

Research Note

**Scanning Electron Microscopic Observation of Undetermined Zoonotic Filaria
Found from a Man with Creeping Eruption**

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Lately, larva migrans has aroused increasing attentions of parasitologists, because the way of infection had closely related with recent trends of human life style. Dirofilariasis is one of the most popular larva migrans in Japan, while several cases of larva migrans caused by a few new causative agents have been reported in the last decade (Maclean *et al.*, 1979; Yoshimura *et al.*, 1983; Ando *et al.*, 1988; Nawa *et al.*, 1989; Hashimoto *et al.*, 1990; Ando *et al.*, 1992). The accurate identification of the causative worm is very important in order to explain the mechanism of the host-parasite relationship in each larva migrans and to prevent its further spreading as well. It is necessary for diagnosing larva migrans to rely on the immunological or histopathological examinations, since the worms migrate through the host tissues, keeping the stage of larva or immature adult at most. It is often difficult to confirm the worm species because of insufficient information on the morphological characteristics,

not only when the worm was degenerated in the tissue, but also when it was unfamiliar with the human tissue. Actually, the species of causative worms could not be identified in a few of the recently reported cases (Ando *et al.*, 1985; Kagei, 1991).

In our present case developed creeping eruption, a worm was removed from the lesion at the right shoulder on the occasion of a biopsy, and several sections of the worm were revealed in the tissue examined. Furthermore, the rest parts of worm were detected in the residual paraffin block, of which some were examined with a scanning electron microscope (SEM). As the result of these observation, a new finding of morphological characteristics was demonstrated on the lateral field of the worm cuticle. Geraert and Raski (1986) observed the lateral field of nematode cuticles in details, and emphasized that morphological characteristics of the cuticle in the lateral field had shown taxonomic significance in some species. It is suggested that a morphological observation with a SEM on even a small part of normal cuticle in preserved materials could present the useful information for identifying the worm species of nematode larva migrans. Although the finding of morphological characteristics on the lateral field of the cuticle could not lead up to the definite identification of the species in this case, this report expects to serve as a future reference to diagnostic procedure for zoonotic filaria.

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For SEM preparation some pieces of the worm were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, for 2 hrs at room temperature, washing with the same buffer, and postfixed in 1% osmium tetroxide for 1.5 hrs at 4°C before and after intermediate treating with 2% tannic acid solution. They were then dehydrated with an alcohol series, soaking in amylacetate, and dried by the critical point drying method. The dried samples were sputter-coated with gold-palladium alloy, and examined with a Hitachi S-450 SEM. A mature adult of *D. immitis* preserved in our laboratory was also examined with SEM for morphological comparison.

The patient, 32-year-old man, resident of Yonago City, Japan, has never been abroad all these several years. He presented the edematous creeping eruption with itch in the range of 2mm. The eruption moved a few centimeter per day from his right upper extremity to the shoulder, and marked light pigmentation, disappearing in about four days. The leukocyte count was 13,800 with 10% eosinophils. The seroimmunological examination was negative against helminths antigens such as *Gnathostoma*, *Anisakis*, *Toxocara*, *Dirofilaria*, *Ascaris*, *Taenia*, *Fasciola*, *Paragonimus* and *Spirometra* in the Ouchterlony and the immunoelectrophoresis test. The patient has never shown any eruption since the biopsy carried out. Eight sites of sections of a coiled filarial worm were observed in the median layer of dermis surrounded by granulomatous, fibrous agents with cellular infiltration such as eosinophils, lymphocytes, histocytes and plasma cells (Fig. 1). In some of these sections, transverse section of thick-walled genital tubes as well as the large genital tube filled with the contents were detected, while in others the worm organs were degenerated (Figs. 2, 3). The worm, measuring 70 μm in diameter, had the cuticle, approximately 3 μm in thickness, which consists of three or four layers without the median fibrous layer. Two small grooves on the cuticle outer surface were observed in the both lateral field of the body (Fig. 2). The lateral cords with the broad base extended inward to the body cavity. The ventral and dorsal cord were vague. No internal longitudinal cuti-

cular ridge was detected in the side of lateral cord. Muscle cells were tall, showing the polymyarian coeromyarian type. Eight pieces of the worm fragments, of which the total length was 11.5mm, were detected from the residual paraffin block. Three pieces of them including a head were examined with a SEM. Materials surrounding the body surface was regarded as a remnant of the tissue when they observed with a light microscope, but they have been proved to be the worm slough as the results of observation with a SEM (Fig. 4). The characteristics of the head could not confirmed due to the specimen covered with the slough-remnant. The worm was presumed as the fifth stage larva of some zoonotic filaria judging from its size, measuring more than 11.5mm, and from the presence of dirofilaria-like ecdysis.

The transverse striations of the cuticular outer surface were deep and clear, measuring 3 μm in width. They were interrupted in each side of the lateral field with densely distributed short slits observed through SEM observation, which coincide with the grooves recognized by the side of lateral cord under a light microscope (Fig. 5). Such a lateral field was not detected in the mature adult worm of *D. immitis* (Fig. 6).

In Japan, sparganosis, ancylostomiasis caninum and gnathostomiasis spinigerum have so far been known as the helminth-zoonosis developing creeping eruption due to larva migrans. As for gnathostomiasis, *G. hispidum* has recently been recognized as the cause of creeping eruption in the imported case of infection (Akahane *et al.*, 1982), and *G. nipponicum* and *G. doloresi* were found causing creeping eruption in the intrinsic cases of infection (Ando *et al.*, 1988; Nawa *et al.*, 1989). Besides, several cases of creeping eruption as larva migrans caused by *Spirurina* were added lately. In our case, the histological examination of a biopsy demonstrated the transverse section of a filarial worm as the causative agent, though it was clinically suspected of spiruroidea. Some cases of dirofilariasis (*D. immitis* and *D. depens*), onchocerciasis, and a case of *Dipetalonema* infection have so far been known as zoonotic filariasis in Japan, where they have never presented creeping eruption. However, a case of zoonotic oncho-

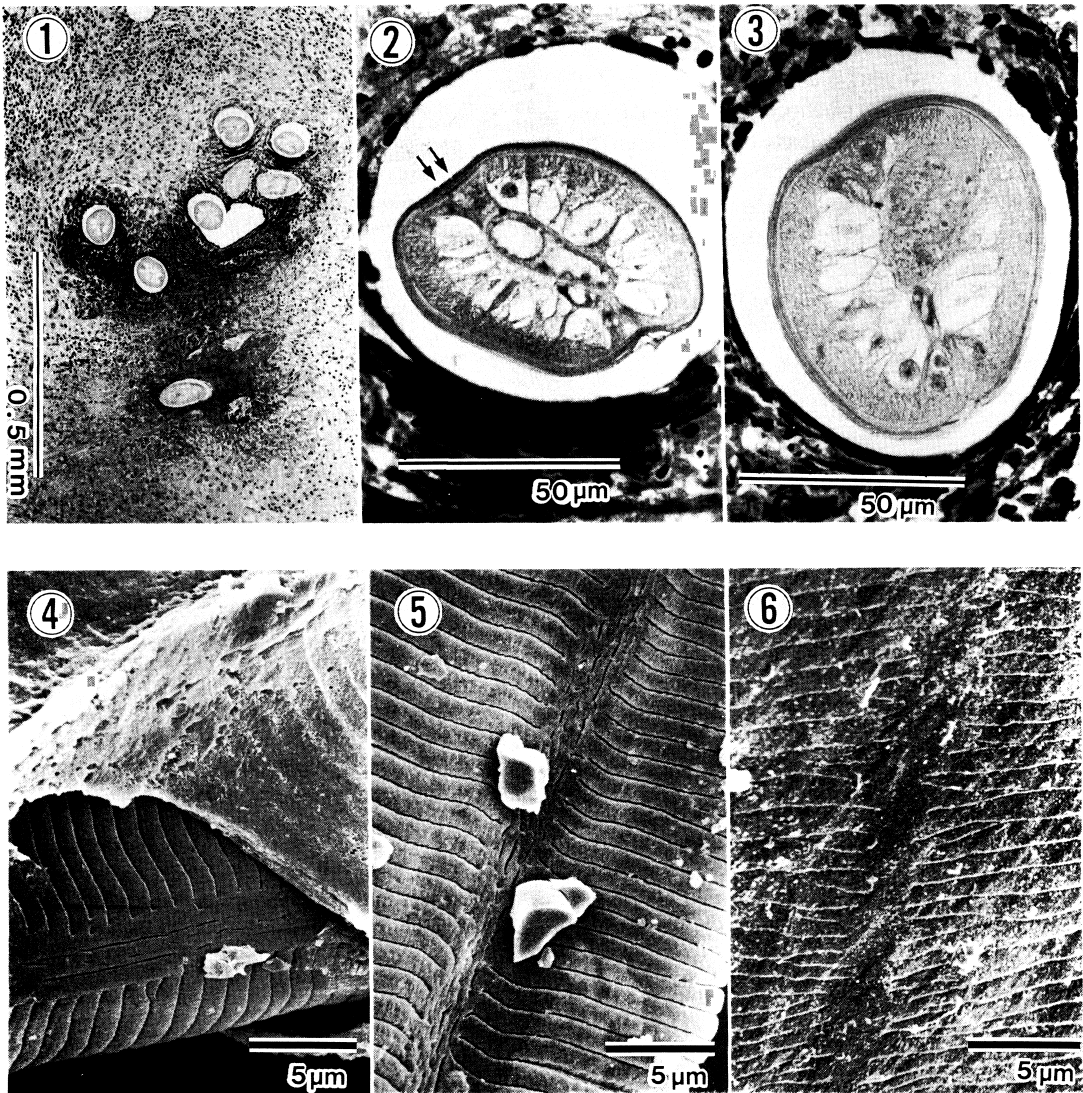


Fig. 1 Eight sites of transverse sections of a filarial worm in the cutaneous tissue.

Fig. 2 Transverse section through the genital tube.

Two small grooves on the cuticle outer surface are shown (arrows).

Fig. 3 Transverse section through the genital tube.

Fig. 4 Cuticle in the lateral field. A piece of slough of the fourth stage worm is shown (scanning electron micrograph).

Fig. 5 Cuticle in the lateral field. Interruption by a longitudinal groove with densely distributed small slits is shown.

Fig. 6 Cuticle in the lateral field of the mature adult worm of *D. immitis*.

cersiasis, where bites of black flies suspected of its infection route, was developed a nodule at the left foot of a two-year-old Japanese girl (Hashimoto *et al.*, 1990). So onchocerciasis should not be excluded out of objects for differential diagnosis. If the causative worm of our case were identical with *D. immitis*, it should be the immature immediately after the fourth ecdysis. It was confirmed that the worm is different from the mature adult of *D. immitis* in the morphological characteristics of the cuticle in the lateral field of body, but a further comparison between the immatures of them on this point is required.

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