NEWLY RECOGNIZED CAUSATIVE LARVAL NEMATODE (SUBORDER : SPIRURINA) OF CREEPING ERUPTION

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INTRODUCTION

Gnathostoma are parasites of wild mammals and larval stages of 3 native species, *G. spinigerum*, *G. doloresi* and *G. nipponicum* have been found in cold blooded vertebrates. Moreover, larval *G. hispidum* has been found in loaches imported to Japan from east Asia (Akahane *et al*, 1982). Among these species, *G. spinigerum* had been considered to be the only causative agent of gnathostomiasis until the discovery of *G. hispidum* (Kitajima *et al*, 1981), *G. nipponicum* (Ando *et al*, 1988) and *G. doloresi* (Nawa *et al*, 1989) from specimens biopsied from human cases in Japan. However, since 1985, the number of patients with creeping eruption who had not eaten the presently known intermediate host of gnathostomiasis are increasing (Otaki *et al*, 1995). In addition, many human cases of ileus have been reported (Kagei *et al*, 1992; Morita *et al*, 1994). Causative larval worms from sections obtained by biopsy from patients resembled the larvae of Spirurina "type x" named by Hasegawa (1978). It was shown that most patients had eaten raw firefly squid and we detected type x larva in the firefly squid. This article describes the present status of creeping eruption by these larvae and morphological features of type x larvae.

HUMAN CASES

Clinical features

As of May 1995, 48 cases have been reported (Otaki *et al*, 1995), namely 31 cases of creeping eruption, 16 cases of ileus and 1 case of invasion into anterior chamber of the eye (Chuang *et al*, 1993). Patients were reported to have never eaten raw snakes, frogs or fresh water fishes which are known agents of transmission of gnathostomiasis. Almost all patients visited the hospital from March to July when firefly squid is sold in Japan. The period from ingestion to onset is approximately 2 weeks. Clinical findings are different from those of gnathostomiasis because the linear lesion is narrower and longer and usually contains vesicle (Fig 1). Eosinophil count is usually in the normal range or slightly higher. It is considered that larvae move in the superficial part of the skin because the worm sections are usually detected by biopsy from upper corium or epidermis.

Diagnosis

Patients were diagnosed by examination of morphological features of worm sections in biopsied specimens as described below. We established an immunofluorescence antibody test using a section of esophagus of type x larvae (Fig 2) (Taniguchi *et al*, 1994). Dehydrated larvae were embeded in resin, Technovit 7100, and cut 3 μm thick with a glass knife. IgG antibody of patients was positive for sera dilutions of over 1:32, where normal control sera and sera from the patients of human gnathostomiasis did not react with the sections of these larvae.

Morphological features of type x larvae

Since patients were seen from March to July, it was during this time that we examined by the digestive method of marine fish, shrimp and squid which were eaten raw including viscera. Four type x larvae (2.5%) were detected from the viscera of 162 firefly squids, *Watasenia scintillans* (Hotaru-ika) but not detected from fish, *Salangichthys microdon* (Shira-uo), *Leucopsarian petersi* (Shiro-uo) and shrimp, *Pandalus borealis* (Hokkoku aka-ebi)(Ando *et al*, 1992a).

Observation of external morphology of larvae by scanning electron microscope

Detected type x larvae were 6.5-8.5mm long and 80-105μm wide, and had almost transparent muscular esophagus (about 20 μm wide), slightly light brown glandular esophagus (about 45 μm wide) and almost

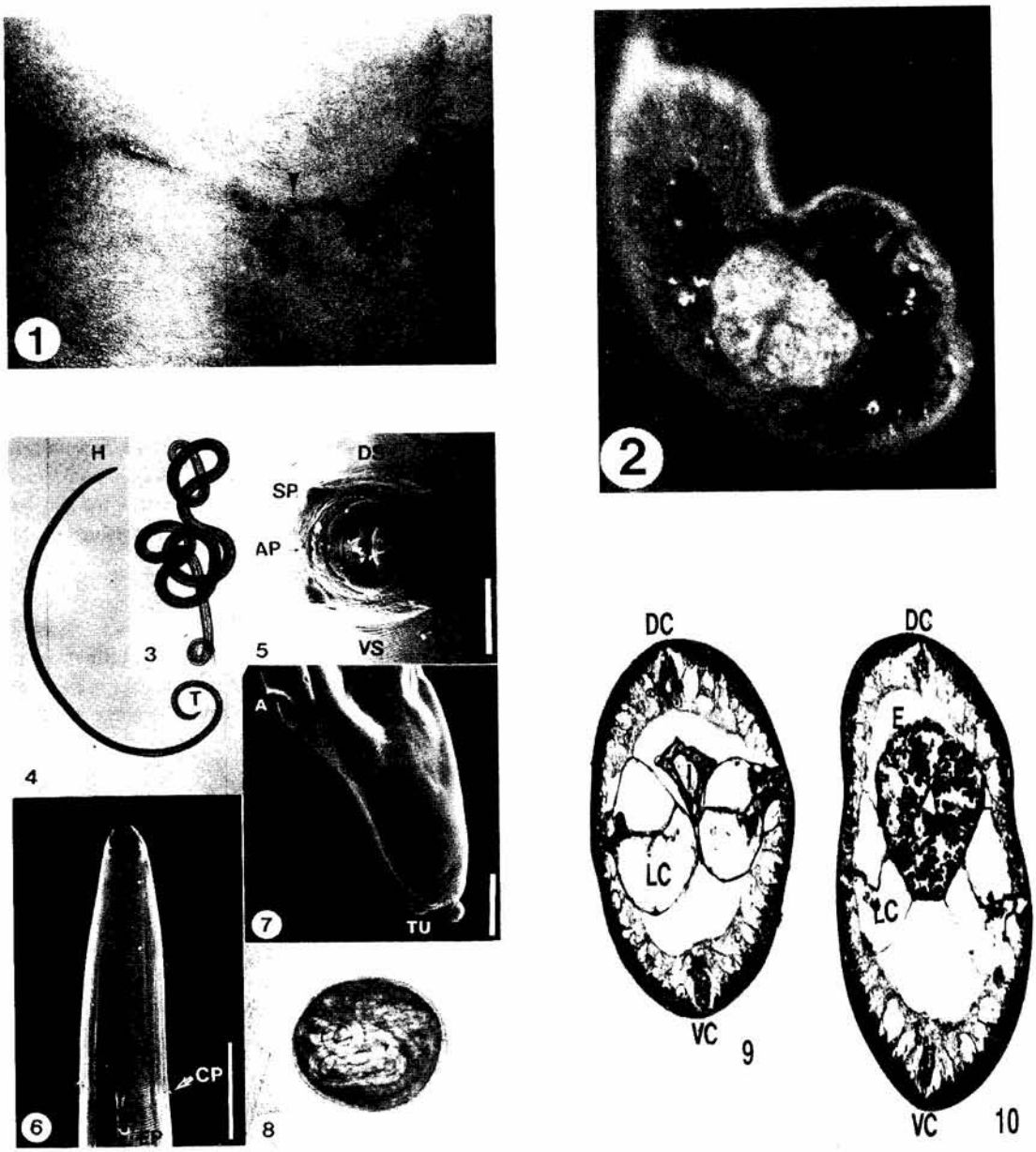

Fig 1 - Clinical findings of patient. Note vesicle (arrow).

Fig 2 - Immunofluorescence test using the section of larva type x. Esophageal part has positive fluorescence.

Figs 3-8 -Light microscopy (3,4 and 8) and scanning electron microscopy (5-7) of type x larva of the suborder Spirurina.

3. Living larva. 4. Dead larva. 5. Front view of head. Bar = 10 µm

6. Ventral view of anterior part of the body. Bar = 50 µm 7. Ventral view of tail. Bar = 20 µm

8. Larva encapsulated in the stomach wall of cod. 1 mm in diameter.

Fig 9-10 - Transverse section through intestine (9) and slightly oblique section through glandular esophagus (10)

A: anus; AP: amphidial pore; CP: cervical papilla; DS: dorsal surface: DC: dorsal chord; E: esophagus; EP: excretory pore; H: head; I: intestine; LC: lateral chord; SP: submedian papilla; T: tail; TU: tubercle; VC: ventral chord; VS: ventral surface;

transparent intestine (about 20 μm wide). Living larvae moved vigorously but dead ones were bent ventrally forming a coiled or fishhook shape (Fig 3, 4). Head ornamented with two triangular lateral pseudolabia pointed apically. Each lateral pseudolabia had two submedian papillae (Fig 5), and an amphidial pore was located anterior to the submedian papillae. The mouth was elongated and enlarged dorsoventrally. A pair of cilium-like cervical papillae was located laterally at a distance of 143 μm from the cephalic apex (Fig 6). The excretory pore was located on the ventral midline at a distance of 164 μm from the cephalic apex. Anus opened at a distance of 90 μm from tail tip (Fig 7). Two large tubercles were present, one (about 7 μm in diameter) at the tail tip and the other slightly smaller than the former on the ventral surface. A pair of phasmidial pores was clearly visible near the smaller turbercle on the ventral surface. Transverse striation was visible from the end of the pseudolabia to the tail tip. The intervals of the transverse striation were 1.4 μm at the anterior part of the body, 2.5 μm at the middle and 1.8 μm near the anus. Lateral alae were absent.

Morphological features in cross section of larvae

At the level of the middle part of the intestine, width of the cuticle was about 2.0 μm without lateral alae and spines (Fig 9). Musculature consisted of 28-31 muscle cells of polymyarian coelomyarian type in 1 quadrant. Dorsal and ventral chords were small but clearly visible. Lateral chords consisted of 2 unequal large lobes, projected conspicuously into the pseudocoelom and attached to each other. A nucleus existed mainly at the base of each lobe. The intestine was small and was pushed dorsally by lateral chords. Intestinal wall consisted of one layer of 5 to 6 epithelial cells. These cells were so small that nuclei in the cells were indistinguishable. At the level of the glandular esophagus, extremity of lateral chords was attached to esophagus and the lumen was triradiate (Fig 10) (Ando *et al*, 1992b).

DISCUSSION

The first 2 human cases (ileus) due to Spirurina larvae occurred in 1966 and 1969 and first reported by Otsuru *et al* in 1974. No more human cases were reported until 1985 when patients with creeping eruption and ileus caused by these larvae were seen. We first recognized a patient with creeping eruption different from that of gnathostomiasis in March of 1988, then one case in April of 1989, one case in March and one case in April of 1990 at our University Hospital. The causative agent was unknown until the 1991 report by Kagei (1991).

Type x larvae were detected in the stomach and intestinal wall of cod (Fig 8), *Theragra chalcogramma* (Suketou-dara)(Hasegaswa, 1978; Ando *et al*, 1992b) and sandfish, *Arctoscopus japonicus*(Hata-hata) (Otsuru *et al*, 1974). However, the viscera of these fish are very rarely eaten raw. As outbreak of patients was limited from March to July, we investigated the marine products eaten raw including viscera within this period and detected type x larvae from firefly squid (about 9cm long) for the first time. After that, it was found that most patients had eaten raw firefly squid (Okazaki *et al*, 1992; Otaki *et al*, 1995). Therefore, the period in which cases occurred coincided with the period in which firefly squid were sold in the market. Type x larvae have been detected in fish such as cod, *T. chalcogramma* sandfish, *A. japonicus*, and *Pleurogrammus azonus* (Hasegawa, personal communication) squid, *Todarodes pacificus* (Surume-ika) (Okazawa *et al*, 1993) and firefly squid, *W. scintillans*.

The natural final host of type x larva has been considered to be marine birds or mammals (Hasegawa, 1978; Hasegawa and Otsuru, 1982). However, with the absence of knowledge about the adult stage, its superfamily has not been specified. The suborder Spirurina consists of 10 superfamilies, namely Gnathostomatoidea, Physalopteroidea, Rictularioidea, Thelazioidea, Spiruroidea, Habronematoidea, Acuarioidea, Filarioidea, Aploctoidea and Diplotriaenoidea (Chabaud, 1974). We are presently attempting to determine the phylogenetic position of this worm based on partial 18S-rDNA sequences.

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