AN OUTBREAK OF GNATHOSTOMIASIS AMONG KOREAN EMIGRANTS IN MYANMAR

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INTRODUCTION

Gnathostomiasis is a food-borne parasitic zoonosis caused by several species of the genus *Gnathostoma* (Nematoda), particularly *Gnathostoma spinigerum*.1 Dogs, cats, and wild mammals are known to serve as definitive hosts, but humans can be accidental or paratenic hosts.2 In humans, the nematode larvae typically cause intermittent subcutaneous migratory swellings, but less commonly involve the internal organs.2 A total of 12 *Gnathostoma* species have been reported,3 with the most important species causing human infection being *G. spinigerum*.2 A few human cases infected with *G. hispidum*, *G. doloresi*, *G. nipponicum*, and *G. binucleatum* have been reported in Thailand, Japan, and Mexico.1,4

The general life cycle is identical among all *Gnathostoma* species, with only slight variations in the second, paratenic, and definitive hosts.1 The adult parasites are found in the stomach or esophageal wall of definitive hosts that consume raw fish.1 When the host's feces containing eggs are deposited in fresh water, free-swimming first-stage larvae are liberated and ingested by the minute crustacean, *Cyclops*, where they molt twice to become the early third-stage larvae (L3).1 They then develop into the advanced L3 in the second intermediate hosts, namely fishes and amphibia.1,2 They are passed to a wide spectrum of paratenic hosts including fishes, amphibians, reptiles, birds, and mammals.2

Humans usually acquire the infection through the consumption of raw or undercooked flesh of the paratenic hosts containing the infective L3. Most human gnathostomiasis cases are reported in Asia, particularly in Thailand and Japan.4 Over the last 30 years, however, the geographic range of this disease has extended to countries along the Pacific coasts of East Asia and the Americas.3 In Myanmar, two cases of human gnathostomiasis were reported involving the eyes.5,6 No further reports of gnathostomiasis have been published in Myanmar. In the Republic of Korea, only one imported case has been reported in a Thai woman with meningoecephalitis; the worm was removed from her brain and it was identified as *G. spinigerum*.7 Larval worms have been reported in the fresh water fishes and snakes caught in the Republic of Korea,8,9 and also in a species of fresh water fish imported from China.10,11

We report here an apparent outbreak of gnathostomiasis among Korean emigrants residing in Yangon, the capital of Myanmar, which was previously unrecognized as an endemic area for this parasite infection.

MATERIALS AND METHODS

Study subjects and treatment. A total of 60 Korean emigrants residing in Yangon, Myanmar who had eaten raw fresh water fish in a local Korean restaurant one (n = 55) or two (n = 5) times during January and February 2001 were selected for this study. Thirty-eight of these 60 individuals, 22 men and 16 women with ages ranging from 5 to 54 years, developed migratory swellings and creeping eruptions, with pain and itching in the cutaneous regions (designated hereafter as cases; Table 1). The remaining 22, 17 men and 5 women with ages ranging from 6 to 51 years, were grossly healthy when interviewed, but several complained of nervousness and insomnia, seemingly due to fear of developing the disease (designated as asymptomatic counterparts; Table 1). In all 60 cases, clinical histories, including the onset, progression, and severity of the disease were obtained, and physical examinations were performed by two of the authors (J-YC and MH).

The patients recalled that they had eaten several kinds of fresh water fish (in sashimi-style, raw flesh, 50–200 grams per person) including catfish, fresh water bream, and snake-headed fish, which were served at a restaurant, especially during the last week of January and the first week of February 2001. No other special risk factors at the infection source were reported. Under the impression of cutaneous larva migrans due to migrating nematode larvae, the patients were treated with albendazole, 400 mg, three times a day for three weeks, together with a single dose of ivermectin, 200 μg/kg of body weight.

Histopathologic examinations. Just before the start of chemotherapy, skin biopsies were performed in two patients with...
recent cutaneous lesions after informed consent was obtained. Skin snips with an area of 1–3 cm² were obtained under local anesthesia. The tissues were immediately fixed in 10% buffered formalin, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5-μm thickness were prepared, stained with hematoxylin and eosin, and examined using a light microscope.

**Collection of blood and sera.** Blood and serum samples were obtained from 110 people, including 38 cases, 22 asymptomatic counterparts (who consumed raw fish), and 50 healthy controls (living in Japan, without history of migratory swellings). The samples were taken two times (March 15 and April 2, 2001) from the cases and asymptomatic counterparts, and only once from the healthy controls. The blood was drawn by venipuncture, and thin blood smears were prepared, stained with hematoxylin and eosin, and examined using a light microscope.

**Enzyme-linked immunosorbent assay (ELISA).** The details of the antigen preparation and the ELISA procedure for detection of gnathostomiasis have been previously described. Briefly, the antigen was obtained as the crude somatic extract from the infested fish. The lyophilized worms were homogenized and sonicated in 0.15 M phosphate-buffered saline (PBS), pH = 7.6, containing p-methylsulfonylfluoride (Sigma, St. Louis, MO), 0.1% p-hydroxymercuribenzoate (Sigma), 10 mM EDTA (Sigma), and 0.2% antibiotics-fungizone solution (Gibco/BRL Life Technologies, Gaithersburg, MD). The extract was centrifuged at 6,400 × g for 20 minutes at room temperature. The supernatant was filtered through a 0.25-mm Millipore (Billerica, MA) membrane, and the protein content measured by the Lowry method. The antigen, at a concentration of 200 ng/100 mL, was coated onto a flat-bottomed, 96-well microtiter plate (Corning Inc., Corning, NY). The plate was incubated overnight at 4°C, and rinsed once with PBS containing 0.5% bovine serum albumin (Gibco/BRL Life Technologies) and 0.5% Tween, and twice with PBS containing 0.5% Tween. The serum samples were diluted 1:200 with PBS, incubated at 37°C for 90 minutes, and washed with PBS containing 0.5% Tween. Horseradish peroxidase–conjugated goat anti-human IgG (Caltag Laboratory, Burlingame, CA) was added at a dilution of 1:1,000 and incubated at 37°C for 90 minutes. The plates were then washed with PBS containing 0.5% Tween, and the substrate, o-phenylenediamine (Sigma), added to the wells for 30 minutes. The reaction was stopped by the addition of 8 M sulfuric acid, and the plates read at a wavelength of 490 nm in an ELISA reader (MR 600; Dynatech, Diversified Equipment Co., Lorton, VA). A group of 50 healthy volunteers with no history of migratory swellings served as negative controls. The cut-off value for a positive criterion was determined as the mean optical density (OD) plus two standard deviations of the negative controls. Samples showing OD values ≥ 0.30 were regarded as positive.

**Examination of Gnathostoma larvae in fish.** In an attempt to identify the infection source, several species of local fish were collected and examined for the presence of Gnathostoma larvae. A total of 10 fresh water fish, including 6 Parasilurus sp. (catfish), 3 Tilapia sp. (fresh water bream), and 1 Ophiocephalus sp. (snake-headed fish), were purchased from a local market in Yangon, Myanmar. The flesh of each fish was ground in a meat grinder, and incubated in an artificial gastric juice containing 6 grams of pepsin (1:10,000; Sigma) per liter of 0.08% HCl at 37°C for 4–5 hours. The sediment was rinsed twice in running water and examined using a stereomicroscope. The larvae were vigorously washed three times with PBS, fixed in 2.5% glutaraldehyde (Sigma), pH = 7.4, for 24 hours, and then further washed with PBS. The larvae were examined

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic, clinical, and serologic characteristics of the study population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Cases (n = 38)</td>
</tr>
<tr>
<td>0–15</td>
<td>6 (15.8%)</td>
</tr>
<tr>
<td>16–30</td>
<td>2 (5.3%)</td>
</tr>
<tr>
<td>31–45</td>
<td>22 (57.9%)</td>
</tr>
<tr>
<td>≥46</td>
<td>8 (21.1%)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>35.4 (12.2)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Male</td>
<td>22 (57.9% /56.4%) †</td>
</tr>
<tr>
<td>Female</td>
<td>16 (42.1% /76.2%) †</td>
</tr>
<tr>
<td>History of eating raw fish</td>
<td>Yes</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Eosinophilia (March 15, 2001)</td>
</tr>
<tr>
<td>No. examined</td>
<td>Positive ‡</td>
</tr>
<tr>
<td>0–3%</td>
<td>16 (55.2%)</td>
</tr>
<tr>
<td>4–7%</td>
<td>5 (17.2%)</td>
</tr>
<tr>
<td>≥8%</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td>ELISA (March 15, 2001)</td>
<td>No. examined</td>
</tr>
<tr>
<td>No. examined</td>
<td>28</td>
</tr>
<tr>
<td>Positive ‡</td>
<td>20 (71.4%)</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (28.6%)</td>
</tr>
</tbody>
</table>

* ELISA = enzyme-linked immunosorbent assay.
†Percent of the total number of males and females who consumed raw fresh water fish. The proportion who developed symptoms and became cases was higher in females than in males, but the sex difference was not statistically significant (P = 0.17).
‡ People who showed an optical density (OD) > 0.30 in the ELISA were considered positive. The mean ± SD OD of the sera from healthy controls, who had never been in Myanmar, was 0.12 ± 0.09 (n = 50).
using a light microscope, and then carefully dehydrated and processed for scanning electron microscopic observations.

Statistical analysis. The Student’s $t$-test or Fisher’s exact test was performed to evaluate the significance of differences between groups, with a value of $P < 0.05$ regarded as statistically significant.

RESULTS

Demographic characteristics of the patients. The majority (32 of 38, 84.2%) of the cases were adults 16–54 years old and the other six (15.8%) were children 5–14 years old. The mean age of the cases was 35.4 years (Table 1). The age distribution of the asymptomatic counterparts was similar to that of the cases (mean age = 35.5 years). A higher proportion of females (76.2%, 16 of 21) than males (56.4%, 22 of 39) ($P = 0.17$) among those who consumed the raw fish developed creeping eruptions (Table 1).

Clinical manifestations of the patients. The chief complaints were migratory swellings and creeping eruptions (Figure 1) on the back ($n = 24$, Figure 1A and C), abdomen (14, Figure 1B), flanks (6), buttocks (6), chest (2), neck (2), thighs (2), arms (2), hands (1), and lower jaw (1). Common symptoms included itching ($n = 28$), nodule formation (22), fatigue (16), urticaria (12), fever (10), pain on the skin (10), and erythematous plaques (10). Minor symptoms were anorexia.
(4), blurred vision (3), indigestion (2), vomiting (2), headache (2), and diarrhea (1). All the cases had a history of eating raw fresh water bream, snake-headed fish, or catfish at the same restaurant between the end of January and early February 2001. Symptoms appeared sporadically from 1 to 10 weeks after eating the raw fresh water fish. The incubation periods from the day of the raw fish consumption (in 5 of 38 cases from the time of the first of two consumptions) to the onset of the skin symptoms were <10 days (n = 19), 10–19 days (12), and ≥20 days (7), as revealed by their histories.

Relapse and re-treatment. After treatment with a combination of albendazole and ivermectin, the patients' symptoms quickly subsided in most cases. However, relapse of the symptoms was observed in five cases from one to three months following treatment. Reinfection was ruled out because none of the cases consumed raw fresh water fishes again; the responsible Korean restaurant in Yangon, Myanmar was closed directly after the disease outbreak. The relapsed cases were re-treated with albendazole alone, with the same daily dose, for 7–14 days. The results were satisfactory, with complete resolution of the symptoms in all cases, and no further relapses were reported.

Biopsy findings. No parasites were found in the two biopsy specimens. However, there was evidence of acute inflammatory reactions, with diffuse infiltration of eosinophils and histiocytes. Tunnel formations were observed along the connective tissues or muscle layers, which were suggestive of the migration routes of the larva.

Eosinophilia. The mean ± SD percentages of eosinophils in the white blood cells of peripheral blood samples were 6.3 ± 6.5% (n = 29, range = 0–23%) and 9.0 ± 9.8% (n = 26, range = 0–44%) on March 15 (Table 1) and April 2, 2001, respectively, among the cases. These values were significantly higher than the 2.9 ± 1.8% (n = 18, range = 0–6%) and 2.9 ± 1.1% (n = 10, range = 0–28%), respectively, observed in the asymptomatic individuals (P < 0.05 by non-paired t-test) (Table 1).

ELISA titers. The titers of antibodies to *Gnathostoma* were compared between the sera of the cases, asymptomatic persons (who consumed the raw fish), and the healthy controls (Figure 2). The sera of the healthy controls showed a mean ± SD OD value of 0.12 ± 0.09 (n = 50), and the cut-off value was arbitrarily determined as 0.30 (Table 1 and Figure 2). The OD values for the sera of the cases were 0.47 ± 0.29 (n = 28) and 0.32 ± 0.20 (n = 30) on March 15 and April 2, 2001, respectively, and both were significantly higher (P < 0.05) than that of the controls. Twenty-four (63.2%) of the 38 cases showed OD values higher than 0.30 in at least one of the two examinations. In the two consecutive examinations, 20 (71.4%) of 28 and 12 (40.0%) of 30 of the cases examined showed positive results (Table 1 and Figure 2). The sera of the asymptomatic persons had OD values of 0.19 ± 0.24 (n = 16)

**FIGURE 2.** Enzyme-linked immunosorbent assay (ELISA) absorbances of the sera of gnathostomiasis patients shown as optical density (OD) values. Sera were obtained twice, on March 15, 2001 (A) and April 2, 2001 (B), and showed similar results. A crude extract of *Gnathostoma doloresi* was used as the antigen, horseradish peroxidase-conjugated goat anti-human IgG was used as the secondary antibody, and o-phenylenediamine was used as the substrate. The reaction was read in an ELISA reader at a wavelength of 490 nm. The cut-off value for a positive reaction was 0.30 (dashed horizontal lines) based on the mean OD plus two standard deviations of the negative controls, 0.12 ± 0.09 (n = 50).
and $0.20 \pm 0.19$ ($n = 10$) for the respective examinations (Figure 2). These were significantly lower ($P < 0.05$) than that of the cases. One and three asymptomatic persons, in the respective examinations, had positive OD values (Figure 2).

Recovery of nematode larvae from the fish. Two Gnathostoma larvae were detected in two catfish (Parasilurus sp.) examined; one from the muscle and one from the viscera. They had an average length of 3.4 mm, an average width of 0.3 mm, and were morphologically compatible with G. spinigerum. Four rows of single-pointed hooklets were observed on the head bulb of the larvae (Figure 3), and the average number of hooklets in each row was 43, 43, 44, and 51, respectively (Table 2). These larvae were regarded as the advanced third-stage larvae of G. spinigerum.

**DISCUSSION**

The confirmatory diagnosis of gnathostomiasis requires isolation of the larvae from the lesions. However, such an opportunity rarely arises. In this study, no larval or adult parasites were detected in the skin biopsy specimens of the two cases; thus, the etiologic agent of the current outbreak of creeping eruptions remains unknown. Other than gnathostomes, the major agents responsible for creeping eruptions in humans include human (Necator americanus) or animal (Ankyloloma caninum or A. braziliense) hookworms. However, it is suggested that the outbreak was due to infection with G. spinigerum because of the severe clinical features in the cases, their ELISA results, their histories of consuming the raw fresh water fish, and the detection of the larvae in the fish. The source of the infection is presumed to be the fish the cases stated they consumed in the Korean restaurant in Yangon.

**FIGURE 3.** Light (A and B) and scanning electron (C) micrographs of advanced third-stage larvae of Gnathostoma spinigerum that were recovered from a catfish, Parasilurus sp., purchased from a local market in Yangon, Myanmar. A. Whole worm showing its typical head bulb with spines, esophagus, intestine, and anus. Scale bar = 0.5 mm. B. Head bulb, equipped with four transverse rows of spines. Scale bar = 20 μm. C. View of the head bulb with spines. M = mouth; arrowhead = dome type labial papillae. Scale bar = 20 μm.

In Yangon, Myanmar, human gnathostomiasis has been uncommon, with only two cases reported, one in 1960 and the other in 1968. This is probably because people in Myanmar rarely eat raw fish. Thus, this region had never been identified as an endemic area for human gnathostomiasis. Interestingly, in both the Myanmar cases, it was reported that the patients complained of eye symptoms, such as intraocular hemorrhages, anterior uveitis, red eye with defective vision, and a probable foreign body in the eye. In both cases, surgery was performed on the lesions, and the worms removed from the eye; they were identified as G. spinigerum. The presence of G. spinigerum adults in cats and dogs has occasionally been observed in Myanmar, but no proper investigation has been done on the intermediate hosts, such as fish, chickens, pigs, and cyclops. However, in this study, G. spinigerum larvae were found in the fresh water fish purchased in Yangon, Myanmar. Therefore, it can be concluded that the life cycle of G. spinigerum is maintained around Yangon, Myanmar, and there is a potential risk of other outbreaks of human gnathostomiasis in this country.

In addition to G. spinigerum, the presence of G. hispidum has also been observed in Myanmar, although no human cases have been reported. The possible presence of another Gnathostoma species has also been reported. Two Japanese individuals who visited Myanmar and consumed raw fresh water shrimp developed creeping eruptions, and the third-stage larva of G. malaysiae was detected in the subcutaneous tissue of one of the two patients. Surveys of the fresh water shrimps for G. malaysiae infection are required.

Because the definitive diagnosis of gnathostomiasis through identification of parasites is rarely possible, immunodiagnosis, particularly ELISA, is often used. With respect to the antigen, the third-stage larvae of G. spinigerum have been used with satisfactory results. However, G. doloresi larvae were shown to be useful for the diagnosis of gnathostomiasis due to G. hispidum and G. spinigerum in Japan, although the sensitivity or specificity was lower than when using homologous antigens. The diagnostic usefulness of the G. doloresi antigen was further verified in human gnathostomiasis in Mexico, which was previously known to be due to G. spinigerum, but later verified to be due to a distinct species, G. binucleatum.

The present study shows that G. doloresi antigen is generally useful in the serodiagnosis of gnathostomiasis presumably due to G. spinigerum. However, two points must be taken into considerations. First, the sensitivity of the test may be low. Only 63.2% (24 of 38) of the cases with visible skin lesions showed positive ELISA OD values, and there were 14 false-negative individuals. For example, the case shown in Figure 1A was included among those who showed false-negative results. Second, the specificity of the test may also be low because a crude extract of G. doloresi, a heterologous antigen, was used. However, the four seropositive asymptomatic persons (Figure 2) may have been infected and positive for antibodies, but had undergone a subclinical course.

Eosinophilia is a characteristic condition in tissue-invading helminthic infections, including gnathostomiasis. In this study, the average values of eosinophils were 6.3% and 9.0% in two consecutive examinations of the cases with symptoms, which were significantly higher than that of their asymptomatic counterparts. The four ELISA-positive asymptomatic
persons had 0%, 5%, 11%, and 28% eosinophils, supporting the suggestion in the latter three cases.

With the exception of albendazole, which is given at a daily dose of 400 mg for 21 days,3,22 there had been no effective chemotherapeutic regimen for human gnathostomiasis.2 Recently, however, ivermectin was used to treat gnathostomiasis patients, and showed a reported cure rate of 95.2% in a single dose (with 0.2 mg/kg).23 Conversely, in the treatment of infection with Wuchereria bancrofti, a combination therapy of ivermectin and albendazole was used and compared with that of ivermectin alone.24 The use of the two drugs in combination produced no increase in the frequency or severity of adverse reactions. Therefore, we used the combination therapy of ivermectin and albendazole, which was shown to be satisfactory, with no serious adverse reactions. With the exception of five relapsed cases (not re-infections because these individuals did not consume raw fish again), all became free from symptoms after the full 21-day course of treatment. The relapsed cases were re-treated with albendazole alone for reduced periods of 7–14 days, after which no further relapses occurred. Thus, the combination therapy of ivermectin and albendazole is recommended for the treatment of human gnathostomiasis.

Based on the clinical manifestations of the cases, their histories of eating raw fish, their ELISA results, and the detection of the larvae in the fish, the current outbreak of creeping eruptions among Korean emigrants in Myanmar is suggested to have been caused by infection with a species of gnathostome, most probably G. spinigerum. The fresh water fishes the cases consumed in the Korean restaurant in Yangon are presumed to be the source of the infection. Health education on the avoidance of consuming raw fresh water fish is required for the Korean people residing in Myanmar.

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REFERENCES


Table 2
Comparative body length and hooklet numbers in the advanced third-stage larvae of four species of Gnathostoma

<table>
<thead>
<tr>
<th>Species</th>
<th>Body length (mm)</th>
<th>No. of hooklets on the head bulb (mean)</th>
<th>First row</th>
<th>Second raw</th>
<th>Third raw</th>
<th>Fourth raw</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. nipponicum</em></td>
<td>1.0–1.5</td>
<td>29–36 (32)</td>
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<tr>
<td><em>G. dolores</em></td>
<td>1.8–4.0</td>
<td>34–42 (38)</td>
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</tr>
<tr>
<td><em>G. hispidum</em></td>
<td>1.2–3.5</td>
<td>32–38 (36)</td>
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</tr>
<tr>
<td><em>G. spinigerum</em></td>
<td>2.6–4.1</td>
<td>40–47 (43)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><em>G. spinigerum</em></td>
<td>3.2–3.4</td>
<td>42–44 (43)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Miyazaki and Ishi (1952).† Koga and others (1985).‡ This study.