

Cross-Section Morphology of *Dirofilaria ursi* in Comparison with *D. immitis*

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Introduction

Since the original description of *D. ursi* was made by Yamaguti (1941) on the specimens from the Japanese black bear, *D. ursi* and *D. immitis* have been known to us as the native *Dirofilaria* species in Japan. All cases of zoonotic filariasis reported until now were caused by *D. immitis* on the main island of Japan (Nishimura *et al.*, 1964; Yoshimura and Yokogawa, 1970). Recently the human cases from Canada were considered to be caused by *Dirofilaria* resembling *D. ursi* (Beaver and Samuel, 1977). In the present report a comparative study of *D. ursi* and *D. immitis* will be undertaken on the histological sections for contributing to identify the sections of a filaria species in human cases in future.

Materials and Methods

D. ursi (2 females, 1 female fragment and 6 males) were collected from the subscapular connective tissue and the perirenal adipose tissue of two Japanese black bears (*Selenarctos thibetanus japonicus*) from Tamba district in western part of Japan, and they were preserved in ethyl alcohol (70%). One female, one male of the specimens and one blood smear containing the microfilariae

were deposited in the collection of the National Science Museum, Tokyo, (NSMT-As-1440 and 1441) (Uni, 1979).

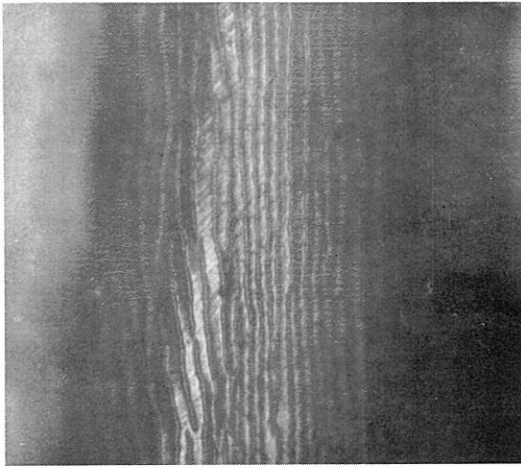
D. immitis (82 worms) were collected from hearts of five dogs in the animal laboratory of this medical school, and were preserved in ethyl alcohol.

The material (1 female, 198 mm long, and 2 males, 76 and 78 mm long, of *D. ursi*, and 2 females, 285 and 300 mm long, and 2 males, 130 and 145 mm long, of *D. immitis*) was sectioned by routine methods. The sections were stained with hematoxylin and eosin, and were examined by light microscopy. Supplementarily, the surface of the worms was observed by metallurgical microscopy (MM) and scanning electron microscopy (SEM). The preparations for SEM study have been described in the previous paper (Uni, 1978).

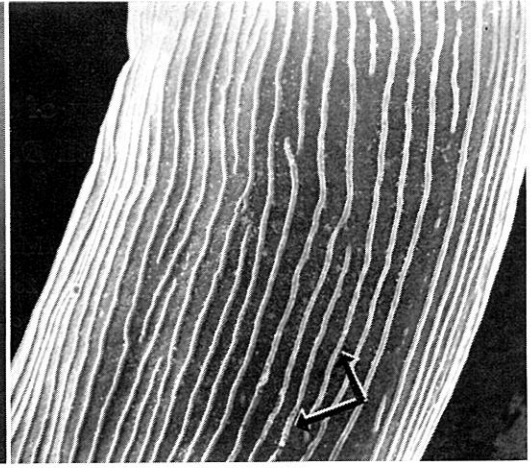
Results

Morphology of D. ursi on the histological sections

Integument. (The outer surface of the cuticle was shown by MM and SEM in Text-Figs. 1, 2, respectively). The cuticle showed prominent, straight longitudinal ridges and fine transverse striations (Text-Fig. 1), although the striations were hardly



Text-Fig. 1 Longitudinal ridges at midbody of *D. ursi* by MM, $\times 90$.



Text-Fig. 2 Scanning electron micrograph of longitudinal ridges of *D. ursi*. The ridges are composed of the small beads (arrows), $\times 220$.

recognizable in low magnification (see Uni, 1978). The appearance of the ridges around the body was clearly seen by SEM with larger focal depth, and the ridges were cut into small beads by the transverse striation (Text-Fig. 2). The ridges were unrecognizable at the anterior part of both sexes (Fig. 1). At the ventral side of the posterior part of the male, the ventral ridges (according to Chitwood and Lichtenfels, 1972, *i.e.*, rugae) appeared instead of the longitudinal ridges. At midbody 65 to 77 ridges were recognizable around the body, and were spaced at intervals of 16 to 33 microns (Fig. 2). The cuticle was 4 to 7 microns thick, and appeared to consist of five layers—a cortex with the longitudinal ridges, two median fibrous layers, and two thin basal lamellae (Fig. 3). The ventral ridges, numbering 20 ridges in all, were seen at the coiled posterior part of the male, and their crests were sharply pointed (Fig. 4). The interior projections of the cuticle were recognizable at both lateral chords (Fig. 4). The thickness of cuticle of the lateral side became larger toward the posterior part of the male. This thickened cuticle appeared

to be the beginning of the caudal alae, which were recognizable, as the cuticular inflation on both lateral sides, most distinctly at the level anterior to the part with the caudal papillae (Fig. 5).

Hypodermis. Thin hypodermis was situated between the musculature and the cuticle. It formed broad lateral chord on either lateral side. The combined width of both lateral chords was comparable to one-fourth of the circumference of the worm (Fig. 2), but sometimes was compressed as seen in other sections. The chord nuclei were present in the center or near the periphery of each cell. The dorsal and ventral chords were tall and slender, being situated at the middle of each side.

Musculature. The coelomyarian musculature divided into dorsal and ventral fields. The muscle cell was composed of a contractile muscle fiber zone and a protoplasmic area with a nucleus, and 33 to 45 cells were countable in each of the four sectors at the sections of the anterior or midbody level (Figs. 2, 6). At the level of the dilatation of the anterior part (see Uni, 1978) or the vulva (385 to 500 microns in body width),

the muscle cells were 99 to 115 microns thick, the fiber zone of which was 33 to 37 microns thick (Figs. 6, 7). In midbody (400 to 554 microns in body width) the muscle cells were 99 to 140 microns thick, the fiber zone of which was 58 to 74 microns thick. Thus, the relative ratio of fiber zone to body width was 12 to 16% (Figs. 2, 8). *Digestive system.* The esophagus divided into muscular and glandular parts, and the base of the latter became large. The intestine was surrounded with the cuboidal epithelial cells containing nuclei near the periphery. The microvilli were situated on the inner surface of the lumen. Thirty cells were seen at a transverse section of the intestine just posterior to the esophagus (Fig. 9). The intestine was often round and was smaller than the reproductive ducts. The body width of the anus level was 160 microns.

Reproductive organs. In the female specimen, the vulva was situated near the esophagus end level. The vagina was surrounded with well-developed muscle layers (Fig. 7). Paired uteri were seen at midbody level, containing embryos (Fig. 2). The wall of the uterus was lined with a low, squamous epithelium (Fig. 10). The ovaries ran extended posteriorly, finally turning ascendingly. At a section of the posterior part, six sections of the ovaries were recognizable, containing oogonia (Fig. 11).

In the male specimens, a single genital tube ran anterior to the cloaca. It was lined with thin epithelial cells, and was packed with sex cells at the level of the seminal vesicle at midbody (Fig. 8). The vas deferens was lined with a thick epithelium, containing spermatozoa (Fig. 13). The epithelium was thickened toward the ejaculatory duct in the coiled posterior part (Fig. 15). Two spicules were seen at the section just anterior to the cloaca, and at this level a pre-cloacal central papilla and other caudal papillae were recognizable (Fig. 14).

Special structure. The postdeirid was found

only on the left side of a longitudinal section on the coiled posterior part, located at the distal end level of the retracted left spicule (Figs. 15, 16).

Cross-section morphology of D. immitis in comparison with D. ursi

The body width of *D. immitis* was larger than that of *D. ursi* in almost all levels, except at the dilatate level (see Uni, 1978) of the anterior part of large specimens of *D. ursi*.

The outer surface of the cuticle of *D. immitis* showed the presence of minute projections of longitudinal ridges in the transverse sections of midbody in high magnification (Fig. 17). Lateral interior projections of the cuticle were clearly recognizable in some sections (Fig. 18). The ventral ridges, numbering 13 in all, were recognized at the coiled posterior part of the male, and the crests were sharply pointed and were larger than in *D. ursi* (Fig. 19). The lateral chords were broad, and in the gravid female they were flattened between the uteri and the cuticle (Fig. 21). The fiber zone of the musculature was 17 to 24 microns thick at the level of the esophagus or the vulva, the body width of which was 450 to 500 microns (Fig. 20). At midbody the fiber zone was 21 to 57 microns thick, and the body width of the same level was 710 to 770 microns (Fig. 21). The relative ratio of the zone to the body width was 3.2 to 8.0%. Thus, the fiber zone appears to be thinner than that of *D. ursi* in each section, although the musculature varied conspicuously in different fixatives. The seminal vesicle and the vas deferens were larger and stouter than in *D. ursi*. The ejaculatory duct was clearly seen in Fig. 18. At the sections of the posterior part of the female, five to six sections of ovaries were seen, the sizes of which were larger than in *D. ursi*. The postdeirid of the female (see Uni, 1978) was found in sections, being situated only on the right lateral side of the level near 20 mm anterior to the terminal tip (Figs. 22, 23). Near the

terminal end of the female, the areas on both lateral sides were differently stained, which were assumed to be the phasmids (Fig. 24).

Discussion

The outer and inner structures of the male and female specimens of *D. ursi* have been revealed on the histological sections, and the size of body and organs, the appearance of musculature and the markings of cuticle were conspicuously different from those of *D. immitis*. Especially, the markings of the cuticle appear to be important characters for identifying the species, because they showed specific characters at each body level of the specimens of each species.

In sections of the immature worms of *D. immitis* (Ohmori *et al.*, 1977), the ratio of muscle fiber zone to body width seems to be larger than that of the adult worms shown in the present report, but it may not be over that of *D. ursi*.

According to Orihel and Beaver (1965), the longitudinal ridges of *D. tenuis*, which caused commonly human dirofilariasis in Florida, were shown to be relatively low, rounded at the crests and irregularly wavy. The number of the ridges appears to be more numerous than in *D. ursi*, but the thickness of the muscle fiber zone to be less than in *D. ursi*. Beaver and Orihel (1965) suggested in a case of dirofilariasis from Washington by Welty *et al.* (1963), that the causative species was different from *Dirofilaria* species of the common cases in the United States. Later, three more human cases were added from Canada (Anderson *et al.*, 1968; Meerovitch *et al.*, 1976; Beaver and Samuel, 1977), and according to the description by Meerovitch *et al.* (1976), the appearance of muscle fiber, the thickness of its zone and the number of the longitudinal ridges and its appearance in the sections of the worm from the human case appear to be comparable to our present description of

D. ursi. Anderson (1952) reported *D. subdermata* from Canadian porcupines, and he suggested that it morphologically resembled *D. ursi*.

Recently, Maclean and Beaver (1979) reported *D. repens* from a man in Okinawa, Japan. The number of longitudinal ridges denoted at the sections of their case seems to be more numerous than in *D. ursi*.

Due to the high incidence of *D. immitis* among dogs in present Japan, the cases of human dirofilariasis should be much more than reported and more are anticipated to be found in the future. Although the chance of transmission from bears to humans is suspected to be rare in Japan, the differential diagnosis of worms between *D. immitis* and *D. ursi* should be taken into account.

Summary

The morphological features of the specimens of *D. ursi* were examined on the histological sections, and were compared with that of *D. immitis*. The body size, thickness of musculature and the cuticular markings were different between the both species. The ventral ridges of the posterior part of the male were also characteristic for each species. The postdeirids of the male and the female were demonstrated in the sections. These findings will be useful to diagnose the species in the tissue sections of the human infection by *Dirofilaria* species.

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Addendum

After finishing this manuscript, histological sections of the nodule from a patient in Quebec, reported by Meerovitch *et al.* 1976 (Canad. J. Pub. Health 67: 333-335), were kindly sent for our examination from the senior author. At the sections of the

worm, the body was 140–199 μm wide, the muscle fiber zone was 20–30 μm thick, and the cuticle had 59–67 longitudinal ridges with 2.5 μm wide and 2.5–3.8 μm apart. The ridges appeared to be straight and continuous in the slant sections. Thus, the number and the appearance of the ridges, the thickness of the fiber zone and the appearance of the muscle fiber in the worm sections of the Canadian human case were remarkably similar to those of adult *D. ursi* taken out directly from the Japanese black bear, although the sizes of the former were smaller than those of the latter.

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Dirofilaria ursi および *D. immitis* の切片標本における比較研究

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ニホンツキノワグマの結合組織に寄生する糸状虫, *D. ursi*, の切片標本における特徴を明らかにし, 犬糸状虫, *D. immitis*, との鑑別上の相違点を示した. *D. ursi* において, その体幅は *D. immitis* より各部位において小さい. ただし, 虫体前方の dilatation 部位の体幅は *D. immitis* より大きいことがある. 筋線維層は *D. immitis*

より肥厚している. 角皮に顕著な longitudinal ridges が存在するのが特徴であるが, 一方, *D. immitis* においても高倍率ではそれに相当する微小な突起が認められる. 雄虫体尾部には ventral ridges が存在し, その数は両種で異なっている. これらの知見は糸状虫症の人体例における虫種同定に有用であると考えられる.

Explanation of Figures

Figs. 1-16. Transverse sections (1-14) and longitudinal sections (15 and 16) of *D. ursi*.

1. Cuticle at esophagus level of male, $\times 360$.
2. At midbody of female, $\times 90$.
3. Longitudinal ridges at midbody of male, $\times 360$.
4. At coiled posterior part of male, ventral ridges (*) and interior projection of cuticle (arrow), $\times 180$.
5. Caudal alae at male posterior part, $\times 90$.
6. At esophagus level of male, $\times 90$.
7. At level of vagina uterina, $\times 90$.
8. At midbody of male, $\times 90$.
9. Intestine, $\times 360$.
10. Uterus containing embryos, $\times 360$.
11. At posterior part of female, $\times 90$.
12. At posterior part of male, $\times 180$.
13. Vas deferens, $\times 360$.
14. At level near cloaca, precloacal central papilla (*), $\times 360$.
- 15 and 16. Postdeirid (*) at coiled posterior part of male (15), $\times 90$, and the area enlarged (16), $\times 360$.

Figs. 17-24. Transverse sections of *D. immitis*.

17. Cuticle of midbody level in high magnification, minute projections (arrows), $\times 900$.
18. At coiled posterior part of male, ventral ridges (*) and interior projection of cuticle (arrow), $\times 150$.
19. Ventral ridges and ejaculatory duct enlarged, $\times 360$.
20. At vagina uterina, $\times 90$.
21. At midbody of female, $\times 75$.
22. Postdeirid (arrows) at posterior part of female, $\times 150$.
23. Postdeirid enlarged, $\times 360$.
24. At level near terminal end of female, phasmid (*), $\times 180$.

