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Original article

Pathological changes of stomach in ringed seal (*Pusa hispida*) from Arviat (North Canada) caused by anisakid nematodes

Z. Sołtysiak[1], M. Simard[2], J. Rokicki[3]

[1] Parasitology Unit of the Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wrocław University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wrocław, Poland
[2]Nunavik Research Centre, Makivik Corporation, Kuujjuaq, Quebec, J0M 1C0
[3] Department of Invertebrate Zoology and Parasitology University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

Abstract

This is a report of lesions associated with the nematodes (Anisakidae) from the stomach of ringed seals (*Pusa hispida*). On thirty one examined ringed seals from Arviat, thirteen were infected by 1 to 24 anisakids. Identification of nematodes from their stomachs showed two species; *Contracaecum osculatum* (sensu lato) (79 specimens) and *Pseudoterranova decipiens* (sensu lato) (11 specimens). In the gastric sections with parasites, larvae and adults of nematodes were present mainly in the fundic portion of the stomach. The anterior parts of the nematodes were embedded in mucosa and submucosa. Anisakids were associated with ulcerous gastric lesions and raised inflammatory areas in the stomachs. The histological examination of a sample taken from the tissue surrounding anisakids revealed the presence of more or less confluent focal necrotic areas. Most small petechial hemorrhages were located in the mucosal layer of the gastric wall and were surrounded by inflammatory mononuclear cells such as lymphocytes, histiocytes, eosinophils and fibroblasts. The Anisakidae larvae in the stomach caused atrophy of glands, hemorrhaging and eosinophilic infiltrations that consequently healed, creating fibrotic scars.

Key words: ringed seal, *Pseudoterranova decipiens*, *Contracaecum osculatum*, parasitic gastritis

Introduction

Anisakidae nematodes are parasite species of veterinary, medical, and economic importance, living in marine fish and mammals from the northern and southern hemispheres (Smith and Wootten 1978). Adults of most of these parasites live in the alimentary tract of marine vertebrate hosts (cetaceans and pinnipeds). *Delphinidae* are the main *Anisakis* spp. final host. Adults of *Pseudoterranova decipiens* (sensu lato) and *Contracaecum osculatum* (sensu lato) are found mainly in *Otariidae* and *Phocidae* (Smith and Wootten 1978).

Ringed seal (*Pusa hispida*) has a circumpolar distribution and is generally found in close association with sea ice. During the winter, ringed seals occupy

Correspondence to: J. Rokicki, e-mail: rokicki@ug.edu.pl, tel.: +48 58 523 61 92

land-fast ice for breeding and molting and the rest of the year they spend time in open waters where they mostly feed. The diet of ringed seals in Arviat is dominated by sandlance with a few invertebrate species (Chambellant 2010).

Research conducted on helminth parasites in ringed seals that reported stomach clusters are from Johansen et al. (2010) in Svalbard and from Geraci (2003) in the western Canadian Arctic. Other reports on anisakids in ringed seals are by Adams (1988), Vik (1986), Popov et al. (1993), Treshchev and Popov (1993), Measures and Gosselin (1994), Lucas et al. (2003), Johansen et al. (2010) and from *Pusa hispida botnica* in Bothnian Bay (Valtonen et al. 1988). Many parasitic nematodes create some damage to the tissue of intermediate and definitive hosts, causing pathology during their prepatent and patent phases. Arctic sea mammals are often infected by anisakids, where adult and larval stages live in the gastric and intestine parts of the digestive tract.

The size of pathological changes recorded in ringed seals caused by various species of anisakids is not clear. It is caused by the fact that usually observation was made on the occasion of other studies and often nematodes found were identified to genus. The purpose of this work was to determine the influence of larval and adult stages of anisakids on the stomach wall of ringed seals.

Materials and Methods

Thirty one ringed seals (*Pusa hispida*) were collected from Arviat (61° 63' N and 94° 32' W) on the west coast of Hudson Bay between October 17 and November 1, 2007. All ringed seals from the Arctic area of Canada (Arviat – a hamlet in the territory of Nunavut) were examined. Stomach samples with nematodes were collected for histopathological examination. All samples were fixed in 10% buffered formalin, dehydrated in graded alcohol and xylene and embedded in paraffin, six fm thick sections were stained with hematoxylin and eosin. Nematodes recovered from the stomach were fixed in 70% ethanol with 5% glycerol, cleared by evaporation of the ethanol/glycerin mixture and examined using light microscope.

Results

Thirteen examined seals were infected by 90 anisakids (intensity 1-24, average 7,61 individuals). Identification of nematodes from the stomach by microscopy showed two species; *Contracaecum osculatum* s.l. (79 specimens) and *Pseudoterranova decipiens* s.l. (11 specimens). Fourth stage larvae (L_4) of *C. osculatum* dominated and the rest were adult females, whereas the number of *P. decipiens* L_4, females and males was similar. The L_4 were often attached to the mucosa of the stomach and the adults were commonly found in the lumen. Histology examination showed L_4 and adult parasite attached to the gastric wall. The anterior parts of anisakids attached to the gastric mucosa and submucosa were ruptured and were also associated with ulceration. A mucosa surrounding the anisakids revealed the presence of more or less confluent focal necrotic areas (Fig. 1a). Most small petechial hemorrhages were located in the mucosa layer of the gastric wall (Fig. 1b) and were surrounded by inflammatory mononuclear cells such as lymphocytes, histiocytes, eosinophils and fibroblasts. At the centre of the necrotic areas, single or multiple parasitic elements of irregular shape, with a thick segmented cuticle covering the dorsal and ventral musculature and the lateral, dorsal and ventral chords were observed. Inside the pseudocoel, the elements resembling gastroenteric-like structures and the lateral chords of the parasite were detected. The worms provoked a surrounding granulomatous reaction, containing a central core of necrotic and cellular debris and large numbers of eosinophils. Lesions exhibited an inflammatory response of the lamina mucosa (Fig. 2a) and submucosa (Fig. 2b), but did not reach the gastric muscularis. The gastric glands near the attachment of the parasites were damaged. The *Anisakidae* larvae penetrated deeply into the stomach wall and led to hemorrhages and eosinophil infiltrations causing atrophy and sometimes formation of small cysts, consequently creating tissue scars with multiple fibroblasts (Fig. 2c). Necrotic foci were sometimes calcified.

Discussion

Parasite-associated lesions are severe and cause ulcerative gastritis in adjacent areas. Our study showed that the inflammation caused by *Anisakidae* are limited to the mucosa and submucosa of the stomach. Damage to the muscle was not observed in the sections examined. These results are similar to that of McClelland (1980) who reported that anterior extremities of *Pseudoterranova decipiens* and *Contracaecum osculatum* were embedded in the mucosa and submucosa of the stomach of ringed seals, harbour (*Pusa vitulina*) and grey seal (*Halichoerus grypus*).

Anisakids, especially larval stages, are protected from host immune system by lipids of the cuticle

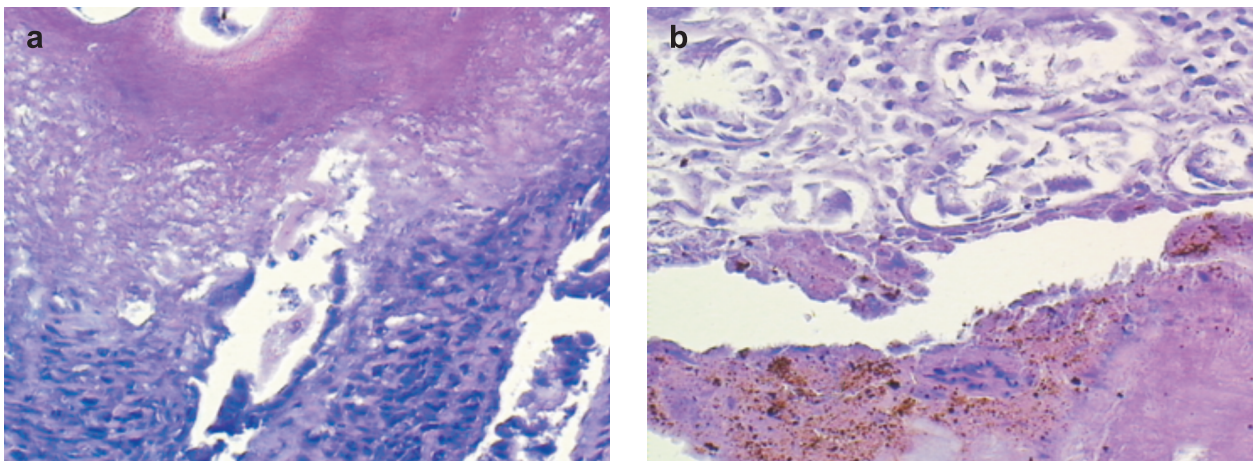

Fig. 1a,b. The site of attachment of the nematodes *Contracaecum osculatum* and *Pseudoterranova decipiens*, with necrosis of stomach mucosa (a), and haemosiderin, a consequence of hemorrhage (b) ringed seals *(Pusa hispida),* H-E, magnification x 200.

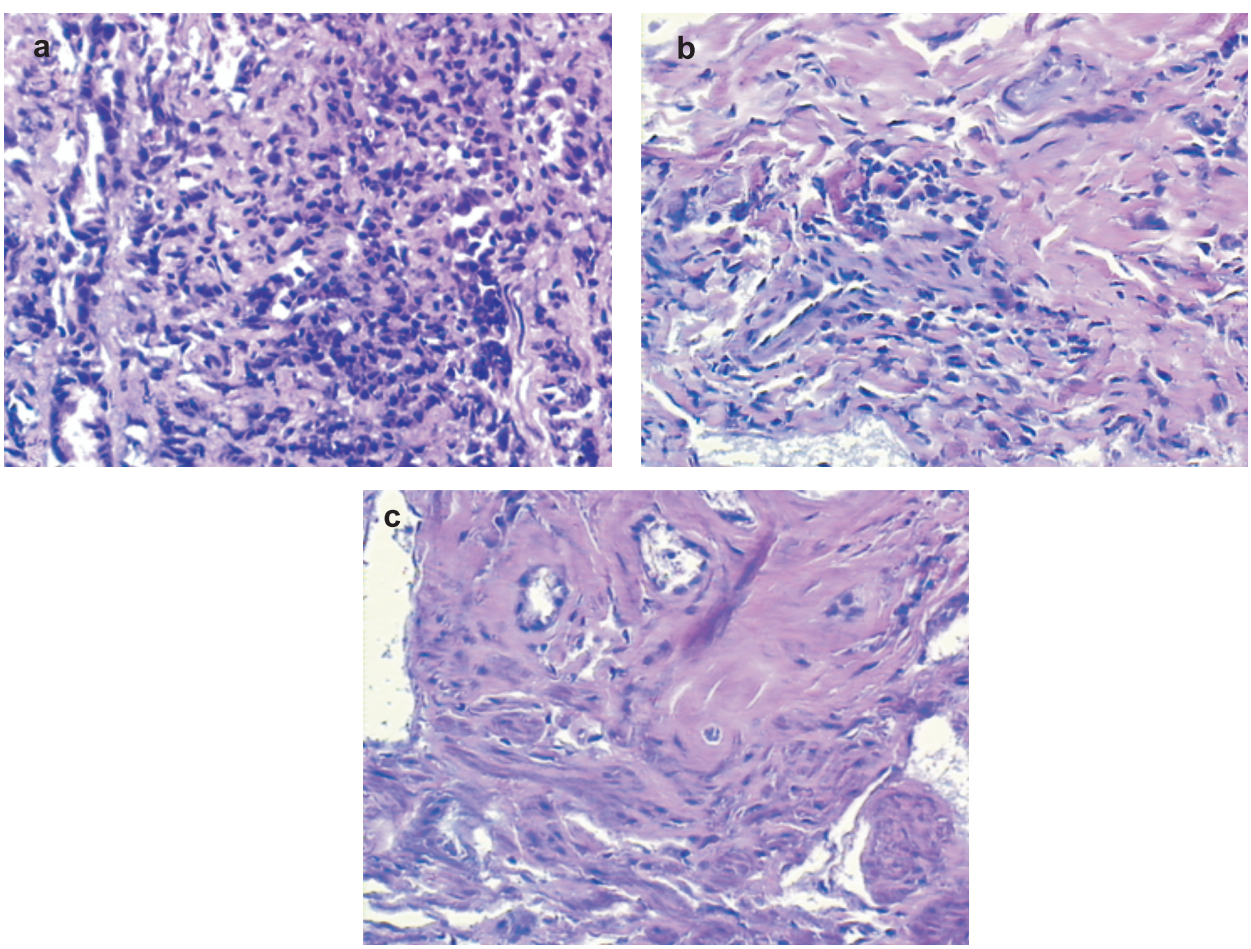

Fig. 2a,b,c. Inflammatory infiltration such as lymphocytes, histiocytes, eosinophils and fibroblasts in stomach mucosa (a) and submucosa (b). Diffuse atrophy of glands consequently creating tissue scars with multiple fibroblasts (c). ringed seal *(Pusa hispida)*, H-E, magnification x 200.

(Blaxter 1993, Mika et al. 2010). Partially embedded fragments of nematodes or cuticle in Arviat ringed seals' stomach caused an increase of eosinophils. Similarly, a few grossly visible granulomas caused by anisakids were observed in stomach of harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) from Sable Island, Nova Scotia, Canada. Harp seals parasites were identified as adult *Con-*

tracaecum osculatum and 3rd and 4th stage larvae of *Contracaecum* sp. (Lucas et al. 2003). Histological examinations revealed eosinophilic granulomas at the lesions and inflammatory areas. In gray seals conspicuous pools of lymphocytes often occurred at the periphery of granulation masses (McClelland 1980).

Pathological changes caused by anisakids seemed to be also a reflection of size of infection which was moderate in ringed seals from Arviat. Larvae of *Phocascaris*/*Contracaecum* sp(p.) in ringed seals from Svalbard were observed in large clusters attached to the stomach wall (Johansen et al. 2010). Comparatively, L_4 and adults anisakids from Arviat ringed seals were embedded in ulcers, singly attached to the stomach wall or aggregated and attached to the mucosa. In Arviat ringed seal infestation was smaller than in Svalbardm's seals, therefore, it could possibly cause weaker pathological changes. This might be explained by the host's different diet, which is mostly composed of highly infected Atlantic cod (*Gadus morhua*) in Svalbard ringed seals (Mouritsen et al. 2010). In Arviat, the ringed seal diet is mainly composed of sand lance (86.2%) and 13.8% of other invertebrate and fish species (Chambellant 2010). This is the first documented case showing that seal diet in Hudson Bay may have an impact on stomach pathology.

In gray seals (*Halichoerus grypus*) the nematodes neither occurred in clusters nor in association with gastric lesions but McClelland (1980) described clusters of adults *P. decipiens* as associated with ulcerous gastric lesions in harbour seals (*Pusa vitulina*) and raised inflammatory areas in the stomach of gray seals. In harbour seals from Wadden Sea, *P. decipiens* (sensu lato) and *C. osculatum* (sensu lato) were observed to create, in most cases, diffuse catarrhalic and lymphocystic gastritis. Only one seal of 107 examined had ulcers associated with nematodes (Lehnert et al. 2007). *P. decipiens* seemed to be more pathogenic in harbour seals than they were in grey seals.

The high degree of pathogenicity of *P. decipiens* exhibited in harbour seals was perhaps indicative of a poor host-parasite relationship (McClelland 1980). Size of currently found pathological changes in the stomach of ringed seal caused by anisakid nematodes may also be a result of host sensitivity. Such changes are different even within parasite species. For example, in the experimental infestation of pigs by L_3 *Anisakis simplex* B from the Baltic Sea, sensitivity of host was different. In the case of sensitive pigs, much greater reactive changes were found in the stomach submucosa than in that of resistant pigs. In this group of pigs nematode larvae have also been traced to the submucosa of the same organ (Rokicki et al. 1993).

Adult specimens of *P. decipiens* and *C. osculatum* found in ringed seals from Arviat confirm that they are the final host of those nematodes. Pathological changes in ringed seal stomachs may be influenced by the proportions of anisakid species, therefore their identification is necessary. Another factor is the level of activity of the excretory-secretory products of both adult nematodes and L_4 larvae which contain a number of hydrolase enzymes that can damage host tissue. Dziekońska-Rynko and Rokicki (2005) suggest that it is *Contracaecum rudolphii* that secretes enzymes and not the mouth apparatus that participates in tissue damage. Some of the enzymes can be responsible for penetration and nutrient uptake during parasite migration in the hosts. Bonner (1990) reported seeing: grey seals in excellent condition, with ample blubber reserves whose stomachs contained many nematodes and determined that parasites did not cause apparent significant harm to the animal, but we argue that the issue is more complex. Factors such as host diet and immunity, nematode intensity and species (with their specific enzymes), may contribute to pathological changes in ringed seal stomachs. Further investigations on parasite species sensitivity for each seal species by regions and seal immunity to each parasite may deepen our current knowledge.

Conclusions

Presence of *Contracaecum osculatum* and *Pseudoterranova decipiens* in *Pusa hispida* from Arviat causes necrotic-ulcerative changes in the stomach. Fibrotic scars showed that these ulcers were healing.

Acknowledgements

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