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Morphological Identification of Parasites in Biopsied Specimens from Creeping Disease Lesions

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Abstract

The most common pathogenic parasite causing creeping disease in humans in Japan is the larva of *Gnathostoma* spp. There are also 4 species of hookworms, including exotic species, that are known to infect humans and lead to creeping eruption in Japan. This report presents the results of a parasitological investigation during a 5-year study of creeping eruption in cases suspected of suffering from gnathostomiasis. The stumps of the infecting parasites were observed in some of the histological samples, enabling identification of the causative organisms in those cases. Among the 8 subjects in this study, one case each were identified to be caused by *G. spinigerum* and *G. hispidum,* one case by filarioid nematoda and one case could not be definitively identified. In the remaining 4 cases, the parasites were identified as Spiruroid nematoda, because of the absence of spines in the cuticle, the presence of a polymyarian coelomyarian type muscle layer, a low number of intestinal cells, and because of the characteristics of the lateral chords including how far it extensively projected into the body cavity. These 4 cases were the examples of creeping disease caused by a previously unidentified parasite different from the known pathogens of creeping eruption in Japan. I intend to conduct further studies to determine the life cycle of this parasite in order to identify its species and the source of infection.

Key words: Creeping eruption, Tissue sections, Morphology, Spiruroid nematoda

Introduction

Parasites commonly causing creeping diseases include various parasitic worms of the genus *Gnathostoma,* hookworms, genus *Strongyloides,* filarioid nematoda, sparganum, and arthropodes. In Japan, creeping diseases caused by parasitic *Gnathostoma* species have occurred most commonly.

I conducted a morphological identification on the parasitic stumps found in the histological samples from lesions removed from 8 subjects exhibiting the symptoms of creeping diseases over the past 5 years. In addition to the parasite species described above, I discovered cases caused by the larvae of Spiruroid nematoda (Spiruroidea Raillet et Henty, 1915; Nematoda). Although I have not yet identified the genus of this pathogenic parasite, I am reporting my findings because I believe that contribute to the identification of the parasite in future cases.

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Materials and Methods

As Table 1 shows, histological samples were obtained from 8 patients that had received treatment at Gifu University School of Medicine, Tokyo Women's University School of Medicine (2 cases), Shibata Clinic, National Defence Medical College School of Medicine (2 cases), Tokyo Medical and Dental University, and Tokyo Metropolitan Komagome Hospital. The patients, 5 males and 3 females, were between 38 and 50 years old. The creeping-eruption sites included the chest (2 cases), abdomen (2 cases), and the forearm, shoulder, thigh, and buttocks (one case each). Clinical findings in all but one case, revealed eosinophilia (5–75%), providing a circumstantial evidence for a diagnosis of gnathostomiasis. Accordingly, serological examinations were performed, but all cases were proven to be

Table 1 Human Cases with Creeping Eruption

Case No.	Age	Sex	Location of creeping	White blood corpuscle	Eosino-phil	Serodiagnosis Oucht.	Serodiagnosis ELISA	Client
1.	36	♂	Right breast	11,600	10%	–	–	Gifu-Univ.
2.	43	♂	Right abdomen	7,200	5	–	–	Tokyo-Women's Med. College
3.	49	♂	Abdomen	ND*	ND	ND	ND	Shibata Clinic
4.	40	♀	Right thigh	6,100	5	ND	ND	Tokyo-Women's Med. College
5.	50	♀	Left pre-breast	6,100	20	–	–	Nat. Defense Med. College
6.	38	♀	Left forearm	4,300	10	–	–	Nat. Defense Med. College
7.	42	♂	Right buttock	8,700	1	–	–	Tokyo Med. Dent. Univ.
8.	49	♂	Left shoulder	27,900	75	–	–	Tokyo-Komago-me Hospital

*: ND: Not done

negative for the antigen of *G.doloresi*.

I was asked to conduct a morphological identification of the parasitic stumps found in histological sections of the biopsied specimen from the anterior extremity of the eruption in 8 patients. In the 7th case, a portion of the parasitic stump was picked up from the paraffin block with a needle tip. This assisted in its identification.

Morphological Description of the Parasitic Stump Within the Tissue

Case 1

This patient experienced pruritus on the right side of the chest, followed by the development of a small erythema, which developed a linear form when scratched. A biopsy of the tip of the erythema revealed the subcutaneously located parasitic stump (Fig. 1). The dimensions of the stump were found to be 92–97 μm×94–105 μm. The cuticle of the parasite was smooth with a thickness of 1.7–2.6 μm, and the subcuticular muscle layer was of the polymyarian (with about 7–8 muscule bundles in 1/4) coelomyarian type. A small intestine of 15–20 μm consisting of 5 mononuclear cells was found in the body cavity. The parasite also possessed the lateral chords which extended with a bilobular form into the body cavity and exhibited a narrow base with

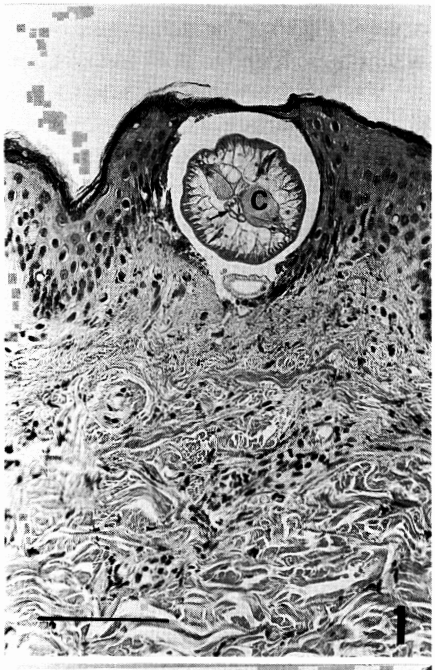
Fig. 1 Cross-section of larval nematoda in the cutaneous tissue of Case 1. Arrow: intestine; C: well-developed lateral chord. Scale: 100 μm.

Fig. 2 This figure shows the creeping eruption in Case 2 where four sections (A, B, C and D) of a larval nematoda migrating under the skin are visible. Scale: 200 μm.

Fig. 3 Enlargement of a section-A of the larval nematoda in Fig. 2. I: intestine; LC: lateral chord. Scale: 100 μm.

Fig. 4 Enlargement of a section-C of the larval nematoda in Fig. 2. G: grandular part of the esophagus. Scale: 100 μm.

Fig. 5 This figure shown the eosinophilic granuloma in Case 3 where sections of larval nematoda (arrow) migrating into the skin are visible. Scale: 500 μm.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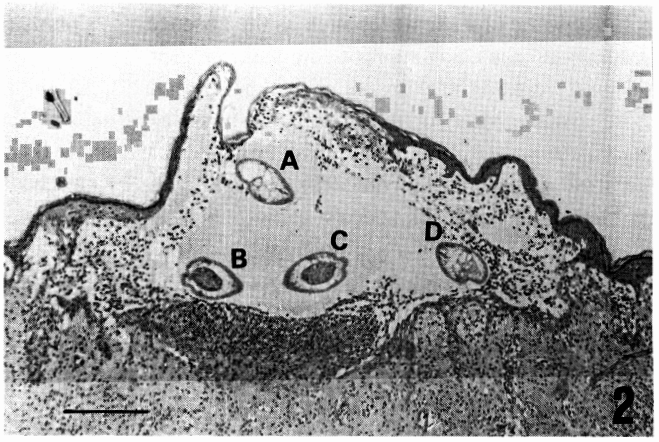
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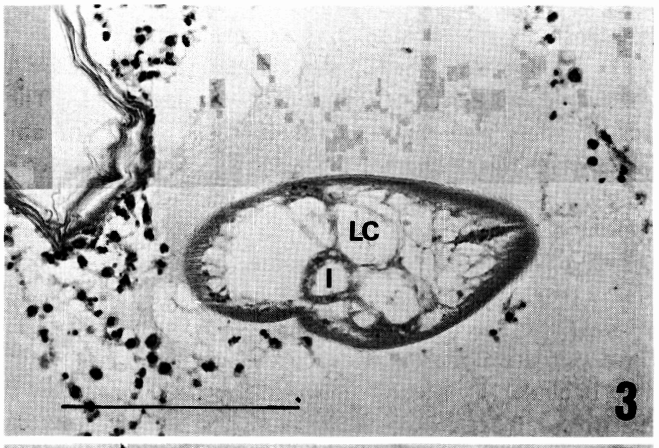
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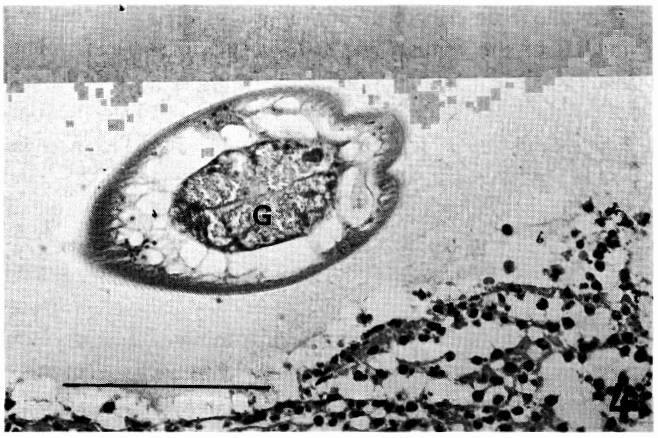
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considerable bilateral variability in size.

Since some of its features such as the absence of spines on the body surface, the small size of the intestine, and the existence of the large lateral chords were very different from those of *Gnathostoma,* I identified this parasite as Spiruroid nematoda (Spiruroidea), though I was unable to determine its species.

Case 2

A linear eruption with a width of 2 mm and a length of 15 cm accompanied by pruritus was seen in the left abdominal region. As gnathostomiasis was suspected, the front of eruption was excised. Four parasitic stumps were observed in the subcutaneous tissue (Fig. 2). The dimensions of the stumps were 76–97 μm × 118–180 μm. The cuticle had a thickness of 1.7–2.0 μm and cross-striations were visible on the surface. The muscle layer was of the polymyarian (with 5–6 muscle bundles in 1/4) coelomyarian type. An intestine with a diameter of 25–30 μm consisting of 4–6 cells was observed in 2 stumps (Fig. 3), while a esophagus measuring 47–53 μm × 70–75 μm was visible in the other 2 stumps (Fig. 4). There were lateral chords with a narrow base and large bilobular form extending into the body cavity.

The above-described morphology differed from that of the genus *Gnathostoma,* and the parasite was identified as a Spiruroid nematoda larva.

The patient was fond of raw fish dishes (sashimi) and had consumed raw fishes such as conger, prawn, squid and tuna 2 weeks before the onset of the disease.

Case 3

This patient visited a hospital complaining of an abdominal eruption accompanied by blisters and serpiginous erythema with pruritus. An area measuring 2.5 cm × 3.5 cm × 0.5 cm was excised and histopathological sections were prepared. However, I received only one section for identification. The parasitic stump was located in a deep subcutaneous tissue (Fig. 5), closely adhering to the host tissue, and a large number of eosinophils were accumulated at the periphery of the parasite (Fig. 6). The dimensions of the parasitic stump were found to be 84 μm × 91 μm. The cuticle was relatively thick at 3.0–4.3 μm, and it had a smooth surface. Its muscle layer was of the polymyarian (with 30 muscle bundles in 1/4) coelomyarian type. There was a esophagus with dimensions of 43 μm × 45 μm in the body cavity, but the presence of a lateral chord was unclear.

From the morphological characteristics described above, the parasite was identified as filarioid nematoda (Filarioidea Weinkand, 1985; Nematoda).

The patient had consumed slices of raw carp that had been washed in cold water.

Case 4

The patient came to a hospital complaining of an eruption in the right thigh. A biopsy revealed a subcutaneously located parasite measuring 84–96 μm × 106–220 μm (Fig. 7). The cuticle had a thickness of 1.7–2.2 μm, and a smooth surface. The muscle layer was of the polymyarian (with 10 muscle bundles in 1/4) coelomyarian type. Its intestine was small, measuring 27 μm × 36 μm, and consisting of 7 cells. The lateral chords had a narrow base and projected its bilobular form extensively into body cavity (Fig. 8).

The above-described findings conformed closely to the stump picture of Spiruroid nematoda type 10 (Spiruroidea) reported by Hasegawa (1978).

The patient did not consume raw fish.

Case 5

A biopsy was performed on the patient who visited the hospital complaining of linear serpiginous erythema with pruritus, which appeared in this case one month after a trip to Mexico. A parasitic stump was observed at a relatively deep subcutaneous tissue in the histopathological specimen. The dimensions of the stump were 90–183 μm × 102–198 μm, and chitinous spines were observed in the cuticle. The muscle layer was of the polymyarian (9–11 muscle bundles in 1/4) coelomyarian type. The intestine in the body cavity was large and consisted of cells with 2–6

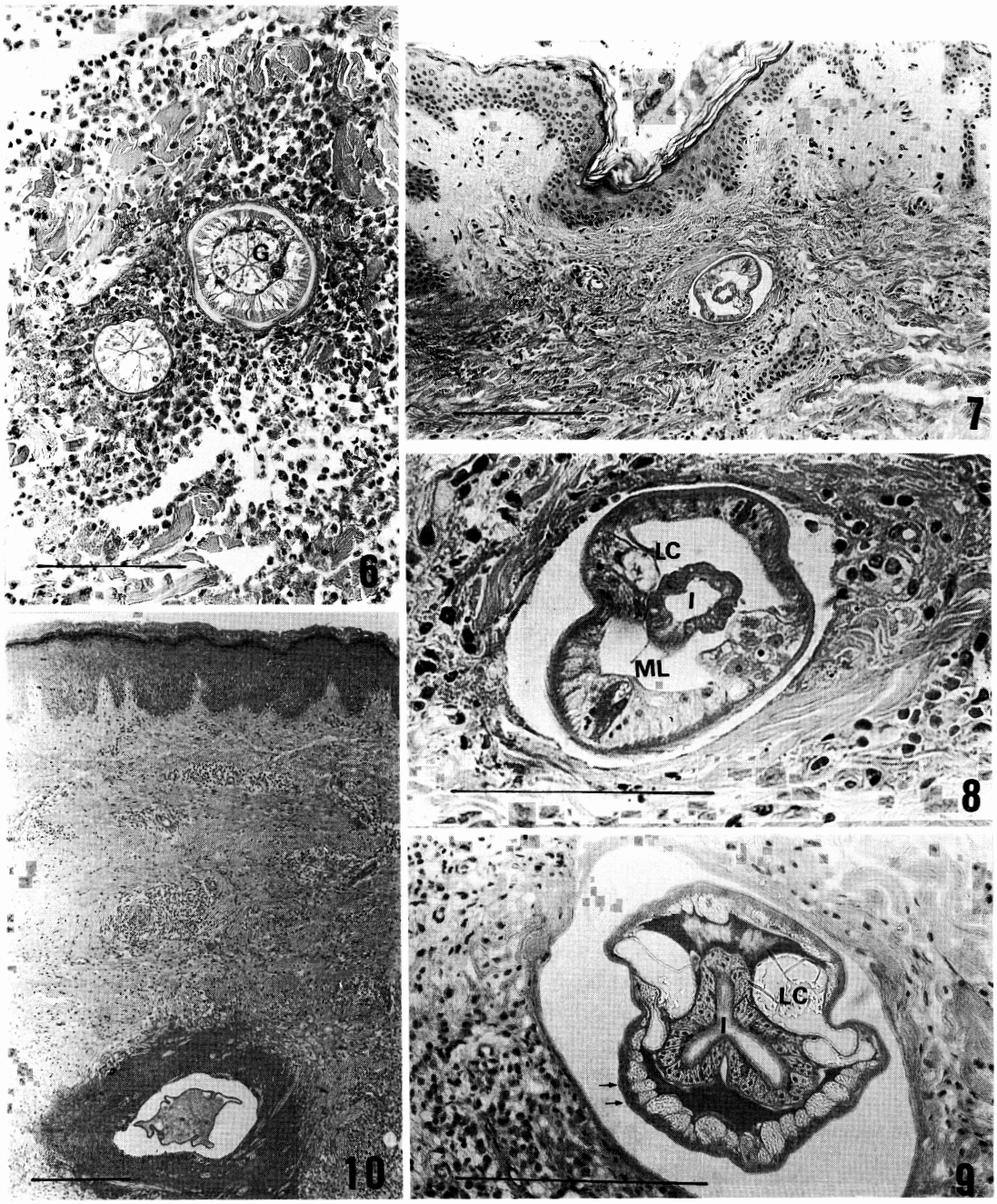


Fig. 6 Enlargement of a cross section of the larval nematoda in Fig. 5. G: grandular part of the esophagus. Scale: 100 μm.

Fig. 7 Cross-section of the larval nematoda in the cutaneous tissues of Case 4. Scale: 200 μm.

Fig. 8 Enlargement of a cross section of the larval nematoda in Fig. 7. I: intestine; ML: middle line; LC: lateral chord. Scale: 100 μm.

Fig. 9 Cross-section of the larva of *Gnathostoma spinigerum* in the cutaneous tissue of Case 5. I: intestine; LC: lateral chord; arrow: spine. Scale: 100 μm.

Fig. 10 This figure shows a section of worm-like migrations into the skin in Case 6. Scale: 1 cm.

nuclei. The lateral chords were also large and projected into the body cavity (Fig. 9).

As of the presence of spines on the body surface, a large number of intestinal cell nuclei within a range of 2 to 6, the parasite was identified as the larva of *Gnathostoma spinigerum* (Akahane *et al.*, 1986).

The patient had eaten sushi (vinegared fish and rice) made from raw flesh of a fish in Mexico.

Case 6

An erythema accompanied by pruritus appeared on the forearm. A biopsy of the induration was performed and histopathological sections were prepared. The parasite-like stump (Fig. 10) was large with the dimensions of 543 μm × 933 μm. It had a smooth surface, the inner tissue was uniformly spongiform, and structures like a characteristic muscle layer and/or internal organs were not visible. Hence, it did not appear to be a nematode, and I have not yet been able to identify.

Case 7

After this patient consumed raw turtle meat, a linear eruption appeared on his right buttock. A biopsy revealed the formation of granuloma measuring 0.43–0.61 mm × 0.95–1.39 mm at a 0.61 mm deep in the subcutaneous tissue. In the center of this granuloma, 3–5 parasitic stumps were observed (Fig. 11). This size was 84–89 μm × 101–110 μm. The cuticle had a thickness of 2.0–2.4 μm, and there were cross-striations on its surface. The muscle layer was of the polymyarian (10–13 muscle bundles in 1/4) coelomyarian type. In one parasitic stump, an esophagus measuring 26–68 μm × 23–75 μm was observed in the body cavity (Fig. 12), while in another stump an intestine consisting of a small and indeterminate number of cells was observed (Fig. 13). The lateral chords, surrounding the intestine, were also visible in these stumps. Many nuclei were observed in the narrow base of the lateral chords, and the bilaterally projecting lateral chords did not have a clear boundary. Many small nuclei were aggregated along the median line.

Using a needle, I picked up the remains of this parasite from the paraffin block. However, the anterior and posterior portions, which are important for identification, were absent, so that I could not identify the parasite species. Since it had a body width of 81 μm with only cross-striations with a width of 2.0 μm on the cuticle and no spines (Fig. 14), the parasite was clearly not *Gnathostoma,* but was, in all likelihood, Spiruroid nematoda (Spiruroidea).

Case 8

Ten days after consuming raw loach, this patient visited a hospital complaining of diarrhea and gastralgia. When he was admitted as an inpatient following a diagnosis of eosinophilia (74.5%), a creeping eruption was observed in the right shoulder. This was excised and histopathological sections were prepared.

The parasite, which was sectioned somewhat longitudinally below the epidermis of host (Fig. 15), was found to measure 123–125 μm × 642–734 μm. Several spines were visible on the cuticle surface, and the muscle layer below this was of the polymyarian coelomyarian type. Longitudinal sectioning of the parasite prevented determination of the number of muscle bundles. Intestinal cells were mono-nuclear or more rarely, has 2 large nuclei. Blackish granules were observed in the body cavity (Fig. 16). From the

Fig. 11 Figure shows the eosinophilic granuloma in Case 7 where three sections (A, B and C) of a larval nematoda migrating into the skin are visible. Scale: 500 μm.

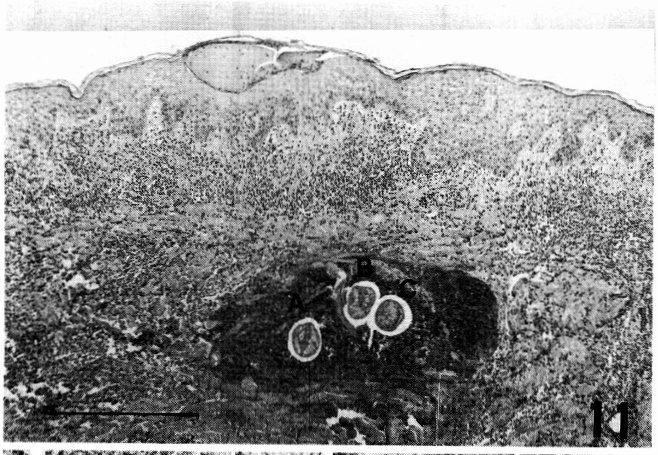
Fig. 12 Enlargement of section-C of the larval nematoda in Fig. 11. G: grandular part of the esophagus. Scale: 100 μm.

Fig. 13 Enlargement of section-A of the larval nematoda in Fig. 11. I: intestine; LC: lateral chord. Scale: 100 μm.

Fig. 14 A part of nematoda with cuticural cross-striation (2.0 μm wide) in which is piked up from the patient's skin by a needle. Scale: 100 μm.

Fig. 15 Longitudinal-section of the larva of *Gnathostoma hispidum* in the cutaneous tissue of Case 8. Scale: 500 μm.

Fig. 16 Enlargement of the longitudinal section of the larva *G. hispidum* in Fig. 15. I: intestine; LC: lateral chord; ML: middle line; arrow: spine. Scale: 100 μm.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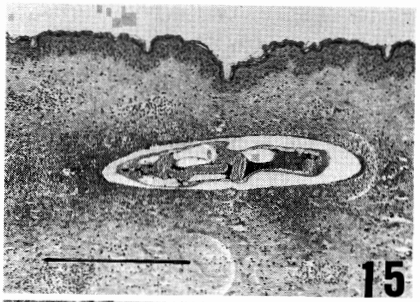
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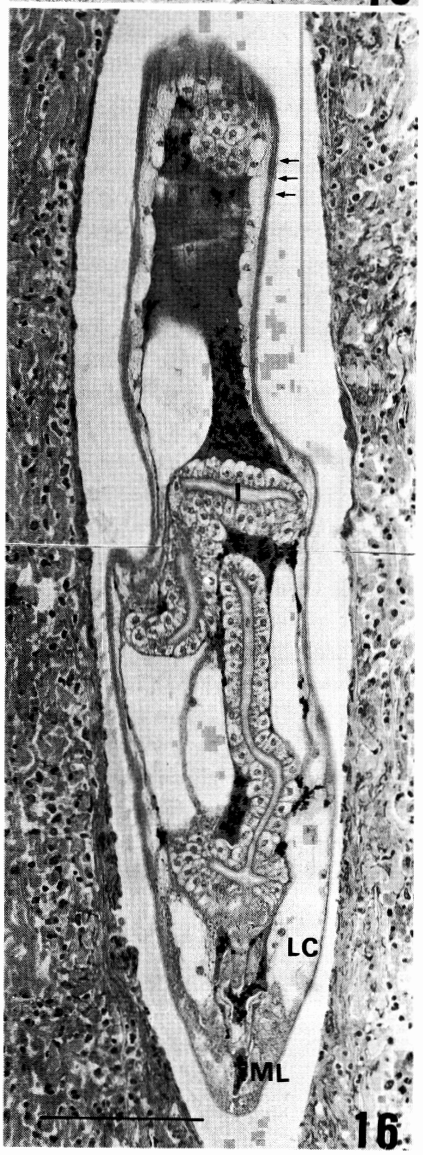
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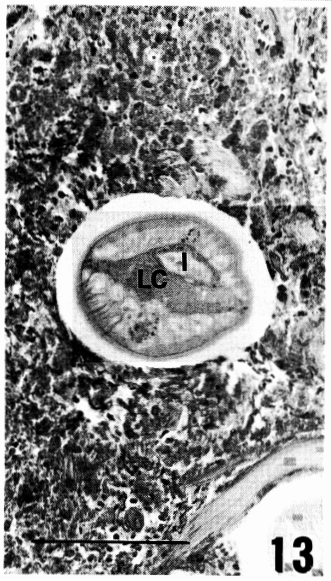
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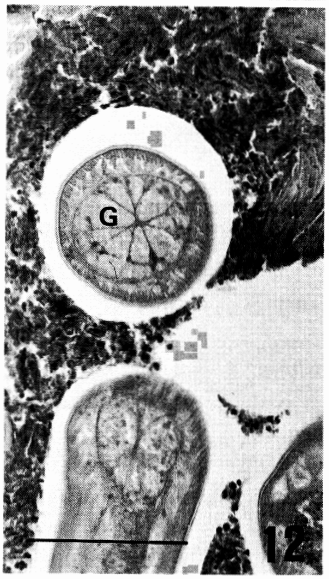
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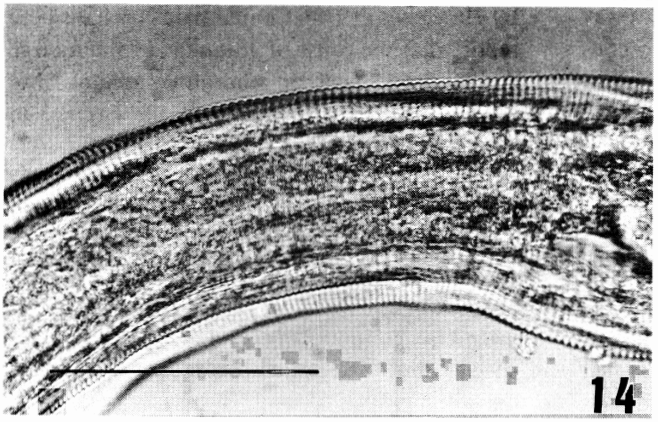
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presence of spines on the body surface, I identified this parasite as *Gnathostoma,* and because of its narrow body width, the presence of only one nucleus in the intestinal cells, and because the patient had consumed raw loach I identified it as *G. hispidum* (Akahane *et al.*, 1986).

Discussion

The findings of my morphological study revealed that the creeping eruption was caused by *Gnathostoma spinigerum* in one case, *G. hispidum* in one case, filarioid nematoda in one case, and an unidentified parasite in one case. The parasites found in the remaining four cases had, as described by Chitwood and Lichtenfels (1972), the polymyarian coelomyarian type muscle layer, an intestine consisting of a small number of cells, and of 4 irregular lateral chords. On the basis of these observations, I identified these four parasites as Spiruroid nematoda (Spiruroidea). This result indicates that some species of Spiruroid nematoda other than *Gnathostoma,* is a newly found parasite causing creeping eruption in Japan.

Creeping-eruption caused by *Gnathostoma* spp. larvae of the Spiruroidea in Japan had previously been attributed to only *G. spinigerum.* However, with the appearance in the 1980s of cases caused by *G. hispidum* existing in exotic loach species (Araki, 1983), and from *G. doloresi* (Ogata *et al.*, 1988; Nawa *et al.*, 1989), and *G. nipponicum* (Ando *et al.*, 1988), a need for a way to identify these species arose. This led to considerable research, resulting in the development by Akahane *et al.* (1986) of an almost complete method of identification using parasitic stumps in tissue sections. However, identification of eruption caused by Spiruroidea nematodes other than *Gnathostoma* species remains unresolved and this group of organisms itself remains unidentified.

Some features of the parasites found in four cases of the present study and which I classified as Spiruroid nematoda located in subcutaneous tissue, which clinically led to form the erythema. Furthermore, the surface of the parasite's cuticle was either smooth or exhibited fine cross-striations. This cuticle was thin, measuring 2–3 μm, and the subcuticular muscle layer was of the polymyarian coelomyarian type in which there were 5–8 or approximately 10 muscle bundles in 1/4 of the entire muscle layer. The basal portion of the lateral chords was narrow within the proximity of the muscle layer, while the portion projecting into the body cavity was large and bilobular, frequently varying in the size of the two lobules. An esophagus or intestine was visible in the body cavity, although the number of cells in the intestine was extremely small. These features are not identical to that of the Spiruroid nematoda species found in Japan to date, and suggest that the parasite causing the creeping eruption should be a new species. These findings also indicate that two or more species may be acting as pathogens of creeping eruption. One of the species appears to closely resemble the Spiruroid-larva type 10 discovered in the pyroric appendage of *Theragra chalcogramma* by Hasegawa (1978). However, the parasite reported by Hasegawa (1978) has not yet been solidly identified, and requires further study.

Reports of humans infected by Spiruroid nematoda in Japan are limited to the two cases described by Otsuru *et al.* (1974), in which the infected parasite was discovered in an eosinophilic phlegmonous inflammation of the ileum after laparotomy for suspected ileus. They subsequently reported that the parasite closely resembled the Spiruroid-larva type 10 described by Hasegawa (1978). Similar parasites have been reported as the cause of granuloma formation in the intestinal wall of laboratory animals, but there have been no reports of the parasite in subcutaneous tissue.

The above-described findings indicate that the larvae of an unidentified species of Spiruroid nematoda seem to cause cutaneous or visceral larva migrans. This represents a new area of interest in the field of human parasitology. I intend to continue my studies to identify the species and determine the life cycle of this parasite.

The content of this paper have been reported at the 2nd (1987) and 4th (1989) Parasitic Diseases Clinical Study

Meetings, the conference of the Japanese Dermatological Association (1987), and the 50th Conference of the Eastern Japan Regional Meeting of the Japanese Society of Parasitology (1990).

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