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Pathological changes in the auditory organs of the harbor porpoise (*Phocoena phocoena* L.) associated with *Stenurus minor* (Kühn, 1829)

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Abstract

The problem of echo disruption has been frequently noted by authors who have studied parasites of harbor porpoise. Six harbor porpoises were examined macroscopically and microscopically for the occurrence of *Stenurus minor*. The present investigation confirmed earlier observations that this nematode may probably cause auditory malfunction, as there were changes to the auditory nerves of the cochlea and middle ear in consequence of chronic or acute inflammation with parasitic infection in animals. Moreover, it can be stated that damage caused by *S. minor* produced severe stress and pain, which, very likely, affected the animal behavior.

Key words

Stenurus minor, Nematoda, *Phocoena phocoena*, marine mammals, pathology, auditory organs

Introduction

The harbor porpoise (*Phocoena phocoena* L., 1758) is the only cetacean permanently occurring in Baltic Sea (Skóra 1991). In Polish waters, the harbor porpoise is, however, a rare species, and is thus protected by Polish law.

Stenurus minor (Kühn, 1829) is the most common parasite infecting the ear of the harbor porpoise (Deliamure 1955, Arnold and Gaskin 1975, Dailey and Stroud 1978, Dailey 1985, Wunschmann *et al.* 2001). Deliamure (1955) was the first to draw attention to the possible connection between *S. minor* and hearing malfunction in harbor porpoises. The presence of this nematode in ears of porpoises was also considered a reason for the atypical behavior of these animals (Ridgway 1972). It was suggested that *S. minor* could account for stranding in these animals (Geraci 1978). However, Dailey and Stroud (1978) concluded that parasites in ears did not appear to cause much harm. Their histological examination of infected porpoises revealed only chronic, low-grade inflammation of the mucous membranes.

The aim of the present investigation was to examine and describe the histopathological changes caused by *S. minor* infection of the Eustachian tubes and the tympanic cavities of six harbor porpoises.

Materials and methods

Six porpoises were examined between 1996 and 1998. One of them was found dead on a Polish beach; fishing vessels off the Polish coast had caught another five porpoises (Table I). The porpoises were stored deep-frozen at –20°C. After thawing, the Eustachian tube, and the middle and inner ears were examined for the presence of parasites which were collected, fixed in Berland's fluid, and stored in 70% ethanol mixed with 5% glycerol. The mean intensity of infection was calculated according to Margolis *et al.* (1982). The species and sex of the collected parasites were determined by light microscopy according to the key of Arnold and Gaskin (1975).

Table I. Time, place, sex and measurements of examined harbor porpoises *Phocoena phocoena*

No.	Date	Localization	Sex	Length (cm)	Weight (kg)
1	19-09-97	Władysławowo	male	110	25
2	25-07-96	Unieście	male	120	25
3	01-12-97	Jantar	female	117	21
4	06-01-98	Gdańsk Bay	female	114	30
5	09-01-98	Gdańsk Bay	male	155	28
6	03-11-98	Ustka	male	134	33

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Samples from five infected and one uninfected porpoise were prepared for histopathological examination. The control sample was taken from healthy white-beaked dolphin *Lagenorhynchus albirostris* (Gray, 1846), which had no parasites in ears.

The fixed tissues were processed in an autotechnicon and embedded in paraffin wax. They were then cut in 5 μm sections, stained in hematoxylin-eosin, mounted in Canada balsam and examined by light microscopy. The tympano-periotic complexes were examined under a stereomicroscope to assess the effect of the parasites on the surrounding bones.

Results

Approximately 6000 *S. minor* were detected in 5 of 6 harbor porpoises; the mean intensity of infection was 779.6 parasites per ear, SD = ± 739.9. The only animal devoid of parasites was a 110-cm long male (no. 1, see Table I), most likely the youngest in the sample of six. All parasites were found in the Eustachian tube, and the middle and the inner ear. However, the differences in intensity of infection between each part of the ear indicated a higher intensity of infection in the middle ear (Figs. 1 and 2). Comparison of intensity of parasitic infection in left and right middle ear shows parasites located in the right ear outnumber parasites found in the left ear (data not shown). The larger numbers of *S. minor* in the right tympanic cavity and inner ear in all the porpoises may be due to the cranial asymmetry described by Mead (1975) and Yurick and Gaskin (1987). This developmental distortion is most conspicuous in the area surrounding the nasal passages and the frontals. It is linked to the functional division of the nasal complex, where the right side is responsible for sound production and the left side for pulmonary ventilation.

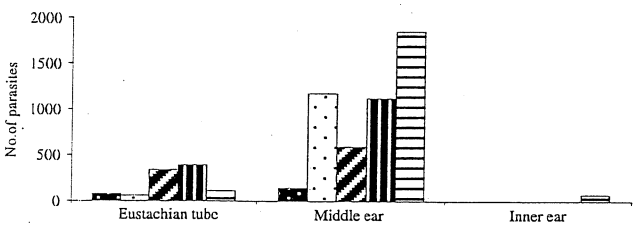


Fig. 1. Number of *Stenurus minor* in various sites within the harbor porpoise *Phocoena phocoena*. Patterns are characteristic for each porpoises

Of the nematodes in the tympanic cavity, 65% were females, 80% of which possessed ova containing first-stage larvae in the uteri. The presence of gravid females of *S. minor* at this site of infection indicates that this species is capable of reproducing not only in the lungs but also in the ears.

In microscopical observations some of changes could be the effect of freezing: loss of staining, extracellular fluid accumulation, cell shrinkage, intracellular vacuolization of epithelial cells and hemolysis. Although these changes are annoying, adequate visualization of the tissues is possible (Baraibar and Schoning 1985). During the examination, changes described above were taken into account and presented data were corrected.

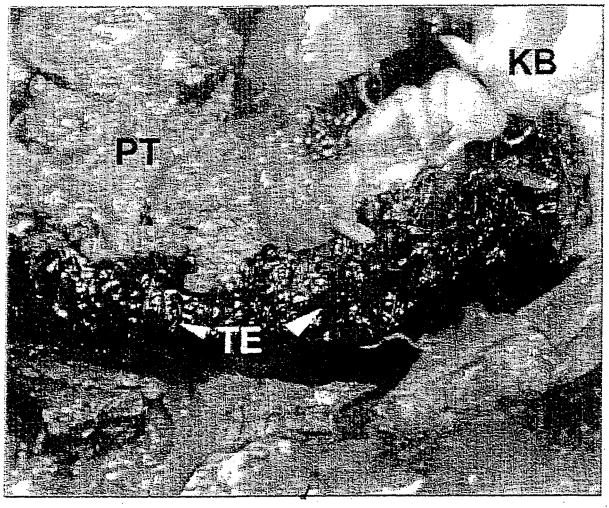


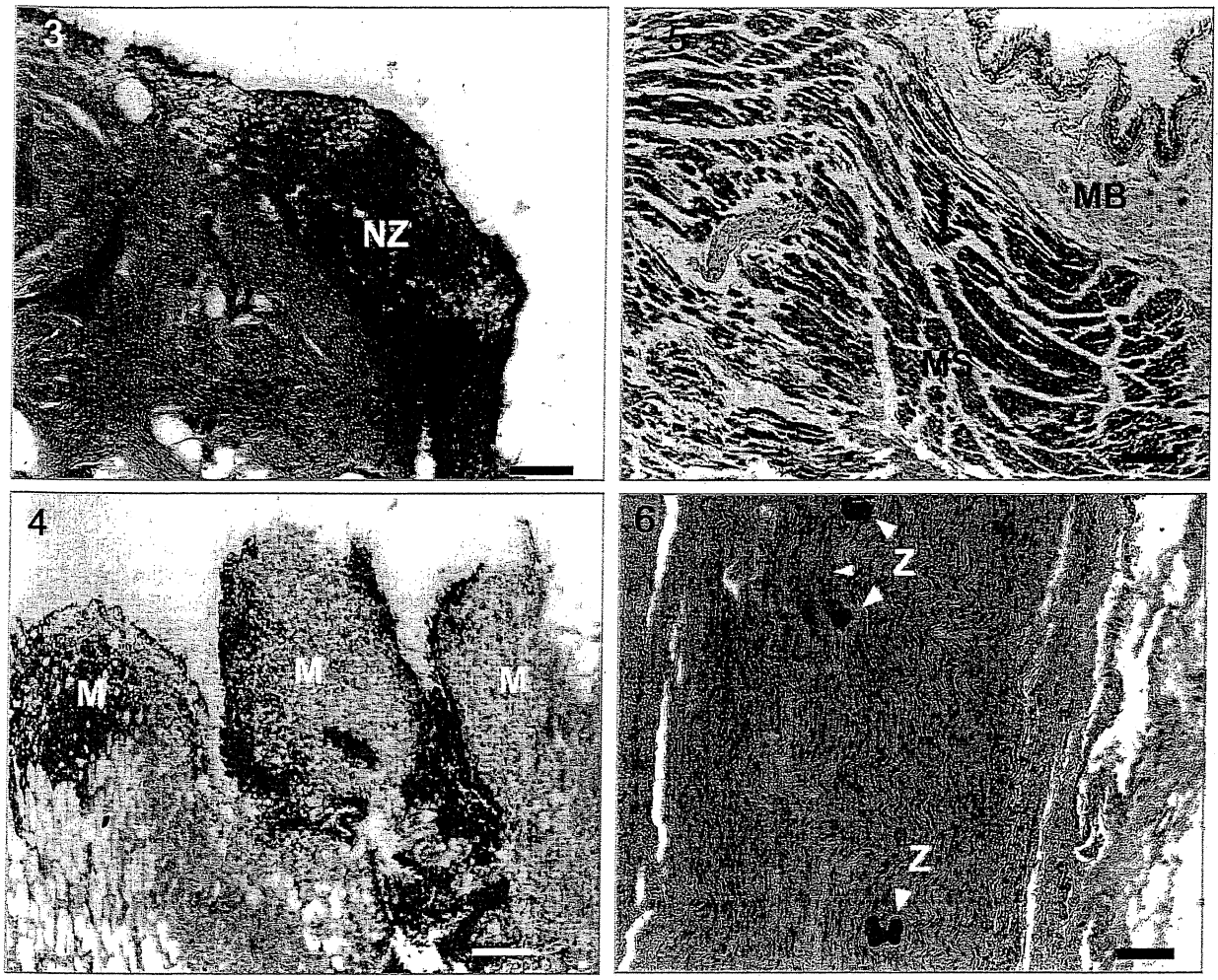
Fig. 2. The anatomical structure of the ear and the presence of parasites within the tympanic bulla and Eustachian tube. The tympanic bulla (KB) and Eustachian tube (TE) with *Stenurus minor*. On the left, the remains of the fatty foam (PT) insulating the ear. Scale = 1.5:1. Photographs made by L. Rolbiecki and M. Pempera

Macroscopic examination from the middle ear of control sample from the healthy harbor porpoise revealed a slightly hyperemic, smooth, elastic mucosa adhered to bone. In three porpoises (number of parasites in both of middle ears did not exceed 1200) the examination revealed a serous, yellow fluid in the lumen of the middle ear, hyperemia, edematous and desquamated mucosa with hemorrhages, granular surfaces, and erosions. In one, the most heavily infected porpoise no. 6 (over 1800 parasites – more than 1100 in right middle ear), the mucous membrane was also gray and frail, and a dense, milky liquid was present within the Eustachian tube.

Microscopic examination of tissues in the middle ear and the Eustachian tube of the control sample revealed the edematous mucosa and fragments of hyalinizing fibrous connective tissue. On the surface, the mucosa was partially covered with the paraepidermal epithelium. Subepithelially, in the mucosa were focal infiltration of the mononuclear cells.

In all infected porpoises (Figs. 3–6) revealed focal subepithelial infiltration of mononuclear cells predominantly lymphocytes, forming lymphoid follicle structures. Fragments of mucosa with diffuse hemorrhages and broadly distended vessels were observed.

All of the tissue sections also displayed a papillary hyperplasia of the mucosa covered by stratified squamous nonkeratinizing epithelium with erosions. Fibrosis and infiltrations were also found in the mucosa. *Stenurus minor* was seen between folds of mucosa. Additionally in the sixth, heavily



Figs. 3–6. Histological sections of the middle ear: **3** – a massive inflammation in the form of dense infiltration of mononuclear cells (NZ) in the mucosa; **4** – inflammation of the mucosa and connective fibrous tissue; the inflammation appears to be an early stage of the abscess (M); **5** – edematous mucosa (MB) adhering to striated muscle (MS) with numerous mononuclear cells (the inflammatory condition of the muscle); **6** – calcifications within the nerve (Z). Scale bars = 250 μm

infected porpoise there were many calcifications in glandular-like structures in the mucosa and in tympanic and facial nerves. Focal fibrosis and inflammatory infiltrations in the striated muscle were noted.

There was no decalcification or erosion on the surfaces of the skull or auditory ossicles.

Analysis of the pathological changes (microscopic examination) in the soft tissues of the middle ear in one heavily infected porpoise suggests that it had been suffering from chronic inflammation in the middle ear (otitis media chronica). This hypothesis is supported by the macroscopic observation of a thick, milky exudate on the surfaces of the Eustachian tube, soft tissue degeneration, and an edematous mucous membrane. Three, severely infected porpoises were suffering from acute inflammation of the middle ear (otitis media acuta), as indicated by edematous mucous membranes and bleeding from peripheral blood vessels. The possibility that the inflammation of the middle ear could spread to adjacent tissues of the inner ear cannot be ruled out.

Discussion

The inflammatory changes to the mucous membrane of the middle ear in four porpoises resulted from irritation induced by the parasites. The nematodes may also exert pressure on the muscles of the tympanic cavity and on the round and oval windows, probably immobilizing them.

Presented observations suggest that the parasites form a soft plug in the middle ear, thereby distorting the transmission of sound waves by muffling them. Furthermore, the discrete inflammation of the skeletal muscles, separated only by a band of edematous mucous membrane, could be due to toxins secreted by the parasites. Through the secretion of such substances, *S. minor* may also affect the inner ear as it was suggested by Geraci (1978).

Calcification of the nerves was observed, but since these injuries had healed, it was impossible to establish their etiology, although the possibility that they were caused by the parasites cannot be excluded. It was likewise not possible to

establish what caused the presence of calcifications – their number was not related to the infection rate but they were noticed in tissues taken from infected porpoises only. By active penetration the mucous membrane, parasites could have damaged or punctured the membrane of the round window, what would explain the fairly high degree of infection in this part of the ear in one porpoise. Perforation of the round window would cause the perilymph to bleed into the middle ear, reducing pressure in the perilymph in the inner ear. With reduced pressure proper functioning of the inner ear would be impaired and, at the very least, severe pain would be produced.

It appears to be likely, that the marked inflammatory reaction of the middle ear, damage of the auditory nerves and round and oval windows occurred. This was probably accompanied by intense pain but neither morphological examination, nor the nature and intensity of the macro- and microscopic changes in ear permit any prediction regarding the degree of hearing impairment. Nonetheless, these changes may expose the animals to severe stress and pain due to the presence of the parasites as it was suggested by Schmidt-Ries (1939), what, in all probability, affects the behavior of porpoises. In extreme cases, according to Ridgway (1972), may cause grounding behavior. Furthermore, the presence of these nematodes in the ears of porpoises could explain why these animals become entangled in fishing nets in accordance with earlier views (Schmidt-Ries 1939, Deliamure 1955, Ridgway 1972).

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