International Journal for Parasitology: Parasites and Wildlife 6 (2017) 331–339

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Morphological keys to advance the understanding of protostrongylid biodiversity in caribou (*Rangifer* spp.) at high latitudes

Pratap Kafle [a], Lisa-Marie Leclerc [b], Morgan Anderson [c, 1], Tracy Davison [d], Manigandan Lejeune [a, 2], Susan Kutz [a, *]

[a] Faculty of Veterinary Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, T2N 4N1, Canada
[b] Department of Environment, Government of Nunavut, Kugluktuk, NU, X0B 0E0, Canada
[c] Department of Environment, Government of Nunavut, Igloolik, NU, X0A 0L0, Canada
[d] Department of Environment and Natural Resources, Government of Northwest Territories, Inuvik, NT, X0E 0T0, Canada

ARTICLE INFO

Article history:
Received 12 July 2017
Received in revised form
24 August 2017
Accepted 25 August 2017

Keywords:
Parelaphostrongylus andersoni
Varestrongylus eleguneniensis
Diagnostic parasitology
Morphological diagnosis
Dorsal spined larvae
Canadian Arctic
Dolphin and Union caribou

ABSTRACT

The Protostrongylidae is a diverse family of nematodes capable of causing significant respiratory and neuromuscular disease in their ungulate and lagomorph hosts. Establishing the species diversity and abundance of the protostrongylid fauna has been hindered because the first stage larvae, commonly referred as dorsal spined larvae (DSL), that are shed in the feces are morphologically very similar among several genera. We aimed to determine the protostrongylid diversity and distribution in caribou (*Rangifer tarandus groenlandicus* and *R. t. pearyi*) in the central and high Canadian Arctic. We first developed, tested and validated a morphological diagnostic guide for the DSL of two important protostrongylids, *Parelaphostrongylus andersoni* and *Varestrongylus eleguneniensis,* and then applied this guide to determine the prevalence and intensity of infection of these parasites in fecal samples from 242 caribou. We found that DSL of *V. eleguneniensis* and *P. andersoni* can be differentiated morphologically based on the structural differences at the caudal extremity. The presentation and morphology of the dorsal spine, and caudoventral bulging at the start of the tail extension were identified as the key identifying features. The two species were found in caribou on the arctic mainland and southern Victoria Island in single and co-infections, but the prevalence and intensity of infection was low. No protostrongylids were detected in caribou from the high arctic islands. Through this study, we provide a simple, efficient, and robust method to distinguish the DSL of the two protostrongylids, and present the current status of infection in different herds of caribou of the central Canadian Arctic. We report new geographic and host records for *P. andersoni* infection in Dolphin and Union caribou herd.

1. Introduction

Climate warming in the Arctic is impacting various biological processes, influencing the health of wildlife (Burek et al., 2008; Altizer et al., 2013; Post et al., 2013; Van Hemert et al., 2015; Descamps et al., 2017), and altering the ecology and distribution of parasites of Arctic ungulates (Kutz et al., 2005, 2013; Laaksonen et al., 2010). Caribou (*Rangifer* spp.), a keystone species in the Canadian Arctic, have recently experienced substantial population declines, which are attributed to cumulative impacts of various environmental and anthropogenic changes, and their indirect consequences on caribou health (Vors and Boyce, 2009; Fauchald et al., 2017). Climate-mediated ecological changes, and the concomitant emergence of novel diseases and parasites in northern caribou and sympatric ungulates, has highlighted the need for increased monitoring and comprehensive disease surveillance to help in efficient and proactive management (Hoberg et al., 2008b; Kutz et al., 2009). Limiting the efficacy of health monitoring, however, is the lack of robust, pertinent and easily accessible monitoring tools to diagnose and accurately assess the severity of the problems. This is particularly true for some parasitic infections

* Corresponding author.
E-mail addresses: pkafle@ucalgary.ca (P. Kafle), LLeclerc@GOV.NU.CA (L.-M. Leclerc), Morgan.Anderson@gov.bc.ca (M. Anderson), Tracy_Davison@gov.nt.ca (T. Davison), ml872@cornell.edu (M. Lejeune), skutz@ucalgary.ca (S. Kutz).
[1] Present address: Fish and Wildlife, British Columbia Government, Prince George, BC.
[2] Present address: Department of Population Medicine and Diagnostics, Cornell University, Ithaca, NY.

http://dx.doi.org/10.1016/j.ijppaw.2017.08.009

such as helminths, where high morphological and molecular similarity within a group often makes diagnosis challenging (Jenkins et al., 2005; Kutz et al., 2007; Kafle et al., 2015).

Protostrongylids are parasitic nematodes that infect ungulates and lagomorphs globally (Anderson, 2000). Depending on the species, their predilection sites can be the neuro-muscular or pulmonary system, and infection can result in mild to fatal disease (Foreyt and Jessup, 1982; Lankester, 2001; Kutz et al., 2012). All protostrongylids have indirect life cycles involving gastropod intermediate hosts, where the first stage larvae (L1) passed along with the feces develop to infective third stage larvae (L3), which finally infect the definitive host (Anderson, 2000). The development rate of L1 to L3 depends on the ambient temperature, which makes the transmission of these parasites sensitive to climate warming (Kutz et al., 2005; Jenkins et al., 2006). Five species of protostrongylids have been reported from North American caribou: *Parelaphostrongylus andersoni*, *V. eleguneniensis, Parelaphostrongylus odocoilei*, *Parelaphostrongylus tenuis* (aberrantly), and *Elaphostrongylus rangiferi*. Only the first three have been reported in caribou of the Canadian Arctic (Kutz et al., 2007, 2012; Verocai et al., 2014). *Parelaphostrongylus andersoni*, a muscle worm, was first reported in white-tailed deer (*Odocoileus virginianus*) but also infects caribou and moose (*Alces alces*), covering an extensive geographical area in northern North America (Lankester and Hauta, 1989; Verocai, 2015). *Varestrongylus eleguneniensis*, a recently described lungworm, is found in caribou and muskoxen (*Ovibos moschatus*), and occasionally moose, and has a wide geographic distribution from arctic to boreal regions of North America (Verocai et al., 2014). These two species of protostrongylids co-occur in caribou throughout much of this host's range (Kutz et al., 2007; Verocai, 2015). The third protostrongylid, *P. odocoilei,* is a muscle worm that infects a broad spectrum of hosts, but appears relatively uncommonly in woodland caribou (*Rangifer tarandus caribou*) and has not been confirmed in barrenground caribou to date (Gray and Samuel, 1986; Kutz et al., 2012). *Parelaphostrongylus tenuis* and *E. rangiferi* are limited to temperate regions of central and eastern Canada, and the island of Newfoundland, respectively, and cause mild to severe neurological disease in caribou (Kutz et al., 2012).

Three out of four protostrongylid subfamilies (Muelleriinae, Elaphostrongylinae and Varestrongylinae) produce dorsal spined larvae (DSL) that are morphologically very similar; this has hindered the accurate documentation of protostrongylid diversity. For instance, historically, when only microscopy was available, DSL detected in caribou were generally assumed to be *P. andersoni* (Lankester, 2001). The introduction of polymerase chain reaction (PCR) and sequencing of Internal Transcribed Spacer-2 (ITS-2) region of the L1 proved to be a valuable tool to better define parasite diversity and demonstrated a novel species of *Varestrongylus, V. eleguneniensis*, in caribou across their range (Jenkins et al., 2005; Kutz et al., 2007; Verocai, 2015). However, this method is still limited in its ability to quantify the larval abundance in mixed infections, or identify rare species, because it is based on sequencing of PCR-amplified DNA of individual larvae from a sample (Gajadhar et al., 2000; Huby-Chilton et al., 2006; Kutz et al., 2007). More recently, a novel high-throughput sequencing method applying deep amplicon sequencing to study the nematode species composition has been developed (Avramenko et al., 2015). This method can be used to determine and quantify co-infections by two or more species, and although its application in wildlife monitoring is in the initial stages, it has a considerable potential in this field. However, even when it is developed, the need for highly specialized equipment and personnel makes its use in the typical wildlife laboratory unrealistic. Similar DNA based diagnostic assays are limited by their technical complexities, cost, and applications (Yang and Rothman, 2004; Perkins et al., 2011).

Traditional microscopy-based parasitological diagnosis is still relevant and widely used, as it can be performed with minimal equipment and lab expertise. Recently, Kafle et al. (2015) demonstrated that DSL produced by the two protostrongylids, *Umingmakstrongylus pallikuukensis* (subfamily: muelleriinae) and *Varestrongylus eleguneniensis* (subfamily: varestrongylinae), can be morphologically differentiated, despite having a high degree of structural similarity. Although morphological features of DSL of some protostrongylids have been described in the past (Prestwood, 1972; Demartini and Davies, 1977; Lankester and Hauta, 1989; Hoberg et al., 2005; Kutz et al., 2007), there are few studies that characterize the morphological differences as diagnostic features.

The objectives of this study were twofold: i) to develop a morphological identification guide for differential diagnosis of DSL produced by two common species of protostrongylids, *V. eleguneniensis* and *P. andersoni*, infecting caribou, and ii) by using the developed identification guide, determine the diversity, prevalence, and intensity of infection of protostrongylids in caribou of the central and high Canadian Arctic.

2. Materials and methods

This research occurred in two phases. The first was to describe and validate the morphological features to differentiate the DSL of *P. andersoni* and *V. eleguneniensis*. The second phase was to use these validated morphological features to do a broader geographic survey for these parasites in caribou.

2.1. Morphological differentiation of DSL

2.1.1. Sample acquisition and processing

Caribou fecal samples, collected during capture and collaring activities in 2015 and 2016 in the vicinity of Bathurst Inlet, Nunavut (67°13′27.0″N, 108°49′05.6″W), were used to identify the morphological features that could differentiate *V. eleguneniensis* from *P. andersoni*. Fecal samples were first processed using the modified Baermann method (Forrester and Lankester, 1997), DSL were isolated, and caudal morphology of all DSLs were observed under 400× magnification. *Varestrongylus eleguneniensis* was identified morphologically as per Kafle et al. (2015). On careful observation, DSLs with different caudal morphology were also observed in these samples (Fig. 1). These non-*V. elegueniensis* DSL had consistent morphological features, suggesting a single species. Ten DSLs from three different hosts, representing the DSL with different caudal morphology, were subsequently identified as *P. andersoni* through PCR and sequencing of ITS-2 (Kutz et al., 2007). This established that this group of samples could be used for subsequent studies on defining diagnostic features for morphological differentiation.

2.1.2. Identification and familiarization with the diagnostic morphological features

Fifty DSLs that differed morphologically from *V. eleguneniensis* were isolated from six fecal samples and individually heat fixed in approximately 50 μl water on a glass slide. These were examined under differential interference contrast (DIC) settings at 400× magnification (Olympus, Massachusetts, USA). Three consistent morphological features around the caudal extremity (dorsal spine morphology, caudoventral bulging at the start of tail extension, and tail spike morphology) (Fig. 2) were identified as features that consistently differentiated them from *V. eleguneniensis* DSL.

2.1.3. Validation of identified morphological keys and morphometric analysis

Sixty DSLs were randomly isolated from three caribou fecal

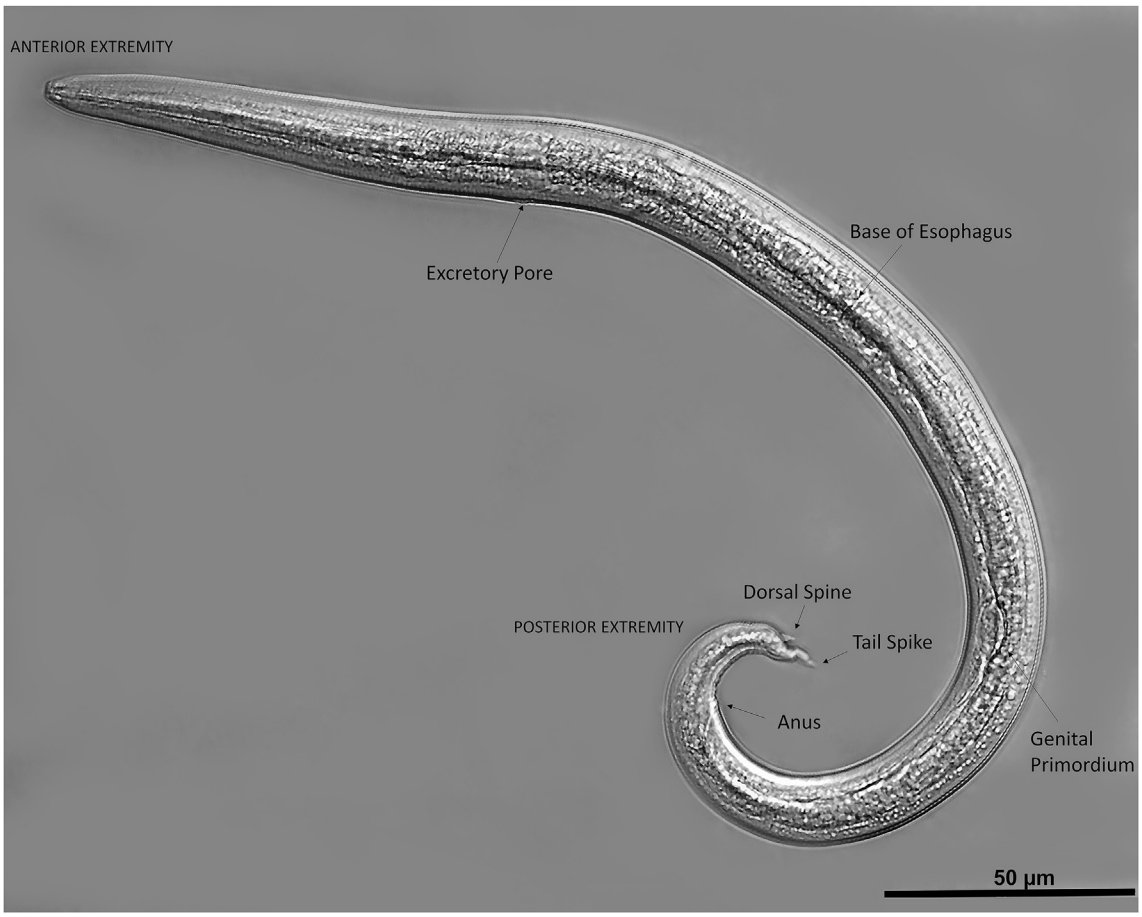

Fig. 1. Dorsal spine larva of *Parelaphostrongylus andersoni* at 400× magnification in differential interference contrast, depicting important anatomical features.

samples containing both types of larvae, individually heat fixed (as above), and observed under DIC settings (400× magnification) of the microscope. The DSLs were identified as *V. eleguneniensis* or *P. andersoni* based on morphological features described above and those described by Kafle et al. (2015) for *V. eleguneniensis*. Morphometric measurements were taken for each of the DSL using special software (CellSens; Olympus Soft Imaging Solutions GmbH) (Table 1). Photomicrographs of the DSLs were taken for future reference with the camera head mounted on the microscope (Olympus BX53 fitted with a digital camera, Olympus DP73, Olympus®). Each studied DSL was then recovered individually, and molecular confirmation of species was done by sequencing the ITS-2 of nuclear ribosomal DNA (Kutz et al., 2007), in order to confirm the morphological diagnosis.

2.2. Determining protostrongylid diversity and abundance in caribou

2.2.1. Study species and area

Two subspecies of caribou - *Rangifer tarandus groenlandicus*, or barren-ground (BG) caribou, and *Rangifer tarandus pearyi,* or Peary caribou, inhabit the central Canadian Arctic/high Arctic (Gunn et al., 2011; Ray et al., 2015). A genetically and behaviorally distinct group of caribou, the Dolphin and Union herd (DU) (Zittlau, 2004), belonging to the subspecies *R. t. groenlandicus,* also occurs in the west-central Canadian Arctic. The BG and DU herds are listed as 'threatened' and 'special concern', respectively, by the Committee on the Status of Wildlife in Canada (COSEWIC, 2016). Peary caribou were listed as 'Endangered' under Canada's Species at Risk Act (SARA) following drastic population declines in the 1990s (Miller and Gunn, 2003), although COSEWIC recently re-assessed their status as 'threatened' (COSEWIC, 2016). The BG caribou do not cross the sea ice, but DU caribou migrate seasonally between the mainland and Victoria Island (Poole et al., 2010). Peary caribou occur on northern Victoria Island and throughout most of the Canadian Arctic Archipelago and migrate among these islands (Jenkins and Lecomte, 2012).

Between 2013 and 2015, 242 caribou fecal samples were collected from the mainland of the central Canadian Arctic (n = 50), Victoria Island (n = 14), King William Island (n = 5), and the High Arctic Islands (n = 173) in Nunavut (NU) (Fig. 3). Most of the mainland samples were collected around Bathurst Inlet during the capture of caribou for deployment of satellite radio collars, hence, the subsequent movement of these caribou was tracked. The fecal samples collected on the mainland and King William Island were sent to Wildlife Genetics International, Nelson, BC, Canada, for herd identification. The fecal samples obtained from the high arctic islands (Melville, Bathurst, and Byam Martin Islands) were assumed to be from Peary caribou. Six samples collected opportunistically on the mainland near Kugluktuk, NU, where both DU and BG caribou occur, and seven samples whose herd identity could not be confirmed by genetics, were classified as unknown herd.

2.2.2. Sample processing and diagnosis

Fecal samples were processed using the modified Baermann technique (Forrester and Lankester, 1997). The final concentrate

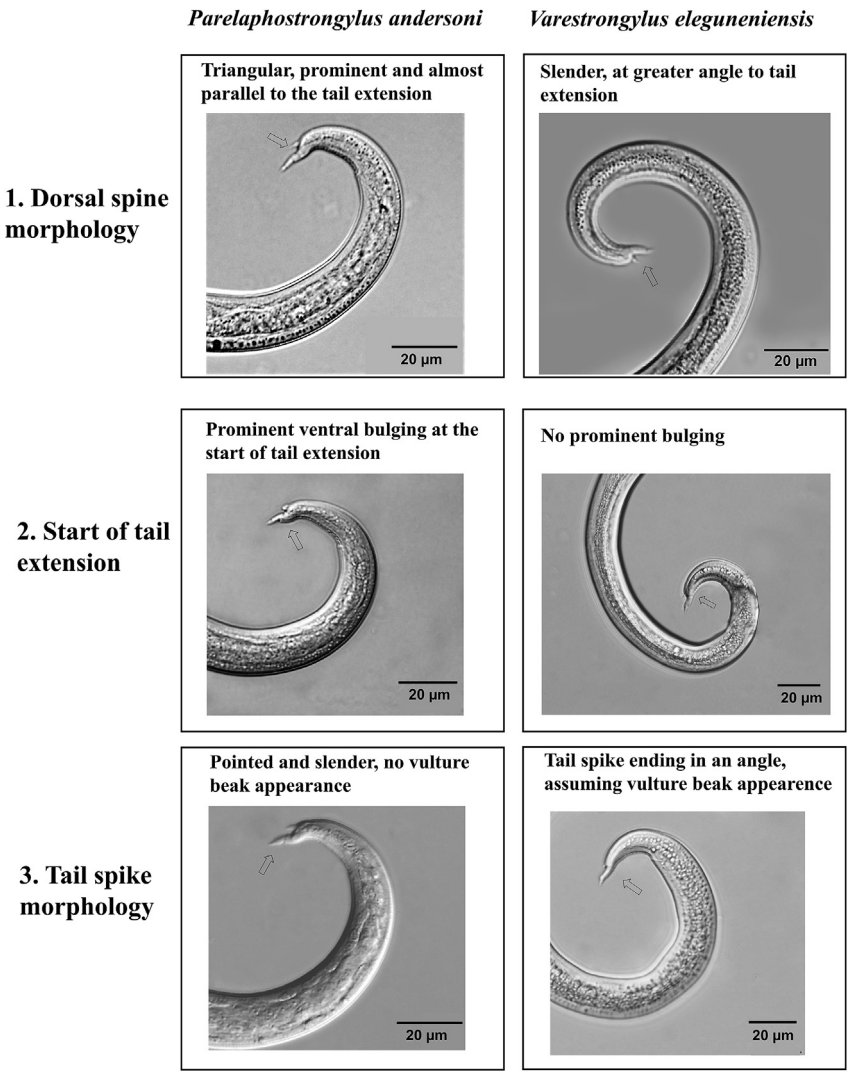

Fig. 2. Identification guide for differential diagnosis of dorsal spine larvae of *Parelaphostrongylus andersoni* and *Varestrongylus eleguneniensis.*

Table 1
Morphometric measurements of dorsal spine larvae of *Parelaphostrongylus andersoni* and *Varestrongylus eleguneniensis* from this study and previous studies. All measurements are expressed in micrometers (mean ± SD).

	P. andersoni [a] (*n* = 30)	*V. eleguneniensis* [a] (*n* = 20)	*V. eleguneniensis* [c] (Kafle et al., 2015)	*P. andersoni* [d] (Prestwood, 1972)
Total length	368.26 ± 16.26[b]	344 ± 9.54[b]	378 ± 9.84	351 (308–382)
Width	17.35 ± 1.37	17.02 ± 0.88	17.98 ± 0.83	17 (17–18)
Excretory Pore	90.53 ± 5.48	89.62 ± 4.59	97.34 ± 3.48	94 (66–109)
Esophagus	168.9 ± 6.62	157.01 ± 13.38	NA	175 (163–183)
Genital Primordium	240.95 ± 10.80	231.03 ± 5.68	244.15 ± 5.38	234 (216–249)
Anus	333.29 ± 18.07	312.58 ± 7.07	341 ± 9.32	NA
Tail extension length	9.49 ± 4.80	7.48 ± 1.15	8.47 ± 0.82	NA
Dorsal Spine	2.50 ± 0.37[b]	1.78 ± 0.26[b]	1.70 ± 0.34	NA
Tail	34.96 ± 5.89	31.95 ± 8.76	37.65 ± 4.19	32 (27–36)

[a] Statistical comparison was made only between the two DSL types measured in this study.
[b] Statistically significant ($p < 0.01$).
[c] In muskoxen.
[d] In white-tailed deer.

(1.5 ml) obtained after the centrifugation step of the Baermann technique, was heat fixed by passing the test tube through the Bunsen burner ten times. Total counts were determined by quantifying DSL found in three 0.5 ml/aliquots in a six-well plate using an inverted microscope (Olympus CKX53, Massachusetts) at 200 and 400× magnification. Differential diagnosis of DSL was performed using the morphological keys for *P. andersoni* identified in this study and the morphological keys for *V. eleguneniensis* described by Kafle et al. (2015). The intensity of infection is reported as larvae per gram of feces (LPG).

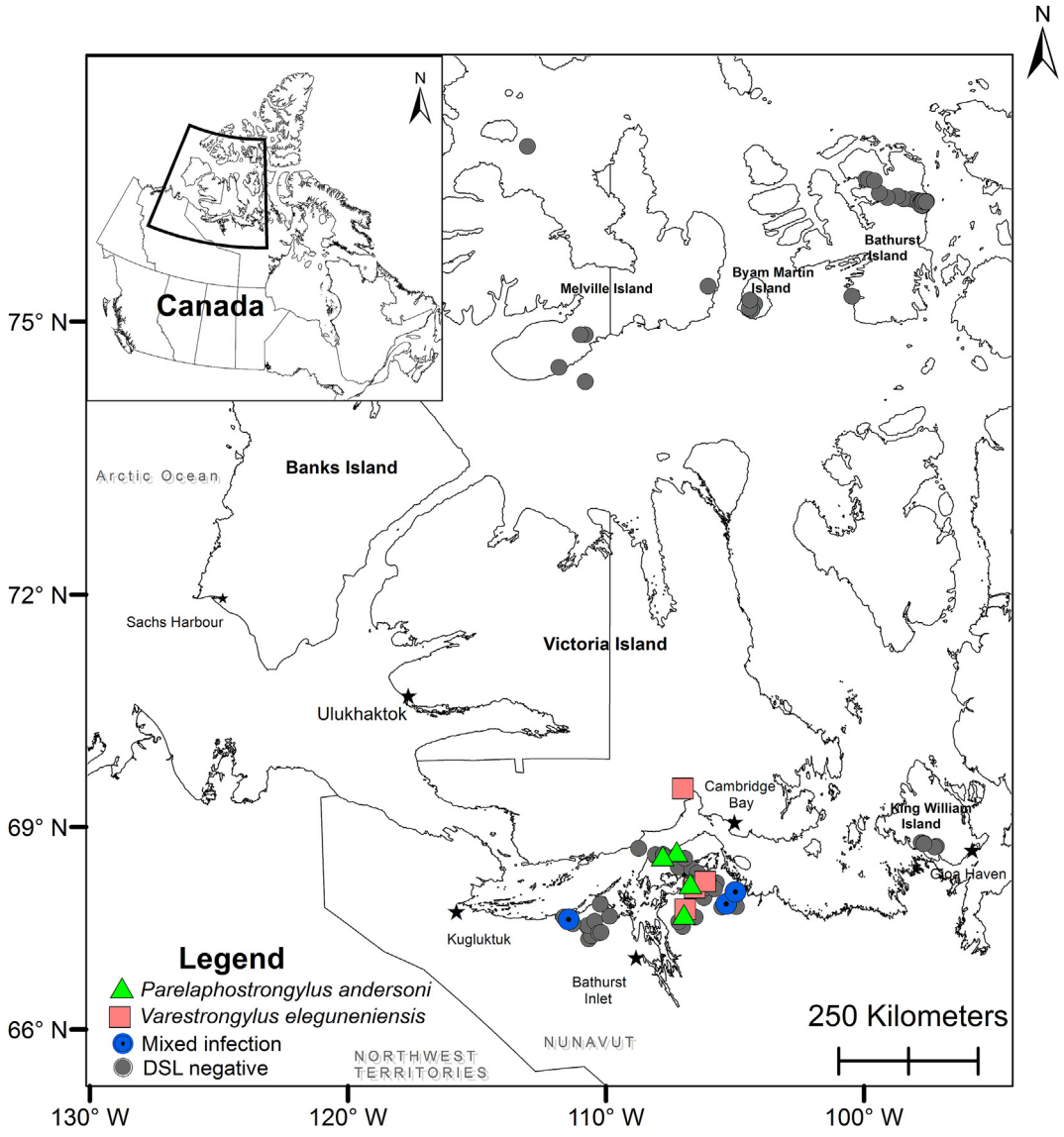

Fig. 3. Map showing the location of samples and results from the fecal survey. Each point on the map represents a group of 2–10 samples.

Voucher specimens of larval *P. andersoni* and *V. eleguneniensis* were deposited and archived in the Museum of Southwestern Biology (MSB), Division of Parasites, University of New Mexico, Albuquerque, NM, USA. (**MSB:Para:25805** and **MSB:Para:25806** respectively), and DNA sequences were deposited in GenBank under various accession numbers (**MF001306** – **MF001313**) (Table 2).

2.2.3. Statistical analysis

Morphometric measurements of *P. andersoni* and *V. eleguneniensis* DSLs were compared using permutation tests performed in R software version 3.2.2 (R Core Team, 2016). The statistical significance was assessed at $P \leq 0.01$.

3. Results

3.1. Morphological differentiation of DSL

3.1.1. Unique morphological features of P. andersoni DSL

Three characteristic structural features at the caudal extremity of *P. andersoni* DSL that discriminated it from *V. eleguneniensis* DSL were identified (Fig. 2). The first was the morphology and the presentation of its dorsal spine. The dorsal spine of *P. andersoni* DSL has a characteristic triangular shape with a wider base, compared to the thin and slender dorsal spine of *V. eleguneniensis* DSL. Similarly, the dorsal spine consistently appears parallel to the tail extension in *P. andersoni* DSL, unlike *V. eleguneniensis* where the dorsal spine occurs at a greater angle to the tail extension (Figs. 2 and 3). The second identifying feature was present in the ventral aspect of the tail, at the region where the tail extension starts. In all *P. andersoni* DSLs examined, a characteristic ventral bulging at the beginning of the tail extension was evident; this was absent from *V. eleguneniensis*. The third feature identified as diagnostically relevant, was the morphology of the tail spike. *Parelaphostrongylus andersoni* DSL did not have the vulturebeak-like structure at the end of the tail, which is observed in the DSL of *V. eleguneniensis,* as described in Kafle et al. (2015); rather the tail spike of *P. andersoni* DSL was straight, slender and pointed, somewhat similar to the distantly-related protostrongylid *Umingmakstrongylus pallikuukensis* (Hoberg et al., 1995; Kafle et al., 2015). Although the vulture beak appearance of the tail spike was not prominent in a very few confirmed *V. eleguneniensis* DSL (less than 5%), the tail spike morphology (particularly shorter and sharply pointed tail tip, and

Table 2
Summary of caribou fecal samples analyzed in this study.

Caribou herd	Sampling dates	Sampling area	n	DSL[b] Positive (PA/VE)	Coinf.	Range of LPG[c]		Accession Numbers
						PA	VE	
Peary	Aug 2013	North Bathurst Island	48	0				
	Apr 2014	Melville Island	11	0				
	May 2015	South Bathurst Island	7	0				
	July 2015	Airstrip Point-Bathurst Island	66	0				
	July 2015	Byam Martin Island	41	0				
Dolphin and Union	Oct 2015	Wellington Bay, Victoria Island	14	1(0/1)			148.31	MF001312
	Apr 2015, Oct 2016	Bathurst Inlet	21	4(3/2)	1	0.2–0.8	3.75–4.8	MF001306, MF001307, MF001311, MF001313
Barren Ground	Apr 2015, Oct 2016	Bathurst Inlet	10	5(4/3)	2	0.35–8.6	0.2–1.4	MF001308, MF001309, MF001310
	Mar 2016	King William Island	5	0				
Unknown[a]	Apr 2015, Oct 2016	Bathurst Inlet	7	1(0/1)			3.2	
	Apr 2015	Kugluktuk	6	0				

PA – *Parelaphostrongylus andersoni*, VE – *Varestrongylus eleguneniensis*, Coinf. – Number of coinfections.
[a] No confirmed herd identity.
[b] Dorsal spined larvae.
[c] Larva per gram of feces.

less distinct segments in the tail extension) was clearly different from that of *P. andersoni*.

Morphometrically, DSL of *P. andersoni* was significantly longer than *V. eleguneniensis* ($P < 0.01$). However, there was overlap in the total length of a few DSLs of the two species, and hence, total length was not selected as a diagnostic feature. The dorsal spine of *P. andersoni* DSL was significantly longer than that of *V. eleguneniensis* ($P < 0.01$), but due to practical difficulty in measuring the length of the dorsal spine, it was not retained as a diagnostic feature. The length of the tail extension, which was identified as a major diagnostic feature differentiating *V. elegueneniensis* DSL from *U. pallikuukensis* DSL (Kafle et al., 2015), was not considered diagnostically important while discriminating the two species studied here because of their similar length ($P > 0.01$). Other morphometric features, such as the esophagus length, tail length, tail spike length, and body width, overlapped between the DSL of the two species. Comparisons of the morphometric measurements from this study to earlier studies are presented in Table 1.

3.1.2. Validation and establishment of the identification guide

Using the morphological diagnostic features described above and in Fig. 2, the morphological identification of 49 out of 50 DSLs matched the species identities determined through sequencing of the ITS-2. All 36 DSLs diagnosed as *P. andersoni* morphologically were confirmed as such by PCR, and out of 14 DSLs diagnosed morphologically as *V. eleguneniensis*, 13 were confirmed as the same species through PCR. One DSL diagnosed as *V. eleguneniensis* morphologically but *P. andersoni* based on ITS-2 sequence had a distorted tail spike and slightly deformed dorsal spine morphology. No other protostrongylid species were suspected based on DSL morphology, nor were any detected based on sequencing of the ITS-2.

3.2. Protostrongylid diversity and abundance in caribou

Dorsal spined larvae were isolated from 11 of the total 242 fecal samples tested (Table 2). The majority of samples were collected from High Arctic (Peary Caribou) during 2013–2015 (n = 172), and they were all DSL negative (Fig. 3, Table 2). Ten out of 50 samples, collected on the mainland, were DSL positive, and one sample out of 14, collected near Wellington Bay, Victoria Island, NU was DSL positive. Five out of 35 DU caribou tested were infected by protostrongylids, and *P. andersoni* was reported in three samples, the northernmost and first record of the parasite in DU caribou. Five out of 15 BG caribou tested were DSL positive. The number of samples analyzed, samples positive for DSL and the range of larva per gram of feces for the two protostrongylid species in different herds of caribou at different sampling events are presented in Table 2.

4. Discussion

We have developed a morphological guide for differential diagnosis of two protostrongylids, *P. andersoni* and *V. eleguneniensis*, and applied this guide to further describe the biodiversity of parasites of caribou in the central Canadian Arctic. Analysis of 242 fecal samples, with species-level identification using the developed guide, revealed a low prevalence and intensity of infection of protostrongylids in caribou. Nevertheless, we demonstrate new host and geographic records for *P. andersoni*.

Dorsal spine larvae of *P. andersoni* and *V. eleguneniensis* were considered to be morphologically and morphometrically indistinguishable prior to this study. Prestwood (1972) in the original description of *P. andersoni* DSL indicated the prominent dorsal spine with a triangular shape and pointed tail spike (Fig. 4). However, this character was never considered as a diagnostic feature. Similarly, Demartini and Davies (1977) had mentioned that DSL of genus *Parelaphostrongylus* could possibly be differentiated from DSL of other protostrongylids based on their larger and broader dorsal spine morphology. Our observations of the prominent triangular dorsal spine as the most striking feature (feature 1), and the slender and pointed tail spike (feature 3), corroborates these earlier descriptions. The features of *P. andersoni* DSL not pointed out, but visible in the original line drawings, were the presentation of the dorsal spine (parallel to tail extension) and the ventral bulging just proximal to the tail spike (Fig. 4). For *V. eleguneniensis* DSL morphology, the greater angle of the dorsal spine (feature 1) and the vulture beak like pointing of the tail spike (feature 3) were also reported as diagnostic features differentiating them from the DSL of another co-infecting protostrongylid *U. pallikuukensis,* in muskoxen (Kafle et al., 2015). These morphological features were not explicitly discussed in the original description of *V. eleguneniensis* DSL (Verocai et al., 2014), but are distinctly visible in the original line drawing (Fig. 4). The other diagnostic features of *V. eleguneniensis* DSL described in Kafle et al. (2015), such as - post anal cuticular striations (not distinct) and caudal curvature (assuming spherical shape when heat fixed), did not differ consistently from *P. andersoni* DSL in this study, hence, these features were not included in the identification guide.

Despite the statistically significant difference in total DSL length

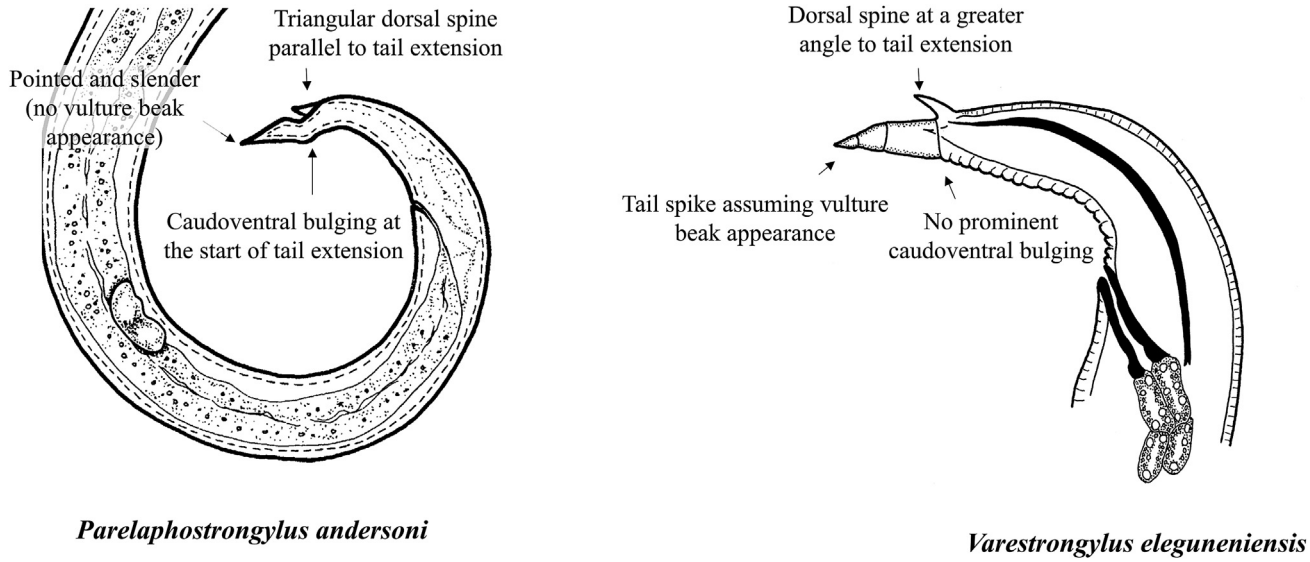

Fig. 4. Line drawings of caudal extremities of *Parelaphostrongylus andersoni* and *Varestrongylus eleguneniensis* dorsal spine larvae from Prestwood (1972) and Verocai et al. (2014). The line drawings clearly show the characteristic morphological features identified in this study.

between the two species (*P. andersoni* longer) (Table 1), this was not included as a diagnostic feature because of overlaps in length. Interestingly, the average length of *V. eleguneniensis* DSL was substantially smaller in this study compared to that measured by Kafle et al. (2015). All measurements were performed using the same fixation procedures, observer, and equipment, thus differences may be associated with the different final hosts. The Kafle et al. (2015) measurements were based on muskox derived DSL. This finding supports Harrison's rule, which states that when a parasite genus is present in different but nearly related host species, parasite size tends to correlate with host size (Harrison, 1915; Johnson et al., 2005). Muskoxen weigh between 218 and 266 kg on average, whereas caribou are much smaller, around 87–99 kg (Klein, 1992). Although they are not closely related species (Bovidae and Cervidae, respectively), they are both artiodactyls, and in this case Harrison's rule appears to still be relevant. The length of the dorsal spine, although significantly longer in *P. andersoni*, was not included in the diagnostic manual for the same reasons as the total length. It is true, however, that these morphometric features can be used together with the morphological features to aid larval identification and to recognize other, or novel species. For instance, DSL of the muskox lungworm, *U. pallikuukensis,* can be differentiated from that of *P. andersoni* by combining morphological and morphometric features (e.g., triangular dorsal spine and shorter tail extension length of *P. andersoni* DSL, versus angular/slender dorsal spine and longer tail extension of *U. pallikuukensis* DSL) (Hoberg et al., 1995; Kafle et al., 2015).

Among the known caribou protostrongylid fauna, *V. eleguneniensis* and *P. andersoni* appear to be the two most common and widespread species. They are found in three different subspecies of caribou (*R.t. caribou, R. t. granti, R. t. groenlandicus*), with their distribution ranging across the North American mainland from northern Alaska in the west to boreal forests of Quebec in the east, including the provinces of British Columbia, Alberta, Saskatchewan and Ontario (Jenkins et al., 2005; Kutz et al., 2007; Verocai, 2015), and, based on this study, also onto Victoria Island, Nunavut. In contrast, protostrongylids appear to be absent from Peary caribou (*R. t. pearyi*). Although mostly opportunistic, we had a robust sample size across different years and seasons (n = 172) for Peary caribou covering different high Arctic islands; the absence of any DSL from these samples provides the first substantial evidence of absence of protostrongylids in this caribou subspecies.

Parelaphostrongylus odocoilei, common in Dall's (*Ovis dalli*), Stone's sheep (*Ovis dalli stonei*) and mule deer (*Odocoileus hemionus*), and confirmed only twice in woodland caribou, is another differential diagnosis to consider for DSL found in caribou (Gray and Samuel, 1986; Jenkins et al., 2005; Hoberg et al., 2008a). *Parelaphostrongylus odocoilei* has not been reported north of the Arctic Circle and this restricted northern range, according to Jenkins et al. (2006), is either due to climatic constraints on the parasite or biotic and abiotic barriers to introduction (with respect to its occurrence in Dalls' sheep). *Parelaphostrongylus odocoilei* is closely related to *P. andersoni* and the DSL of the two species potentially share similar morphological characters (Pybus and Shave, 1984; Jenkins et al., 2005), hence, if present in co-infections with *P. andersoni*, it may be challenging to differentiate. Hoberg et al. (2005) present the detailed description of *P. odocoilei* DSL derived from Dall's sheep and its caudal polymorphism resulting in two morphotypes of the same species. The morphological description of one of the morphotypes is somewhat consistent with the morphology of *P. andersoni* DSL described in this study. Hoberg et al. (2005) reported the prominent triangular dorsal spine in both types of larvae, which is similar to the dorsal spine morphology of *P. andersoni* reported here, but the tail extension morphology appears to be different. The tail extension of the first morphotype of *P. odocoilei* DSL appears to be more sinuous, and the three tail folds appear to be more distinctly evident compared to the *P. andersoni* tail extension. The second morphotype's tail extension has a characteristic digitate terminal extension at the tail spike, and the tail extension lacks clearly visible tail folds, which is different from *P. andersoni* tail extension. The morphological differences, however, need to be tested in order to establish them as diagnostic features. In our study, we did not detect any DSL morphologically similar to *P. odocoilei,* and despite sequencing 70 DSLs from all the positive animals, we did not detect *P. odocoilei*, which suggests that this parasite is extremely rare or absent in the study area. Nevertheless, we recommend a detailed morphological investigation to characterize the diagnostic features of the DSL of *P. odocoilei,* as well as ongoing surveillance. Morphometric measurement, in particular total DSL length of *P. odocoilei* (334–428 μm) (Prestwood, 1972),

overlaps with that of *P. andersoni* (Table 1), thus cannot be used to differentiate the two species.

Unprecedented warming is greatly impacting Arctic host-parasite systems by altering the geographical ranges of hosts and parasites, driving parasitic invasions to the higher latitudes and leading to host switching (Dobson and Carper, 1992; Harvell et al., 2002; Kutz et al., 2005, 2013; Brooks and Hoberg, 2007; Hoberg et al., 2008a). For instance, Arctic warming has already released the climatic constraints for the muskox lungworm, *U. pallikuukensis*, facilitating its invasion onto the Victoria Island (Kutz et al., 2013). A similar mechanism is hypothesized to be responsible for the establishment of *V. eleguneniensis* in muskoxen and caribou on southern Victoria Island (Kafle, unpublished). Our finding of *P. andersoni* in DU caribou demonstrates that this parasite is also transported to Victoria Island, likely regularly and repeatedly with the annual migration of the DU caribou from the mainland. Whether it establishes a transmission cycle on the island, similar to the recently established *U. pallikuukensis* and *V. eleguneniensis* (Kutz et al., 2013), will depend on a variety of factors, including temperature constraints on the lifecycle of the parasite and the abundance and behavioral ecology of the definitive hosts.

Under the current regime of rapid Arctic warming, effective parasitic surveillance is important to anticipate shifts in species ecology and distribution. The value of the morphological keys that we provide lies not just in identifying particular parasitic species, but as a tool to increase our vigilance against any cryptic species, which, if suspected, can later be confirmed with more sophisticated molecular tools. Results from this study contribute to our existing knowledge on diagnosis and diversity of protostrongylid fauna in the ungulates at high latitudes and provide tools for ongoing biodiversity assessment. Simple methodologies such as these can be implemented with minimal equipment, training and cost, and can easily be applied for routine monitoring of existing pathogens as well as early recognition of any newly emerging pathogens.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The work was supported by funding to SK from NSERC Discovery, Research Tools and Instruments (#RGPIN/04171-2014), and Northern supplement grants, ArcticNet Network Centre of Excellence (#03650-GF099007), Polar Knowledge Canada (#1516-166), Canada North Outfitting, and the Government of Nunavut; PK was supported by a scholarship from the University of Calgary NSERC-CREATE Host-Parasite Interactions (HPI) graduate training program.

We gratefully acknowledge the considerable effort involved, particularly in remote locations during the collection of fecal samples. We acknowledge Myles Lamont, Etuangat Akeeagok, Tabitha Mullin, Samson Simeonie, James Iqaluk, Tommy Salluviniq for their invaluable assistance during fecal sampling. Many thanks to James Wang for technical support in the lab, summer student Cassandra Bruce and Katrine Gillet who helped to process the samples, and Elizabeth (Libby) Redman and Susan Stasiuk for providing reagents and offering technical assistance while performing PCR. Special thanks to Guilherme Verocai, University of Georgia, for insights and stimulating discussions.

References

Altizer, S., Ostfeld, R.S., Johnson, P.T., Kutz, S., Harvell, C.D., 2013. Climate change and infectious diseases: from evidence to a predictive framework. Science 341, 514–519.

Anderson, R.C., 2000. Order Strongylida (The Bursate Nematodes). in: Nematode Parasites of Vertebrates. Their Development and Transmission, seco[nd] ed. C.A.B. International, University Press, Cambridge, pp. 41–229 (Chapter 3).

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S., 2015. Exploring the gastrointestinal "Nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One 10, 1–18.

Brooks, D.R., Hoberg, E.P., 2007. How will global climate change affect parasite–host assemblages? Trends Parasitol. 23, 571–574.

Burek, K.A., Gulland, F.M., O'Hara, T.M., 2008. Effects of climate change on Arctic marine mammal health. Ecol. Appl. 18 (Suppl. l.), 126–134.

COSEWIC, 2016. Canadian Wildlife Species at Risk. Committee on the Status of Endangered Wildlife in Canada. http://www.registrelep.gc.ca/virtual_sara/files/species/Csar-2015-v002016Nov04-Eng.pdf.

Demartini, J., Davies, R., 1977. An epizootic of pneumonia in captive bighorn sheep infected with *Muellerius* sp. J. Wildl. Dis. 13, 117–124.

Descamps, S., Aars, J., Fuglei, E., Kovacs, K.M., Lydersen, C., Pavlova, O., Pedersen, Å.Ø., Ravolainen, V., Strøm, H., 2017. Climate change impacts on wildlife in a High Arctic archipelago–Svalbard. Nor. Glob. Change Biol. 23, 490–502.

Dobson, A., Carper, R., 1992. Global warming and potential changes in host-parasite and disease-vector relationships. In: Peters, R.L., Lovejoy, T.E. (Eds.), Global Warming and Biodiversity. Yale University Press, New Haven, pp. 201–217.

Fauchald, P., Park, T., Tømmervik, H., Myneni, R., Hausner, V.H., 2017. Arctic greening from warming promotes declines in caribou populations. Sci. Adv. 3, e1601365.

Foreyt, W.J., Jessup, D.A., 1982. Fatal pneumonia of bighorn sheep following association with domestic sheep. J. Wildl. Dis. 18, 163–168.

Forrester, S.G., Lankester, M.W., 1997. Extracting protostrongylid nematode larvae from ungulate feces. J. Wildl. Dis. 33, 511–516.

Gajadhar, A., Steeves-Gurnsey, T., Kendall, J., Lankester, M., Steen, M., 2000. Differentiation of dorsal-spined elaphostrongyline larvae by polymerase chain reaction amplification of ITS-2 of rDNA. J. Wildl. Dis. 36, 713–722.

Gray, J.B., Samuel, W.M., 1986. *Parelaphostrongylus odocoilei* (Nematoda, Protostrongylidae) and a protostrongylid nematode in woodland caribou (*Rangifer tarandus caribou*) of Alberta, Canada. J. Wildl. Dis. 22, 48–50.

Gunn, A., Russell, D., Eamer, J., 2011. Northern Caribou Population Trends in Canada. Canadian Councils of Resource Ministers, Ottawa, ON.

Harrison, L., 1915. Mallophaga from *Apteryx*, and their significance; with a note on the genus *Rallicola*. Parasitology 8, 88–100.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158–2162.

Hoberg, E., Polley, L., Jenkins, E., Kutz, S., 2008a. Pathogens of domestic and free-ranging ungulates: global climate change in temperate to boreal latitudes across North America. Rev. Sci. Tech. OIE 27, 511–528.

Hoberg, E.P., Jenkins, E.J., Rosenthal, B., Wong, M., Erbe, E.F., Kutz, S.J., Polley, L., 2005. Caudal polymorphism and cephalic morphology among first-stage larvae of *Parelaphostrongylus odocoilei* (Protostrongylidae: Elaphostrongylinae) in Dall's sheep from the Mackenzie mountains, Canada. J. Parasitol. 91, 1318e1325.

Hoberg, E.P., Polley, L., Gunn, A., Nishi, J.S., 1995. *Umingmakstrongylus pallikuukensis* gen nov et sp nov (Nematoda: Protostrongylidae) from muskoxen, *Ovibos moschatus*, in the central Canadian Arctic, with comments on biology and biogeography. Can. J. Zool. 73, 2266–2282.

Hoberg, E.P., Polley, L., Jenkins, E.J., Kutz, S.J., Veitch, A.M., Elkin, B.T., 2008b. Integrated approaches and empirical models for investigation of parasitic diseases in northern wildlife. Emerg. Infect. Dis. 14, 10–17.

Huby-Chilton, F., Chilton, N.B., Lankester, M.W., Gajadhar, A.A., 2006. Single-strand conformation polymorphism (SSCP) analysis as a new diagnostic tool to distinguish dorsal-spined larvae of the Elaphostrongylinae (Nematoda: Protostrongylidae) from cervids. Vet. Parasitol. 135, 153–162.

Jenkins, D., Lecomte, N., 2012. All about ice: Peary caribou movements in the Bathurst Islands complex. unpublished report. Department of Environment, Government of Nunavut, Pond Inlet, Nunavut, 22pp.

Jenkins, E.J., Appleyard, G.D., Hoberg, E.P., Rosenthal, B.M., Kutz, S.J., Veitch, A.M., Schwantje, H.M., Elkin, B.T., Polley, L., 2005. Geographic distribution of the muscle-dwelling nematode *Parelaphostrongylus odocoilei* in North America, using molecular identification of first-stage larvae. J. Parasitol. 91, 574–584.

Jenkins, E.J., Kutz, S.J., Hoberg, E.P., Polley, L., 2006. Bionomics of larvae of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in experimentally infected gastropod intermediate hosts. J. Parasitol. 92, 298–305.

Johnson, K.P., Bush, S.E., Clayton, D.H., 2005. Correlated evolution of host and parasite body size: tests of Harrison's Rule using birds and lice. Evolution 59, 1744–1753.

Kafle, P., Lejeune, M., Verocai, G.G., Hoberg, E.P., Kutz, S.J., 2015. Morphological and morphometric differentiation of dorsal-spined first stage larvae of lungworms (Nematoda: Protostrongylidae) infecting muskoxen (*Ovibos moschatus*) in the central Canadian Arctic. Int. J. Parasitol. Parasites. Wildl. 4, 283–290.

Klein, D.R., 1992. Comparative ecological and behavioral adaptations of *Ovibos moschatus* and *Rangifer tarandus*. Rangifer 12, 47–55.

Kutz, S., Hoberg, E.P., Polley, L., Jenkins, E., 2005. Global warming is changing the dynamics of Arctic host–parasite systems. Proc. R. Soc. B 272, 2571–2576.

Kutz, S.J., Asmundsson, I., Hoberg, E.P., Appleyard, G.D., Jenkins, E.J., Beckmen, K., Branigan, M., Butler, L., Chilton, N.B., Cooley, D., Elkin, B., Huby-Chilton, F., Johnson, D., Kuchboev, A., Nagy, J., Oakley, M., Polley, L., Popko, R., Scheer, A., Simard, M., Veitch, A., 2007. Serendipitous discovery of a novel protostrongylid

(Nematoda : Metastrongyloidea) in caribou, muskoxen, and moose from high latitudes of North America based on DNA sequence comparisons. Can. J. Zool. 85, 1143–1156.

Kutz, S.J., Checkley, S., Verocai, G.G., Dumond, M., Hoberg, E.P., Peacock, R., Wu, J.P., Orsel, K., Seegers, K., Warren, A.L., Abrams, A., 2013. Invasion, establishment, and range expansion of two parasitic nematodes in the Canadian Arctic. Glob. Change Biol. 19, 3254–3262.

Kutz, S.J., Ducrocq, J., Verocai, G.G., Hoar, B.M., Colwell, D.D., Beckmen, K.B., Polley, L., Elkin, B.T., 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. In: Advances in Parasitology, vol. 79. Elsevier Academic Press Inc, San Diego, pp. 99–252.

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, 217–228.

Laaksonen, S., Pusenius, J., Kumpula, J., Venalainen, A., Kortet, R., Oksanen, A., Hoberg, E., 2010. Climate change promotes the emergence of serious disease outbreaks of filarioid nematodes. Ecohealth 7, 7–13.

Lankester, M.W., 2001. Extrapulmonary Lungworms of Cervids. Parasitic Diseases of Wild Mammals, second ed., pp. 228–278

Lankester, M.W., Hauta, P.L., 1989. *Parelaphostrongylus andersoni* (Nematoda: Protostrongylidae) in caribou (*Rangifer tarandus*) of northern and central Canada. Can. J. Zool. 67, 1966–1975.

Miller, F.L., Gunn, A., 2003. Catastrophic die-off of Peary caribou on the western queen Elizabeth Islands, Canadian High Arctic. Arctic 381–390.

Perkins, S., Martinsen, E., Falk, B., 2011. Do molecules matter more than morphology? Promises and pitfalls in parasites. Parasitology 138, 1664–1674.

Poole, K.G., Gunn, A., Patterson, B.R., Dumond, M., 2010. Sea ice and migration of the Dolphin and Union caribou herd in the Canadian Arctic: an uncertain future. Arctic 414–428.

Post, E., Bhatt, U., Bitz, C.M., Brodie, J., Fulton, T.L., Hebblewhite, M., Kerby, J., Kutz, S., Stirling, I., Walker, D.A., 2013. Sea ice as driver of ecological responses to climate change in the Polar Regions. Science 341.

Prestwood, A.K., 1972. *Parelaphostrongylus andersoni* sp. n. (Metastrongyloidea: Protostrongylidae) from the musculature of white-tailed deer (*Odocoileus virginianus*). J. Parasitol. 897–902.

Pybus, M., Shave, H., 1984. *Muellerius capillaris* (Mueller, 1889)(Nematoda: Protostrongylidae): an unusual finding in rocky Mountain bighorn sheep (*Ovis canadensis canadensis* Shaw) in South Dakota. J. Wildl. Dis. 20, 284–288.

R Core Team, 2016. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ray, J.C., Cichowski, D.B., St-Laurent, M.-H., Johnson, C.J., Petersen, S.D., Thompson, I.D., 2015. Conservation status of caribou in the western mountains of Canada: protections under the species at Risk Act, 2002-2014. Rangifer 35, 49–80.

Van Hemert, C., Flint, P.L., Udevitz, M.S., Koch, J.C., Atwood, T.C., Oakley, K.L., Pearce, J.M., 2015. Forecasting wildlife response to rapid warming in the Alaskan Arctic. BioScience 65, 718–728.

Verocai, G.G., 2015. Contributions to the Biodiversity and Biogeography of the Genus Varestrongylus Bhalerao, 1932 (Nematoda: Protostrongylidae), Lungworms of Ungulates, with Emphasis on a New Nearctic Species. University of Calgary.

Verocai, G.G., Kutz, S.J., Simard, M., Hoberg, E.P., 2014. *Varestrongylus eleguneniensis* sp. n. (Nematoda: Protostrongylidae): a widespread, multi-host lungworm of wild North American ungulates, with an emended diagnosis for the genus and explorations of biogeography. Parasit. Vect 7, 556.

Vors, L.S., Boyce, M.S., 2009. Global declines of caribou and reindeer. Glob. Change Biol. 15, 2626–2633.

Yang, S., Rothman, R.E., 2004. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect. Dis. 4, 337–348.

Zittlau, K.A., 2004. Population Genetic Analyses of North American Caribou (Rangifer tarandus). PhD dissertation. Department of Biological Sciences, University of Alberta, Edmonton, Alberta.