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Anisakids (Nematoda: Anisakidae) from ringed seal, *Pusa hispida*, and bearded seal, *Erignathus barbatus* (Mammalia: Pinnipedia) from Nunavut region

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As many Arctic fish species are intermediate hosts of anisakids, they are present in the diet of the ringed seal, Pusa hispida, *and the bearded seal,* Erignathus barbatus. *Parasitic nematodes from the stomachs of 66 seals caught in the Nunavut region (Canada) from two communities (Arviat and Sanikiluaq) from October 2007 to January 2008 have been examined in order to identify the epidemiological risk for Inuit communities who consume traditional food. In Arviat 2428 anisakids were observed in 37 seals, while in Sanikiluaq 316 Anisakidae were isolated from 29 seals. The worms were treated with a host tissue, washed in deionized water and stored until analysis in 70% ethanol. The parasites were divided into three parts. The anterior and posterior parts were stored in 70% ethanol containing 5% glycerol and were examined using a light microscope by evaporation of the ethanol/glycerin mixture. The central parts were prepared for molecular identification by fixing in 70% ethanol. Using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method, the following members of the family Anisakidae were identified:* Contracaecum osculatum *A and C and* Pseudoterranova bulbosa. *In the studied material, more adult worms were noted than larval stages. The most numerous nematodes were* P. bulbosa*, and mixed infection was observed. The mean prevalence of anisakids infection was 43.2% in the Arviat and 37.9% in the Sanikiluaq communities.*

Keywords: ringed seal, bearded seal, *Pseudoterranova bulbosa*, *Contracaecum osculatum* A and C, North Canada

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INTRODUCTION

Hudson Bay is a subarctic ecosystem in which seals play a significant role. The ringed seal, *Pusa hispida* (Schreber, 1775) and the bearded seal, *Erignathus barbatus* (Erxleben, 1777) are both important species in the marine ecosystem, being the major consumers of marine fish and invertebrates. The listed seal species have a significant role in the diet of coastal Inuit communities (Hopping *et al.*, 2010), who consume semi-dried flesh, which carries a risk of anisakiasis, zoonosis caused by the third-stage larvae of nematodes from the Anisakidae family. Also, as definitive hosts for anisakids, seals play an important role in the transmission of these parasites to fish, which are traditionally consumed raw, or prepared in ways that do not kill the larvae, such as smoking or light salting. Potentially dangerous for humans, anisakid nematodes, *Contracaecum osculatum* s.l. and *Pseudoterranova decipiens* s.l. are the only common members of the family Anisakidae in pinnipeds from the northern hemisphere. In Canada, not many cases of anisakiasis are documented (Couture *et al.*, 2003), mainly because clinical symptoms, such as diarrhoea, vomiting, a tingling sensation in the throat, are similar to other diseases and are often overlooked in differential diagnostics. The success of any parasitic disease control depends on the accurate identification of the parasites causing the diseases. For anisakid nematodes the life cycle stage identification is central to the diagnosis of anisakiasis in humans and animals, and therefore is an important step for disease surveillance and control (Chen *et al.*, 2008).

The parasite fauna of the ringed seal, *Pusa hispida*, from Arctic waters is known from studies performed by Myers (1957), Adams (1988) and Measures & Gosselin (1994). The most abundant parasites of *P. hispida* are helminths of the gut tract (Johansen *et al.*, 2010). Ringed seal is described as a host for *C. osculatum* s.l. (Valtonen *et al.*, 1988), a common parasite in other Arctic seal species, such as *Phoca groenlandica*, *Cystophora cristata* and the bearded seal, *E. barbatus* (Adams, 1988; Measures & Gosselin, 1994). Another anisakid worm, *Pseudoterranova decipiens* s.l., has a more boreal distribution and is more common in grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) (McClelland, 1980; Brattey & Stenson, 1993). Both nematode species listed above constitute a complex of sibling species, which are morphologically almost indistinguishable, but are different genetically, so that molecular characterization is necessary.

The bearded seal is the main definitive host so far detected for *Pseudoterranova bulbosa* (species from *P. decipiens* complex) (Mattiucci *et al.*, 1998). Also, *Contracaecum osculatum* A,

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species from *C. osculatum* s.l., which occurs in the Norwegian and Barents Seas, Canadian Atlantic and Pacific waters, Icelandic waters and the Sea of Japan, is known as a parasite of *E. barbatus* and the grey seal, *H. grypus* (Brattey & Stenson, 1993). *Halichoerus grypus* is also a definitive host for *C. osculatum* C (Nascetti *et al*., 1993), but this helminth was not recorded in *E. barbatus* (Mattiucci & Nascetti, 2008).

The purpose of this work was to study the occurrence of parasitic nematodes, potentially dangerous for humans, in two species of seals at two sites in the Canadian Arctic. The occurrence of Anisakidae in ringed and bearded seals was studied because, as definitive hosts of *C. osculatum* s.l. and *P. decipiens* s.l., they play an important role in the transmission of these parasites to fish used as food source by the Inuit. Also, the infection mechanisms were investigated by looking into the developmental stages of the parasites in seals to assess the risk of zoonotic diseases in Inuit traditional foods.

MATERIALS AND METHODS

Parasite materials

Pusa hispida and *Erignathus barbatus* seal species, commonly consumed in Inuit communities, were hunted by professional hunters from October 2007 to January 2008 in two Nunavut communities (Arviat and Sanikiluaq, Figure 1). Hunts were not regular, but seasonal, dependent on temperature, time and funds available for hunting. Hunters were trained in sample collection techniques, provided with sampling kits and asked to record on standardized forms where the animal was caught. Basic morphometrics, e.g. body length, weight and sex, were recorded for 28 of 66 caught seals. The samples were frozen until analysis in the laboratory. Seal stomach contents and stomach walls were macroscopically examined by Inuit students for lesions and anisakid parasites, as an important source of human infection. Seals were systematically investigated only for the presence of nematodes, because of their high zoonotic potential. Anisakids from the stomachs of 59 ringed and seven bearded seals were isolated, counted and collected. After isolation from host tissues, worms were washed in deionized water and stored until analysis in 70% ethanol. Parasites were divided into three parts. Anterior and posterior parts were stored in 70% ethanol with 5% glycerol and examined using a light microscope by evaporation of the ethanol/glycerin mixture. The systematic classification of the worms was established on the basis of features described by Fagerholm (1991). Central parts were prepared for molecular identification by fixing in 70% ethanol.

DNA isolation and amplification

Random samples of nematode species identified morphologically were also checked using a molecular key. The central parts of the parasites, including 45 of *Contracaecum osculatum* s.l. and 45 of *Pseudoterranova decipiens* s.l., taken randomly from hosts and locations, were used for the DNA isolation.

Genomic DNA from nematodes was isolated using a procedure described by Hoarau *et al.* (2002). Amplification of rDNA markers was done according to the procedure described by Zhu *et al.* (1998). Each reaction mixture (total volume of 16 μl) contained 1 μl solution of isolated genomic DNA, 1U DyNAzyme II DNA Polymerase (Finnzymes, Finland), dNTPs (250 μM each), 100 μM of each primer (NC2 and NC5; Zhu *et al.*, 1998) and buffer 1 × (10 mM Tris-HCl (pH = 8.4), 50 mM KCl, 0.1% Triton X-100 and 1.5 mM

Fig. 1. Study area.

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$MgCl_2$). Amplification was carried out in a Techne Progene (UK) thermocycler. The cycling protocol was 5 min at 94°C, 30 cycles with 30 s at 94°C, 30 s at 60°C and 30 s at 72°C, followed by 5 min at 72°C. Polymerase chain reaction (PCR) products were separated electrophoretically on 1% agarose gels and visualized by staining with ethidium bromide.

Restriction fragment length polymorphism

The amplified DNA samples were digested with AluI, RsaI, TaqI, HhaI, HinfI and BsuRI (Fermentas, Lithuania) restriction enzymes and the products were separated on 4% agarose gels and visualized by staining with ethidium bromide (Sambrook *et al.*, 1989). The pUC Mix Marker (0.5 µg/µl; Marker 8, Fermentas) was used as a marker. Product sizes were compared to rDNA ITS1–5.8S–ITS2 digestion patterns keys (D'Amelio *et al.*, 2000; Kijewska *et al.*, 2002).

In the case of unclear digestion patterns, the rDNA ITS1–5.8S–ITS2 region was amplified, sequenced and used as a control. Primers Nc13 (Zhu *et al.*, 1998) and Anc13 (Kijewska *et al.*, 2009) were used. Sequencing was performed by Macrogen Inc. (Seoul, Korea).

In this paper, whenever we talk about the results of genetic tests, we use the names of particular sibling species from the complex (e.g. *P. bulbosa*). When we describe the results of morphological studies, we name the species complex (e.g. *P. decipiens* s.l.), because not all specimens have been examined molecularly.

Statistical analysis

The basic parasitological parameters were calculated according to the Rózsa *et al.* (2000) definition. Only certain species data were subjected to statistical analysis, based on adequate sample sizes and distribution of samples in both Arviat and Sanikiluaq. The non-parametric tests (Mann–Whitney *U*-test and Spearman's rank correlation) were used because of irregular distribution of parasites caused by accumulation of larvae and the diet preferences of each seal. The data were checked according to differences between the intensity of parasite infection in two areas of hunting.

RESULTS

The Sanikiluaq seals stomach contents were thoroughly investigated. In bearded seal stomachs, Sticheidae spp. and a few shrimps were observed. In the case of ringed seals, the shrimps and Ammodytidae were found in 85% and 57% of stomachs, respectively. The stomach content of seals from Arviat was not investigated.

In the Arviat community, 2428 anisakids were observed in 31 ringed and six bearded seals, while in Sanikiluaq, 316 Anisakidae were isolated from 28 ringed and one bearded seal. In one ringed seal from Sanikiluaq a few specimens of cestodes were found and used as a teaching material for Inuit students to learn the different preservation and collection techniques. Unlike the the anisakids, not all cestodes were systematically collected.

Nematodes morphologically of two species, *Pseudoterranova decipiens* s.l. and *Contracaecum osculatum* s.l., were observed. Among these species, the most numerous in Arviat was *P. decipiens* s.l., while in Sanikiluaq *C. osculatum* s.l. was present in greater numbers (Table 1). Accordingly, mixed infection with *C. osculatum* s.l. and *P. decipiens* s.l. was observed, especially in the Arviat community. Note that *P. decipiens* s.l. in ringed seals was not reported in Sanikiluaq but was observed in Arviat (intensity 11.00). Mean prevalence of anisakids infection was 43.2% and 41.4% in Arviat and Sanikiluaq, respectively.

Bearded seals in both communities have a greater number of nematodes than ringed seals (Figure 2). Furthermore all *Erignathus barbatus* specimens are hosts for *P. decipiens* s.l. (prevalence 100%), while in *Pusa hispida, C. osculatum* s.l. is the dominant species. Moreover, ringed seals in Arviat are characterized by a greater intensity of *C. osculatum* s.l. infection (6.00) than in Sanikiluaq (2.18) (Table 1). There are significant differences between the intensity of parasite infection between ringed and bearded seals in the two areas of hunting (Mann–Whitney *U*-test; $P < 0.0001$).

Generally, more adult worms were noted than L4 larval stages, while females were more numerous than males in the two communities (Figure 2). Exceptionally, in Arviat ringed seals, L4 dominated, with 98.3% prevalence. There was a high predominance of female *C. osculatum* s.l. and *P. decipiens* s.l. in bearded seals from Arviat.

Molecular determination confirmed the affiliation of nematodes to the identified species' complexes. Genetically, *C. osculatum* A (in both seal species and communities), *C. osculatum* C (in ringed seals from Sanikiluaq) and *P. bulbosa* (in one ringed seal from Arviat and bearded seals from both localities) were identified. All of *P. decipiens* s.l. studied molecularly were *P. bulbosa*.

DISCUSSION

Anisakids are cosmopolitan parasites with a wide geographical distribution. In Canadian Arctic waters, so far,

Table 1. Number, abundance, prevalence (%), intensity and mean intensity of infection of the ringed seal (*Pusa hispida*) and bearded seal (*Erignathus barbatus*) from the Nunavut region.

Region	Host (*N*)	Species (s.l.)	*N*	Abundance	Prevalence (%)	Mean intensity (intensity)
Arviat	*P. hispida* (31)	*P. decipiens*	11	0.4	3.23	11 (11)
		C. osculatum	59	1.9	32.26	6 (1–24)
	E. barbatus (6)	*P. decipiens*	1590	265.0	100.00	265 (116–641)
		C. osculatum	768	128.0	66.67	192 (55–359)
Sanikiluaq	*P. hispida* (28)	*P. decipiens*	0	–	–	–
		C. osculatum	24	0.9	39.29	2.18 (1–7)
	E. barbatus (1)	*P. decipiens*	57	57.0	100.00	57 (57)
		C. osculatum	235	235.0	100.00	235 (235)

P. decipiens, Pseudoterranova decipiens; C. osculatum, Contracaecum osculatum.

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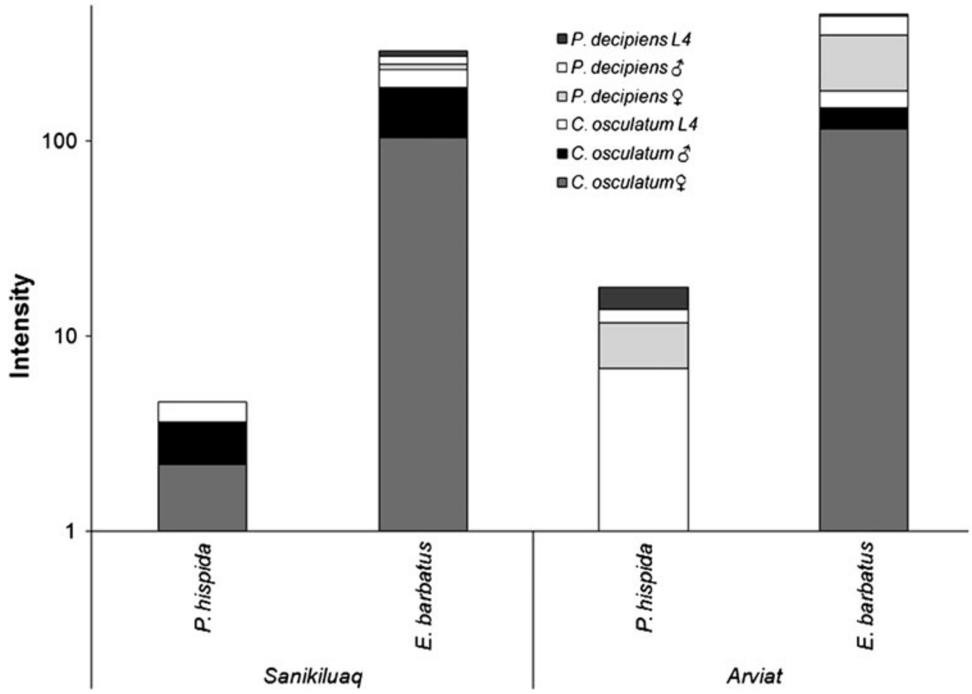

Fig. 2. Developmental stage intensity of *Pseudoterranova decipiens* s.l. and *Contracaecum osculatum* s.l. of ringed and bearded seals in Sanikiluaq and Arviat.

Pseudoterranova bulbosa has been recorded in bearded seal and *Contracaecum osculatum* A in bearded and grey seals (Mattiucci & Nascetti, 2008). In the present study, seals from the Nunavut region were infected with three anisakids species: *P. bulbosa*, *C. osculatum* A and C. Interestingly, *C. osculatum* A occurred in ringed and bearded seals from the two communities, while *C. osculatum* C was noted only in *Pusa hispida* from Sanikiluaq. It is the first record of *C. osculatum* A and C in Hudson Bay. *Contracaecum osculatum* C in *P. hispida* has so far been observed in the waters around Iceland and the Baltic Sea, but never reported in the western part of the Atlantic or Pacific waters (Mattiucci & Nascetti, 2008). It is important to investigate the occurrence of anisakid nematodes in seals because high anisakid prevalence in definitive hosts can potentially contribute to stronger infection in fish.

Contracaecum osculatum s.l. is the dominant species in *P. hispida*, and it has greater mean intensity of infection in Arviat (6.00) than in Sanikiluaq (2.18). Note that *Pseudoterranova bulbosa* in ringed seals occurred in only one specimen in Arviat and in none in Sanikiluaq. Notes taken from this study showed that the stomachs of ringed seals from Sanikiluaq were dominated by shrimps and a large number of Ammodytidae, which are a major component of ringed seal autumn diet (Chambellant, 2010). Both zooplankton and fish (anisakid intermediate hosts) are planktonic, which explains the absence of *P. bulbosa* in *Pusa hispida* from Sanikiluaq. In Sanikiluaq's bearded seals stomachs, benthic organisms such as Sticheidae and shrimps were found, which confirms the food preferences of *Erignathus barbatus,* which feed on the bottom, primarily on crustaceans, molluscs, clams, squid and fish (Finley & Evans, 1983). Different food preferences of the two studied seal species have an impact on the state of their parasitic fauna. Bearded seals, which feed on benthic organisms, are much more strongly infected in both localities ($P < 0.0001$).

Based on this survey, we can conclude that the food preferences of seals have an impact on anisakid species infection. Finding only the Anisakidae L4 larval forms and adult stages leads us to a conclusion that the consumption of semi-dried seal intestines does not pose such a threat of anisakiasis infection for humans as the fish-living invasive stages of L3 larvae. Nevertheless we should still remember that seals play an important role in the transmission of anisakid parasites to fish used as a prominent food source by the Inuit.

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