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Toxoplasma gondii and related Sarcocystidae parasites in harvested caribou from Nunavik, Canada

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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.

1. 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 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 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 et al., 2016). The Leaf River herd in

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Nunavik is classified as a migratory ecotype and exhibits long-term fluctuations in population numbers. Between 1975 and 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 et al., 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.

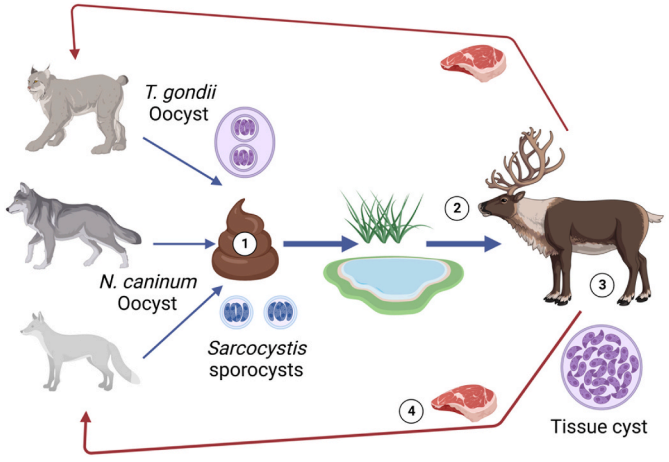

Fig. 1. General life cycle of Sarcocystidae parasites. 1, For *Toxoplasma gondii* and *Neospora caninum*, oocysts are shed in feces by definitive hosts (DH, felids and canids, respectively), sporulate and become infective in the environment, whereas for *Sarcocystis* spp., sporulation occurs in the intestine 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, 2007a). 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. Material and methods

2.1. 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).

2.2. 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 g each of heart, brain and skeletal muscle were collected by local hunters between 2018 and 2022, and stored at −20 °C 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. Serological tests

Heart fluid was collected from the storage bags containing the heart while thawing and centrifuged at 1200×*g* for 5 min; the supernatant was

subsequently transferred to a 1.5 mL Eppendorf tube and stored at −20 °C 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.

2.3.1. 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 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 suspect.

2.3.2. 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.

2.4. Molecular tests

2.4.1. 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 2.5×10^5/mL and 2.5×10^4 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.

2.4.2. 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 −20 °C 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 94 °C for 7 min, 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min, and a final extension of 72 °C 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 94 °C for 4 min, 40 cycles of 95 °C for 45 s, 61 °C for 1 min and 72 °C for 1 min, and a final extension of 72 °C 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′-ATTTCTCA-TAAGGTGCAGGAG-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 95 °C for 4 min, 40 cycles of 94 °C for 40 s, 59 °C for 30 s and 72 °C for 1min, and a final extension of 72 °C 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 et al., 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 et al., 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.

2.5. 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.

3. Results

3.1. Detection of antibodies against T. gondii and N. caninum

Heart fluid from 16 of 88 (18%; 95% CI: 11.5, 28. Fig. 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

Table 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

was no statistical difference between communities.

3.2. 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. 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.

3.3. 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 ($\chi^2 = 14$, df = 1, $p < 0.001$, n = 88). There was no statistical difference for *N. caninum* between serology and molecular ($\chi^2 = 1.3$, df = 1, $p = 0.248$, n = 88) and only a fair agreement ($\kappa = 0.39$).

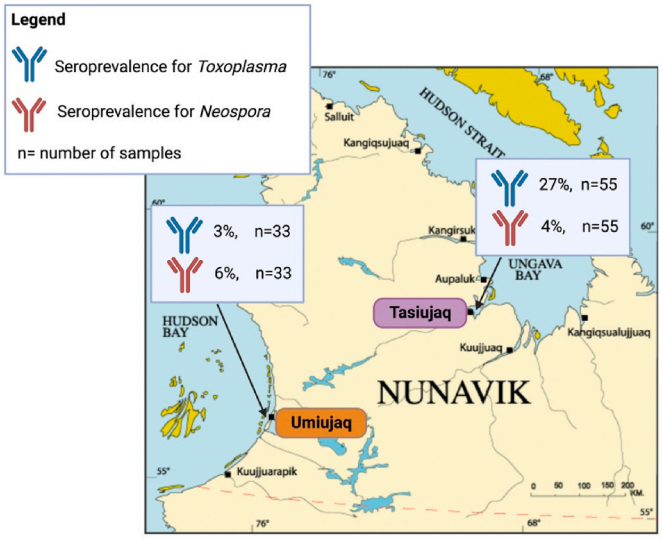

Fig. 2. Observed seroprevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in caribou harvested from 2 communities in Nunavik. Source: Nunavik Research Centre, Makivik Corporation.

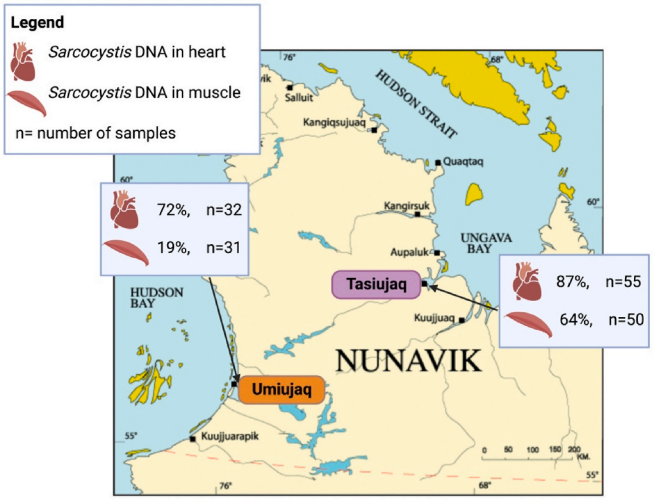

Fig. 3. Sarcocystis DNA prevalence in heart and muscle of caribou harvested by 2 communities in Nunavik, Québec, Canada.

3.4. 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 (Figs. 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 among 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 Figs. 4 and 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

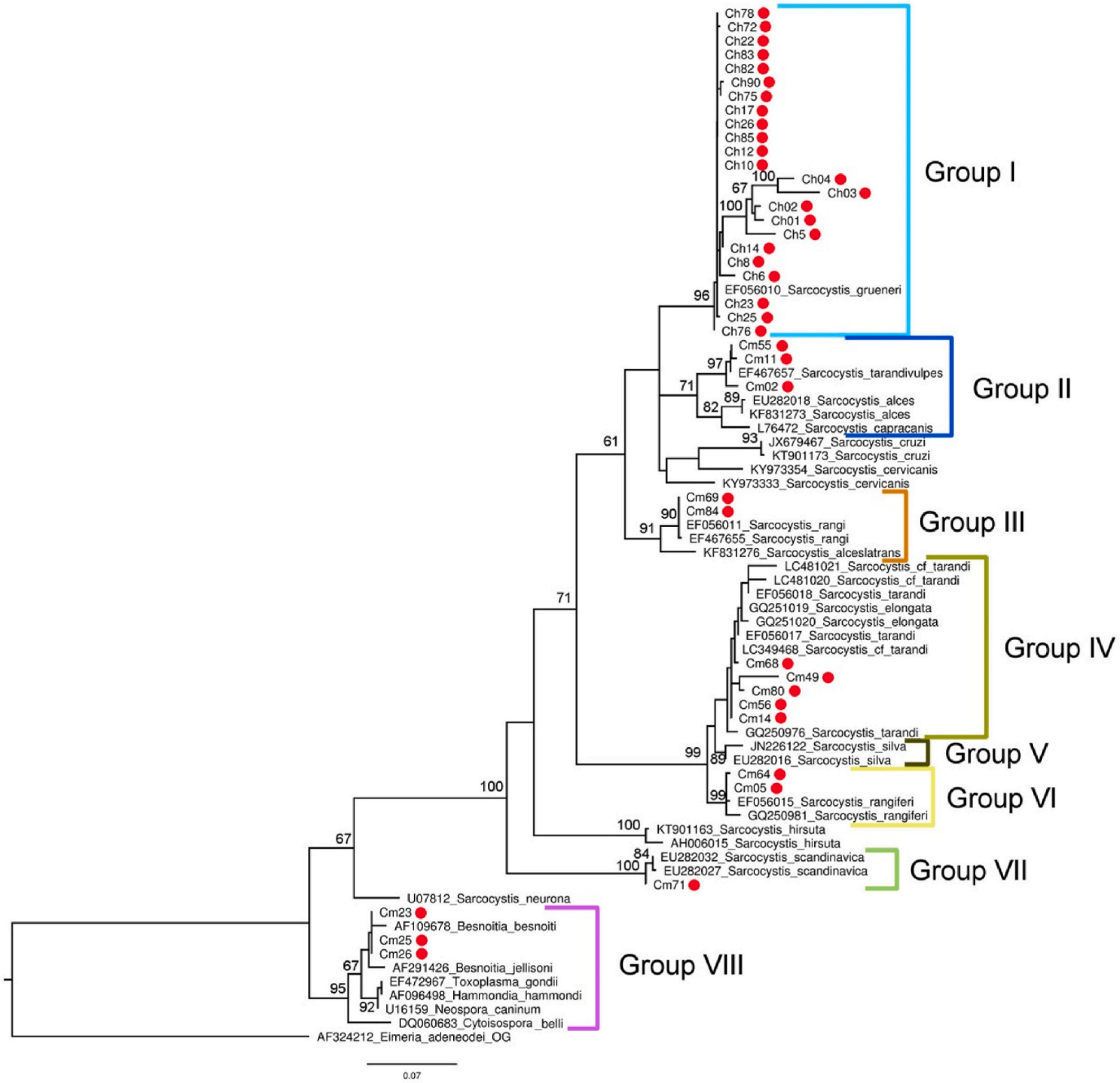

Fig. 4. Maximum likelihood topology for tissue dwelling coccidians (mostly *Sarcocystis* spp.) generated from 18S rDNA sequence data analyzed in RAxML 8 under the GTRCAT approximation. Group names bear no taxonomic designation but merely assigned for discussion purposes. Bootstrap values > 60 are displayed above branches as branch/node support. Red dots indicate sequences generated in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

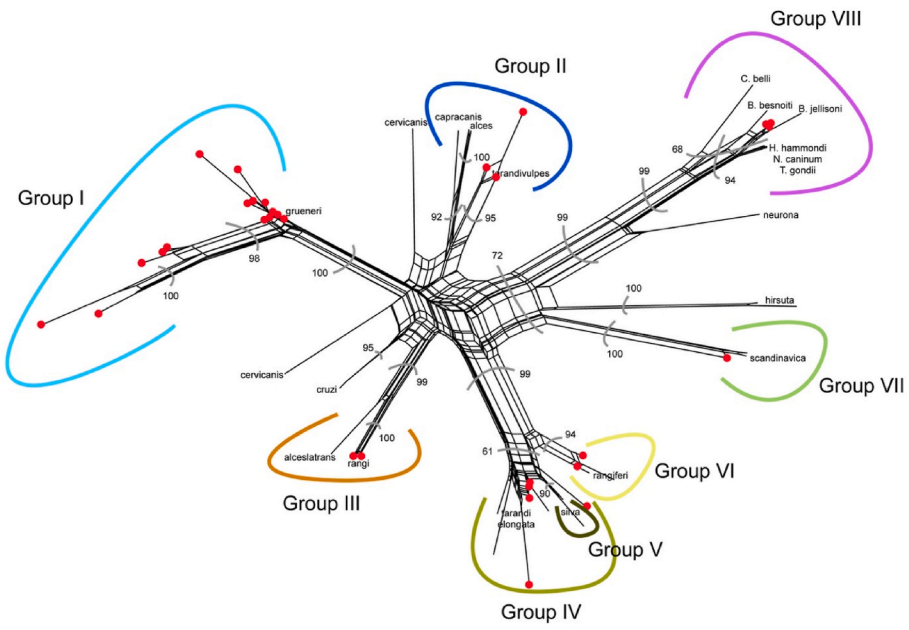

Fig. 5. A data-display network constructed from uncorrected 18S rDNA p-distances, using all characters, for tissue dwelling coccidians (mostly *Sarcocystis* spp.). Group names bear no taxonomic designation but merely assigned for discussion purposes. Bootstrap supports are displayed by the gray curves and associated values imposed on the network. Red dots indicate sequences generated in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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 excel file).

4. 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, 2007a). 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, 2007a; 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 et al., 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 et al., 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 et al., 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.

5. 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* spp. 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 *Rangifer* 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.06.008.

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