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Source: Journal of Wildlife Diseases, 25(2) : 218-224

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-25.2.218

Journal of Wildlife Diseases, 25(2), 1989, pp. 218–224

PREVALENCE AND PATHOLOGY OF NEMATODE INFECTIONS IN THE LUNGS OF RINGED SEALS (*PHOCA HISPIDA*) OF THE WESTERN ARCTIC OF CANADA

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ABSTRACT: Two genera of lung nematodes were found in a sample of 382 wild ringed seals (*Phoca hispida*). *Otostrongylus circumlitus* were found mainly in young of the year where they occupied the main stem bronchi causing extensive mucus production, mucosal hyperplasia and peribronchitis. Immature stages of the nematode were found in pulmonary vessels causing endarteritis. There was only a slight reduction in respiratory parenchyma in infected seals and no correlation between infection and size or body condition. However, the sharp decline of *O. circumlitus* infection from 32% in young of the year to 0.5% after the first winter suggests that this parasite may play a role in the population dynamics of ringed seals. *Filaroides* (*Parafilaroides*) *hispidus* was found in alveoli of seals throughout all age classes causing no significant lesions. The infection rate increased from 44 to 71% in the 7 to 13-yr-old animals and declined to 38% in older seals. Concurrent infections occurred in 28% of young of the year seals.

Key words: Lung nematode infection, ringed seal, *Phoca hispida*, *Otostrongylus circumlitus*, *Filaroides* (*Parafilaroides*) *hispidus*, verminous pneumonia.

INTRODUCTION

Most pinnipeds appear to be infected by one or several genera of lungworms (Dougherty and Hermann, 1947; Van Den Broek and Wensvoort, 1959; Menschel et al., 1966; Sweeney, 1974). Most of the animals surveyed were in captivity or found debilitated or dead along shorelines. The primary significance of lung nematodes and their associated lung lesions are difficult to evaluate under these circumstances. Ringed seals are reported to be hosts for three genera of lung nematodes: *Otostrongylus* sp., *Dipetalonema* sp. (Delyamure, 1955), and *Parafilaroides* sp. (Delyamure and Alexiev, 1966). The present study reports the prevalence and pathology of lungworm infection in wild populations of ringed seals (*Phoca hispida*).

MATERIALS AND METHODS

A total of 382 ringed seals were collected by netting or during native hunting from two locations in the Amundsen Gulf in the eastern Beaufort Sea. Seals netted at Cape Parry (70°05'N, 124°22'W) were available for complete necropsy while only lungs, jaws and body measurements were obtained from seals shot in the Holman area (70°43'N, 117°43'W).

Body length was determined from the tip of the nose to the end of the tail. The girth was measured immediately behind the front limbs and blubber thickness was taken on the midline on midsternum. These data were expressed as a condition factor using the ratio of girth to body length multiplied by 100.

From fresh lungs, large bronchial parasites found by dissection were fixed in 95% ethanol (50 parts): formalin (10): acetic acid (2): water (40). They were cleared in Berleses fluid (Esbe Laboratory Supplies, Markham, Ontario, Canada L3R 3V6) and identified with reference to the description by Delyamure (1955). Several specimens were sent to L. F. Khalil (Commonwealth Institute of Helminthology, St. Albans, Hertfordshire, England AL1 3EW) for verification of the identification. Microscopic sections of these nematodes were prepared by placing segments of the worms in warm 1% agar. After cooling, the gelled agar was processed routinely for paraffin embedding and 8 μm serial sections stained with hematoxylin/eosin. Three fresh lungs were shipped frozen to the Veterinary Laboratory (Alberta Agriculture, Edmonton, Alberta, Canada T6H 4P2) where small parenchymal parasites were fixed in a solution of 10% glycerin in 70% ethanol for identification (Kennedy, 1986).

Entire lungs collected by hunters were fixed and shipped in 10% formalin before they were dissected for microscopic examination and to recover bronchial nematodes. Ten lungs from young of the year seals with bronchial parasites

were matched with an equal number of lungs from the same age group free of these large lungworms for a quantitative assessment of lung lesions using a modification of methods described by Weibel (1963). The lungs were divided into 10 transverse sections of equal thickness and individually labelled. A point grid with 5-mm spacing was laid over the anterior surface of each slice. The number of points laying over healthy parenchyma were compared to points over non-respiratory lung tissue such as bronchi, connective tissue and consolidating lesions (Fig. 1). Subsequently, samples were obtained for microscopic examination by laying a numbered grid onto the lung slices and selecting 1-cm^2 areas according to a random number table. In order to be selected, at least three-fourths of a square had to be filled with tissue. Four squares or a minimum of 20% from each lung slice were examined. The relationship of *O. circumlitus* infection to condition factor and body length was examined using a *t*-test. The effect of *O. circumlitus* infection on the percent of respiratory parenchyma was tested with a *t*-test after angular transformation. Seals examined for *F. hispidus* were divided into three age classes: ≤6 yr, 7 to 13 yr and >13 yr. Data were analyzed with a Chi-square test for independence. For age determination, jaws were sent to T. G. Smith (Department of Fisheries and Oceans, Arctic Biological Station, Sainte-Anne-de-Bellevue, Quebec, Canada H9X 3L6). Type specimens of *F. hispidus* are deposited in the National Museum of Natural Sciences (Invertebrate Zoology Division, Ottawa, Ontario, Canada K1A 0MA; Accession numbers 1986-0034 to 1986-0050). Specimens of *O. circumlitus* are deposited in the CAB International Institute of Parasitology) 395a Hatfield Road, St. Albans, Hertfordshire, England AL1 3EW; Accession number 5881).

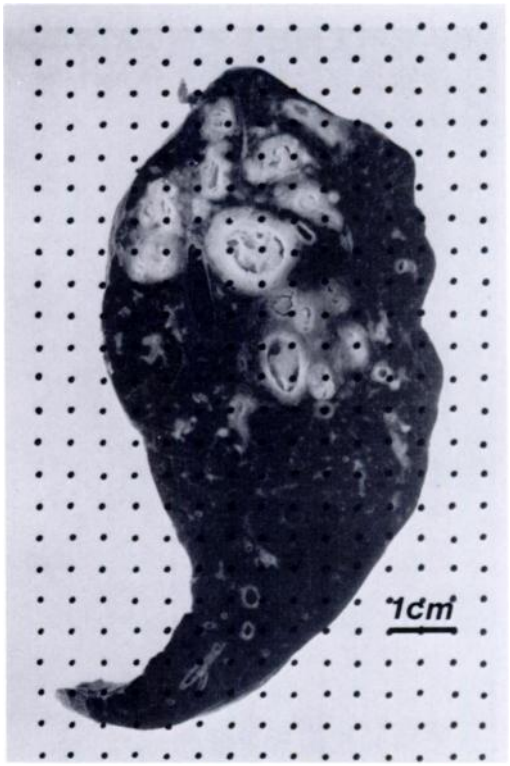

FIGURE 1. Quantitation of lesions in a transverse section of lung from a young ringed seal with an infection of *Otostrongylus circumlitus*. Note the pale areas of inflammation and fibrosis around airways which are counted as non-respiratory parenchyma. (Formalin fixed specimen.)

RESULTS

The large bronchial nematodes were identified as *Otostrongylus circumlitus*. Figure 2 shows the prevalence of this lungworm superimposed on the age distribution profile. It is primarily a parasite of seals <1 yr of age (35/108 = 32%) while only two 1-yr-olds, a 5-yr-old and an 8-yr-old seal in thin body condition were found to be infected. The overall prevalence was 39 of 382 (10%). *Otostrongylus circumlitus* were attached deep in the bronchi of the caudal lobes where they penetrated the mucosa so that the anterior 5 to 10 mm were in the peribronchial tissue. Histologic examination of the nematode intestinal tract showed them to ingest inflammatory cells from peribronchitic tissue (Fig. 3). The airways reacted to the parasite by extensive mucosal hyperplasia infiltrated by lymphocytes mixed with a few esosinophils and mucus production from greatly dilated mucous glands (Fig. 4). There was an increase in peribronchial fibrous tissue. The posterior end of the parasites usually reached the tracheal bifurcation where copious amounts of mucus accumulated; this resulted in obliterative bronchitis (Fig. 5). Interlobular connective tissue and alveoli were infiltrated by neutrophils and eosinophils and flooded with proteinaceous exudate.

The average number of nematodes per

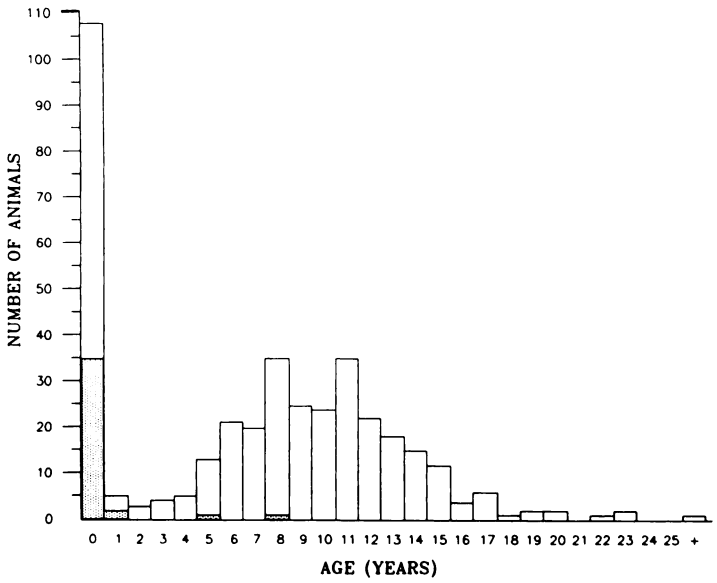

FIGURE 2. Age profile and prevalence of *Otostrongylus circumlitus* infection (shaded areas) in ringed seals (n = 382).

lung was 14 with a range of 1 to 73. Assuming a birthdate of mid-April (Smith, 1973), the age of the young seals ranged from 2 to 5 mo. The earliest *O. circumlitus* infection was at 3.5 mo. All seals infected by this large nematode had variable degrees of pulmonary arteritis characterized by hyperplasia of the tunica media with infiltration of the tunica intima by neutrophils mixed with lymphocytes (Fig. 6). Occasionally, periarteritis was seen. In two cases, immature *O. circumlitus* in the lumen of vessels were associated with these lesions. In one case, the worm was mineralized and occluded the vessel. Areas of consolidation were found in most of the lung lobes; however, there was only a slight tendency for infected seals to have less respiratory tissue (P = 0.099). The condition factor for 13 seals infected with *O. circumlitus* was 81 ± 5.53 while that of 26 uninfected seals of the same age group was 81.4 ± 4.29. Body length was 87.5 ± 9.47 and 91.4 ± 6.84, respectively.

Small alveolar nematodes were identified as *Filaroides (Parafilaroides) hispidus*, a new species in ringed seals from Canada, recently described by Kennedy (1986). They were found in 53% of a subsample of 79 ringed seals examined. The age distribution and prevalence of infection are shown in Figure 7. The prevalence of this infection was related to age class (P = 0.043). In the 7 to 13-yr class 71% were infected compared to 44% in the ≤6-yr and 38% of the >13-yr class.

The nematodes sometimes formed small firm nodules in the parenchyma. This was due to granuloma formation associated with degenerating parasites. In most cases, the nematodes were found throughout the lung by microscopic examination and caused no or only mild lymphocyte reaction. Occasionally, adults were seen in lymph vessels of interlobular septa. In a subsample of 25 young of the year, 28% also were infected with *O. circumlitus*.

Other incidental lesions observed were gastric ulcers in one mature seal and fibrosing wounds in another. Most seals were infected with acanthocephalans and cestodes.

DISCUSSION

In this survey, ringed seals were found infected with two species of lung nema-

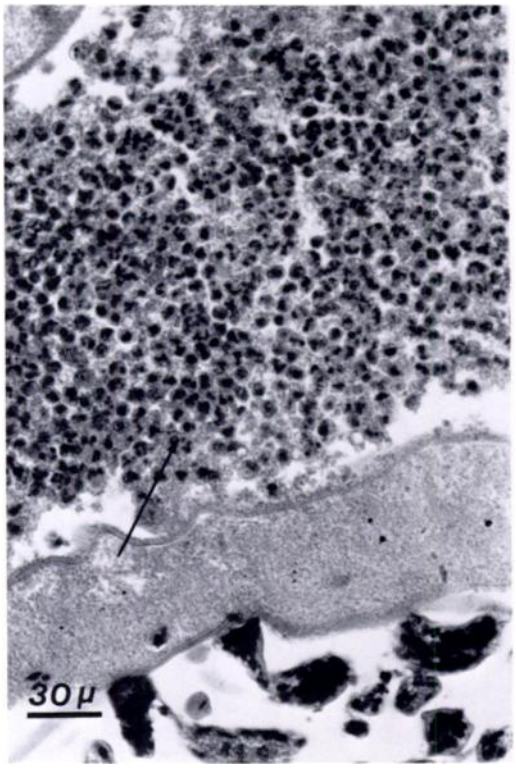

FIGURE 3. Longitudinal section of *Otostrongylus circumlitus* showing ingested inflammatory cells in the intestine (arrow). H&E.

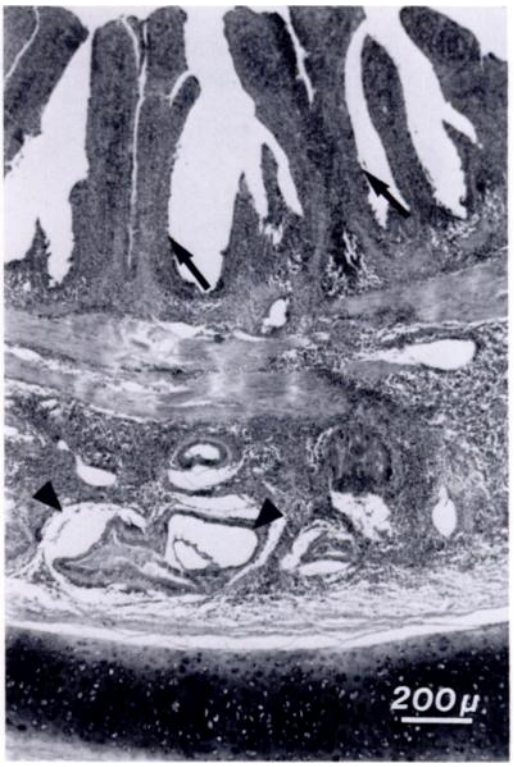

FIGURE 4. Bronchus from young ringed seal with *Otostrongylus circumlitus* infection. The mucosa is extensively hyperplastic and infiltrated with inflammatory cells (arrows). Mucous glands are greatly dilated (arrowheads). H&E.

todes. *Otostrongylus circumlitus* was found predominantly in young of the year. The infection was associated with fairly extensive lesions in the airways and surrounding parenchyma. The peribronchial inflammatory reaction is probably a response to substances from the excretory aperture of the nematode. The aperture is at the level of mid esophagus of the parasite and is in alveolar tissue when the head penetrates the wall of small bronchi. Similar lesions were described by Van Den Broek and Wensvoort (1959), Menschel et al. (1966) and Schroeder et al. (1973) in harbor seals (*Phoca vitulina*). The pulmonary endarteritis associated with immature *O. circumlitus* suggests that this parasite enters the lungs via the pulmonary circulation. This confirms observations by Menschel et al. (1966) who found young adults and male nematodes in the right ventricle and pulmonary artery of harbor seals. The most striking clinical finding was the extensive mucus production and mucosal hyperplasia in bronchi. A young seal observed in a holding pen for several days and subsequently found to have 73 *O. circumlitus* only occasionally produced a short cough. Weak cough reflexes to such an extensive irritation might be an important adaptation in a diving marine mammal.

Despite locally extensive pulmonary consolidation, only a slight reduction in respiratory parenchyma resulted. However, respiratory difficulties may be greatly enhanced by the presence of copious amounts of mucus reducing the bronchial lumina. These seals may have shorter diving and feeding times which might affect

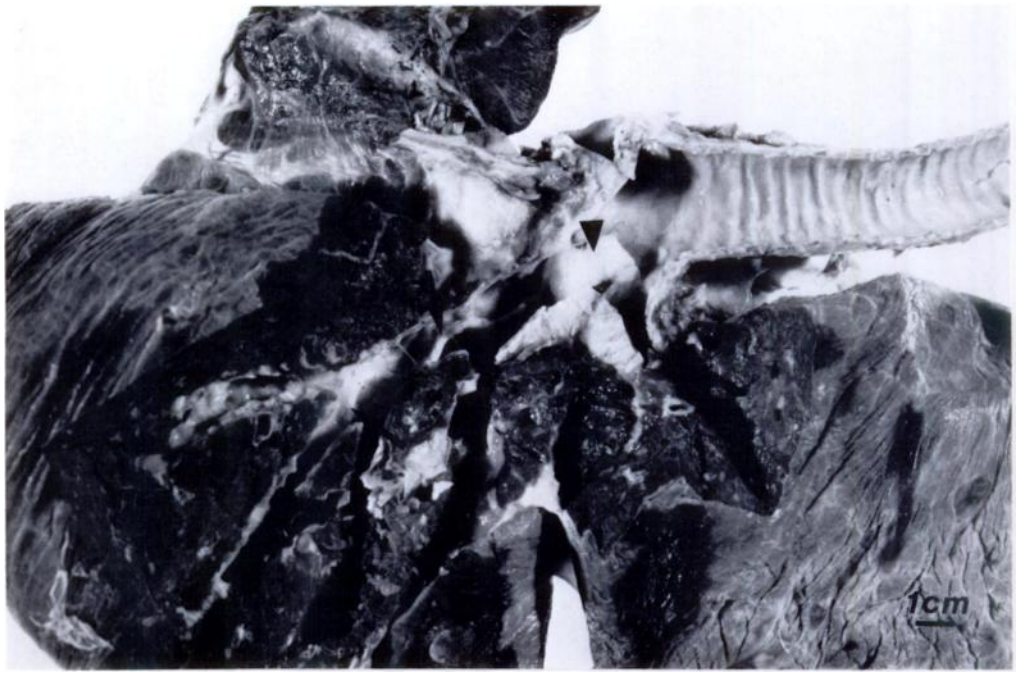

FIGURE 5. *Otostrongylus circumlitus* in mainstem bronchus of diaphragmatic lobe (arrows). The posterior end of the parasite, surrounded by a mucous plug (arrowhead), is in the tracheal bifurcation. (Fixed specimen.)

their growth. Alternatively, condition factor and body length did not significantly differ from uninfected seals ($P > 0.90$). Field observations of stunted or "short" seals (Smith, 1987) cannot be explained on the basis of lung parasitism. Nonetheless, survival of these seals may be reduced during the first winter where they are obliged to stay under the ice utilizing only breathing holes. The prevalence of *O. circumlitus* sharply declines in seals ≥1-yr-old. Microscopic examination of lungs from these older seals showed no evidence of the peribronchial fibrosis which should persist as a healed parasite lesion (Dungworth, 1985), suggesting that the young of the year infected with *O. circumlitus* were no longer represented in the ≥1-yr-old age class.

Filaroides hispidus was found commonly in all age classes. However, there was a relationship between age class and the prevalence of this infection ($P = 0.043$). It seems that this nematode is gradually acquired until >70% of the seals are infected during their middle age (7 to 13 yr). In subsequent years, the prevalence declines to 38%; this may result from immunological clearance. *Filaroides hispidus* seems to be a well adapted lung parasite in the ringed seal causing an occasionally focal tissue reaction to degenerating nematodes. This parasite is viviparous. Therefore it does not produce antigenic debris from embryonating eggs and this may account for the limited host reaction. By comparison, *Protostrongylus stilesi*, a common nematode in the lungs of Rocky Mountain bighorn sheep occupying the same microhabitat, causes distinct nodules as a result of extensive focal lymphocyte infiltration in response to hatching eggs (Honess, 1942). However, other members of the genus *Filaroides* are known to cause mucopurulent bronchitis, bronchiolitis, lobar catarrhal pneumonia and extensive granulomas in California sea lions (*Zalophus californianus*) (Johnston and Ridgeway, 1969; Fleischmann and Squire, 1970; Migaki et al., 1971; Morales and Helmboldt, 1971;

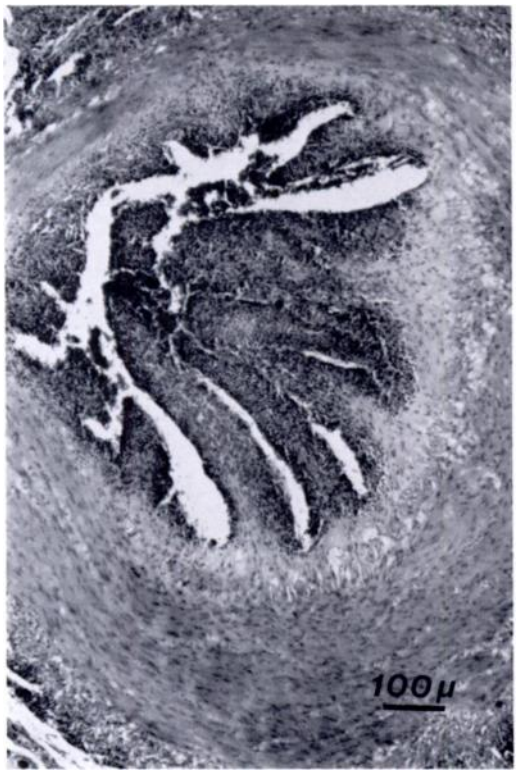

FIGURE 6. Purulent endarteritis and hyperplasia of tunica intima in the pulmonary artery of a young ringed seal with *Otostrongylus circumlitus* infection. H&E.

Sweeney and Gilmartin, 1974) and harbor seals in European coastal waters (Van Den Broek and Wensvoort, 1959; Borst et al., 1972). These differences in parasite–host interactions should be examined in areas where different species of seals are sympatric; this would provide an opportunity to study cross infections.

Except for *Filaroides* (*Parafilaroides*) *decorus* in California sea lions (Daily, 1970), the life cycles of lung nematodes of seals are unknown. Based on evidence from feeding studies in the Beaufort Sea (Smith, 1987), crustaceans may be an intermediate host for the lung nematodes of ringed seals.

ACKNOWLEDGMENTS

This work was supported by the Department of Pathology, Ontario Veterinary College, Guelph, Ontario under the chairmanship of R. G. Thomson. I thank J. R. Geraci, M. A. Fernando and M. Beverley-Burton for their guidance and T. G. Smith and H. Wright for their help in obtaining the specimens. I thank M. J. Kennedy whose identification of the *Filaroides hispidus* led to the description of a new species.

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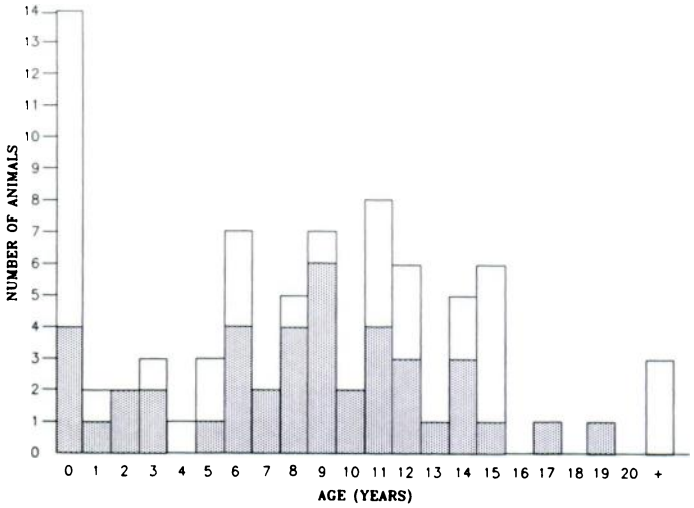

FIGURE 7. Age profile and prevalence of *Filaroides hispidus* infection (shaded areas) in ringed seals (n = 79).

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Received for publication 25 January 1988.