

Pulmonary dirofilariasis in a Japanese Hare, *Lepus brachyurus angustidens*

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ABSTRACT. A case of pulmonary dirofilariasis in a Japanese hare, *Lepus brachyurus angustidens* captured in Aomori Prefecture, was first reported in Japan. Two dead worms recovered from the pulmonary artery and detected within an organized arterial thrombus in the lung, were identified as a male and a female young adult *Dirofilaria immitis*, respectively. Principal lesions in the lung were associated with pulmonary arterial degeneration and consisted of rugose and villous intimal fibrosis, endarteritis with infiltration by numerous eosinophils and mononuclear inflammatory cells, and thrombosis.—**KEY WORDS:** *Dirofilaria immitis*, hare, pulmonary dirofilariasis.

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The canine heartworm, *Dirofilaria immitis*, is a parasitic nematode with cosmopolitan distribution and has a wide range of hosts. Even though the main hosts are carnivores, primarily dogs and wild Canidae, a number of other species of mammals have been also reported to be infected with this nematode [8, 13]. Additionally, it is a wellknown zoonotic parasite causing pulmonary or extra-pulmonary dirofilariasis in man [2, 4, 20].

In Japan, this parasite frequently occurs in dogs and rarely in other species of animals. However, spontaneous parasitism with this species of nematode in Leporidae is rarely encountered in the literatures. Narama *et al.* [10] reported the first infection case of a laboratory rabbit, *Oryctolagus cuniculus domesticus* in 1982. Up to now, the Japanese hare, *Lepus brachyurus angustidens*, has not been reported to be infected with *D. immitis*.

A female Japanese hare, 2.4 kg in body weight was captured in Hashikami-machi, Aomori Prefecture and necropsied in February, 1990. A filarial nematode removed from the pulmonary artery was washed in physiologic saline, fixed in hot 10% formalin, cleared and mounted in lactophenol for morphological observation. For histological examination, tissues of the lung and other organs were fixed in 10% buffered formalin, embedded in paraffin and sectioned serially, and sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Heidenhain's azocarmine (Azan) and Masson's trichrome stains.

At necropsy, two nodular lesions with hemorrhagic foci were palpated in the lower lobe of left lung. These lesions were located adjacent to each other and were about 15 × 10 × 8 mm and 10 × 7 × 5 mm in size, respectively. One filarial nematode was recovered from the larger hemorrhagic focus in pulmonary artery (Fig. 1-a). The other focus was well-defined, grayish-white in color, and relatively solid in consistency. Other organs showed no pathological changes except for the parasitism by many cysticerci in the abdominal viscera on the surface and by other species of nematodes in the alimentary tract.

The worm recovered from the pulmonary artery was dead and slightly degenerated. However, it had still the normal architecture of body, so it could be identified as a young

male adult of *D. immitis* by the following morphological features that the body was slender, string-like, 100.4 mm in length and 456 μm in width and white in color, and had the cuticle provided with fine transverse striations. The anterior end of worm was bluntly rounded and had 4 pairs of the small cephalic papillae and the small mouth without lips (Fig. 1-b). The esophagus was 1,100 μm in length and the nerve ring was located 408 μm from the anterior end. The posterior end of body was spirally coiled and provided with the bluntly conical tail and small caudal alae. Five finger-like precloacal papillae, 4 of which were paired, 2 large paired and 6 small paired conical postcloacal papillae were situated near the tip of tail (Fig. 1-c). The left spicule was 383 μm in length and pointed, while the right one was 253 μm in length and ended in a blunt tip.

Histologically, in the organized thrombus in the pulmonary artery, were found sections of the worm which were surrounded by a large number of lymphocytes, macrophages, fibroblasts, and a small number of eosinophils or multinucleated giant cells (Fig. 2-b). Although the worm was partially mineralized, the fundamental histological structures were considerably well preserved. The worm ranged from 188 μm to 244 μm in the diameter of transverse sections. The cuticle had prominent fine transverse striations on the surface, but no external longitudinal ridges. The cuticle was 10 μm thick and laminated with four distinct layers. The innermost layer was extended interiorly to form internal longitudinal ridges at the lateral cords. Muscle layers were well developed, 28 μm thick and of polymyarian-type. In the body cavity, a single digestive tract and 2 to 6 genital tubular structures were recognized, and were moderately stained by PAS staining (Fig. 2-b).

In the other areas of host body, the large to small-sized pulmonary arteries with the thickened wall showed chronic endarteritis composed of rugose and villous intimal fibrosis. However, the marked pathological changes were present in the branches of pulmonary artery containing unorganized worm. The arterial lesions were characterized by intimal thickening and hyperplasia of lining endothelial cells, and infiltration with eosinophils, lymphocytes and histiocytes. No endocarditis and other significant lesions associated with *D. immitis* adult or microfilaria infection were observed in

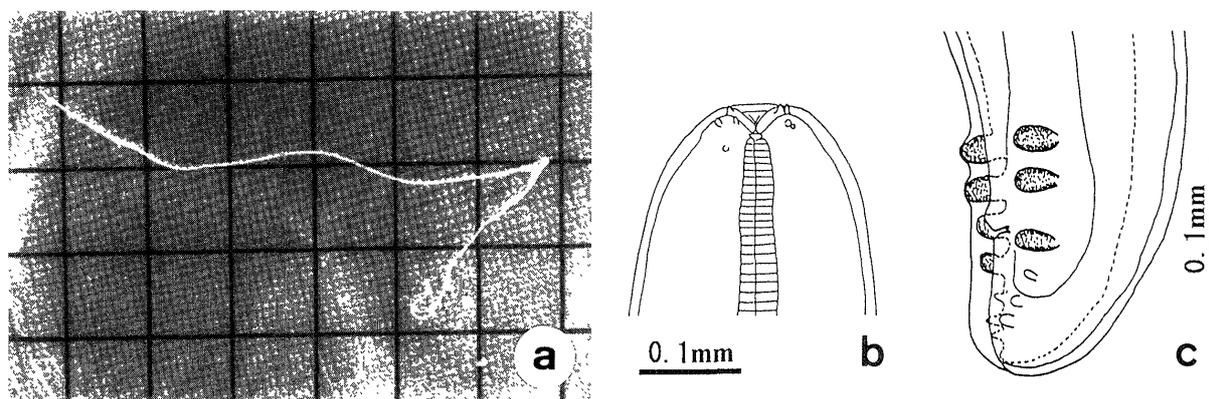


Fig 1. A young male adult of *D. immitis* recovered from Japanese hare. a: Lateral view of the whole body, b: anterior end, lateral view, and c: posterior end, latero-ventral view.

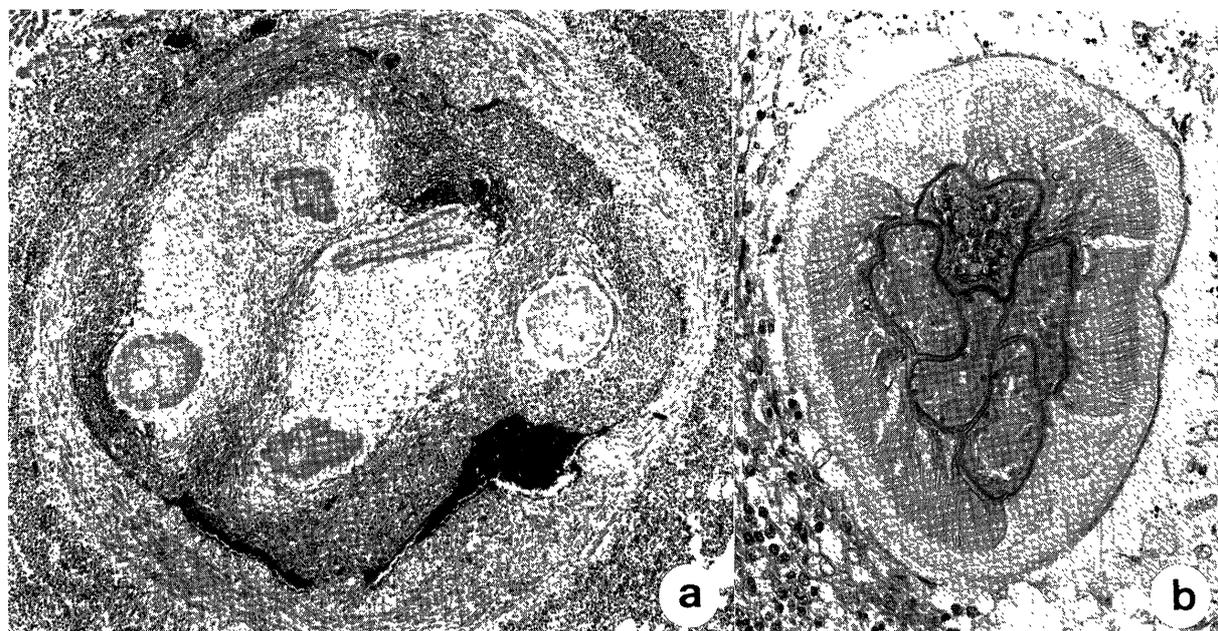


Fig. 2. a: Transverse sections of *D. immitis* within organized thrombus in the pulmonary artery. Worm sections were surrounded by inflammatory cells. Trichrome stain, $\times 56$. b: Magnification of the sectioned worm, showing a thick laminated cuticle, internal longitudinal cuticular ridges, thick musculature, a single digestive tract and a few genital canals. PAS stain, $\times 280$.

this host animal.

Pulmonary infection with *D. immitis* of a laboratory rabbit, *Oryctolagus cuniculus domesticus*, was histologically diagnosed by Narama *et al.* in Japan [10]. In this study, we could detect this nematode in the lung of a Japanese hare, *Lepus brachyurus angustidens*. This paper is the first and the second reports of *D. immitis* infection in the Japanese hare and Leporidae respectively in Japan. The worm recovered from the pulmonary artery was identified as a young male adult of *D. immitis*. Morphological features of this worm coincided with those described by previous authors except for the number of caudal papillae [8, 12], although some variation is noticeable in the number of caudal papillae of this species of parasite [16].

In the present case, the pulmonary nodular lesions were almost similar in histopathological features to those due to

D. immitis infection in man [2, 9, 14, 18] and other animal species [1, 10, 15]. The histological characteristics in the sections of *D. immitis* have been clearly given in the previous reports [2, 9, 17, 19]. In human pulmonary dirofilariasis, Neafie and Piggott [11] state that even the worms poorly preserved can be identified to the genus level of *Dirofilaria* with such morphological characteristics as thick, multilayered cuticle, prominent transverse striations, internal longitudinal cuticular ridges, and abundant somatic muscle. The morphological findings of worm in the present case exactly coincided with those of previously reported cases. In addition, a single digestive tract and a few tubular structures suggestive of the female genitalia were recognized, although no microfilariae was observed in these genital tubes. From these findings, the worm observed in the sections was diagnosed as a young female adult of *D.*

immitis.

The Japanese hare developed severe proliferative endarteritis and thrombosis in the pulmonary artery. The histopathological findings were essentially the same as of carnivores and man infected with *D. immitis* [1-3, 5-7, 15, 18]. Conclusively, the present Japanese hare harbored at least two young adults of *D. immitis* in the lung and they caused pathogenic changes in this animal. Furthermore, it was suggested that the Japanese hare is an accidental and unsuitable host for *D. immitis* as well as man.

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