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Gnathostomiasis Possibly Caused by *Gnathostoma malaysiae*

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Gnathostomiasis is rarely reported in travelers, although the disease remains a major public health problem in Southeast Asia. A creeping eruption and Quincke's edema (slowly migrating erythema with pruritus) appeared in two Japanese men who had eaten raw freshwater shrimp in Myanmar. A *Gnathostoma* larva was found in subcutaneous tissue from one of the men. Four species causing human gnathostomiasis, *G. hispidum*, *G. doloresi*, *G. nipponicum* and *G. spinigerum*, can be distinguished based on the number of nuclei in intestinal epithelial cells of infected larvae, in cross-section. In *G. hispidum*, only a single large nucleus is found. Morphologically, our larva was initially identified as *G. hispidum*. However, since the number of epithelial cells was greater and the body width was larger than those of a "large-type" 3rd-stage larva of *G. hispidum*, the larva was then identified as a 3rd-stage larva of *G. malaysiae*, Miyazaki and Dun, 1965, as reported by Setasuban *et al*, (1991). Since no human cases caused by this species of *Gnathostoma* have previously been encountered, this appears to be the first report of gnathostomiasis due to *G. malaysiae*.

Key words : gnathostomiasis, Myanmar, *Gnathostoma hispidum*, *G. malaysiae*

INTRODUCTION

Since being described by Owen in 1836, at least 12 species of the genus *Gnathostoma* have so far been recorded from various animals in different localities [9, 11, 14, 17]. Six *Gnathostoma* species, *G. spinigerum*, *G. doloresi*, *G. nipponicum*, *G. vietnamicum*, *G. malaysiae* and *G. hispidum*, are normally found in domestic cats and dogs and in wild felines, such as leopards and tigers, in Asia. Adult worms are found in the stomach wall, esophageal wall, and intestinal lumen of the final hosts, whereas the larval worms are found in the subcutis of the second intermediate (or paratenic) hosts [9, 11]. Human gnathostomiasis, caused by migration of *Gnathostoma* larvae, is an important foodborne parasitic zoonosis that is endemic mainly in Asian countries where people consume raw freshwater fish. Infection in man is by the ingestion of parasitized raw, poorly cooked, fermented, or marinated freshwater fish (second intermediate or paratenic host). Common sources are "somfak", a fermented fish delicacy, and "sashimi", slices of raw fish. Among Japanese, eating "sashimi" prepared from freshwater fish is the most frequent cause of creeping disease due to gnathostomids [9, 11]. Imported cases of gnathostomiasis have rarely been observed [7]. Among Thai people, creeping disease after eating "somfak", is very common with more than 800 cases per a year recorded [15]. However, man is a dead end host in which larvae can persist but not develop further, giving rise to typical "cutaneous larva migrans" or "visceral larva migrans" syndromes [9]. Only the larvae of four species, *G. spinigerum*, *G. doloresi*, *G. nipponicum* and *G. hispidum*, have been reported as causing human infection [9, 11, 17].

Since 1989, the military government of Myanmar has begun to liberalize domestic economy and to accept economic aid from foreign countries. In addition to Japanese companies returning to Myanmar, many Japanese have visited or are working there.

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Although human gnathostomiasis is also endemic among Myanmar people eating fermented or raw freshwater fish or shrimp, little statistical information is available on the distribution of *Gnathostoma* and on human infection. We identified gnathostomiasis, possibly caused by *G. malaysiae*, in 2 Japanese men returning from a trip to Myanmar.

CASE REPORTS

Case 1 is a 45-year-old, healthy Japanese business man, who had resided for 30 days in Yangon, the capital of Myanmar. Usually, he ate only well-cooked fresh fish, but in October, 1995, on several occasions, he ate a salad containing roast pork and raw freshwater shrimp (species unknown) at a restaurant. One month later, a hard lump with associated mild pruritus (30 × 17 mm; erythematous region including the hard lump, 55 × 17 mm) appeared on his abdomen, which then migrated to his left breast (Fig. 1). On December 14, he came to our hospital because of the creeping eruption. A mild eosinophilia (12.1%) and a

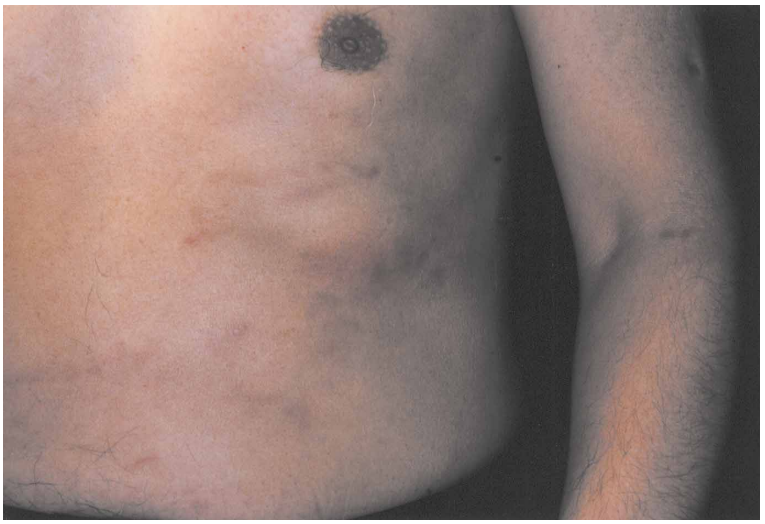

Fig. 1 Creeping eruption with pigmentation observed on the chest of case 1.

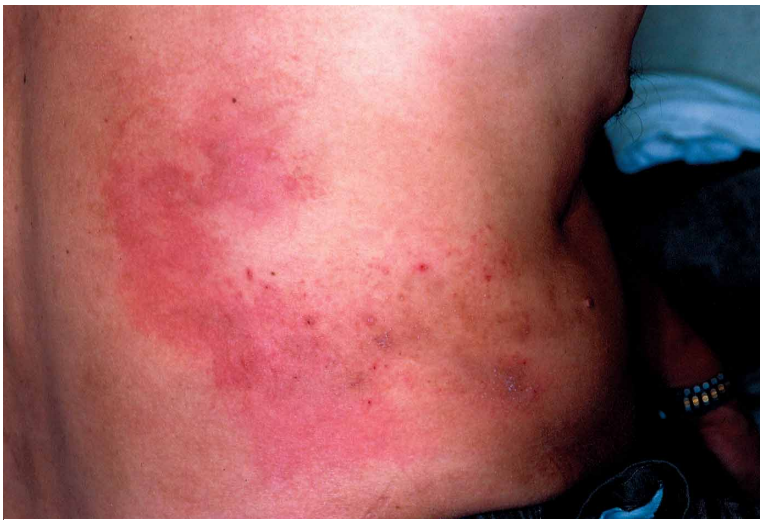

Fig. 2 Quincke's edema (slowly migrating erythema with pruritus) observed on the right abdominal wall of case 2.

high level of IgE (1,800 IU/ml) were detected. Further clinical examination and routine laboratory tests were negative. As gnathostomiasis was suspected, the hard lump was surgically removed in December 21. After the surgery, there has been no recrudescence. Ten months later, the patient's eosinophilia and the level of IgE had decreased to normal limits.

Case 2 is a 51-year-old, healthy Japanese business man. He is a long-time resident in Yangon, Myanmar. Similar to case 1, he had often eaten marinated raw freshwater shrimp in restaurants. In September 1995, he noted the migration of a hard lump, with pruritus, from the right to left hip, then to the left abdominal wall, and finally to the right abdominal wall (Fig. 2). At consultation, the patient showed Quincke's edema (slowly migrating erythema with pruritus) with a mild eosinophilia (10.0%). Other clinical and laboratory data were in the normal range. Because we suspected gnathostomiasis, Mebendazole (300 mg/day, for 3 days) was administered to the patient. The results of the treatment are unknown because the patient soon returned to Myanmar.

Both patients were examined serologically. By ELISA, after absorbing both sera with *Toxocara canis* antigen, case 1 was judged as negative, but case 2 was positive for *G. hispidum*. Neither serum reacted with *G. doloresi* antigen.

PATHOLOGIC FINDINGS

A cross section of a parasite (227 × 321 μm) was found in deep subcutaneous tissue (4.34 mm beneath the skin surface) in H & E stained slides from case 1. The parasite, closely adhering to host tissue, was cross-sectioned at the level of the esophagus and a large number of eosinophils were found along the wall of the parasite. The esophagus (125 × 78 μm) revealed four spherical neck bladders. A blackish-stained fluid was observed in the body cavity (Fig. 3). The parasite was morphologically identified as a *Gnathostoma* larva; body width 189–284 × 347–599 μm at the shoulder region between esophagus and intestine, the presence of a surface cuticle with chitinous hard spines, polymerian (about 21–26 muscle bundles in 1/2 of the body) type subcuticular muscle layer, and the existence of giant lateral cords. However, the head region, including the bulb, was not obtained. Further microscopic examination showed that the intestine of the larva consisted of 20–26 spherical epithelial cells, each with a single large nucleus (diameter, 4.6–5.0 μm).

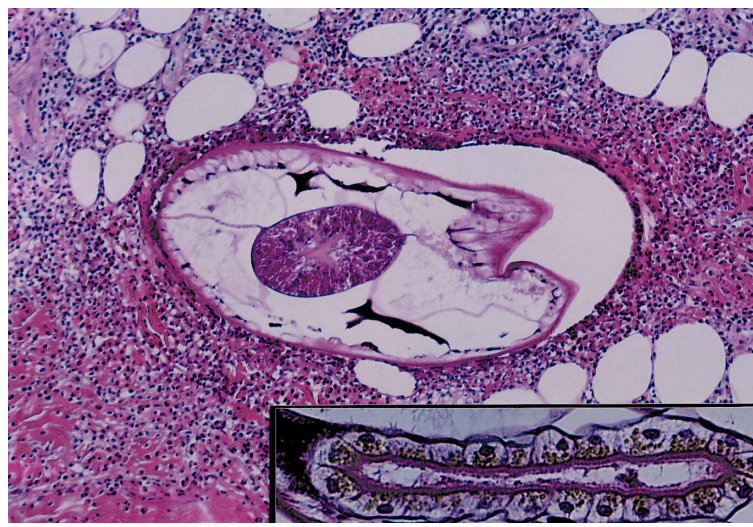

Fig. 3 Cross-section though the esophagus of the larva surrounded by an eosinophilic infiltration (HE, × 100). The parasite contains a round, dark structure which is the esophagus, four neck bladders, and blackish-colored body fluid. Insert: the intestine of the parasite. 25 spherical epithelial cells, each with a single large nucleus, can be seen. (HE, × 400)

The larva was first identified as a 3rd-stage larva of *G. hispidum* Fedtschenko, 1872, with intestinal epithelial cells containing a single large nucleus. The intestine of a 3rd-stage "small-type" larva of *G. hispidum* consists of 19–31 epithelial cells, whereas that of a "large-type" larva consists of 17–18. The body width of the present larva (189–284 × 347–599 μm) appeared to be too large to be a mature 3rd-stage larva from the "small-type" of *G. hispidum* (mean value; 214 μm) and to that reported for "large-type" larva of *G. hispidum* (mean value; >300 μm), However, since the larva consisted of 20–26 spherical epithelial cells, was different from that the mature 3rd-stage "large-type" larva of *G. hispidum*. On the other hand, the body width of the 3rd-stage larva of *G. malaysiae* (maximum; 400 μm) is significantly larger than that of *G. hispidum*, and the epithelial cells of the intestine contain 1–2 nuclei. Thus, our larva was finally identified as a mature 3rd-stage larva of *G. malaysiae* Miyazaki and Dun, 1965, rather than *G. hispidum*.

DISCUSSION

Parasites causing creeping disease include helminths of the genera *Gnathostoma*, *Spirurina*, and *Strongyloides*, and hookworms, filarioid nematodes, sparganum, and arthropods. The major causative agents are *Gnathostoma* and *Spirurina* larval worms.

Gnathostomiasis is an important parasitic disease in the Far East, giving rise to a "cutaneous larva migrans" or "visceral larva migrans" syndrome. Thailand and Japan are highly endemic for the disease, with more than 800 cases per a year in Thailand and more than 1,000 cases in Japan being recorded [11, 15].

Creeping eruption caused by *Gnathostoma* spp. larvae in Japan had previously been attributed only to *G. spinigerum* [11]. However, with the appearance in the 1980s of gnathostomiasis caused by *G. hispidum*, introduced in loachs imported from Taiwan, Korea, and Mainland China [1], and by *G. doloresi* [10, 13], and *G. nipponicum* [5], a need for methods to distinguish between the several species arose.

Since the absolute identification of *Gnathostoma* must be based on the morphology and DNA analysis of the adult worm, in that regard, we cannot specifically identify the larva. However, we need to identify the species of larval *Gnathostoma* found in biopsied specimens, although the taxonomic characteristics of the larvae are imperfectly known. Miyazaki reported that the mature 3rd-stage larvae of the *Gnathostoma* could be identified by the number of rows and features of the hooklets [9]. However, these taxonomic characteristics are rarely observed in biopsied specimens. On the other hand, Akahane *et al.* reported that the mature 3rd-stage larvae of *G. spinigerum*, *G. hispidum* and *G. doloresi* were distinguishable in cross-section by the number of nuclei in the intestinal epithelial cells [3]. Ando *et al.* reported that the intestinal epithelial cells of *G. nipponicum* contained 1–3 nuclei per cell, although a single nucleus is present in 50% of the larvae [5]. On this basis, we can identify the four species found in humans.

Because the parasite in one of our cases revealed 20–26 spherical epithelial cells with a single large nucleus, the larva was first identified as a 3rd-stage larva of *G. hispidum* Fedtschenko, 1872. This diagnosis was supported by serological data using *G. hispidum* and *G. doloresi* antigens. In loachs from Mainland China, there are at least two types of *G. hispidum* larvae, a "small-type", measuring about 610 × 93 μm in body length and width; and a "large-type", measuring about 2–4 × 0.3 mm in length and width. It is well recognized that the "small-type" 3rd-stage larva remains in the same stage without any subsequent development, and wanders in human tissue [2, 18]. On the other hand, the intestinal wall of the "large-type" 3rd-stage larva in loachs consists of a single layer of 17–18 spherical epithelial cells, each possessing only a single nucleus (a few cells contain more than 2 nuclei), similar to the mature 3rd-stage larva in rats [4]. Therefore, our larva was different from the 3rd-stage larva of *G. hispidum*, which consists of spherical epithelial cells with a single large nucleus.

Most human cases in Thailand have been caused by *G. spinigerum* [15]. Since the presence of *G. malaysiae* was confirmed in Thailand and Malaysia [8], all five *Gnathostoma* species, (*G. spinigerum*, *G. hispidum*, *G. dorolesi*, *G. vietnamicum* and *G. malaysiae*), have been detected [15]. In 1991, Setasuban *et al.* obtained five unidentified 3rd-stage larvae of *Gnathostoma* from fresh-

water eels (*Fluta alba*) in Nakho Nayok, Thailand [16]. The body length and width (5.2×0.4 mm) was significantly larger than the mature or "large-type" 3rd-stage larvae of *G. hispidum*. The larvae were peculiar in possessing four rows of hooklets with complicated branches at the base. Microscopic examination of the intestinal epithelial cells of the larvae showed spherical cells with 1–2 nuclei, suggesting a mature 3rd-stage larvae of *G. malaysiae* Miyazaki and Dunn, 1965 [12, 16]. Larval gnathostome taxonomy, based on the morphology and number of nuclei in the intestinal epithelial cells of the mature 3rd-stage larvae of the five *Gnathostoma* species, is summarized in Table 1 [10].

According to Table 1, our larva is a 3rd-stage larva of *G. malaysiae* Miyazaki and Dunn, 1965, although we cannot absolutely identify the larva. At present, there is a very strong possibility that our helminth is a 3rd-stage larva of *G. malaysiae*. Since no human cases caused by this species of gnathostome have previously been reported, this appears to be the first report of gnathostomiasis due to *G. malaysiae*. Although gnathostomiasis is endemic among Myanmar people eating fermented or raw freshwater fish or shrimp, little statistical information is available on the distribution of *Gnathostoma* and on human infection. Thus, this appears to be the first report of gnathostomiasis acquired in Myanmar.

Formerly, a slowly migrating erythema with pruritus ("Rangoon edema") was known and feared by the Myanmarese. Still prevalent, however, are the cutaneous symptoms of a creeping eruption and Quincke's edema, similar to the present cases, in people eating fermented or raw freshwater fish and shrimp. Since larvae 1 cm from the surface are killed by placing parasitized fresh fish in boiling water for 5 minutes, effective prevention is best achieved by avoiding poorly cooked fish [6]. Therefore, travelers to Myanmar should be cautioned about the danger of eating uncooked freshwater fish and shrimp.

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Table 1 Characteristics of five species of 3rd-stage *Gnathostoma* larvae found in human tissues

Species	parasite distribution	body width (μm)	intestinal epithelial cells			References
			type	No. of cells	no. of nuclei	
G. spinigerum	Southeast Asia	355	columnar	21–29	3–7	
G. hispidum						
"small-type"	Southeast	214	spherical	19–31	1	
"large-type"	Asia	>300	spherical	17–18	1	4
G. doloresi	Southeast Asia	239	spherical	18–28	2	
G. nipponicum	Japan	110	columnar	11–14	1–2	
G. malaysiae	Malaysia Thailand	400	spherical	20–26	1	12, 16

This table is modified from the table shown in Ref. 10.

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