International Journal for Parasitology: Parasites and Wildlife 26 (2025) 101030

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Widespread geographic distribution of filarioid nematodes in caribou (*Rangifer tarandus* sspp.) in Canada

Aparna Mariyam Thomas [a], Guilherme G. Verocai [b], John Soghigian [a], Fabien Mavrot [a], Naima Jutha [c], Jan Adamczewski [c], Tracy Davison [d], Gwen Duytschaever [e], Arthur Fernandes [e], Allicia Kelly [c], Matthew R. Kulpa [b], Eve Lamontagne [f], Lisa-Marie Leclerc [g], Sara McCarthy [h], Ashley McLaren [i], Amanda D. Melin [e,j,k], Susan J. Kutz [a,*]

[a] *Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada*
[b] *Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, 77843, USA*
[c] *Department of Environment and Climate Change, Government of Northwest Territories, PO Box 1320, Yellowknife, NT, X1A 2L9, Canada*
[d] *Department of Environment and Climate Change, Government of Northwest Territories, PO Box 2749, Inuvik, NT X1E 0T0, Canada*
[e] *Department of Anthropology and Archaeology, University of Calgary, Calgary, Alberta, Canada*
[f] *Department of Environment and Climate Change, Government of Northwest Territories, PO Box 240, Fort Simpson, NT, X0E 0N0, Canada*
[g] *Department of Environment, Government of Nunavut, P.O. Box 377, Kugluktuk, NU, X0B 0E0, 11, Canada*
[h] *Department of Fisheries, Forestry and Agriculture, Wildlife Division, Government of Newfoundland and Labrador, P.O. Box 3014, Goose Bay, NL, A0P 1E0, Canada*
[i] *Department of Environment and Climate Change, Government of Northwest Territories, Highway 5, PO Box 900, Fort Smith, NT, X0E 0P0, Canada*
[j] *Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada*
[k] *Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada*

ARTICLE INFO

Keywords:
Droplet digital PCR
Molecular parasitology
Host-parasite interactions
Disease ecology
Cervidae
Nematode
Filarioidea
Onchocercidae
Vector-borne diseases
Simuliidae
Culicidae
Setaria yehi
Onchocerca cervipedis

ABSTRACT

The caribou (*Rangifer tarandus* sspp.) is a keystone wildlife species in northern ecosystems that plays a central role in the culture, spirituality and food security of Indigenous People. The Arctic is currently experiencing an unprecedented rate of climate change, including warming temperatures and altered patterns of precipitation. These environmental changes can facilitate the transmission of arthropod-borne parasites, such as filarioid nematodes.

Filarioids are an important cause of morbidity and occasional mortality in *Rangifer* in Fennoscandia, however, much of the ecology and epidemiology of these parasites in caribou in North America, including Canada, remains unknown. We aimed to determine the parasitic diversity and geographic distribution of filarioid nematodes in three Canadian designatable units (DU) of caribou (barren-ground, boreal and Dolphin & Union) from Northwest Territories, Nunavut and Newfoundland & Labrador. Genomic DNA extracted from 768 blood samples was screened for filarioid nematodes using real-time PCR. The positive samples were Sanger sequenced to identify the parasite present. Based on the sequencing results, we identified *Setaria yehi* and *Onchocerca cervipedis* s.l. We then standardized a TaqMan probe based duplex droplet digital PCR (ddPCR) protocol for the simultaneous detection of *S. yehi* and *O. cervipedis* s.l. Based on real-time PCR results, 8/768 samples were positive. *Setaria yehi* and *O. cervipedis* s.l. were present in 4 separate samples (0.5%) each. Using ddPCR, 68/192 samples were positive (35.4%). *Setaria yehi* DNA was detected in 57/192 positive samples (29.7%), *O. cervipedis* s.l. DNA was present in 22/192 samples (11.5%) and 11/192 samples (5.7%) had co-infections. *Setaria yehi* was detected in all three DUs tested. *Onchocerca cervipedis* s.l. were found in barren-ground and boreal caribou, but not from the Dolphin and Union caribou.

Through this broad-based survey and through developing and implementing advanced molecular methodologies, we have documented the apparent distribution and diversity of *S. yehi* and *O. cervipedis* s.l. in parts of three Canadian DUs of caribou. The knowledge gained from this study provides baseline data and methodology for the further elucidation of the epidemiology of these parasites in North America.

* Corresponding author. Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta. AB T2N 4Z6, Canada.
E-mail address: skutz@ucalgary.ca (S.J. Kutz).

https://doi.org/10.1016/j.ijppaw.2024.101030
Received 4 October 2024; Received in revised form 5 December 2024; Accepted 6 December 2024
Available online 7 December 2024

1. Introduction

Climate change, occurring at an unprecedented rate in the Arctic, is dramatically altering the habitat, flora and fauna of this ecosystem (Boggs, 2016; Pörtner et al., 2022) and driving the expansion and amplification of pathogens and emergence of disease in the Arctic and sub-arctic regions (Deksne et al., 2020; Haider et al., 2018b; Hoberg et al., 2008; Kafle et al., 2020; Kutz et al., 2009; Kutz et al., 2013; Laaksonen and Oksanen, 2007; Laaksonen et al., 2010). Vector-borne pathogens, including nematodes, are considered some of the most sensitive to climate change. Biting arthropods and the pathogens they vector are particularly sensitive to warmer temperatures which generally shorten the pathogen's incubation period, increase development and biting rates of their vectors and may prolong host exposure to biting insects (Benedict and Barboza, 2022; Caminade et al., 2019; Culler et al., 2015). A warming climate may thus increase the transmission of vector-borne diseases (Kutz et al., 2014; Laaksonen et al., 2010; Polley and Thompson, 2009) and exacerbate other insect harassment concerns for people and wildlife at high latitudes (Gillespie et al., 2020; Hoye, 2020; Koltz and Culler, 2021).

Caribou and reindeer (*Rangifer tarandus* sspp.) are keystone wildlife species across the Arctic and sub-arctic regions (Hummel and Ray, 2008). They play a central role in the lives of Indigenous people as a cultural icon, a source of income and provide food, and warm clothing (Hanke et al., 2024). As such, the health and well-being of the Indigenous People across the Arctic is directly linked to that of *Rangifer*. Caribou are highly sensitive to insect harassment, and the impacts of this harassment on energy expenditure, feeding rates and fitness are well-described (e.g., Benedict and Barboza, 2022; Johnson et al., 2022; Joly et al., 2020; Koltz et al., 2022; Raponi et al., 2018). In addition, these insects can transmit parasites to caribou resulting in a range of clinical and subclinical impacts (Kutz et al., 2012; Laaksonen and Oksanen, 2007).

Rangifer are host to a variety of vector-borne parasites, including several genera of filarioid nematodes (Spirurida: Filarioidea) (Kutz et al., 2012; Paulsen et al., 2017). Filarioids are elongate and slender, white, parasitic worms that live in the tissues or body cavities and can be highly pathogenic to humans as well as animals (Lefoulon et al., 2017; Paulsen et al., 2017). The filarioids reported in *Rangifer* across the Holarctic include *Setaria* spp. (Dieterich and Luick, 1971; Laaksonen et al., 2009a,b), *Onchocerca* spp. (Bylund et al., 1981; Verocai et al., 2012) and *Rumenfilaria andersoni* (Laaksonen et al., 2015). Filarioids have an indirect life cycle which involves a mammal definitive host, including cervids, and a hematophagous dipteran insect intermediate host, which are mosquitoes (Culicidae) for *Setaria* (Anderson, 2000; Laaksonen et al., 2009a,b) and black flies (Simuliidae) and biting midges (Ceratopogonidae) for *Onchocerca* (Anderson, 2000; Roe et al., 2023). Laaksonen et al. (2015) hypothesized hematophagous arthropods to be the vectors for *R. andersoni*, but the specific family, genus or species remain unknown. The definitive host becomes infected with a filarioid nematode when an infected insect intermediate host deposits the infective third-stage larvae (L3) in the sub-cutaneous tissues of the host during the blood meal. The larvae moult to the L4 stage, migrate to the predilection site and develop into adults. Microfilariae are released by adult female nematodes into the blood stream or surrounding host tissues and are ingested by another susceptible insect vector during a blood meal. Within the insect vector, the microfilariae develop to L3 and the cycle continues (Tryland and Kutz, 2018).

Setaria tundra emerged as a significant cause of disease in *Rangifer* in Finland with outbreaks occurring following two consecutive warm summers (Laaksonen et al., 2009a,b). The adults are commonly seen in the peritoneal cavity of ungulates, and the clinical symptoms may include mild to severe peritonitis with ascites, green fibrin deposits and adhesions on the liver (Tryland and Kutz, 2018). *Onchocerca cervipedis*, or the "leg worm", infect the distal extremities of cervids as adult worms, often from the tibio-tarsal joint to the hoof. In caribou periostitis, cellulitis and granulomatous lesions are reported; these syndromes may affect host mobility making them more susceptible to predation (Verocai et al., 2012). Although *O. cervipedis* was originally described as a single species, molecular analysis has shown there is at least four to five cryptic species that have yet to be formally described (Benedict et al., 2023; Kulpa et al., 2021; McFrederick et al., 2013; Verocai et al., 2018). *Rumenfilaria andersoni* are lymphatic-dwelling filarioid nematodes which cause chronic, persistent infection in their moose (*Alces alces*) (Lankester and Snider, 1982), white-tailed deer (*Odocoileus virginianus*) (Grunenwald et al., 2018) and reindeer (Laaksonen et al., 2015) definitive hosts. Although not considered highly pathogenic, the high eosinophilic count and microfilariae may affect the overall performance of the host (Laaksonen et al., 2015).

The literature on the diversity and distribution of filarioids in *Rangifer* in North America is limited. *Setaria yehi* was originally described by Desset in 1966 from deer (*O. hemionus* and *O. virginianus*) and caribou from the United States and Canada (Becklund and Walker, 1969). An early report of *Setaria* in *Rangifer* in North America preceded the description of *S. yehi* (Erickson and Highby, 1942). Dieterich and Luick (1971) reported *S. yehi* in a domestic reindeer in Alaska, and Fruetel and Lankester, 1989 reported it in boreal caribou from Ontario. *Onchocerca cervipedis* was first described in white-tailed deer and black-tailed deer (*O. hemionus*) from Montana and British Columbia, respectively, in 1935 (Wehr and Dikmans, 1935). The first presumed report of *Onchocerca* sp. in Northern Mountain caribou is from Tweedsmuir Provincial Park, British Columbia in 1976 (Low, 1976), with subsequent reports from Grant's caribou (Verocai et al., 2012) in Alaska. *Onchocerca* is widespread in Fennoscandian reindeer (Bylund et al., 1981). Several more recent studies in North America have described *O. cervipedis* as a species complex with cryptic species of *O. cervipedis* s.l. in white-tailed deer (McFrederick et al., 2013; Verocai et al., 2012), mule deer (*O. hemionus*) (Kulpa et al., 2024), blackflies (Kulpa et al., 2021; Verocai et al., 2018) and moose (Benedict et al., 2023; Verocai et al., 2012, 2024). Given these recent advances in the knowledge of *Onchocerca* diversity in caribou and other North American cervids, we refer to it as *O. cervipedis sensu lato* (s.l.).

To increase our understanding of vector-borne filarioid nematodes in caribou in Canada, we conducted a biospecimen survey using molecular techniques. Caribou are classified into 12 discrete and significant designatable units (DU) for conservation in Canada, with a DU defined as a unit that is spatially, ecologically, or genetically discrete and evolutionarily significant and an irreplaceable component of biodiversity (COSEWIC, 2011). Opportunistic samples were collected from three of these DUs including: barren-ground caribou, boreal caribou and the Dolphin & Union caribou from the Northwest Territories (NWT), Nunavut (NU) and Newfoundland & Labrador (NL) in Canada (Fig. 1). We expected to find all three filarioid genera *Setaria*, *Onchocerca*, and *Rumenfilaria*, in the sampled herds, and predicted that the three genera would be more common in caribou from lower latitudes than those from further north because of the temperature dependent transmission of these parasites. This work serves as a requisite first step in understanding the ecology, epidemiology and impacts of these pathogens in *Rangifer* in a rapidly changing Canadian Arctic.

2. Materials and methods

2.1. Sample acquisition and DNA extraction

We obtained 768 whole blood samples in K2-EDTA tubes from caribou collected during capture and collaring operations over the previous 15 years from the inventories of the Governments of the NWT (GNWT), Nunavut (GNU) and Newfoundland & Labrador (GNL). The samples were stored at −80 °C (GNWT), or at −20 °C (GN&GNL) until they were shipped to University of Calgary and then stored at −20 °C until processing. Blood samples were from 3 DUs, including: 465 from barren-ground caribou (Bathurst, Beverly, Bluenose East, Bluenose

West, Cape Bathurst and Tuktoyaktuk Peninsula herds in NWT &NU); 247 from boreal caribou (from Big Island, Pine Point-Buffalo Lake, Hay River Lowlands, Mackenzie, North Slave, North Dehcho and South Dehcho study areas in the NWT; Joir River, Lac Joseph, Mealy Mountain, Red Wine Mountain from NL); and 56 from the Dolphin & Union caribou from NU (Supplementary materials Table 1).

Blood tubes were gently inverted 3 times and then 200 µl whole blood was taken for DNA extraction. Genomic DNA was extracted and purified with QI Amp DNA Blood Mini Kit (Qiagen) using the manufacturer's protocol. The DNA was extracted and diluted in 200 µl kit elution buffer and then stored at −20 °C until further use.

As a broad screening approach to capture nematode worms of the family Onchocercidae, all samples were screened with real-time PCR using a general primer targeting a 450 base pairs (bp) fragment of the cytochrome oxidase c subunit 1 (CO1) region of mitochondrial DNA of filarioid nematodes (Lefoulon et al., 2012). Positive samples were sequenced using Sanger's sequencing to identify the parasite to the species. Using these results, we then designed a species-specific droplet digital PCR (ddPCR) assay for further investigation of a subsample of blood samples representing all the herds from three DUs.

2.2. Amplification using real-time PCR

We used a modified protocol from Kronefeld et al. (2014)'s SYBR Green Chemistry real-time PCR assay for the detection of filarioid parasites in the sample. We used published primers targeting the CO1 region of filarioid nematodes (Lefoulon et al., 2012) (Table 1). The 25 µl PCR reaction mixture contained 10 µl SYBR green Master mix (Sso-Advanced Universal), 9 µl molecular grade water (Corning), 5 µl DNA template and 1 µl (10 µM) primer mix (forward and reverse). The cycling conditions were 95 °C for 15 min for enzyme inactivation, 95 °C for 45 s, 58 °C for 30 s and 72 °C for 45 s; repeated for 35 cycles for amplification; and a final extension 72 °C for 45 s followed by a cooling at 4 °C. The amplification was done using a CFX96 thermocycler (Bio-Rad, Mississauga, Ontario, Canada). We used genomic DNA samples of *S. yehi* from an Alaskan moose obtained from the lab of G. Verocai at Texas A&M University as a positive control (Verocai et al., 2024) to standardize the real-time PCR assay along with the no-template control. We interpreted the results using melting curve and amplification curve analysis.

Table 1
Primers and probes used in the study.

Name	Target species	Sequence	T_m (°C)
COIintF	Filarioids	TGA TTG GTG GTT TTG GTA A	49.35
COIintR	Filarioids	ATA AGT ACG AGT ATC AAT ATC	43.97
Syehi-F	*S. yehi*	TGGTGGACCTGGTAGAAGT	55
Syehi-R	*S. yehi*	CCCTAACAAAGAACCAATACCAAC	54
Syehi-Probe	*S. yehi*	AAGGGTTGAAGGTCAGCCTGAGTT	60.4
ocervi5F_28-50	*O. cervipedis* s. l.	TTATTGGAGGTGGTCCTGGTAG	55.9
ocervi5R_129-154	*O. cervipedis* s. l.	CCAACAAAGAACCAATACCTACAGT	55.1
ocervi5P_83-107	*O. cervipedis* s. l.	TCCAAAGACAACTCCGGTTGACCC	61.1

*T_m: Melting temperature.

2.3. Sequencing and analysis

To determine species identity, the positive samples were amplified with conventional PCR using the protocol from Casiraghi et al. (2001) with the same primers (Table 1). The PCR products of the expected size (450 bp) obtained in the agarose gel were then gel-purified with a kit (E.Z.N.A. Cycle Pure Kit (V-spin), Nucleic acid purification kit) using the manufacturer's protocol. The purified products were sent directly for Sanger's sequencing to the UCDNA services at the University of Calgary. Sequences obtained were edited and aligned using Geneious Prime software (Geneious Prime, 2023.0.4) and the CO1 region was analysed and compared to the sequences available in NCBI by BLAST (Basic Local Alignment Search Tool) sequencing.

2.4. TaqMan probe-based duplex droplet digital PCR (ddPCR)

Based on the sequencing results, the parasites were identified as *S. yehi* and *O. cervipedis* s.l. We focused the ddPCR on these two species and designed primer and probe sets targeting the CO1 region of these parasites using Geneious Prime software (Geneious Prime, 2023.0.4) (Table 1). The *O. cervipedis* primers and probes were custom designed from the sequence data obtained from Sangers sequencing of the positive isolates in qPCR. The *S. yehi* and *O. cervipedis* probes were labelled with FAM and HEX dyes, respectively. The *S. yehi* primers and probes as well as the positive controls for both these parasites were obtained from Verocai et al. (2024). *Setaria yehi* DNA was extracted from adult female

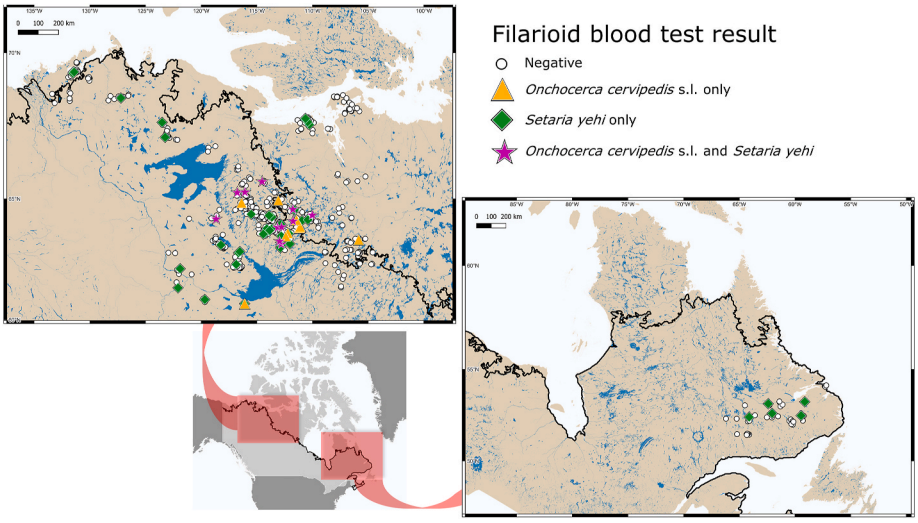

Fig. 1. Map showing the sites of samples collected and the distribution of *S. yehi* (SY) and *O. cervipedis* s.l. (OC) in caribou in Canada based on ddPCR of whole blood samples; circles represent negative samples; triangles represent *O. cervipedis* s.l.; diamonds represent *S. yehi*; stars represent individuals with co-infections. The black line is the latitudinal tree limit based on a percentage cover of less than 5% (derived from CCRS, 2020).

worms isolated from an Alaskan moose and *O. cervipedis* s.l. DNA was from microfilariae in the ear tissue of white-tailed deer.

The duplex ddPCR protocol was standardized by doing a temperature gradient and concentration gradient single plex ddPCR for the respective parasites first. Annealing temperature was finalised at 55 °C and the concentrations of positive control for *S. yehi* and *O. cervipedis* s.l. were fixed as 1/10,000 x and 1/10 x, respectively (x is the DNA concentration of the positive control; 3 ng/µl for both the positive controls). We then ran a duplex ddPCR combining primer and probe sets of both the parasites at the above-mentioned annealing temperature and concentration and finalised the protocol.

The 24 µl ddPCR reaction mixture contained 10 µl ddPCR supermix for probes (no dUTP) (Bio-Rad), 2.25 µl (10 µM) of each primer and 0.625 µl (5 µM) of each probe, 2 µl of DNA sample and 1.75 µl of molecular biology grade water (Corning). After mixing, 24 µl of the reaction mixture was transferred to the second column of a DG8 cartridge (Bio-Rad) and 70 µl of droplet generating oil for probes (Bio-Rad) was added to the first column. The last two wells of the cartridge were loaded with positive control and no-template control (molecular biology grade water). The loaded column was closed using DG8 Gasket (Bio-Rad) and then placed in a QX200 Droplet Generator (Bio-Rad) where 12,000–20,000 droplets were generated per experiment. The droplets generated in the third column of the DG8 cartridge were transferred to a 96 well semi-skirted ddPCR plate (Bio-Rad) and the plate was heat-sealed with a pierceable aluminium foil (Bio-Rad). The cycling conditions were 95 °C for 10 min for enzyme inactivation, followed by 40 cycles of a two-step thermal profile of 30 s at 94 °C for denaturation, 60 s at 55 °C with a 1°C ramp for annealing and extension and a final hold of 10 min at 98 °C for droplet stabilization followed by cooling at 12 °C. We used C100 Touch Thermal Cycler (Bio- Rad, US) machine for the amplification. After amplifications, the plate was read using QX200 Droplet Reader (Bio-Rad, USA). The droplets were read sequentially, and the results were read using Quanta soft program QX Manager Standard Edition (1.2.345). The droplets in the first and second channels of each well indicated the presence of *S. yehi* and *O. cervipedis* s.l., respectively. The positive droplets of the samples were compared with the positive control to finalise the results. The last well (no template control) contained negative droplets with no amplification.

We tested 192 of the original 768 blood samples by ddPCR (Fig. 2). These were initially selected based on: i) if they had been positive by conventional PCR (n = 8) and; ii) if they were suspect positive based on potential activity in the qPCR (n = 63). The suspect positive samples were those where a small but distinctive peak was present, but below the threshold line in our melt-curve analysis ($C_q > 29$, melt curve peak below threshold line). Such blips can be either due to low concentration of DNA or failure to replicate in the reaction. Finally, we tested an additional 121 samples spanning the diversity of our herds that initially tested negative with qPCR. Droplet digital PCR offers advantages in that it is highly sensitive to detect a lower concentration of target DNA (Taylor et al., 2017). The 121 negative samples were selected from different herds of each DU to get a wide geographic representation. For these, two negative samples were pooled (1 µl of each sample DNA was added to the reaction mixture) and screened first; if the pool tested positive then the samples were screened separately afterwards. Pooling was done to make the technique time and cost-effective.

3. Results

3.1. Real-time PCR results and sequence analysis

Eight of 768 samples, representing animals from the barren-ground and boreal caribou DUs, tested positive using real-time PCR. Sixty-three additional samples showed amplification after cycle number 29 ($C_q > 29$) with a melt curve peak below the threshold line (Fig. 3). The remaining samples showed no evidence of amplification. Of the positive samples, four were identified as *Setaria yehi* and four as *Onchocerca cervipedis* s.l. by BLAST sequencing and comparing the pairwise identity of the sequences in the GenBank (details of the top 5 hits are given in Supplementary materials Tables 2 and 3). All eight sequences generated were deposited in NCBI GenBank (Accession numbers: PQ282506-12, PQ318212)

3.2. Droplet digital PCR results

Setaria yehi was detected by ddPCR from all the three DUs in the NWT, Nunavut and Newfoundland & Labrador, Canada; *O. cervipedis* s.l. was detected in barren-ground and boreal caribou but not the Dolphin & Union caribou (Figs. 1 and 4). *Setaria yehi* was the most common with 29.7% (57/192) sample prevalence; *O. cervipedis* s.l. was present in 11.5% (22/192) samples including co-infections in 5.7% (11/192) samples (Fig. 1). *Setaria yehi* was detected in samples from all herds of barren-ground caribou, 9 of 12 boreal caribou areas and from the Dolphin & Union caribou (Appendix Table 1; Fig. 1). Of 118 barren-ground caribou samples tested, 34 (28.8%) were positive for *S. yehi* (18 from Bathurst herd, four from Bluenose East, three each from Ahiak and Bluenose West, two each from Tuk Peninsula and animals without assigned herds and one each from Beverly and Cape Bathurst); 21 samples (8.5%) were positive for *O. cervipedis* s.l. (eight from Bathurst, five from Bluenose East and four each from Ahiak and unassigned animals), including 11 samples (9.3%) with co-infections, four each from Bathurst and Bluenose East, two from Ahiak and one from an unassigned animal. Of 62 boreal samples tested, 19 (30.6%) were positive for *S. yehi*: five from Labrador (two each from Mealy Mountain and Red Wine Mountain and one from Lac Joseph), four each from Hay River Lowlands and North Slave, two each from South Dehcho and Pine Point-Buffalo Lake and one each from Mackenzie and North Dehcho, NWT; *Onchocerca cervipedis* s.l. was detected in only one sample (1.6%), from Pine Point-Buffalo Lake in NWT and no co-infections were found (Fig. 5). Of 12 Dolphin & Union samples tested, four (33.3%) were positive for *S. yehi*. No *O. cervipedis* s.l. were found in Dolphin & Union caribou or boreal caribou from Newfoundland and Labrador (Table 2).

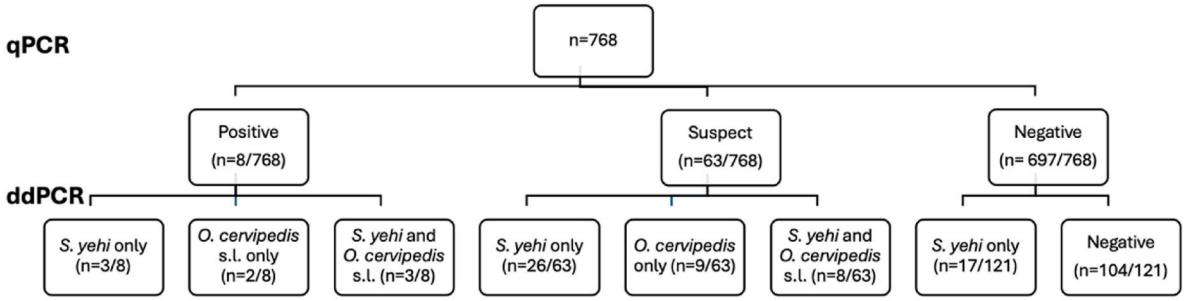

Fig. 2. Flowchart showing the number of samples screened using real-time PCR & ddPCR, and the results.

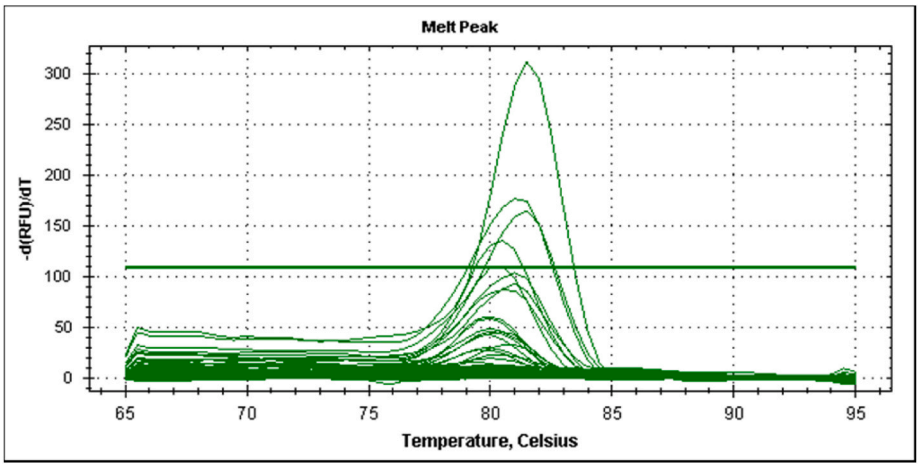

Fig. 3. A real-time PCR melt curve showing positive samples and suspect samples (peaks below threshold line). The x-axis indicates the temperature; y-axis indicates the fluorescence; the bold green line at 110 fluorescence indicates the threshold line; the peaks above the threshold line are positive; the peaks below the threshold line are suspected positive, the lines parallel to x-axis are negative.

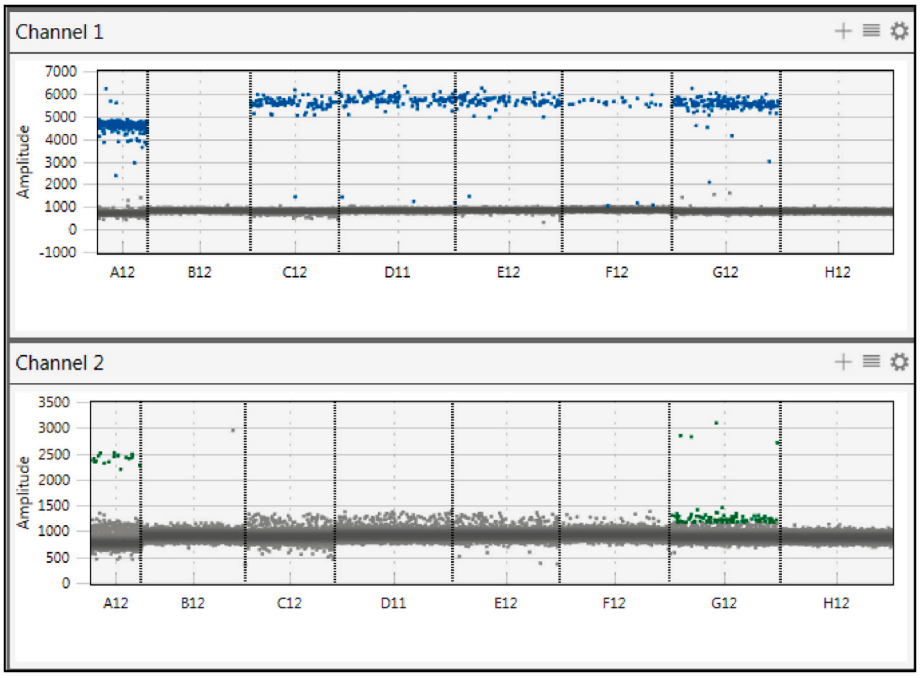

Fig. 4. A duplex droplet digital PCR plot showing positive droplets: *S. yehi* (blue), *O. cervipedis* s.l. (green) and negative droplets (black). Channels A12 to F12 represent individual samples; G12 is the positive control; H12 is the negative control.

4. Discussion

Through the use of sensitive molecular methodologies and convenience sampling, this first broad-scale biodiversity survey for filarioid nematodes in caribou expands our understanding of the diversity and distribution of filarioids in caribou in Canada. The opportunistic nature of our study design, based on convenience samples collected over multiple years and a vast geographic range, did not allow us to evaluate spatial or temporal trends. Nevertheless, we did detect presence of both the *O. cervipedis* and *S. yehi* in the majority of herds tested.

We confirmed the species of *Setaria* present in caribou in Canada as *S. yehi,* different from *S. tundra,* reported from reindeer in Finland (Laaksonen et al., 2009a,b; Oloś et al., 2019). *Setaria yehi*, Desset 1966 was first described from mule and white-tailed deer and subsequently reported in caribou and moose in North America (Becklund and Walker, 1969; Schurr and Rabalais, 1983; Walker and Becklund, 1970; Weinmann et al., 1973b; Weinmann and Shoho, 1975; Yeh, 1959). Dieterich and Luick (1971) reported *S. yehi* in a domestic reindeer in Alaska, and there are unpublished reports of *Setaria* sp. from barren-ground caribou in NWT by hunters in 1990s (Pers. Comm Brett Elkin). We detected *S. yehi* in all three DUs of caribou that we tested in the NWT, Nunavut and Labrador, Canada, indicating wide distribution of this parasite. Our findings, however, do not preclude that *S. tundra* is present in North America. *Setaria tundra* and *S. yehi* are morphologically very similar (Becklund and Walker, 1969). Both species have been reported from multiple cervid host species in the sub-arctic zone (Laaksonen et al., 2009a,b, Dieterich and Luick, 1971) and multiple reindeer introductions from Fennoscandia and Russia in the late 1800s/early 1900s could have introduced *S. tundra* to Alaska and/or Newfoundland (Truede, 2009).

We detected the legworm, *O. cervipedis* s.l., from a more restricted geographic distribution in two DUs, barren-ground and NWT boreal

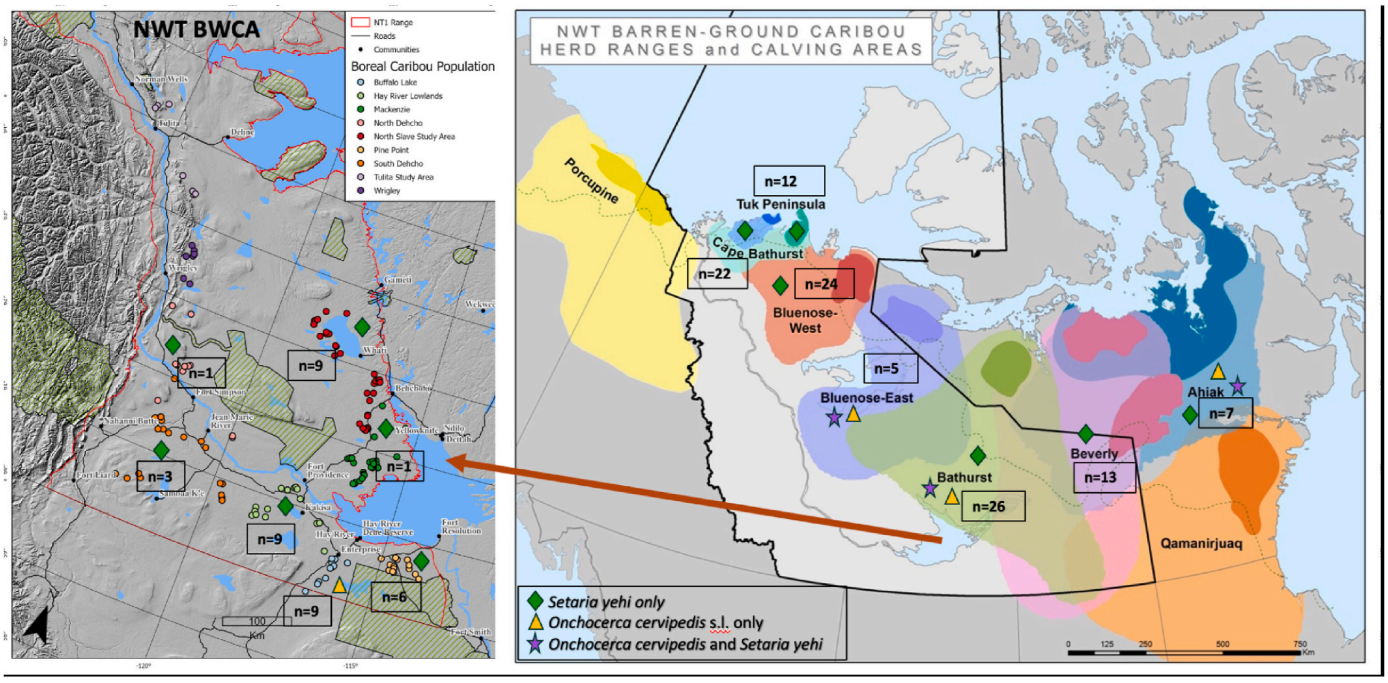

Fig. 5. Distribution of *S. yehi* and *O. cervipedis* s.l. in (a) Boreal (study location wise) and (b) Barren-ground Caribou (herd wise) [triangle indicates *S. yehi*; star indicates *O. cervipedis* s.l.; square indicates co-infection; 'n' indicates the number of samples from each herd/location tested using ddPCR] (Picture credits for base maps: (a) Nick Wilson, Environment and Climate Change, GNWT; (b) Environment and Climate Change, GNWT).

caribou, but did not detect it from the contiguous barren-ground herds in the northwestern Canadian Arctic: Cape Bathurst (n = 22), Bluenose West (n = 24) and Tuktoyaktuk Peninsula (n = 12). The apparent absence of this parasite from these western herds may be linked to a small sample size or there may be other ecological drivers limiting its distribution.

Onchocerca cervipedis has been reported in caribou, moose (Verocai et al., 2012) elk and white-tailed and mule deer (Wehr and Dikmans, 1935) from North America and is considered a cryptic species complex (Benedict et al., 2023; Kulpa et al., 2021; McFrederick et al., 2013; Verocai et al., 2018). Kulpa et al. (2024) reported that there are at least four and most likely five distinct species comprise this species complex so far. The *O. cervipedis* s.l. isolated from caribou in the current study was not included in these analyses, however, preliminary data suggest that this is closely related to the New York specimens in white-tailed deer (McFrederick et al., 2013).

The detection of *S. yehi* in the Dolphin and Union Caribou herd was unexpected. These caribou, the most northerly tested, summer on Victoria Island, Nunavut above 70° N latitude. The presence of *S. yehi* in the Dolphin and Union caribou indicates that these mosquito-borne parasites are likely being transmitted during the short summer on Victoria Island. While the vector mosquito species for *S. yehi* is unknown, *Aedes* spp. are known vectors for the related *S. tundra* in Finland (Kronefeld et al., 2014). A recent report identified *Aedes* spp. (*Ae. impiger, Ae. nigripes* and *Ae. hexodontus)* mosquitoes near Cambridge Bay on Victoria Island (Villeneuve et al., 2021). The vector potential of these species for *S. yehi* is unknown. We did not detect *O. cervipedis* in the Dolphin and Union caribou. This absence may be real or may reflect lower sensitivity of our sample type (blood) for this parasite (see below) and/or the low sample size.

Overall, we detected a higher occurrence of *S. yehi* (57/192 samples) than *O. cervipedis* s.l. (22/192 samples) in caribou. There are several possible explanations for this difference. Although these vector-borne filarioid nematodes have similar life cycles, *S. yehi* microfilariae circulate in the blood stream, whereas microfilariae of *O. cervipedis* are distributed primarily in the dermis near where they are deposited by the adult female worm (Anderson, 2000; Tryland and Kutz, 2018). However, microfilariae of other *Onchocerca* species have been detected from blood. *Onchocerca volvulus* microfilariae were detected in human blood in Cameroon (Fuglsang and Anderson, 1974) and a study in Italy reported the incidental detection of *Onchocerca* microfilariae in donkey blood (Papini et al., 2020). Thus, while blood is not the predilection site for *O. cervipedis,* our work illustrates that this parasite can be detected through analyses of blood, though the sensitivity of this technique is not known. The contamination of blood samples with dermal microfilariae, while possible, is a less likely explanation for detecting the parasite in blood.

Another reason for the higher occurrence of *S. yehi* may be the non-specificity for vectors. Paulsen et al. (2017) suggested that *S. tundra* is not a highly vector specific parasite, which may enhance its ability for geographic range expansion. The known vectors of *S. tundra* in Europe are the mosquitoes *Aedes vexans, Ae. geminus* and the *Culex pipiens* complex (Czajka et al., 2012; Kronefeld et al., 2014). Among these, *Ae. vexans* and *C. pipiens* are prevalent in North America (Cansado-Utrilla et al., 2020; Hongoh et al., 2012; Outammassine et al., 2022). Tran et al. (2022) detected *S. yehi* in *Ae. sierrensis, Anopheles franciscanus*, *An. freeborni* and *Cx. stigmatosoma* from northwest California. In 1979, the above-mentioned mosquitoes were not identified in NWT, Nunavut or Newfoundland & Labrador (Wood et al., 1979). However, a recent report identified *Aedes* spp. mosquitoes from the Canadian Arctic and Subarctic, including Yellowknife, NWT and Cambridge Bay, Victoria Island, Nunavut (Villeneuve et al., 2021), the predominant species being *Ae. impiger, Ae. nigripes* and *Ae. hexodontus*. The vector potential of these mosquitoes for *S. yehi* remains unknown.

The vectors of *O. cervipedis* s.l. reported in North America are black flies *Prosimulium impostor* (Weinmann et al., 1973b), *Simulium decorum, S. venustum* (Pledger, 1978), *S. tescorum*, *S. vittatum* and *S. clarum* (Kulpa et al., 2021; Verocai et al., 2018). However, previous reports of *O. cervipedis* in black flies in North America without molecular characterization should be carefully interpreted due to the mounting evidence of cryptic diversity within this species complex (Benedict et al., 2023; Kulpa et al., 2021; McFrederick et al., 2013; Verocai et al., 2012, 2018). A study done by Currie and Adler. (2000) identified four genera and 29 species of black flies from the Horton River valley in NWT and Nunavut,

Table 2
Distribution of the DU/Herd, year of sample collection and results after screening using real-time PCR and ddPCR (%).

Caribou DU/Herd/Location	CollectionYear	qPCR n	qPCR resultsqPCR results			ddPCR n	ddPCR results		
			S. yehi	*O. cervipedis*	Suspect		*S. yehi* only	*O. cervipedis* only	Co-infections
BGCA									
Ahiak	2012–2020	94	0	0	5	7	1	2	2
Bathurst	2011–2022	119	2	2	21	26	14	4	4
Beverly	2017–2022	44	0	0	1	13	1	0	0
Bathurst/Beverly	2022	6	0	0	0	2	0	0	0
Bluenose East	2011–2022	116	0	1	4	5	0	1	4
Bluenose West	2021	24	0	0	0	24	3	0	0
Cape Bathurst	2021	22	0	0	3	22	1	0	0
Tuk Peninsula	2021	12	0	0	0	12	2	0	0
Unassigned	2014–2022	28	0	1	5	7	1	3	1
Overall BGCA		**465**	**2 (0.4)**	**4 (0.9)**	**39 (8.4)**	**118**	**23 (19.5)**	**10 (8.5)**	**11 (9.3)**
BCA_NWT									
Big Island	2021	2	0	0	0	2	0	0	0
Pine Point-Buffalo Lake	2018–2022	47	0	0	2	9	2	1	0
Hay River Lowlands	2018–2021	49	0	0	9	9	4	0	0
Mackenzie	2018–2021	26	0	0	1	1	1	0	0
North Slave	2017–2022	63	2	0	4	9	4	0	0
North Dehcho	2017–2022	8	0	0	1	1	1	0	0
South Dehcho	2022	7	0	0	3	3	2	0	0
Overall BWCA_NWT		**202**	**2 (0.9)**	**0 (0.0)**	**20 (9.9)**	**34**	**14 (41.2)**	**1 (2.9)**	**0 (0.0)**
BCA_Labrador									
Joir River	2005–2009	7	0	0	0	5	0	0	0
Lac Joseph	2008–2009	15	0	0	3	6	1	0	0
Mealy Mountain	2008	9	0	0	0	7	2	0	0
Red Wine Mountain	2008–2009	14	0	0	0	10	2	0	0
Overall BWCA_Labrador		**45**	**0 (0.0)**	**0 (0.0)**	**3 (6.7)**	**28**	**5 (17.9)**	**0 (0.0)**	**0 (0.0)**
D&U									
D&U	2016–2021	**56**	**0 (0.0)**	**0 (0.0)**	**1 (1.8)**	**12**	**4 (33.3)**	**0 (0.0)**	**0 (0.0)**$_{cervipe}$
Total		**768**	**4 (0.5)**	**4 (0.5)**	**63(8.2)**	**192**	**46 (23.4)**	**11 (5.7)**	**11 (5.7)**$_{dis\ s.l}$

*DU: Designatable Unit; *D&U: Dolphin and Union Caribou *BGCA: Barren-ground Caribou; *BCA: Boreal Caribou; *NWT: Northwest Territories; SY: *Setaria yehi*; OC: *Onchocerca cervipedis* s.l.

including *S. venustum*, a potential vector for *Onchocerca*. Some recent reports identified black flies from the Canadian Arctic and subarctic areas including Yellowknife, NWT (Villeneuve et al., 2021) and Nunavut (Franke et al., 2016; Lamarre et al., 2018). The presence of a rich fauna of potential vectors in higher latitudes of Canada suggests that *Onchocerca* can be propagated and transmitted in these locations (Adler and Crosskey, 2024; Olejníček, 2005).

Our results clearly demonstrated that droplet digital PCR is far more sensitive approach than real-time PCR and is a powerful tool for the detection and quantification of parasites (Pomari et al., 2019; Wichianchot et al., 2022). For example, we found parasite DNA using ddPCR in 60 samples that tested either negative or suspected using qPCR, including 11 samples with co-infections (see Table 2 for detailed results). The results are consistent with Taylor et al.'s (2017) findings, who concluded that ddPCR technology is more accurate for samples with low levels of nucleic acid ($C_q > 29$) compared to real-time PCR. A limitation of this approach, however, is that it is specific to the species that we looked for and it could not reveal potential for a broader diversity of filarioid nematodes in the samples. Out of the 63 suspected-positive samples from real-time PCR, 43 samples were positive for either *S. yehi* or *O. cervipedis* s.l. with ddPCR and 20 were negative. DdPCR could not inform on what was present in the other 20 samples. *Rangifer* are host to other filarioid nematodes such as *O. tarsicola, R. andersoni and S. tundra,* (Paulsen et al., 2017*)*. In future, the suspected positive samples could be shot-gun sequenced (e.g., illumina massive parallel sequencing) or alternatively, other ddPCR panels specific to each filarioid known to infect *Rangifer* could be developed.

Our sampling strategy, which was an opportunistic sample design using archived samples collected primarily during capture and collaring operations that occur during late winter, was not optimal and likely underestimates the abundance and distribution of these parasites. The sample size was non-uniform in time, geography and demographics and the sampling season was not ideal for detecting these vector-borne parasites. *Setaria* have long prepatent period of four to six months (Laaksonen et al., 2008), and while the prepatent period of *O. cervipedis* s.l. is unknown; *O. volvulus* has a prepatent period of 10–20 months (Anderson, 2000). This means that animals infected late the previous summer and sampled in March may have few, or no circulating microfilariae or microfilaridermia at this time. Rather, the peak microfilaremia is likely to occur during the summer when suitable vectors for transmission are present. Ideally, blood samples would be collected during the summer, when the likelihood of detecting microfilaremia is highest. Even though summer is the preferred time of sample collection, getting blood samples from caribou during summer can be challenging. Both the summer heat and the fact that caribou calve in May or June increase the risk to caribou at this time of year and the benefits of sampling during this time would not outweigh the risks. The time of day for sampling may also be relevant if there is a diurnal pattern to the microfilaremia (Laaksonen et al., 2008). Nevertheless, despite these sampling limitations, we detected the parasites in blood in the second screening using ddPCR.

5. Conclusion

Filarioid nematodes are important disease-causing agents in wild and semi-domesticated *Rangifer* (Kutz et al., 2012; Verocai et al., 2012). Climate-linked disease outbreaks in Finland have illustrated how these parasites can have devastating consequences for semi-domestic reindeer and the related food industry (Laaksonen and Oksonen, 2007; Paulsen et al., 2017). Under the current climate change scenarios, it is likely that vectors, and these vector-borne parasites, will increase in abundance and distribution in the North American Arctic (Koltz and Culler, 2021), with consequences for caribou conservation, but also for food security and quality for Indigenous people who depend on caribou (Hanke et al., 2021). The ddPCR developed in this study allows for rapid and sensitive detection, even in sub-optimal samples and can contribute to supporting northern communities, food security and wildlife conservation initiatives.

CRediT authorship contribution statement

Aparna Mariyam Thomas: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Guilherme G. Verocai:** Writing – review & editing, Methodology, Investigation. **John Soghigian:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Fabien Mavrot:** Writing – review & editing, Visualization. **Naima Jutha:** Writing – review & editing, Resources, Methodology. **Jan Adamczewski:** Writing – review & editing, Resources, Methodology. **Tracy Davison:** Resources, Methodology. **Gwen Duytschaever:** Methodology. **Arthur Fernandes:** Methodology. **Allicia Kelly:** Writing – review & editing, Resources, Methodology. **Matthew R. Kulpa:** Writing – review & editing, Methodology. **Eve Lamontagne:** Methodology. **Lisa-Marie Leclerc:** Writing – review & editing, Resources, Methodology. **Sara McCarthy:** Writing – review & editing, Resources, Methodology. **Ashley McLaren:** Methodology. **Amanda D. Melin:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Susan J. Kutz:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements AND FUNDING

This study was funded by Polar Knowledge Canada; NSERC Discovery (SK and ADM) and NSERC Northern Supplement (SK) and the Canada Research Chairs program (SK and ADM). The authors are thankful to Judy Williams (GNWT), Ty Pan, Angie Schneider, James Wang, Ben Padilla (UofC). We also acknowledge those who collected the samples for the study from NWT, NU and NL. We acknowledge that efforts to collect samples contributing to this work took place on the traditional territories and with the support of the Indigenous peoples who have lived with and depended on these caribou for generations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.101030.

References

Adler, P.H., Crosskey, R.W., 2024. World Blackflies (Diptera: Simuliidae): a Comprehensive Revision of the Taxonomic and Geographical Inventory [2024]. Clemson University.

Anderson, R.C., 2000. Nematode Parasites of Vertebrates: Their Development and Transmission, second ed. CABI Publishing, Wallingford.

Becklund, W.W., Walker, M.L., 1969. Taxonomy, hosts, and geographic distribution of the *Setaria* (nematoda: Filarioidea) in the United States and Canada. J. Parasitol. 55 (2), 359–368. https://doi.org/10.2307/3277411.

Benedict, B.M., Barboza, P.S., 2022. Adverse effects of Diptera flies on northern ungulates: *Rangifer, Alces*, and Bison. Mamm Rev. 52 (3), 425–437. https://doi.org/10.1111/mam.12287.

Benedict, B.M., Barboza, P.S., Crouse, J.A., Groch, K.R., Kulpa, M.R., Thompson, D.P., Wiener, D.J., 2023. Sores of boreal moose reveal a previously unknown genetic lineage of parasitic nematode within the genus *Onchocerca*. PLoS One 18 (1), e0278886. https://doi.org/10.1371/journal.pone.0278886.

Boggs, C.L., 2016. The fingerprints of global climate change on insect populations. Curr. Opin. Insect Sci. 17, 69–73. https://doi.org/10.1016/j.cois.2016.07.004.

Bylund, G., Fagerholm, H.P., Krogell, C., Nikander, S., 1981. Studies on *Onchocerca tarsicola* bain and schulz-key, 1974 in reindeer (*Rangifer tarandus*) in northern Finland. J. Helminthol. 55 (1), 13–20. https://doi.org/10.1017/S0022149X00025402.

Caminade, C., McIntyre, K.M., Jones, A.E., 2019. Impact of recent and future climate change on vector-borne diseases. Ann. N. Y. Acad. Sci. 1436 (1), 157–173. https://doi.org/10.1111/nyas.13950.

Canada Centre for Remote Sensing (CCRS), 2020. 2020 Land Cover of Canada. Geodata. https://open.canada.ca/data/en/dataset/ee1580ab-a23d-4f86-a09b-79763677eb47.

Cansado-Utrilla, C., Jeffries, C.L., Kristan, M., Brugman, V.A., Heard, P., Camara, G., Walker, T., 2020. An assessment of adult mosquito collection techniques for studying species abundance and diversity in Maferinyah, Guinea. Parasites Vectors 13 (1). https://doi.org/10.1186/s13071-020-04023-3, 150-150.

Casiraghi, M., Anderson, T.J.C., Bandi, C., Bazzocchi, C., Genchi, C., 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of Wolbachia endosymbionts. Parasitology 122 (1), 93–103. https://doi.org/10.1017/S0031182000007149.

Cosewic, 2011. Designatable units for caribou (*Rangifer tarandus*) in Canada. In: Committee on the Status of Endangered Wildlife in Canada, vol. 88.

Culler, L.E., Ayres, M.P., Virginia, R.A., 2015. In a warmer arctic, mosquitoes avoid increased mortality from predators by growing faster. Proc. Biol. Sci. 282 (1815). https://doi.org/10.1098/rspb.2015.1549, 20151549-20151549.

Currie, D.C., Adler, P.H., 2000. Update on a survey of the black flies (Diptera: Simuliidae) from the Northwest territories and Nunavut project. Arctic Insect News 11, 6–9.

Czajka, C., Becker, N., Poppert, S., Jöst, H., Schmidt-Chanasit, J., Krüger, A., 2012. Molecular detection of *Setaria tundra* (Nematoda: Filarioidea) and an unidentified filarial species in mosquitoes in Germany. Parasites Vectors 5 (1). https://doi.org/10.1186/1756-3305-5-14, 14-14.

Deksne, G., Davidson, R.K., Buchmann, K., Kärssin, A., Kirjušina, M., Gavarāne, I., Jokelainen, P., 2020. Parasites in the changing world – ten timely examples from the Nordic-Baltic region. Parasite Epidemiol. Control 10, e00150. https://doi.org/10.1016/j.parepi.2020.e00150.

Dieterich, R.A., Luick, J.R., 1971. The occurrence of *Setaria* in reindeer. J. Wildl. Dis. 7 (4), 242–245. https://doi.org/10.7589/0090-3558-7.4.242.

Environment and Climate Change, 2023. Map downloaded. https://www.gov.nt.ca/ecc/en/services/barren-ground-caribou.

Erickson, A.B., Highby, P.R., 1942. Parasites of the woodland caribou. J. Parasitol. 28 (5). https://doi.org/10.2307/3272989, 423-423.

Franke, A., Lamarre, V., Hedlin, E., 2016. Rapid nestling mortality in arctic peregrine falcons due to the biting effects of black flies. Arctic 69 (3), 281–285. https://doi.org/10.14430/arctic4580.

Fruetel, M., Lankester, M.W., 1989. Gastrointestinal helminths of woodland and barren ground caribou (*Rangifer tarandus*) in Canada, with keys to species. Can. J. Zool. 67 (9), 2253–2269. https://doi.org/10.1139/z89-318.

Fuglsang, H., Anderson, J., 1974. Microfilariae of *Onchocerca volvulus* in blood and urine before, during, and after treatment with diethylcarbamazine. J. Helminthol. 48 (2), 93–97. https://doi.org/10.1017/S0022149X00022653.

Gillespie, M.A.K., Alfredsson, M., Barrio, I.C., Bowden, J.J., Convey, P., Culler, L.E., Høye, T.T., 2020. Status and trends of terrestrial arthropod abundance and diversity in the North Atlantic region of the Arctic. Ambio 49 (3), 718–731. https://doi.org/10.1007/s13280-019-01162-5.

Grunenwald, C.M., Butler, E., Wünschmann, A., Armien, A.G., Carstensen, M., Hildebrand, E., Gerhold, R.W., 2018. Emergence of the arterial worm *Elaeophora schneideri* in moose (*Alces alces*) and tabanid fly vectors in northeastern Minnesota, USA. Parasites Vectors 11 (1). https://doi.org/10.1186/s13071-018-3077-0, 507-507.

Haider, N., Laaksonen, S., Kjær, L.J., Oksanen, A., Bødker, R., 2018. The annual, temporal and spatial pattern of *Setaria tundra* outbreaks in Finnish reindeer: a mechanistic transmission model approach. Parasites Vectors 11 (1). https://doi.org/10.1186/s13071-018-3159-z, 565-565.

Hanke, A.N., Angohiatok, M., Leclerc, L.-M., Adams, C., Kutz, S., 2021. A caribou decline foreshadowed by Inuit in the Central Canadian Arctic: a retrospective analysis. Arctic 74 (4), 437–455. https://doi.org/10.14430/arctic73826.

Hanke, A., Niptanatiak Dumond, A., Di Francesco, J., Adams, C.L., Milton, T., Leclerc, L.-M., Kutz, S., 2024. Supporting Inuit food sovereignty through collaborative research of an at-risk caribou herd. Front. Sustain. Food Syst. 7. https://doi.org/10.3389/fsufs.2023.1306521.

Hoberg, E.P., Polley, L., Jenkins, E.J., Kutz, S.J., Veitch, A.M., Elkin, B.T., 2008. Integrated approaches and empirical models for investigation of parasitic diseases in northern wildlife. Emerg. Infect. Dis. 14 (1), 10–17. https://doi.org/10.3201/eid1401.071119.

Hongoh, V., Berrang-Ford, L., Scott, M.E., Lindsay, L.R., 2012. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under climate change. Appl. Geogr. 33, 53–62. https://doi.org/10.1016/j.apgeog.2011.05.015.

Hoye, T.T., 2020. Arthropods and climate change – arctic challenges and opportunities. Curr. Opin. Insect Sci. 41, 40–45. https://doi.org/10.1016/j.cois.2020.06.002.

Hummel, M., Ray, J.C., 2008. Caribou and the North : a Shared Future. Dundurn Press.

Johnson, H.E., Lenart, E.A., Gustine, D.D., Adams, L.G., Barboza, P.S., 2022. Survival and reproduction in arctic caribou are associated with summer forage and insect harassment. Front. Ecol. Evol. 10. https://doi.org/10.3389/fevo.2022.899585.

Joly, K., Couriot, O., Cameron, M.D., Gurarie, E., 2020. Behavioral, physiological, demographic and ecological impacts of hematophagous and endoparasitic insects on an arctic ungulate. Toxins 12 (5), 334. https://doi.org/10.3390/toxins12050334.

Kafle, P., Peller, P., Massolo, A., Hoberg, E., Leclerc, L.-M., Tomaselli, M., Kutz, S., 2020. Range expansion of muskox lungworms track rapid arctic warming: implications for geographic colonization under climate forcing. Sci. Rep. 10 (1), 17323. https://doi.org/10.1038/s41598-020-74358-5.

Koltz, A.M., Civitello, D.J., Becker, D.J., Deem, S.L., Classen, A.T., Barton, B., Ezenwa, V. O., 2022. Sublethal effects of parasitism on ruminants can have cascading consequences for ecosystems. Proc. Natl. Acad. Sci. USA 119 (20), e2117381119. https://doi.org/10.1073/pnas.2117381119.

Koltz, A.M., Culler, L.E., 2021. Biting insects in a rapidly changing Arctic. Curr. Opin. Insect Sci. 47, 75–81. https://doi.org/10.1016/j.cois.2021.04.009.

Kronefeld, M., Kampen, H., Sassnau, R., Werner, D., 2014. Molecular detection of *Dirofilaria immitis, Dirofilaria repens* and *Setaria tundra* in mosquitoes from Germany. Parasites Vectors 7 (1). https://doi.org/10.1186/1756-3305-7-30, 30-30.

Kulpa, M.R., Lefoulon, E., Beckmen, K.B., Allen, S.E., Malmberg, J., Crouse, J.A., Thompson, D.P., Benedict, B.M., Goldsmith, D.G., McCarthy, S., Jones, L.C., Yabsley, M.J., Kutz, S.J., Verocai, G.G., 2024. A footworm in the door: revising *Onchocerca* phylogeny with previously unknown cryptic filarial species in North American wild ungulates. Int. J. Parasitol. submitted for publication.

Kulpa, M., Nelson, K.J., Morales, A.M., Ryan, B.M., Koschik, M.L., Scott, J.J., Verocai, G. G., 2021. Presence of a cryptic *Onchocerca* species in black flies of northern California, USA. Parasites Vectors 14 (1). https://doi.org/10.1186/s13071-021-04990-1, 478-478.

Kutz, S.J., Checkley, S., Verocai, G.G., Dumond, M., Hoberg, E.P., Peacock, R., Wu, J., Orsel, K., Seegers, K., Warren, A., Abrams, A., 2013. Invasion, establishment, and range expansion of two protostrongylid nematodes in the Canadian Arctic. Global Change Biol. 19 (11), 3254–3262. https://doi.org/10.1111/gcb.12315.

Kutz, S.J., Ducrocq, J., Verocai, G.G., Hoar, B.M., Colwell, D.D., Beckmen, K.B., Hoberg, E.P., 2012. Parasites in ungulates of Arctic North America and Greenland. A view of contemporary diversity, ecology, and impact in a world under change. Adv. Parasitol. 79, 99–252. https://doi.org/10.1016/B978-0-12-398457-9.00002-0.

Kutz, S.J., Hoberg, E.P., Molnár, P.K., Dobson, A.P., Verocai, G.G., 2014. A Walk on the Tundra: host-parasite interactions in an extreme environment. Int. J. Parasitol. Parasites Wildl. 3, 198–208.

Kutz, S.J., Jenkins, E.J., Veitch, A.M., Ducrocq, J., Polley, L., Elkin, B., Lair, S., 2009. The Arctic as a model for anticipating, preventing, and mitigating climate change impacts on host–parasite interactions. Vet. Parasitol. 163 (3), 217–228. https://doi.org/10.1016/j.vetpar.2009.06.008.

Laaksonen, S., Oksonen, A., 2007. *Setaria tundra* outbreak in reindeer in Finland. Rangifer 27 (3). https://doi.org/10.7557/2.27.3.276.

Laaksonen, S., Oksanen, A., Hoberg, E., 2015. Lymphatic dwelling filarioid nematode, *Rumenfilaria andersoni* (Filarioidea; Splendidofilariinae), is an emerging parasite in Finnish cervids. Parasites Vectors 8 (1). https://doi.org/10.1186/s13071-015-0835-0, 228-228.

Laaksonen, S., Oksanen, A., Orro, T., Norberg, H., Nieminen, M., Sukura, A., 2008. Efficacy of different treatment regimes against setariosis (*Setaria tundra*, Nematoda: Filarioidea) and associated peritonitis in reindeer. Acta Vet. Scand. 50 (1). https://doi.org/10.1186/1751-0147-50-49, 49-49.

Laaksonen, S., Pusenius, J., Kumpula, J., Venäläinen, A., Kortet, R., Oksanen, A., Hoberg, E., 2010. Climate change promotes the emergence of serious disease outbreaks of filarioid nematodes. EcoHealth 7 (1), 7–13. https://doi.org/10.1007/s10393-010-0308-z.

Laaksonen, S., Solismaa, M., Kortet, R., Kuusela, J., Oksanen, A., 2009a. Vectors and transmission dynamics for *Setaria tundra* (Filarioidea; Onchocercidae), a parasite of reindeer in Finland. Parasites Vectors 2 (1). https://doi.org/10.1186/1756-3305-2-3, 3-3.

Laaksonen, S., Solismaa, M., Orro, T., Kuusela, J., Saari, S., Kortet, R., Sukura, A., 2009b. *Setaria tundra* microfilariae in reindeer and other cervids in Finland. Parasitol. Res. 104 (2), 257–265. https://doi.org/10.1007/s00436-008-1184-0, 1987.

Lamarre, V., Legagneux, P., Franke, A., Casajus, N., Currie, D.C., Berteaux, D., Bêty, J., 2018. Precipitation and ectoparasitism reduce reproductive success in an arctic-nesting top-predator. Sci. Rep. 8 (1), 8530–8537. https://doi.org/10.1038/s41598-018-26131-y.

Lankester, M.W., Snider, J.B., 1982. *Rumenfilaria andersoni* n. gen., n. sp. (Nematoda: Filarioidea) in moose, *Alces alces* (L.), from northwestern Ontario, Canada. Can. J. Zool. 60 (10), 2455–2458. https://doi.org/10.1139/z82-313.

Lefoulon, E., Gavotte, L., Junker, K., Barbuto, M., Uni, S., Landmann, F., Martin, C., 2012. A new type F *Wolbachia* from Splendidofilariinae (Onchocercidae) supports the recent emergence of this supergroup. Int. J. Parasitol. 42 (11), 1025–1036. https://doi.org/10.1016/j.ijpara.2012.09.004.

Lefoulon, E., Giannelli, A., Makepeace, B.L., Mutafchiev, Y., Townson, S., Uni, S., Verocai, G.G., Otranto, D., Martin, C., 2017. Whence river blindness? The domestication of mammals and host-parasite coevolution in the nematode genus *Onchocerca*. Int. J. Parasitol. v. 47, 457–470. https://doi.org/10.1016/j.ijpara.2016.12.009.

Low, W.A., 1976. Parasites of woodland caribou in Tweedsmuir provincial Park, British Columbia. *The* can. Field Notes 90, 189–191.

McFrederick, Q.S., Haselkorn, T.S., Verocai, G.G., Jaenike, J., 2013. Cryptic *Onchocerca* species infecting North American cervids, with implications for the evolutionary history of host associations in *Onchocerca*. Parasitology 140 (10), 1201–1210. https://doi.org/10.1017/S0031182012001758.

Olejníček, J., 2005. Book Review: Adler P.H., Currie D.C. & Wood D.M.: the Black Flies (Simuliidae) of North America, vol. 102. Institute of entomology, p. 154, 154.

Oloś, G., Nowakowska, J., Rojewska, S., Welc-Falęciak, R., 2019. New findings *of Setaria tundra* and *Setaria cervi* in the red deer (*Cervus elaphus*) in Poland. Parasitology 146 (10), 1333–1337. https://doi.org/10.1017/S0031182019000568.

Outammassine, A., Zouhair, S., Loqman, S., 2022. Global potential distribution of three underappreciated arboviruses vectors (*Aedes japonicus, Aedes vexans* and *Aedes vittatus*) under current and future climate conditions. Transbound. Emerg. Dis. 69 (4), e1160–e1171. https://doi.org/10.1111/tbed.14404.

Papini, R.A., Lubas, G., Sgorbini, M., 2020. Incidental detection of *Onchocerca* microfilariae in donkeys (*Equus asinus*) in Italy: report of four cases. Front. Vet. Sci. 7. https://doi.org/10.3389/fvets.2020.569916, 569916-569916.

Paulsen, P., Bauer, A., Smulders, F.J.M., 2017. Game Meat Hygiene : Food Safety and Security. Wageningen Academic Publishers, p. 308.

Pledger, D.J., 1978. Black Flies (Diptera, Simuliidae) of the Swan Hills, Alberta as Possible Vectors of *Onchocerca cervipedis* Wehr and Dikmans, 1935 (Nematoda, Onchocercidae) in Moose *(Alces alces Linnaeus)*. ProQuest Dissertations Publishing.

Polley, L., Thompson, R.C.A., 2009. Parasite zoonoses and climate change: molecular tools for tracking shifting boundaries. Trends Parasitol. 25 (6), 285–291. https://doi.org/10.1016/j.pt.2009.03.007.

Pomari, E., Piubelli, C., Perandin, F., Bisoffi, Z., 2019. Digital PCR: a new technology for diagnosis of parasitic infections. Clin. Microbiol. Infect. 25 (12), 1510–1516.

Pörtner, H.O., Roberts, D.C., Adams, H., Adler, C., Aldunce, P., Ali, E., Zaiton Ibrahim, Z., 2022. Climate Change 2022: Impacts, Adaptation and Vulnerability.

Raponi, M., Beresford, D.V., Schaefer, J.A., Thompson, I.D., Wiebe, P.A., Rodgers, A.R., Fryxell, J.M., 2018. Biting flies and activity of caribou in the boreal forest. J. Wildl. Manag. 82 (4), 833–839. https://doi.org/10.1002/jwmg.21427.

Roe, C.C., Holiday, O., Upshaw-Bia, K., Benally, G., Williamson, C.H.D., Urbanz, J., Sahl, J.W., 2023. Biting midges (Diptera: Ceratopogonidae) as putative vectors of zoonotic *Onchocerca lupi* (Nematoda: Onchocercidae) in northern Arizona and New Mexico, southwestern United States. Front. Vet. Sci. 10, 1167070. https://doi.org/10.3389/fvets.2023.1167070.

Schurr, K., Rabalais, F., 1983. A new state record: *Setaria yehi* (Filaria) found in *Odocoileus virginianus*. Ohio J. Sci. 83 (4), 208–209.

Taylor, S.C., Laperriere, G., Germain, H., 2017. Droplet digital PCR versus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data. Sci. Rep. 7 (1). https://doi.org/10.1038/s41598-017-02217-x, 2409-2408.

Tran, T.D., Nelms, B.M., Koschik, M.L., Scott, J.J., Thiemann, T., 2022. Prevalence of filarial parasites in field-caught mosquitoes in northwestern California. J. Vector Ecol. 47 (1), 61–68. https://doi.org/10.52707/1081-1710-47.1.61.

Tryland, M., Kutz, S., 2018. Reindeer and Caribou: Health and Disease, vol. 1. Taylor and Francis, New York, pp. 189–190.

Verocai, G.G., Gomez, J.L., Hakimi, H., Kulpa, M.R., Luksovsky, J.L., Thompson, D.P., Crouse, J.A., 2024. Validation of a species-specific probe-based qPCR for detection of Setaria yehi in Alaskan moose (Alces alces). Int. J. Parasitiol. Parasit. Wildl. "submitted".

Verocai, G.G., Lejeune, M., Beckmen, K.B., Kashivakura, C.K., Veitch, A.M., Hoberg, E.P., Kutz, S.J., 2012. Defining parasite biodiversity at high latitudes of North America: new host and geographic records for *Onchocerca cervipedis* (Nematoda: Onchocercidae) in moose and caribou. Parasites Vectors 5, 242.

Verocai, G.G., Nelson, K.J., Callahan, R.T., Wekesa, J.W., Hassan, H.K., Hoberg, E.P., 2018. A cryptic species of *Onchocerca* (Nematoda: Onchocercidae) in blackflies (*Simulium* spp.) from southern California, USA. Parasites Vectors 11 (1). https://doi.org/10.1186/s13071-018-3133-9, 547-547.

Villeneuve, C.-A., Buhler, K.J., Iranpour, M., Avard, E., Dibernardo, A., Fenton, H., Leighton, P.A., 2021. New records of California serogroup viruses in *Aedes* mosquitoes and first detection in simuliidae flies from northern Canada and Alaska. Polar Biol. 44 (9), 1911–1915. https://doi.org/10.1007/s00300-021-02921-5.

Wehr, E.E., Dikmans, G., 1935. New nematodes (Filariidae) from NorthNorth America ruminants. Zool. Anz. 110, 202–208.

Weinmann, C.J., Anderson, J.R., Longhurst, W.M., Connolly, G.U.Y., 1973. Filarial worms of columbian black-tailed deer in California: 1. Observations in the vertebrate host. J. Wildl. Dis. 9 (3), 213–220. https://doi.org/10.7589/0090-3558-9.3.213.

Weinmann, C.J., Shoho, C., 1975. Abdominal worm infection in newborn deer (Filarioidea: Setariidae). J. Parasitol. 61 (2). https://doi.org/10.2307/3279012, 317-317.

Wichianchot, S., Hongsrichan, N., Maneeruttanarungroj, C., Pinlaor, S., Iamrod, K., Purisarn, A., Rucksaken, R., 2022. A newly developed droplet digital PCR for *Ehrlichia canis* detection: comparisons to conventional PCR and blood smear techniques. J. Vet. Med. Sci. 84 (6), 831–840. https://doi.org/10.1292/jvms.22-0086.

Wood, D.M., Dang, P.T., Ellis, R.A., 1979. The mosquitoes of Canada. Diptera: Culicidae. In the Insects and Arachnids of Canada. Canadian Government Publishing Centre, Hull, QC, Canada, p. 106.