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

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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

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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