatures during our study ranged between –30.0 and –24.9 °C. Environmental exposure to temperatures below –20 °C is suggested to reduce the survival of *T. nativa* and *Trichinella* -T6 larvae in muscle tissue, with optimal long-term survival in the range of 0 to –20 °C (Pozio, 2016). It is therefore possible that under reduced extreme winter cold as seen in our study, the survival of *Trichinella* spp. increased. Furthermore, carcasses freeze more quickly in extreme cold, likely reducing the consumption of muscle tissue by scavengers. Stirling and Øritsland (1995) observed that in –25 °C weather, polar bears will generally abandon a seal carcass after the fat is stripped. Under reduced extreme cold in winter, *Trichinella* parasites may survive longer in the muscle tissue of infected carcasses, and carcasses may remain available for consumption longer, potentially infecting a greater number of bears.

Arctic research has focused on the cold tolerance of *Trichinella* spp. as a limit to their range (Masuoka et al., 2009; Pozio, 2016), but there has been little research on the effect of summer temperature. Warmer summer air temperatures in Hudson Bay likely increases the probability of heat stress for polar bears (Øritsland et al., 1974) that are near the southern end of their range. Heat stress in endotherms alters the endocrine system, increasing corticoids and depressing the inflammatory response (Morley & Lewis, 2014), which may compromise immunity to parasite invasion. We suggest future studies of *Trichinella* spp. in wildlife include a measure of thermal stress to establish its potential role in pathogen transmission.

Both of the bacterial pathogens we surveyed, *F. tularensis* and *B. bronchiseptica*, increased in seroprevalence between 1986-89 and 1995-98. Little research has been conducted for either of these pathogens in polar bears. Atwood et al. (2017) reported low seroprevalences of *F. tularensis*

in southern Beaufort Sea polar bears, with less than 5% of bears sampled having antibodies. In our study, prevalence was 8 to 12 times higher, with more than 60% of polar bears seropositive in 2015-17. The terrestrial life cycle of *F. tularensis*, spread by biting insects such as ticks, mosquitoes, and biting flies (Feldman, 2003), lagomorph and rodent reservoirs such as muskrats (*Ondatra zibethicus*; (Martin et al., 1982), and/or through water-borne transmission in wetland areas (Eliasson et al., 2006). All WH polar bears spend 3-4 months on land annually, whereas only 20% of Beaufort Sea polar bears come to shore, and stay on average for 2 months (Atwood et al., 2016a). The migration ecology of WH polar bears may explain the large differences in pathogen exposure in comparison with polar bears of the Beaufort Sea.

During summer, WH polar bears are found along the coast of Hudson Bay (Derocher & Stirling, 1990a), with bears sighted around the town of Churchill on the coastline, and farther inland in Wapusk National Park (Stapleton et al., 2014). *Francisella tularensis* seroprevalence was lower for WH polar bears that had been previously captured near the town of Churchill. It is possible that polar bears in closer proximity to the coast find refuge from deer flies and horseflies (Tabanidae), which use peatland habitats for key aspects of their life cycle (McElligott & Lewis, 1996). However, although polar bears demographically segregate, with males staying along the coast (Derocher & Stirling, 1990b) and adult females using inland areas (Derocher & Stirling, 1990a), we did not find any significant difference in *F. tularensis* seroprevalence of males (49%) and females (55%).

Increased time on land as a result of more ice-free days in Hudson Bay, was positively correlated with *F. tularensis* seroprevalence. In the Hudson Bay lowlands, mosquitoes (Culicidae) emerge in June, and peak in mid-July, whereas horseflies and deer flies begin to emerge in July and peak in early August (Park, 2017; Twinn et al., 1948). Longer time on land may increase seasonal exposure to biting insects, and polar bears forced onto land earlier may overlap with peak abundances of mosquitoes. Additionally, more time on land likely increases use of terrestrial water bodies, which may expose polar bears to water-borne *F. tularensis* or facilitate close contact with other reservoir hosts, such as rodents. *Francisella tularensis* antibodies were also more likely in polar bears in better body condition. Because body condition and immune function are positively correlated in polar bears (Whiteman et al., 2019), we may be detecting increased antibodies to *F. tularensis* in bears in better condition. Alternatively, these bears could have been more likely to

survive exposure without developing clinical disease. As longer ice-free periods are expected to reduce polar bear body condition (Castro de la Guardia et al., 2013), future declines in sea ice may hinder antibody response against *F. tularensis* and/or lead to more clinical tularemia, which can be a fatal infection.

Bordetella bronchiseptica is a highly infectious respiratory pathogen well documented in laboratory and domestic animals (Goodnow, 1980), but little is known about its presence in wildlife. The high seroprevalence of antibodies to *B. bronchiseptica* in WH polar bears, including the most frequent seroconversion out of the diseases surveyed, suggests regular exposure. *Bordetella bronchiseptica* has been shown to be common in domestic dogs (*Canis familiaris*) from remote communities in Canada (Bryan et al., 2011), and although dogs are kept in Churchill, MB, we found no correlation with bears previously captured in human settlements. *Bordetella bronchiseptica* spreads by aerosol transmission or direct contact; thus transmission through close contact among polar bears is a possible route of infection and maintenance in the population (Ellis et al., Submitted). While adult males tend to aggregate in the summer, most adult females are solitary except for those accompanied by cubs (Derocher & Stirling, 1990b). However, our results are inconsistent with transmission through social aggregation, as *B. bronchiseptica* seroprevalence was five times more likely to occur in females. Environmentally transmitted bacteria are suggested to benefit from a warming Arctic (Bradley et al., 2005), and we found support for this hypothesis, as *B. bronchiseptica* seroprevalence in WH polar bears increased following warmer summers. We recommend that more wildlife studies include serological and direct testing (culture and/or DNA based methods) for *B. bronchiseptica* to confirm species identity and host range, understand how *Bordetella* spp. are maintained and spread in natural systems, and the potential for transmission within and among wildlife, domestic animals, and people.

Seroprevalence of the viruses we surveyed (CDV, CPV) did not change over time. CDV has been commonly studied in polar bears, including one previous WH study reporting 31% seroprevalence (Cattet et al., 2004). Our study is the first to document CPV seroprevalence in polar bears, and WH polar bears had a relatively low prevalence (7%). Both CDV and CPV are cold-tolerant viruses which can remain viable for months in cool, moist environments, and for years if frozen (Appel & Gillespie, 1972; Gordon & Angrick, 1986). The steady CDV and CPV seroprevalence over time may reflect the optimal conditions in Hudson Bay for long-term virus

survival, creating a stable endemic condition, rather than eruptive outbreaks. There is some evidence from other ecosystems of the influence of climate on the prevalence and virulence of CDV and CPV (Kelman et al., 2020; Munson et al., 2008). However, the source of these viruses in polar bears remains unclear and evidence for environmental regulation scant, warranting further investigation.

Between 1987-2011, the WH polar bear population declined from 1187 individuals to 806 (Lunn et al., 2016). However, the change was not linear. Rapid decline occurred between the mid-1980s and mid-1990s, when several measures of polar bear health including body condition, reproduction and survival decreased (Derocher & Stirling, 1995; Regehr et al., 2007). These indices subsequently stabilized in the mid-2000s (Lunn et al., 2016). Additionally, Boonstra et al. (2020) found a shift in the stress axis of polar bears between 1983-1990 and 1991-2015. In our study, we found a similar temporal pattern in the change in the seroprevalence of *T. gondii*, *F. tularensis*, and *B. bronchiseptica*. We do not have direct evidence for the virulence of these pathogens we surveyed in polar bears, and therefore cannot associate pathogen exposure to survival. However, some exposure patterns suggest the potential for negative impacts on the population if exposure leads to disease. For example, co-occurrence between *T. gondii* and *N. caninum* was higher than expected by chance. Both protozoan parasites can cause spontaneous abortions in some species (Dubey, 2010; Dubey et al., 2007), suggesting a potential impact on the reproductive output of polar bears. In addition, these parasites can cause clinical neurological disease and more subtle behavioural changes. We suggest that these protozoans have potential roles in the population health of polar bears, and should be included in conservation management considerations (Atwood et al., 2016a).

All of the zoonotic pathogens we surveyed in WH polar bears increased in seroprevalence between 1986-89 and 1995-98. By 2015-17, on average, each polar bear in our study had antibodies to three of four zoonotic pathogens we surveyed. We consider polar bears to be sentinel species for all pathogens, and a potential source of human exposure for *F. tularensis*, *Trichinella* spp. and *T. gondii*, all of which have been recorded in communities in northern Canada (MacLean et al., 1989; McDonald et al., 1990; Messier et al., 2012). In particular, 70% of bears had antibodies to *Trichinella* spp. in 2015-17, suggesting a risk for human exposure if polar bear meat is consumed raw or undercooked (Dupouy-Camet et al., 2017). Adult male polar bears that were previously

captured in human settlements were four times more likely to be *Trichinella* spp. seropositive. All samples were collected during a period when male bears were preferentially targeted in subsistence hunts as part of a 2:1, male-biased sex ratio harvest management plan (Taylor et al., 2008). Congruently, male polar bears that enter human settlements in the Arctic are at an increased risk of being harvested (Dyck, 2006). We note that serology only indicates exposure rather than active infection; however, larvae of *Trichinella* can survive for years in muscle tissue, and bears are a known source of human exposure to *Trichinella* (Rostami et al., 2017). Therefore, we recommend Hudson Bay communities employ precautionary measures to reduce the risk of exposure to zoonotic pathogens when handling and consuming polar bears, including, but not limited to, handling carcasses with gloves and/or washing hands after handling, disinfecting harvesting tools following use, freezing meat at –20 °C for at least three days prior to consumption (to inactive cysts of *T. gondii*), and eating meat cooked rather than raw.

The potential for increased exposure to zoonotic pathogens for both people and animals highlights the utility of a One Health approach in the Arctic (Ruscio et al., 2015), which considers the interconnectedness between people, wildlife and ecosystem change. Globally, the majority of emerging infectious diseases affecting people are zoonotic and are increasing with time (Jones et al., 2008), mainly as a result of agriculture, forestry, urbanization and other land-use changes (Gibb et al., 2020; Keesing et al., 2010). In the Arctic, direct anthropogenic land use change is minimal, while the effects of climate change are accelerated. The increases in seroprevalence of zoonotic pathogens in polar bears were all associated with environmental conditions undergoing climate change in both the terrestrial and marine ecosystems. This suggests that climate change can alter zoonotic pathogen prevalence in absence of land use change, especially in depauperate systems. As the pathogens and ecosystem pathways we monitored are common to many species globally, polar bears may once again be a harbinger of the coming impacts of climate change to wildlife health.

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Tables

Table 1 Sera sample sizes from adult polar bears of the western Hudson Bay, Canada, 1986-2017.

Year	Female	Male	Total
1986	17	17	34
1987	20	21	41
1988	19	20	39
1989	21	22	43
1995	20	20	40
1996	20	20	40
1997	20	19	39
1998	20	20	40
2015	17	15	32
2016	18	20	38
2017	19	20	39
Total	211	214	425

Table 2 Serology for selected diseases in adult polar bears of western Hudson Bay, Canada, 1986-2017.

Pathogen	Type	Zoonotic	Test	Kit	Cut-off	Antibodies Detected	Negative Control	Positive Control
Toxoplasma gondii	Protozoan	Yes	iELISA	ID Screen® Toxoplasmosis Indirect Multi-species	S/P ≥ 50% =Positive	IgG	Kit Control	Kit Control
Neospora caninum	Protozoan	No	cELISA	Neospora Caninum Antibody Test Kit	% I > 30 = Positive	IgG, IgM	Kit Control	Kit Control
Trichinella spp.	Helminth	Yes	iELISAcE LISA	Developed at the CFAP, CFIA	PP ≥ 13.15% = Positive	IgG, IgM	Polar Bear Sera	Polar Bear Serum
			cELISA	Developed at the CFAP, CFIA	Not established‡	IgG, IgM	Polar Bear Sera	Infected Pig Serum
Francisella tularensis	Bacteria	Yes	MAT	Sato *et al.* 1990	1:128	IgG, IgM	Arctic Fox Sera	Arctic Fox Sera
Bordetella bronchiseptica	Bacteria	Yes	ELISA	Ellis *et al.*, 2011, Ellis *et al.*, Submitted	15 OD units	IgG	Dog Sera	Dog Sera
Canine parvovirus	Virus	No	ELISA	Developed at the DVM, USask	20 OD units	IgG	Dog Sera	Dog Sera
Canine morbillivirus	Virus	No	ELISA	Soma et al., 2001	1/0	IgG	Dog Sera	Dog Sera

‡Test values of the negative control are used for the normalization of test results in cELISA. Samples from animals with infection-free status confirmed by a gold standard method are required for establishing the cELISA cut-off. Matching muscle tissue samples were not available in this study to confirm the presumed negative *Trichinella*-infection status of the negative controls by the artificial digestion method.

Table 3 Covariates used to model the likelihood of pathogen seropositivity in adult polar bears of western Hudson Bay, Canada, 1986-2017.

Name	Range	Description & Source
Biological		
Age	5 – 31	Age of polar bear via tooth histology (Calvert & Ramsay, 1988)
Sex	1/0	Field determination with females as ref. category (0)
Poor*	1/0	Polar bears rated 1 or 2 on 5-point body condition index (Stirling et al., 2008)
Good*	1/0	Polar bears rated 4 or 5 on 5-point body condition index (Stirling et al., 2008)
Weight‡	136 – 602 kg	Calculated weight following Thiemann et al. (2011) matched to temporal equations for WH
Conflict	1/0	Polar bears captured by Manitoba Conservation in the town of Churchill, MB (Kearney, 1989; Towns et al., 2009) prior to sample collection
Climatic†		
IceFree	110 – 152 days	Number of days of sea ice concentration <15% as determined by SSM/I (Cavalieri et al., 1996), within 95% MCP polar bear home range (McCall et al., 2014)
STemp	7.8 – 10.8 °C	Mean air temperature June – September as measured at Churchill Airport, MB (Environment Canada, 2020)
SPrecip	169.0 – 310.6 mm	Total precipitation June – September as measured at Churchill Airport, MB (Environment Canada, 2020)
WMinTemp	-30.0 – -24.9 °C	Mean minimum air temperature December – March as measured at Churchill Airport, MB (Environment Canada, 2020)
ATemp	-7.4 – -5.2 °C	Mean annual air temperature as measured at Churchill Airport, MB (Environment Canada, 2020)
APrecip	344.7 – 507.8 mm	Total annual precipitation as measured at Churchill Airport, MB (Environment Canada, 2020)

*5-point body condition index dummy-coded, with ‘Average’ (3) forming the reference category

‡Weight mean centred within sex prior to modelling

†All climate variables measured the year prior to serum sample

Table 4 Mean values of climatic variables in each time period of investigation in the Hudson Bay, Canada, 1986-2017. See Table 3 for definitions of each variable.

	Units	1986-1989	1995-1998	2015-2017
IceFree	days	114 ± 6	141 ± 16	145 ± 7
STemp	°C	9.1 ± 0.9	10.5 ± 0.6	10.3 ± 0.9
SPrecip	mm	212.5 ± 49.5	274.1 ± 42.4	226.0 ± 16.0
WMinTemp	°C	-27.5 ± 2.0	-27.2 ± 1.9	-25.9 ± 1.3
ATemp	°C	-7.0 ± 1.1	-6.3 ± 1.2	-5.7 ± 1.3
APrecip	mm	431.7 ± 56.9	458.0 ± 45.4	366.4 ± 38.3

Table *5* Co-occurrence of seropositive assays for pathogens examined in adult polar bears of western Hudson Bay, Canada, 1986-2017. Bold values indicate co-occurrences significantly different than expected by chance (P < 0.05).

	N. caninum	*Trichinella* spp.	*F. tularensis*	*B. bronch.*	CPV	CDV
T. gondii	**9.88%**	40.24%	30.12%	40.71%	3.99%	13.41%
N. caninum		5.88%	4.71%	7.53%	0.50%	1.41%
Trichinella spp.			35.53%	48.24%	4.49%	18.59%
F. tularensis				38.35%	3.24%	14.59%
B. bronch.					5.49%	18.82%
CPV						0.94%

Table 6 Observations of seroconversion for pathogens examined for adult polar bears of western Hudson Bay, Canada with repeat samples (n = 41).

	Seroconversion			Years Apart	
	Positive	Negative	Total	Minimum	Maximum
T. gondii	1	0	1	8	8
N. caninum	1	2	3	1	9
Trichinella spp.	9	0	9	1	9
F. tularensis	4	7	11	1	11
B. bronchiseptica	10	5	15	1	21
CPV	1	0	1	2	2
CDV	1	2	3	2	7

Table 7 Comparison of model fit for top biological and climatic factors to the likelihood of seroprevalence of *T. gondii*, *Trichinella* spp., *F. tularensis*, and *B. bronchiseptica* in adult polar bears of western Hudson Bay, Canada, 1986-2017. Models were fit using a binomial logit-link GLMM, with individual Bear ID as a random effect (1|BearID). For *T. gondii*, no biological covariates made the cutoff for inclusion, therefore the combined model is identical to the climatic model. For *Trichinella* spp., *F. tularensis*, and *B. bronchiseptica*, all top models significantly improved fit with inclusion of both biological and climatic covariates (*LL* Ratio Test; $P \leq 0.02$).

Rank	Type	Model	k	LL	AIC_C	ΔAIC_C	R^2_{Cond}
T. gondii							
1	Combined	(1|BearID) + SPrecip + APrecip	4	-274.99	558.08	0.00	0.39
2	Null	(1|BearID)	2	-284.60	573.24	15.16	0.35
Trichinella spp.							
1	Combined	(1|BearID) + Sex + Age + Conflict + STemp + WMinTemp	7	-250.66	515.58	0.00	0.37
2	Biological	(1|BearID) + Sex + Age + Conflict	5	-258.81	524.64	9.05	0.32
3	Climatic	(1|BearID) + STemp + WMinTemp	4	-257.25	525.72	10.13	0.32
4	Null	(1|BearID)	2	-266.27	536.57	20.99	0.25
F. tularensis							
1	Combined	(1|BearID) + Good + Conflict + IceFree	5	-282.06	574.27	0.00	0.19
2	Climatic	(1|BearID) + IceFree	3	-287.43	580.91	6.64	0.16
3	Biological	(1|BearID) + Good + Conflict	4	-291.35	582.74	8.46	0.16
4	Null	(1|BearID)	2	-292.27	588.56	14.29	0.13
B. bronchiseptica							
1	Combined	(1|BearID) + Sex + Age + STemp	5	-222.27	454.68	0.00	0.42
2	Biological	(1|BearID) + Sex + Age	4	-236.79	481.67	26.99	0.31
3	Climatic	(1|BearID) + STemp	3	-247.72	501.50	46.82	0.24
4	Null	(1|BearID)	2	-260.65	525.32	70.64	0.12

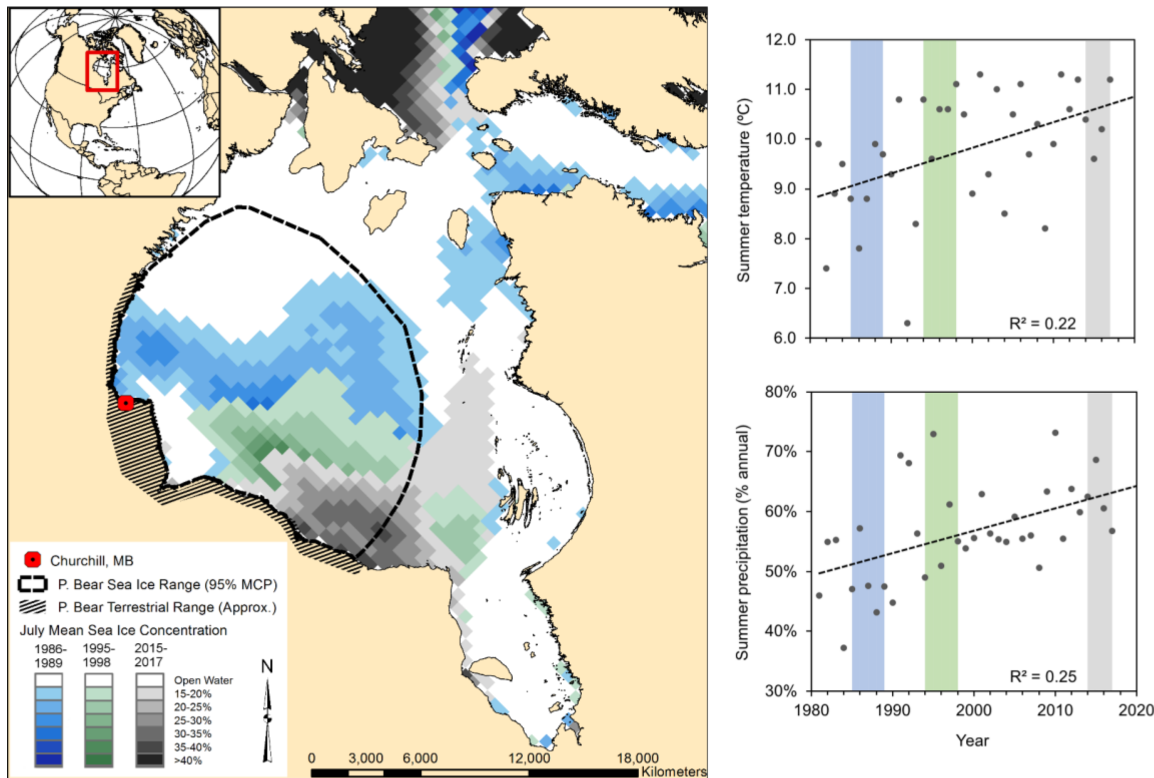

Figure 1 Study area of western Hudson Bay, Canada. Hudson Bay has undergone significant changes in sea ice cover, summer air temperatures and precipitation regimes, 1986-2017. The thick dashed black line is the mean on-ice home range of polar bears based on adult female movement (McCall et al., 2014), with July mean sea ice concentrations during study periods overlaid from oldest to newest. The hatched polygon represents the approximate terrestrial range of polar bears during ice-free months.

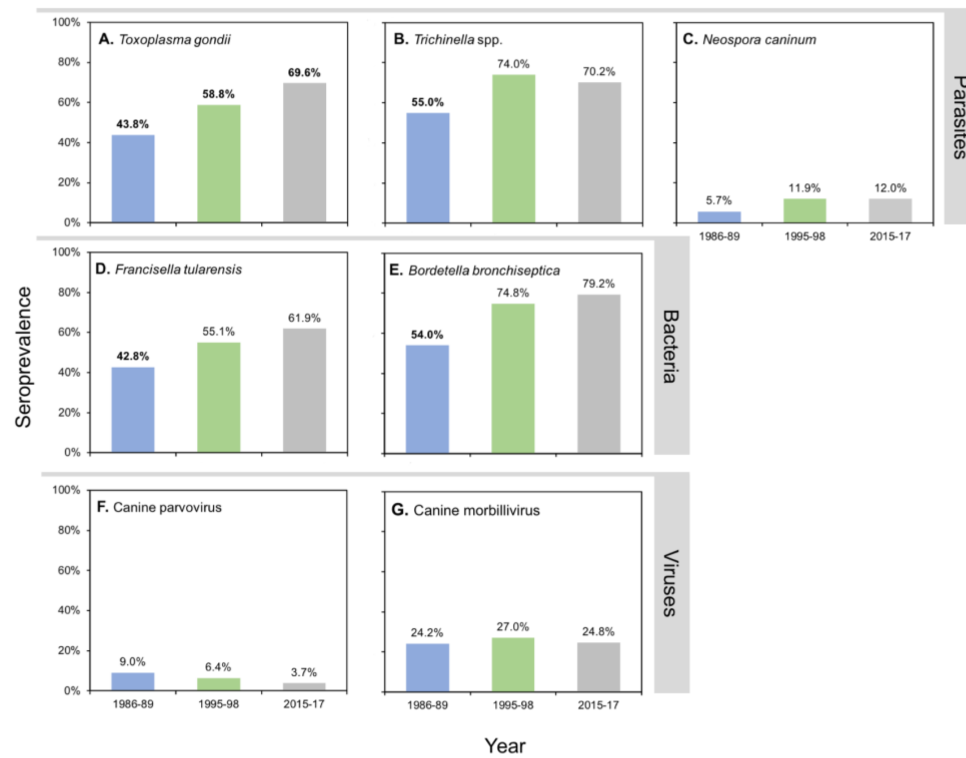

Figure 2 Temporal trends in the seroprevalence of pathogens a) *Toxoplamsa gondii* b) *Trichinella* spp. c) *Neospora caninum* d) *Francisella tularensis* e) *Bordetella bronchiseptica* f) canine parvovirus and g) canine morbillivirus for adult polar bears in western Hudson Bay, 1986-2017. Bold indicates values that are significantly different from all others ($P < 0.05$).

References

Afonso, E., Thulliez, P., & Gilot-Fromont, E. (2010). Local meteorological conditions, dynamics of seroconversion to Toxoplasma gondii in cats (Felis catus) and oocyst burden in a rural environment. *Epidemiology and Infection, 138*(8), 1105-1113. doi:10.1017/S0950268809991270

Aguirre, A. A., Ostfeld, R. S., Tabor, G. M., House, C., & Pearl, M. C. (2002). *Conservation Medicine: Ecological health in practice*. New York: Oxford University Press.

Alexander, K. A., & Appel, M. J. G. (1994). African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. *Journal of Wildlife Diseases, 30*(4), 481-485. doi:10.7589/0090-3558-30.4.481

Almeria, S., Calvete, C., Pages, A., Gauss, C., & Dubey, J. P. (2004). Factors affecting the seroprevalence of *Toxoplasma gondii* infection in wild rabbits (*Oryctolagus cuniculus*) from Spain. *Veterinary Parasitology, 123*(3-4), 265-270. doi:10.1016/j.vetpar.2004.06.010

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: From evidence to a predictive framework. *Science, 341*(6145), 514. doi:10.1126/science.1239401

Appel, M. J., & Gillespie, J. H. (1972). Canine distemper virus. In *Canine Distemper Virus. Virology Monographs / Die Virusforschung in Einzeldarstellungen (Handbook of Virus Research / Handbuch der Virusforschung)* (Vol. 11, pp. 1-96). Vienna: Springer.

Asbakk, K., Aars, J., Derocher, A. E., Wiig, O., Oksanen, A., Born, E. W., . . . Kapel, C. M. (2010). Serosurvey for *Trichinella* in polar bears (*Ursus maritimus*) from Svalbard and the Barents Sea. *Veterinary Parasitology, 172*(3-4), 256-263. doi:10.1016/j.vetpar.2010.05.018

Atwood, T. C., Duncan, C., Patyk, K. A., Nol, P., Rhyan, J., McCollum, M., . . . Hennager, S. (2017). Environmental and behavioral changes may influence the exposure of an Arctic

apex predator to pathogens and contaminants. *Scientific Reports, 7*(1), 13193. doi:10.1038/s41598-017-13496-9

Atwood, T. C., Marcot, B. G., Douglas, D. C., Amstrup, S. C., Rode, K. D., Durner, G. M., & Bromaghin, J. F. (2016a). Forecasting the relative influence of environmental and anthropogenic stressors on polar bears. *Ecosphere, 7*(6), e01370. doi:https://doi.org/10.1002/ecs2.1370

Atwood, T. C., Peacock, E., McKinney, M. A., Lillie, K., Wilson, R., Douglas, D. C., . . . Terletzky, P. (2016b). Rapid environmental change drives increased land use by an Arctic marine predator. *PLoS One, 11*(6), e0155932. doi:10.1371/journal.pone.0155932

Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E., & Jenkins, E. (2019). Serological and molecular detection of *Toxoplasma gondii* in terrestrial and marine wildlife harvested for food in Nunavik, Canada. *Parasites & Vectors, 12*(1), 155. doi:10.1186/s13071-019-3408-9

Baker-Austin, C., Trinanes, J. A., Taylor, N. G. H., Hartnell, R., Siitonen, A., & Martinez-Urtaza, J. (2013). Emerging *Vibrio* risk at high latitudes in response to ocean warming. *Nature Climate Change, 3*(1), 73-77. doi:10.1038/nclimate1628

Baszler, T. V., Knowles, D. P., Dubey, J. P., Gay, J. M., Mathison, B. A., & McElwain, T. F. (1996). Serological diagnosis of bovine neosporosis by *Neospora caninum* monoclonal antibody-based competitive inhibition enzyme-linked immunosorbent assay. *Journal of clinical microbiology, 34*(6), 1423-1428. doi:10.1128/JCM.34.6.1423-1428.1996

Boonstra, R., Bodner, K., Bosson, C., Delehanty, B., Richardson, E. S., Lunn, N. J., . . . Molnár, P. K. (2020). The stress of Arctic warming on polar bears. *Global Change Biology*. doi:10.1111/gcb.15142

Bradley, M. J., Kutz, S. J., Jenkins, E., & O'Hara, T. M. (2005). The potential impact of climate change on infectious diseases of Arctic fauna. *International Journal of Circumpolar Health, 64*(5), 468-477. doi:10.3402/ijch.v64i5.18028

Bryan, H. M., Darimont, C. T., Paquet, P. C., Ellis, J. A., Goji, N., Gouix, M., & Smits, J. E. (2011). Exposure to infectious agents in dogs in remote coastal British Columbia: Possible sentinels of diseases in wildlife and humans. *Canadian Journal of Veterinary Research, 75*(1), 11-17.

Burek, K. A., Gulland, F. M. D., & O'Hara, T. M. (2008). Effects of climate change on marine mammal health. *Ecological Applications, 18*(sp2), S126-S134. doi:10.1890/06-0553.1

Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-theoretic Approach* (2nd ed.). New York: Springer.

Calvert, W., & Ramsay, M. A. (1988). Evaluation of age determination of polar bears by counts of cementum growth layer groups. *Ursus, 10*, 449-453.

Carlsson, A. M., Curry, P., Elkin, B., Russell, D., Veitch, A., Branigan, M., . . . Kutz, S. J. (2019). Multi-pathogen serological survey of migratory caribou herds: A snapshot in time. *PLoS One, 14*(7), e0219838. doi:10.1371/journal.pone.0219838

Castro de la Guardia, L., Derocher, A. E., Myers, P. G., Terwisscha van Scheltinga, A. D., & Lunn, N. J. (2013). Future sea ice conditions in Western Hudson Bay and consequences for polar bears in the 21st century. *Glob Chang Biol, 19*(9), 2675-2687. doi:10.1111/gcb.12272

Castro de la Guardia, L., Myers, P. G., Derocher, A. E., Lunn, N. J., & Terwisscha van Scheltinga, A. D. (2017). Sea ice cycle in western Hudson Bay, Canada, from a polar bear perspective. *Marine Ecology Progress Series, 564*, 225-233. doi:10.3354/meps11964

Cattet, M. R. L., Duignan, P. J., House, C. A., & St. Aubin, D. J. (2004). Antibodies to *Canine distemper* and *Phocine distemper* viruses in polar bears from the Canadian Arctic. *Journal of Wildlife Diseases, 40*(2), 338-342. doi:10.7589/0090-3558-40.2.338

Cavalieri, D., Parkinson, C., Gloersen, P., & Zwally, H. (1996). Sea ice concentrations from Nimbus-7 SMMR and DMSP SSM/I-SSMIS passive microwave data. *Boulder, Colorado*

USA: NASA DAAC at the National Snow and Ice Data Center. doi:10.5067/8GQ8LZQVL0VL

Cecchini, G., Bekele, T., & Kasali, O. B. (1992). The effect of repeated freezing and thawing of serum on the activity of antibodies. *Veterinary Research Communications, 16*(6), 425-428. doi:10.1007/BF01839019

Cherry, S. G., Derocher, A. E., Thiemann, G. W., & Lunn, N. J. (2013). Migration phenology and seasonal fidelity of an Arctic marine predator in relation to sea ice dynamics. *Journal of Animal Ecology, 82*(4), 912-921. doi:10.1111/1365-2656.12050

Clark, D. A., Brook, R., Oliphant-Reskanski, C., Laforge, M. P., Olson, K., & Rivet, D. (2019). Novel range overlap of three ursids in the Canadian subarctic. *Arctic Science, 5*(1), 62-70. doi:10.1139/as-2018-0013

Clausen, B., & Hjort, P. (1986). Survey for antibodies against various infectious disease agents in muskoxen (*Ovibos moschatus*) from Jamesonland, Northeast Greenland. *Journal of Wildlife Diseases, 22*(2), 264-266.

Cohen, J., Screen, J. A., Furtado, J. C., Barlow, M., Whittleston, D., Coumou, D., . . . Jones, J. (2014). Recent Arctic amplification and extreme mid-latitude weather. *Nature Geoscience, 7*(9), 627-637. doi:10.1038/ngeo2234

Connell, F. H. (1949). Trichinosis in the Arctic: A review. *Arctic, 2*(2), 98-107.

Cox, F. E. G. (2001). Concomitant infections, parasites and immune responses. *Parasitology, 122*(S1), S23-S38. doi:10.1017/S003118200001698X

Culler, L. E., Ayres, M. P., & Virginia, R. A. (2015). In a warmer Arctic, mosquitoes avoid increased mortality from predators by growing faster. *Proceedings of the Royal Society B, 282*(1815). doi:10.1098/rspb.2015.1549

Dard, C., Bailly, S., Drouet, T., Fricker-Hidalgo, H., Brenier-Pinchart, M. P., & Pelloux, H. (2017). Long-term sera storage does not significantly modify the interpretation of toxoplasmosis serologies. *Journal of Microbiological Methods, 134*, 38-45. doi:10.1016/j.mimet.2017.01.003

Derocher, A. E., Andriashek, D., & Stirling, I. (1993). Terrestrial foraging by polar bears during the ice-free period in western Hudson Bay. *Arctic, 46*(3), 251-254.

Derocher, A. E., & Stirling, I. (1990a). Distribution of polar bears (*Ursus maritimus*) during the ice-free period in western Hudson Bay. *Canadian Journal of Zoology, 68*(7), 1395-1403. doi:10.1139/z90-208

Derocher, A. E., & Stirling, I. (1990b). Observations of aggregating behavior in adult male polar bears (*Ursus maritimus*). *Canadian Journal of Zoology, 68*(7), 1390-1394. doi:10.1139/z90-207

Derocher, A. E., & Stirling, I. (1995). Temporal variation in reproduction and body mass of polar bears in western Hudson Bay. *Canadian Journal of Zoology, 73*(9), 1657-1665. doi:10.1139/z95-197

Descamps, S. (2013). Winter temperature affects the prevalence of ticks in an Arctic seabird. *PLoS One, 8*(6), e65374. doi:10.1371/journal.pone.0065374

Dick, T. A., & Belosevic, M. (1978). Observations on a *Trichinella spiralis* isolate from a polar bear. *The Journal of Parasitology, 64*(6), 1143-1145. doi:10.2307/3279756

Dubey, J. P. (2010). *Toxoplasmosis of animals and humans* (2nd Edition ed.). Boca Raton, USA: CRC press.

Dubey, J. P., Jenkins, M. C., Rajendran, C., Miska, K., Ferreira, L. R., Martins, J., . . . Choudhary, S. (2011). Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. *Veterinary Parasitology, 181*(2), 382-387. doi:10.1016/j.vetpar.2011.05.018

Dubey, J. P., & Schares, G. (2011). Neosporosis in animals—The last five years. *Veterinary Parasitology, 180*(1), 90-108. doi:10.1016/j.vetpar.2011.05.031

Dubey, J. P., Schares, G., & Ortega-Mora, L. M. (2007). Epidemiology and control of neosporosis and *Neospora caninum*. *Clinical Microbiology Reviews, 20*(2), 323. doi:10.1128/CMR.00031-06

Dudley, J., Hoberg, E., Jenkins, E., & Parkinson, A. (2015). Climate cange in the North American arctic: A One Health perspective. *EcoHealth, 12*(4), 713-725. doi:10.1007/s10393-015-1036-1

Dupouy-Camet, J., Bourée, P., & Yera, H. (2017). *Trichinella* and polar bears: a limited risk for humans. *Journal of Helminthology, 91*(4), 440-446. doi:10.1017/S0022149X17000219

Dyck, M. G. (2006). Characteristics of polar bears killed in defense of life and property in Nunavut, Canada, 1970-2000. *Ursus, 17*(1), 52-62.

Eliasson, H., Broman, T., Forsman, M., & Back, E. (2006). Tularemia: current epidemiology and disease management. *Infectious Disease Clinics of North America, 20*(2), 289-311, ix. doi:10.1016/j.idc.2006.03.002

Ellis, J., Anseeuw, E., Gow, S., Bryan, H., Salb, A., Goji, N., . . . Kutz, S. (2011). Seroepidemiology of respiratory (group 2) canine coronavirus, canine parainfluenza virus, and *Bordetella bronchiseptica* infections in urban dogs in a humane shelter and in rural dogs in small communities. *The Canadian Veterinary Journal, 52*(8), 861-868.

Ellis, J., Gow, S., Lunn, N. J., Richardson, E. S., McGeachy, D., Lacoste, S., . . . Pilfold, N. W. (Submitted). Bordetella bronchiseptica-reactive antibodies in Canadian polar bears. *The Canadian Veterinary Journal*.

Elmore, S. A., Samelius, G., Al-Adhami, B., Huyvaert, K. P., Bailey, L. L., Alisauskas, R. T., . . . Jenkins, E. J. (2016). Estimating *Toxoplasma gondii* exposure in Arctic foxes (*Vulpes lagopus*) while navigating the imperfect world of wildlife serology. *Journal of Wildlife Diseases, 52*(1), 47-56. doi:10.7589/2015-03-075

Elmore, S. A., Samelius, G., Fernando, C., Alisauskas, R. T., & Jenkins, E. J. (2015). Evidence for *Toxoplasma gondii* in migratory vs. nonmigratory herbivores in a terrestrial arctic ecosystem. *Canadian Journal of Zoology, 93*(8), 671-675. doi:10.1139/cjz-2015-0078

Elton, C. (1931). Epidemics among sledge dogs in the Canadian Arctic and their relation to disease in the Arctic fox. *Canadian Journal of Research, 5*(6), 673-692. doi:10.1139/cjr31-106

Environment Canada. (2020). National Climate Data and Information Archive. Retrieved February 2020 https://climate.weather.gc.ca/index_e.html

Feldman, K. A. (2003). Tularemia. *Journal of the American Veterinary Medical Association, 222*(6), 725-730. doi:10.2460/javma.2003.222.725

Forbes, L. B. (2000). The occurrence and ecology of *Trichinella* in marine mammals. *Veterinary Parasitology, 93*(3), 321-334. doi:10.1016/S0304-4017(00)00349-6

Gajadhar, A. A., & Forbes, L. B. (2010). A 10-year wildlife survey of 15 species of Canadian carnivores identifies new hosts or geographic locations for *Trichinella* genotypes T2, T4, T5, and T6. *Veterinary Parasitology, 168*(1), 78-83. doi:10.1016/j.vetpar.2009.10.012

Gamarra, J. A., Cabezon, O., Pabon, M., Arnal, M. C., Luco, D. F., Dubey, J. P., . . . Almeria, S. (2008). Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. *Veterinary Parasitology, 153*(1-2), 152-156. doi:10.1016/j.vetpar.2008.01.028

Gibb, R., Redding, D. W., Chin, K. Q., Donnelly, C. A., Blackburn, T. M., Newbold, T., & Jones, K. E. (2020). Zoonotic host diversity increases in human-dominated ecosystems. *Nature, 584*(7821), 398-402. doi:10.1038/s41586-020-2562-8

Gleason, J. S., & Rode, K. D. (2009). Polar bear distribution and habitat association reflect long-term changes in fall sea ice conditions in the Alaskan Beaufort Sea. *Arctic*, 405-417.

Goodnow, R. A. (1980). Biology of *Bordetella bronchiseptica*. *Microbiological Reviews, 44*(4), 722.

Gordon, J. C., & Angrick, E. J. (1986). Canine parvovirus: Environmental effects on infectivity. *American Journal of Veterinary Research, 47*(7), 1464-1467.

Gormezano, L. J., & Rockwell, R. F. (2013). What to eat now? Shifts in polar bear diet during the ice-free season in western Hudson Bay. *Ecol Evol, 3*(10), 3509-3523. doi:10.1002/ece3.740

Harvell, D., Altizer, S., Cattadori, I. M., Harrington, L., & Weil, E. (2009). Climate change and wildlife diseases: When does the host matter the most? *Ecology, 90*(4), 912-920. doi:10.1890/08-0616.1

Hoar, B. M., Ruckstuhl, K., & Kutz, S. (2012). Development and availability of the free-living stages of *Ostertagia gruehneri*, an abomasal parasite of barrenground caribou (*Rangifer tarandus groenlandicus*), on the Canadian tundra. *Parasitology, 139*(8), 1093-1100. doi:10.1017/S003118201200042X

Hockings, M., Dudley, N., Ellio, W., Ferreira, M. N., MacKinnon, K., Pasha, M., . . . Yang, A. (2020). Editorial essay: COVID-19 and protected and conserved areas. *Parks, 26*(1), 7-24. doi:10.2305/IUCN.CH.2020.PARKS-26-1MH.en

ID Vet. (2020). ID Screen® Toxoplasmosis Indirect Multi-species. Retrieved from https://www.id-vet.com/produit/id-screen-toxoplasmosis-indirect-multi-species/

Jenkins, E. J., Castrodale, L. J., de Rosemond, S. J., Dixon, B. R., Elmore, S. A., Gesy, K. M., . . . Thompson, R. C. (2013). Tradition and transition: Parasitic zoonoses of people and animals in Alaska, northern Canada, and Greenland. *Advances in Parasitology, 82*, 33-204. doi:10.1016/B978-0-12-407706-5.00002-2

Jensen, S. K., Aars, J., Lydersen, C., Kovacs, K. M., & Åsbakk, K. (2010). The prevalence of *Toxoplasma gondii* in polar bears and their marine mammal prey: evidence for a marine transmission pathway? *Polar Biology, 33*(5), 599-606. doi:10.1007/s00300-009-0735-x

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*(7181), 990-993. doi:10.1038/nature06536

Karvonen, A., Rintamaki, P., Jokela, J., & Valtonen, E. T. (2010). Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. *International Journal of Parasitology, 40*(13), 1483-1488. doi:10.1016/j.ijpara.2010.04.015

Kearney, S. (1989). *The Polar Bear Alert Program at Churchill, Manitoba.* Paper presented at the Bear–People Conflict: Proceedings of a Symposium on Management Strategies, Yellowknife, Northwest Territories Department of Renewable Resources.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., . . . Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature, 468*(7324), 647-652. doi:10.1038/nature09575

Kelman, M., Barrs, V. R., Norris, J. M., & Ward, M. P. (2020). Socioeconomic, geographic and climatic risk factors for canine parvovirus infection and euthanasia in Australia. *Preventive Veterinary Medicine, 174*, 104816. doi:10.1016/j.prevetmed.2019.104816

Kirk, C. M., Amstrup, S., Swor, R., Holcomb, D., & O'Hara, T. M. (2010). Morbillivirus and Toxoplasma exposure and association with hematological parameters for southern Beaufort Sea polar bears: potential response to infectious agents in a sentinel species. *EcoHealth, 7*(3), 321-331. doi:10.1007/s10393-010-0323-0

Kutz, S. J., Hoberg, E. P., Polley, L., & Jenkins, E. J. (2005). Global warming is changing the dynamics of Arctic host-parasite systems. *Proceedings of the Royal Society B, 272*(1581), 2571-2576. doi:10.1098/rspb.2005.3285

Labelle, P., Dubey, J. P., Mikaelian, I., Blanchette, N., Lafond, R., St-Onge, S., & Martineau, D. (2001). Seroprevalence of antibodies to *Toxoplasma gondii* in lynx (*Lynx canadensis*) and bobcats (*Lynx rufus*) from Québec, Canada. *The Journal of Parasitology, 87*(5), 1194-1196. doi:10.2307/3285264

Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology, 90*(4), 888-900. doi:10.1890/08-0079.1

Larsen, T., & Kjos-Hanssen, B. (1983). *Trichinella* sp. in polar bears from Svalbard, in relation to hide length and age. *Polar Research, 1*(1), 89-96. doi:10.3402/polar.v1i1.6973

Larsson, C., Comstedt, P., Olsen, B., & Bergström, S. (2007). First record of lyme disease *Borrelia* in the Arctic. *Vector-Borne and Zoonotic Diseases, 7*(3), 453-456. doi:10.1089/vbz.2006.0644

Lecomte, N., Gauthier, G., & Giroux, J.-F. (2009). A link between water availability and nesting success mediated by predator–prey interactions in the Arctic. *Ecology, 90*(2), 465-475. doi:10.1890/08-0215.1

Lindsey, P., Allan, J., Brehony, P., Dickman, A., Robson, A., Begg, C., . . . Tyrrell, P. (2020). Conserving Africa's wildlife and wildlands through the COVID-19 crisis and beyond. *Nature Ecology & Evolution*. doi:10.1038/s41559-020-1275-6

Lunn, N. J., Servanty, S., Regehr, E. V., Converse, S. J., Richardson, E., & Stirling, I. (2016). Demography of an apex predator at the edge of its range: impacts of changing sea ice on polar bears in Hudson Bay. *Ecological Applications, 26*(5), 1302-1320. doi:10.1890/15-1256

MacLean, J. D., Viallet, J., Law, C., & Staudt, M. (1989). Trichinosis in the Canadian Arctic: Report of five outbreaks and a new clinical syndrome. *The Journal of Infectious Diseases, 160*(3), 513-520. doi:10.1093/infdis/160.3.513

Macrae, M. L., Brown, L. C., Duguay, C. R., Parrott, J. A., & Petrone, R. M. (2014). Observed and projected climate change in the Churchill region of the Hudson Bay lowlands and implications for pond sustainability. *Arctic, Antarctic, and Alpine Research, 46*(1), 272-285. doi:10.1657/1938-4246-46.1.272

Martin, T., Holmes, I. H., Wobeser, G. A., Anthony, R. F., & Greefkes, I. (1982). Tularemia in Canada with a focus on Saskatchewan. *Canadian Medical Association journal, 127*(4), 279-282.

Massie, G. N., Ware, M. W., Villegas, E. N., & Black, M. W. (2010). Uptake and transmission of *Toxoplasma gondii* oocysts by migratory, filter-feeding fish. *Veterinary Parasitology, 169*(3-4), 296-303. doi:10.1016/j.vetpar.2010.01.002

Masuoka, P. M., Burke, R., Colaccico, M., Razuri, H., Hill, D., & Murrell, K. D. (2009). Predicted geographic ranges for North American sylvatic *Trichinella* species. *Journal of Parasitology, 95*(4), 829-837. doi:10.1645/GE-1952.1

McCall, A. G., Derocher, A. E., & Lunn, N. J. (2014). Home range distribution of polar bears in western Hudson Bay. *Polar Biology, 38*(3), 343-355. doi:10.1007/s00300-014-1590-y

McDonald, J. C., Gyorkos, T., Alberton, B., MacLean, J. D., Richer, G., & Juranek, D. (1990). An outbreak of toxoplasmosis in pregnant women in northern Québec. *The Journal of Infectious Diseases, 161*(4), 769-774. doi:10.1093/infdis/161.4.769

McDonald, J. L., Bailey, T., Delahay, R. J., McDonald, R. A., Smith, G. C., & Hodgson, D. J. (2016). Demographic buffering and compensatory recruitment promotes the persistence of disease in a wildlife population. *Ecology Letters, 19*(4), 443-449. doi:10.1111/ele.12578

McElligott, P. E. K., & Lewis, D. J. (1996). Distribution and abundance of immature Tabanidae (Diptera) in a subarctic Labrador peatland. *Canadian Journal of Zoology, 74*(7), 1364-1369. doi:10.1139/z96-149

Meerburg, B. G., & Kijlstra, A. (2009). Changing climate-changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitology Research, 105*(1), 17-24. doi:10.1007/s00436-009-1447-4

Menge, B. A., Cerny-Chipman, E. B., Johnson, A., Sullivan, J., Gravem, S., & Chan, F. (2016). Sea star wasting disease in the keystone predator *Pisaster ochraceus* in Oregon: Insights into differential population impacts, recovery, predation rate, and temperature effects from long-term research. *PLoS One, 11*(5), e0153994. doi:10.1371/journal.pone.0153994

Messier, V., Lévesque, B., Proulx, J. F., Rochette, L., Serhir, B., Couillard, M., . . . Déry, S. (2012). Seroprevalence of seven zoonotic infections in Nunavik, Quebec (Canada). *Zoonoses & Public Health, 59*(2), 107-117. doi:10.1111/j.1863-2378.2011.01424.x

Møller, L. N., Koch, A., Petersen, E., Hjuler, T., Kapel, C. M., Andersen, A., & Melbye, M. (2010). *Trichinella* infection in a hunting community in East Greenland. *Epidemiology and Infection, 138*(9), 1252-1256. doi:10.1017/S0950268810000282

Morley, N. J., & Lewis, J. W. (2014). Temperature stress and parasitism of endothermic hosts under climate change. *Trends in Parasitology, 30*(5), 221-227. doi:10.1016/j.pt.2014.01.007

Munson, L., Terio, K. A., Kock, R., Mlengeya, T., Roelke, M. E., Dubovi, E., . . . Packer, C. (2008). Climate extremes promote fatal co-infections during Canine distemper epidemics in African lions. *PLoS One, 3*(6), e2545. doi:10.1371/journal.pone.0002545

Naidenko, S. V., Ivanov, E. A., Mordvintsev, I. N., Platonov, N. G., Ershov, R. V., & Rozhnov, V. V. (2013). Seropositivity for different pathogens in polar bears (*Ursus maritimus*) from Barents Sea Islands. *Biology Bulletin, 40*(9), 779-782. doi:10.1134/s1062359013090082

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*(2), 133-142. doi:10.1111/j.2041-210x.2012.00261.x

Ogden, N. H., & Lindsay, L. R. (2016). Effects of climate and climate change on vectors and vector-borne diseases: Ticks are different. *Trends in Parasitology, 32*(8), 646-656. doi:10.1016/j.pt.2016.04.015

Oksanen, A., Åsbakk, K., Prestrud, K. W., Aars, J., Derocher, A. E., Tryland, M., . . . Born, E. W. (2009). Prevalence of antibodies against *Toxoplasma gondii* polar bears (*Ursus maritimus*) from Svalbard and east Greenland. *Journal of Parasitology, 95*(1), 89-94. doi:10.1645/GE-1590.1

Øritsland, N. A., Lentfer, J. W., & Ronald, K. (1974). Radiative surface temperatures of the polar bear. *Journal of Mammalogy, 55*(2), 459-461. doi:10.2307/1379018

Park, J. S. (2017). A race against time: habitat alteration by snow geese prunes the seasonal sequence of mosquito emergence in a subarctic brackish landscape. *Polar Biology, 40*(3), 553-561. doi:10.1007/s00300-016-1978-y

Parkinson, C. L., Cavalieri, D. J., Gloersen, P., Zwally, H. J., & Comiso, J. C. (1999). Arctic sea ice extents, areas, and trends, 1978–1996. *Journal of Geophysical Research: Oceans, 104*(C9), 20837-20856. doi: 10.1029/1999JC900082

PBSG. (2018). *2016 Status report on the world's polar bear subpopulations*. Retrieved from Gland, Switzerland:

Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution, 22*(3), 133-139. doi:10.1016/j.tree.2006.11.005

Pilfold, N. W., Hedman, D., Stirling, I., Derocher, A. E., Lunn, N. J., & Richardson, E. (2016). Mass loss rates of fasting polar bears. *Physiological and Biochemical Zoology, 89*(5), 377-388. doi:10.1086/687988

Post, E., Bhatt, U. S., Bitz, C. M., Brodie, J. F., Fulton, T. L., Hebblewhite, M., . . . Walker, D. A. (2013). Ecological consequences of sea-ice decline. *Science, 341*(6145), 519-524. doi:10.1126/science.1235225

Pozio, E. (2016). Adaptation of *Trichinella* spp. for survival in cold climates. *Food and Waterborne Parasitology, 4*, 4-12. doi:10.1016/j.fawpar.2016.07.001

Prestrud, K. W., Asbakk, K., Fuglei, E., Mork, T., Stien, A., Ropstad, E., . . . Oksanen, A. (2007). Serosurvey for *Toxoplasma gondii* in arctic foxes and possible sources of infection in the high Arctic of Svalbard. *Veterinary Parasitology, 150*(1-2), 6-12. doi:10.1016/j.vetpar.2007.09.006

Rah, H., Chomel, B. B., Kasten, R. W., Hew, C. H., Farver, T. B., Follmann, E. H., . . . Amstrup, S. C. (2005). Serosurvey of selected zoonotic agents in polar bears (*Ursus maritimus*). *Veterinary Record, 156*(1), 7. doi:10.1136/vr.156.1.7

Regehr, E. V., Lunn, N. J., Amstrup, S. C., & Stirling, I. (2007). Effects of earlier sea ice breakup on survival and population size of polar bears in western Hudson Bay. *Journal of Wildlife Management, 71*(8), 2673-2683. doi:10.2193/2006-180

Reiling, S. J., Measures, L., Feng, S., Boone, R., Merks, H., & Dixon, B. R. (2019). *Toxoplasma gondii*, *Sarcocystis sp*. and *Neospora caninum*-like parasites in seals from northern and

eastern Canada: potential risk to consumers. *Food and Waterborne Parasitology, 17*, e00067. doi:10.1016/j.fawpar.2019.e00067

Richardson, E. S., & Brook, R. K. (2004). Excavation of an Arctic Fox, *Alopex lagopus*, den by a Polar Bear, *Ursus maritimus*. *The Canadian Field-Naturalist, 118*(4), 602-603.

Rijks, J. M., Van de Bildt, M. W. G., Jensen, T., Philippa, J. D. W., Osterhaus, A. D. M. E., & Kuiken, T. (2005). *Phocine distemper* outbreak, The Netherlands, 2002. *Emerging infectious diseases, 11*(12), 1945-1948. doi:10.3201/eid1112.050596

Rostami, A., Gamble, H. R., Dupouy-Camet, J., Khazan, H., & Bruschi, F. (2017). Meat sources of infection for outbreaks of human trichinellosis. *Food Microbiol, 64*, 65-71. doi:10.1016/j.fm.2016.12.012

Ruscio, B. A., Brubaker, M., Glasser, J., Hueston, W., & Hennessy, T. W. (2015). One Health – a strategy for resilience in a changing arctic. *International Journal of Circumpolar Health, 74*(1), 27913. doi:10.3402/ijch.v74.27913

Russell, R. H. (1975). The food habits of polar bears of James Bay and Southwest Hudson Bay in summer and autumn. *Arctic, 28*, 117-129.

Salant, H., Hamburger, J., King, R., & Baneth, G. (2013). *Toxoplasma gondii* prevalence in Israeli crows and Griffon vultures. *Veterinary Parasitology, 191*(1-2), 23-28. doi:10.1016/j.vetpar.2012.07.029

Sasan, F., Graham, L. F., Mukhit, O., Paolo, R., John, F., Donald, P. K., . . . Richard, K. (2019). Mass die-off of saiga antelopes, Kazakhstan, 2015. *Emerging infectious diseases, 25*(6), 1169. doi:10.3201/eid2506.180990

Sato, T., Fujita, H., Ohara, Y., & Homma, M. (1990). Microagglutination test for early and specific serodiagnosis of tularemia. *Journal of clinical microbiology, 28*(10), 2372-2374. doi:10.1128/JCM.28.10.2372-2374.1990

Sciullo, L., Thiemann, G. W., & Lunn, N. J. (2016). Comparative assessment of metrics for monitoring the body condition of polar bears in western Hudson Bay. *Journal of Zoology, 300*(1), 45-58. doi:10.1111/jzo.12354

Sharma, R., Parker, S., Al-Adhami, B., Bachand, N., & Jenkins, E. (2019). Comparison of tissues (heart vs. brain) and serological tests (MAT, ELISA and IFAT) for detection of *Toxoplasma gondii* in naturally infected wolverines (*Gulo gulo*) from the Yukon, Canada. *Food and Waterborne Parasitology, 15*, e00046. doi:10.1016/j.fawpar.2019.e00046

Shereen, M. A., Khan, S., Kazmi, A., Bashir, N., & Siddique, R. (2020). COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research, 24*, 91-98. doi:10.1016/j.jare.2020.03.005

Simon, A., Bigras Poulin, M., Rousseau, A. N., Dubey, J. P., & Ogden, N. H. (2013a). Spatiotemporal dynamics of *Toxoplasma gondii* infection in Canadian lynx (*Lynx canadensis*) in western Quebec, Canada. *J Wildl Dis, 49*(1), 39-48. doi:10.7589/2012-02-048

Simon, A., Chambellant, M., Ward, B. J., Simard, M., Proulx, J. F., Levesque, B., . . . Ogden, N. H. (2011). Spatio-temporal variations and age effect on *Toxoplasma gondii* seroprevalence in seals from the Canadian Arctic. *Parasitology, 138*(11), 1362-1368. doi:10.1017/S0031182011001260

Simon, A., Rousseau, A. N., Savary, S., Bigras-Poulin, M., & Ogden, N. H. (2013b). Hydrological modelling of *Toxoplasma gondii* oocysts transport to investigate contaminated snowmelt runoff as a potential source of infection for marine mammals in the Canadian Arctic. *Journal of Environmental Management, 127*, 150-161. doi:10.1016/j.jenvman.2013.04.031

Soma, T., Ishii, H., Hara, M., Yamamoto, S., Yoshida, T., Kinoshita, T., & Nomura, K. (2001). Comparison of immunoperoxidase plaque staining and neutralizing tests for canine distemper virus. *Veterinary Research Communications, 25*(4), 311-325. doi:10.1023/A:1010682726245

Stapleton, S., Atkinson, S., Hedman, D., & Garshelis, D. (2014). Revisiting Western Hudson Bay: Using aerial surveys to update polar bear abundance in a sentinel population. *Biological Conservation, 170*, 38-47. doi:10.1016/j.biocon.2013.12.040

Stern, H., & Laidre, K. L. (2016). Sea-ice indicators of polar bear habitat. *The Cryosphere, 10*, 1-15. doi:10.5194/tc-10-1-2016

Stirling, I., & Derocher, A. E. (2012). Effects of climate warming on polar bears: a review of the evidence. *Global Change Biology, 18*(9), 2694-2706. doi:10.1111/j.1365-2486.2012.02753.x

Stirling, I., Lunn, N. J., & Iacozza, J. (1999). Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. *Arctic, 52*(3), 294-306.

Stirling, I., Lunn, N. J., Iacozza, J., Elliott, C., & Obbard, M. (2004). Polar bear distribution and abundance on the southwestern Hudson Bay Coast during open water season, in relation to population trends and annual ice patterns. *Arctic, 57*(1), 15-26.

Stirling, I., & Øritsland, N. A. (1995). Relationships between estimates of ringed seal (*Phoca hispida*) and polar bear (*Ursus maritimus*) populations in the Canadian Arctic. *Canadian Journal of Fisheries and Aquatic Sciences, 52*(12), 2594-2612.

Stirling, I., Spencer, C., & Andriashek, D. (1989). Immobilization of polar bears (*Ursus maritimus*) with Telazol® in the Canadian Arctic. *Journal of Wildlife Diseases, 25*(2), 159-168.

Stirling, I., Thiemann, G. W., & Richardson, E. (2008). Quantitative support for a subjective fatness index for immobilized polar bears. *Journal of Wildlife Management, 72*(2), 568-574. doi:10.2193/2007-123

Taylor, M., Larsen, T., & Schweinsburg, R. E. (1985). Observations of intraspecific aggression and cannibalism in polar bears (*Ursus maritmus*). *Arctic, 38*(4), 303-309.

Taylor, M. K., McLoughlin, P. D., & Messier, F. (2008). Sex-selective harvesting of polar bears *Ursus maritimus*. *Wildlife Biology, 14*(1), 52-60. doi:10.2981/0909-6396(2008)14[52:SHOPBU]2.0.CO;2

Thiemann, G. W., Iverson, S. J., & Stirling, I. (2008). Polar bear diets and arctic marine food webs: Insights from fatty acid analysis. *Ecological Monographs, 78*(4), 591-614.

Thiemann, G. W., Lunn, N. J., Richardson, E. S., & Andriashek, D. S. (2011). Temporal change in the morphometry–body mass relationship of polar bears. *The Journal of Wildlife Management, 75*(3), 580-587. doi:10.1002/jwmg.112

Towns, L., Derocher, A. E., Stirling, I., Lunn, N. J., & Hedman, D. (2009). Spatial and temporal patterns of problem polar bears in Churchill, Manitoba. *Polar Biology, 32*(10), 1529-1537. doi:10.1007/s00300-009-0653-y

Twinn, C. R., Hocking, B., McDuffie, W. C., & Cross, H. F. (1948). A preliminary account of the biting flies at Churchill, Manitoba. *Canadian Journal of Research, 26d*(6), 334-357. doi:10.1139/cjr48d-025

Vashon, J. (2016). *Lynx canadensis*. *The IUCN Red List of Threatened Species 2016*, e.T12518A101138963.

Whiteman, J. P., Harlow, H. J., Durner, G. M., Regehr, E. V., Amstrup, S. C., & Ben-David, M. (2019). Heightened immune system function in polar bears using terrestrial habitats. *Physiological and Biochemical Zoology, 92*(1), 1-11. doi:10.1086/698996

Young, B. G., & Ferguson, S. H. (2013). Seasons of the ringed seal: Pelagic open-water hyperphagy, benthic feeding over winter and spring fasting during molt. *Wildlife Research, 40*(1), 52-60. doi:10.1071/wr12168

APPENDIX B

Report sent to the local and regional Nunavimmi Umajulivijiit Katujaqatigininga (LNUK and RNUK)

***Toxoplasma* and related parasites in caribou harvested in Nunavik, Canada.**

At USask: Adrián Hernández-Ortiz and Emily Jenkins.

In Nunavik: Ellen Avard, Géraldine Gouin, and Mikhaela Neelin, at Makivik, RNUK and hunters

The consumption of wildlife has many benefits but may also increase the risk of transmission of food-borne pathogens, such as *Toxoplasma gondii*. People living in some regions of the Canadian North are more exposed to *T. gondii* than the general North American population, and exposure has been linked to the consumption of wildlife. The aim of this study was to determine exposure to *Toxoplasma* in caribou harvested in Nunavik. We also looked for related parasites like *Neospora, Sarcocystis,* and *Besnoitia* spp., which do not infect humans, but could affect caribou health.

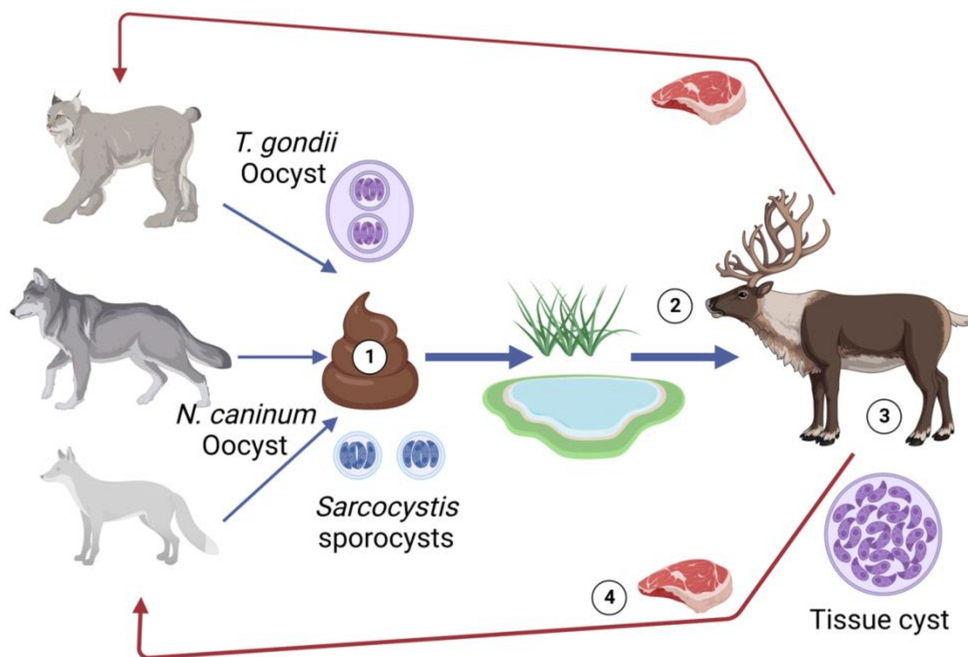

Figure 1 General life cycle of parasites in this study. 1, Microscopic parasites are shed in feces by carnivores 2, Herbivores, like caribou, get infected by ingesting food, water or soil contaminated with the parasites. 3, Microscopic parasites live in cysts in heart, muscle, and brain of the caribou, 4, and infect the carnivore when it eats the caribou.

We received blood, heart, brain, and muscle tissues from 88 caribou harvested between 2018 to 2022 (55 from Tasiujaq and 33 from Umiujaq).

For ***Toxoplasma***, we did not detect DNA in any of the caribou tissue samples. We found antibodies against the parasite in blood in 16 of 88 caribou (almost all from Tasiujaq), indicating that 18% of the animals were exposed at some point in their life to *T. gondii* (Fig. 2). Based on our findings, the risk of transmission to people consuming caribou in Nunavik is considered low, but not zero. Freezing solid for 3 days or thorough cooking inactivates the parasite.
For ***Neospora***, we detected DNA of the parasite in one heart sample. We found antibodies indicating exposure to the parasite in 2 (4%) of 55 caribou from Tasiujaq, and 2 (6%) of 33 samples from Umiujaq (Fig. 2). Further work is needed to understand the parasite life cycle and how it might affect caribou health. It does not transmit to people.

For ***Sarcocystis***, we found DNA of the parasite in 71 (82%) of 87 heart samples, and 38 (47%) of 81 muscle samples (Fig. 3). Species were similar to those reported in reindeer from Norway and Iceland. The high prevalence of *Sarcocystis* in tissue was not surprising, and is generally considered benign in caribou. It does not transmit to people.

Finally, although we were not actively looking for it, we also detected DNA of ***Besnoitia tarandus*** in 3 of 81 (4%) of muscle samples from caribou. This parasite can cause greenish jelly to accumulate under the skin of caribou and has been previously described in caribou in Nunavik. It does not transmit to people.

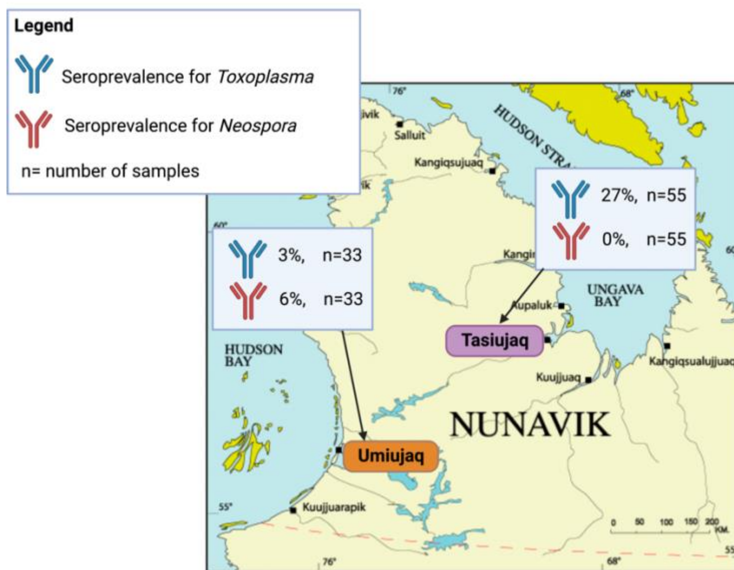

Figure 2 Prevalence for T. gondii and N. caninum in caribou blood samples in Nunavik.

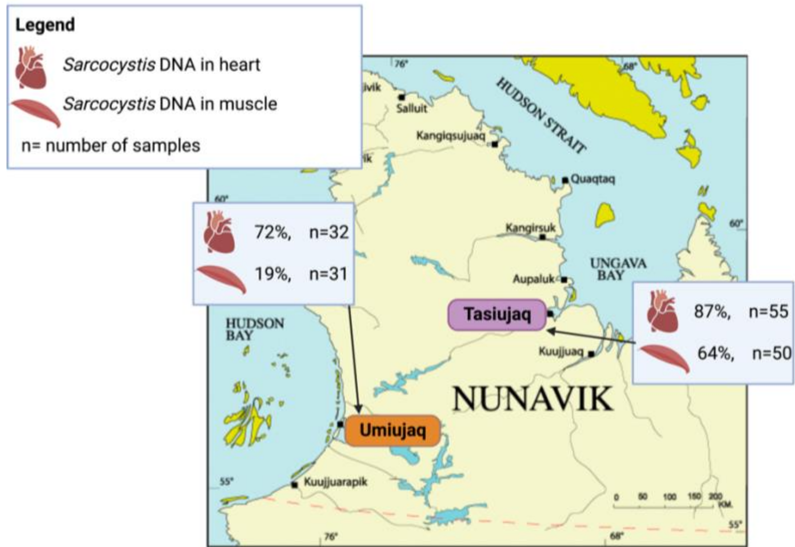

Figure 3 Sarcocystis prevalence in tissue in caribou in Nunavik.

APPENDIX C

ISR Beluga Health Assessment and Food-borne Parasite Testing, Update March 2021.

E. Jenkins, R. Sharma, and A. Hernandez-Ortiz (U of Saskatchewan); Lisa Loseto, Sonja Ostertag, and Shannon MacPhee (DFO); Batol Al-Adhami (CFIA)

Beluga whales (*Delphinapterus leucas*) are important wildlife hunted and harvested as country food by Inuit communities. Thus, they play an important role in alleviating food insecurity, which is disproportionately high in the Canadian North. The consumption of wildlife has many benefits but may also increase the risk of transmission of food borne zoonotic pathogens, for example, *Toxoplasma gondii*. People living in some regions of the Canadian North have much higher seroprevalence of *T. gondii* than the mainstream North American population, and exposure has been linked to consumption of wildlife. Recent reports of *Toxoplasma gondii* in Arctic marine mammals, including beluga in the ISR, have raised concerns that this parasite may affect the health of beluga, or people who harvest beluga.

A total of 112 beluga whales were sampled between 2015 and 2019, 81 whales harvested from Hendrickson Island and 31 from East Whitefish.

Year	**Hendrickson Island**	**East Whitefish**	**Total**
2014	15	13	28
2015	17	7	24
2016	10	6	16
2017	15	5	20
2018	9	0	9
2019	15	0	15
Total	81	31	112

Testing for *Toxoplasma gondii*.

We tested 96 serum samples (blood) using three different serological techniques: a commercially available ELISA kit, modified agglutination test (MAT) and immunofluorescent assay (IFA). With these tests we were looking for antibodies Vs *T. gondii*. The presence of antibodies indicates that the animal was exposed to the parasite. Because beluga sera have high level of lipids and this can interfere with the testing, we treated the samples using Chloroform to remove the lipids, after that, we re-tested the samples.

Antibodies were detected by ELISA and IFA in 1% of the samples (1/96). We obtained the same results after the Chloroform treatment. The sample tested positive for both techniques was from 2015 and identified as AREW-15-06.

We tested tissue with molecular techniques looking for the DNA of the parasite. The magnetic capture (MC) DNA technique we use is the new European Food Safety Authority gold standard for detecting *Toxoplasma* in meat and allows us to analyze 1000 times more meat per animal than previous methods. A total of 97 heart and 68 brain tissues were analyzed by MC technique, only one sample was positive to *T. gondii* DNA from a beluga heart from 2015 identified as ARHI-15-06.

***Trichinella* testing.**

Trichinella is roundworm food-borne parasite that has been reported in marine mammals like walruses. We use a technique that simulates the stomach digestion looking for the presence of the larvae in muscle tissue.

We did not detect *Trichinella* in the 108 tongue and 42 diaphragm tissues examined by the artificial digestion.

APPENDIX D

Beluga Bulletin Health, November 2022.

ISR Beluga Health Assessment and Food-borne Parasite Testing, Update October 2022.

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Beluga whales (*Delphinapterus leucas*) are important wildlife hunted and harvested as country food by Inuit communities. Thus, they play an important role in alleviating food insecurity, which is disproportionately high in the Canadian North. The consumption of wildlife has many benefits but may also increase the risk of transmission of food borne zoonotic pathogens, for example, *Toxoplasma gondii*. People living in some regions of the Canadian North (especially the eastern Arctic) have much higher exposure (as measured in blood) of *T. gondii* than the mainstream North American population, and exposure has been linked to consumption of wildlife. Reports of *Toxoplasma gondii* in Arctic marine mammals, including beluga in the ISR, have raised concerns that this parasite may affect the health of beluga, or people who harvest beluga. In partnership with the community of Tuktoyaktuk, Inuvik, FJMC and DFO samples were collected as part of the beluga health monitoring program.

A total of 147 beluga whales were sampled between 2014 and 2021, 116 whales harvested from Hendrickson Island and 31 from East Whitefish.

Year	Hendrickson Island	East Whitefish	Total
2014	15	13	28
2015	17	7	24
2016	10	6	16
2017	15	5	20
2018	9	0	9
2019	15	0	15
2020	16	0	16
2021	19	0	19
Total	116	31	147

The main objective was to get samples from harvested belugas for food-borne parasite testing (*Toxoplasma gondii* and *Trichinella*) to determine if these parasites were a risk for people consuming beluga. Because of the findings during microscopic examination of tissues from belugas in 2015, we added testing for *Sarcocystis*, a parasite similar to *Toxoplasma*, but not transmitted to humans.

- Diaphragms and tongues were examined for *Trichinella* larvae
- Whole blood was collected (and centrifuged for subsequent serum) testing for exposure to *Toxoplasma*.
- Hearts and brains were collected for *Toxoplasma* testing by molecular techniques looking for DNA of the parasite for *Toxoplasma* and *Sarcocystis*.

Results

For toxoplasma we used several tests to examine multiple and tissues and found extremely low evidence of presence. Specifically, from 107 serum (blood) samples, only one (AREW15-06) tested suspicious for *Toxoplasma* gondii. This indicates previous exposure to the parasite and not necessarily that the animal is actively infected. From 97 heart samples and 68 brain samples tested

with DNA only one heart sample (ARHI-DL-2015-06) was found to have *T. gondii*. No DNA of *T. gondii* was detected by conventional PCR. The detection of *T. gondii* DNA in tissue indicates that the parasite is present, but not necessarily that the parasite is infective for people. Based on our findings (only 2 beluga with evidence of infection of *T. gondii*) risk of transmission of *T. gondii* to people consuming beluga in the ISR is considered low, but not zero.

Trichinella was not detected in tongue and diaphragm muscles examined from belugas harvested in 2015-2019. The risk of transmission of this parasite to people is considered very low.

Note that we did not conduct a formal risk assessment from a public health perspective.

We detected DNA of *Sarcocystis* spp. in 42 heart samples and 25 muscle samples. The *Sarcocystis* DNA we found in these belugas are very close to DNA from *Sarcocystis* reported in fur seal and a sperm whale from other studies.

There are more than 100 species of *Sarcocystis* reported in different animals and humans. So far, very few species are transmitted to humans, and none that we know of in Canada. The normal cycle of the parasite involves a carnivore and an herbivore host. The host (usually a carnivore) sheds the parasite in the feces contaminating food and water sources consumed by an intermediate host (usually an herbivore). The cycle continues when a new definitive host consumes the intermediate host infected with the parasite (figure 1).

Usually, *Sarcocystis* infections in humans and animals do not show symptoms; but brain inflammation has been reported in marine mammals. We know very little of the life cycle of this parasite in marine environments. Our data suggest that belugas are commonly infected, probably by consuming the parasite shed in feces from marine predators, such as killer whales or polar bears.

It is not surprising to find high number of samples positive to *Sarcocystis* if we compared with other animal studies. There are no public health concerns that we are aware of.

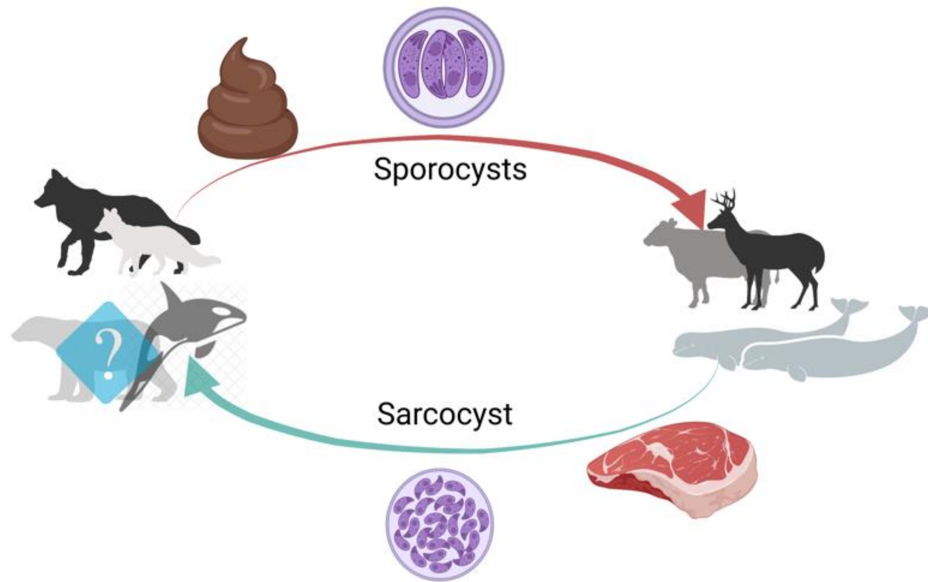

Figure 1 Life cycle of *Sarcocystis* spp. The Sporocysts are the stage of the parasite released in the feces of the definitive host. The sarcocyst is the stage of the parasite in the tissue of the intermediate host.