CLINICAL MANIFESTATIONS AND IMMUNODIAGNOSIS OF GNATHOSTOMIASIS IN CULIACAN, MEXICO

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Abstract. Gnathostomiasis was first described in Mexico in 1970, and endemic areas have been spreading in six states of this country. In Culiacan, Sinaloa, 300 cases of cutaneous larva migans were recorded between January 1992 and December 1995. In addition, a Gnathostoma larva was surgically removed from the eye of one patient. Cutaneous lesions were observed mainly on the face, neck, arms, and legs. About 70% of the patients showed eosinophilia. A skin biopsy was carried out on 35 patients and the parasite was identified in histopathologic sections of 12 of these patients. In four patients, the larva migrated out spontaneously from the skin. An enzyme-linked immunosorbent assay using a crude somatic extract of adult Gnathostoma doloresi worms showed that 93% of the patients were seropositive, confirming the reliability of clinical diagnosis. A total of 14 advanced third-stage Gnathostoma larvae were found in four species of ichthophagous birds captured on dams and dikes near the city of Culiacan. Scanning electron micrographs of human and bird larvae showed that they were morphologically indistinguishable from G. spinigerum. We conclude that the life cycle of Gnathostoma has been established in Sinaloa, and has become a serious public health issue for residents.

Gnathostomiasis is one of the important food-borne parasitic zoonoses caused by infection with larvae of the spirurid nematode, genus Gnathostoma, with the disease being characterized principally as cutaneous larva migans.1 Among 12 distinctive species, only G. spinigerum had been considered as the causative species of human gnathostomiasis until the recent discovery of human cases infected with G. hispidum, G. doloresi, and G. nipponicum in Japan.2-8 The life cycle of Gnathostoma is essentially identical within the genus, with only slight variations in the secondary, paratenic, and definitive hosts. Eggs are released from adult worms that live in the stomach or esophagus wall of the definitive hosts (cats, dogs, and other wild mammals). After being hatched from eggs in fresh water, the first-stage larvae are ingested by copepods where they molt twice to become the early third-stage larva (L3). They then develop into advanced L3 in fish and amphibians, the second intermediate hosts. They are then disseminated into a wide range of paratenic hosts such as large carnivorous fishes, reptiles, and birds along the food chain.

Infection in humans occurs when the second intermediate/paratenic hosts contaminated with the L3 are ingested. The disease is endemic mainly in Japan and the secondary, paratenic hosts the people prefer to eat raw freshwater fish as a delicacy.2,3,9 Sporadic cases have also been described in other Asian countries.2,3,9 In America, native cases were first recorded in Ecuador, later in Mexico.10,11 Subsequently, a large number of patients with cutaneous migratory swellings were identified in the states of Oaxaca and Veracruz in Mexico.12 Endemic areas in Mexico have spread to the states of Guerrero, Nayarit, Tamaulipas, and Sinaloa.13,14 In spite of an increasing number of patients, only limited information is available on Mexican gnathostomiasis.

In this paper we report the study of 300 human cases of gnathostomiasis diagnosed in Culiacan, the capital of the state of Sinaloa, located on the north Pacific coast of Mexico, a geographic area not previously recognized as endemic for this parasite. A serum ELISA for diagnosis was developed and tested on patients and controls. Additionally, an attempt to identify the source of infection in regional paratenic hosts such as freshwater fish and bird species was carried out.

SUBJECTS, MATERIALS, AND METHODS

Subjects. A total of 300 cases (186 women and 114 men) of individuals presenting with intermittent migratory edema of the skin who were diagnosed and treated at the Dermatology Services of the Hospital General Dr. Bernardo Gastelum y Rigoberto Aguilars Pico between January 1992 and December 1995 were retrospectively studied. They were all residents of the city of Culiacan, State of Sinaloa, Mexico. Clinical histories recorded the onset and progression of symptoms, history of consuming raw or undercooked freshwater fish, birds, frogs, and snakes. A group of 240 apparently healthy volunteers, also residents of Culiacan, with no history of migratory edema, served as controls.

Histopathologic examinations. Skin biopsies were obtained from 35 patients with recent cutaneous lesions after informed consent was obtained to perform this diagnostic procedure. Skin snips measuring 1-4 cm² were obtained under local anesthesia. The tissues were fixed immediately in 10% buffered formalin, dehydrated, and embedded in paraffin. Sections 5-µm thick were cut and stained with hematoxylin and eosin.

Hematologic and serologic examinations. Blood samples were obtained by venipuncture. Total white blood cell counts were measured with a hemocytometer. Eosinophil counts were determined by examination of blood smears fixed in methanol and stained with Wright’s solution. Sera
obtained from all patients and controls were kept at −20°C until use.

**Indirect ELISA.** Antigens were obtained from a crude somatic extract of *G. doloresi* adult worms. Lyophilized worms were homogenized and sonicated in 0.15 M phosphate-buffered saline (PBS) pH 7.6, containing 0.2% *p*-methylsulfonylfuoride (Sigma, St. Louis, MO), 0.1% *p*-hydroxymercurobenzoate (Sigma), 10 mM EDTA (J. T. Baker, Philipsburg, NJ), and 0.2% antibiotics-fungizone solution (Gibco/BRL Life Technologies, Gaithersburg, MD). The extract was centrifuged at 6,400×g for 20 min at room temperature. The supernatant was filtered through a 0.25-mm Millipore membrane and the protein content measured by the Lowry method. Antigen at a concentration of 200 ng/100 ml was bound to flat-bottom, 96-well microplates (Immulon II, Dynatech Laboratories, Chantilly, VA) by incubating overnight at 4°C, followed by rinsing once in PBS.

0.5% bovine serum albumin, 0.5% Tween and twice in PBS, 0.5% Tween. Serum samples were diluted 1:200 in PBS, incubated at 37°C for 90 min and washed with PBS, 0.5% Tween. Goat anti-human IgG coupled to alkaline phosphatase (Sigma) was added at a dilution of 1:1,000 and incubated for 90 min at 37°C. The plates were washed in PBS, 0.5% Tween and the substrate p-nitrophenylphosphate (Sigma) was added to the wells for 30 min. The reaction was stopped by adding 3 M sodium hydroxide and the plates were read at a wavelength of 405 nm in an ELISA plate reader (SR-50; Pasteur, Marnes-la-Coquette, France). The cut-off value was determined as the mean optical density (OD) of the controls plus three standard deviations. All samples having OD values > 0.5 were considered positive.

**Search for Gnathostoma larvae in fish and birds.** To identify intermediate/paratenic hosts of *Gnathostoma* in Culiacan, a total of 1,915 freshwater fish specimens were obtained from nearby bodies of fresh water impounded behind dams and dikes, as well as rivers, and lagoons where aquiculture is exploited for local and national consumption of fish. The fish species included *Tilapia* spp., *Oreochromis* spp., *Ictalurus* spp., and *Micropterus salmoides*. From the same area, 57 ichthyophagous birds were caught and included: *Egretta alba* (n = 14), *Pelecanus erythrorhynchos* (3), *Pelecanus occidentalis* (4), *Nictiorax nictiorax* (2), *Ardea herodias* (5), *Phalacrocorax olivaceus* (26), and *Pandion haliaetus* (3). Sex and morphometric data were recorded for each specimen. The muscle masses were dissected, ground in a domestic meat grinder, and compressed between two glass plates (10 × 12 cm). The preparations were observed under a 100 watt light source. The pooled ground tissue was further incubated in artificial gastric juice containing one gram of pepsin in one liter of 0.07% HCl at 37°C for 4–5 hr. The sediment was rinsed twice in running water, and examined in Petri dishes under a stereoscopic microscope following the method of Imai and others. The presence of stray domestic animals, such as cats, dogs, and pigs, feeding on dead fish along the shores, was also recorded, although no systematic observations were made, and it was not possible to obtain feces samples because owners were reluctant to have their pets examined.

**Scanning electron microscopy.** Larvae obtained from human and bird infections were washed in RPMI 1640 medium and immersed in Karnovsky's fixative at 4°C for 24 hr, washed in 0.15 M cacodylate buffer for at least 24 hr, post-fixed in osmium tetroxide for 2 hr, dehydrated, and prepared for scanning electron microscopy by critical point drying and gold coating.

**RESULTS**

**Clinical manifestations of patients.** The majority (61.2%) of the patients were between 30 and 45 years of age. 35% were more than 45 years of age, and only four cases (3.3%) were 7–14 years of age. Most (92%) of the patients had eaten freshwater fish in regional dishes known as ceviche or callos, which are served raw and marinated in lemon juice. Most (78.2%) of the 300 patients presented with typical cutaneous manifestations of intermittent migratory swelling and indurated erythematous plaques (Figure 1A), accompanied by itching and occasional pain. Creeping lesions (Figure 1B) or swelling without erythema (Figure 1C) were less frequently observed. Many patients had experienced previous episodes of migratory swelling.

The location of the lesions in 300 patients was as follows: upper (27.3%) and lower (29.7%) limbs, head (16%), and the trunk (27%) (Figure 2). Ocular involvement was observed in one case, a larva that was successfully removed from the posterior eye chamber by surgery. *Gnathostoma* larvae were found by biopsy in the skin of 12 (34.3%) of 35 patients (Figure 3). In the other 23 biopsies, only eosinophilic panniculitis was observed. In four patients who had been treated with albendazol, the parasite spontaneously migrated out of the skin.

**Hematologic and serologic studies.** Eosinophil counts in peripheral blood were carried out in 240 patients and an equal number of controls. As shown in Figure 4, gnathostomiasis patients had significantly higher eosinophil counts when compared with the control group (P < 0.00005, by χ² analysis with Yates’ correction).

Antibodies to *Gnathostoma* in the sera of 300 patients and 152 controls were measured by ELISA using crude somatic extract of *G. doloresi* adult worms. When the mean OD + 3 SD of 152 samples from healthy volunteers was used as the cut-off point, 279 (93%) patients were identified as positive and 21 (7%) as negative. In the control group, only five cases (3.2%) had ELISA OD values > 0.5. The differences between patient and control groups were statistically significant (P < 0.000000005, by nonpaired t-test). The sensitivity and specificity of the ELISA method were respectively estimated to be 93% and 98.7% (Figure 5).

**Survey for Gnathostoma larvae in fishes and birds.** A total of 14 *Gnathostoma* larvae were found in four species of fish-eating birds: nine from *E. alba*, two from *P. erythrorhynchos*, two from *P. occidentalis*, and one from *A. herodias*. In spite of an extensive search, *Gnathostoma* larvae were not recovered from any fishes examined.

**Morphologic study of the larvae.** To identify the species of *Gnathostoma*, the larva obtained from one patient and five larvae from bird muscle (*E. alba*) were examined by scanning electron microscopy. The morphologic appearance of the larvae obtained from human (Figure 6A) and from birds (Figure 6B) were almost indistinguishable from those reported for *G. spinigerum*. Four rows of single-pointed hooklets were found on the head bulb.
**FIGURE 1.** Skin lesions in three patients with cutaneous larva migrans. A, a serpiginous face lesion in a 42-year-old man with one year history of migratory edema. B, an edematous swelling of left face and upper lip, without erythema, one month after first symptoms in a 24-year-old man. C, a large edematous lesion with erythema on upper thigh of 38-year-old woman with a two-year history of recurrent lesions.

**FIGURE 2.** Location and frequency of skin lesions in 300 patients with cutaneous larva migrans.

of all larvae examined, with an average number of hooklets in each row of bird larvae of 39, 42, 44, and 49, respectively, slightly fewer than those reported previously for *G. spinigerum*. The cervical papilla was located between rows 13 and 15 of the single pointed cuticular spines on the body (Figure 6B).

**DISCUSSION**

The results described here constitute the first evidence of *Gnathostoma* infections in humans and wild animals in Culiacan, Sinaloa, Mexico. In Culiacan, the appearance of gnathostomiasis in humans coincides with the custom of eating raw freshwater fish in the form of ceviche or callos, a custom that began about 20 years ago after the construction of three nearby dams and the formation of lakes that produce 700–900 tons of freshwater fish annually. Earlier cases of human
gnathostomiasis in Mexico reported in Oaxaca, Veracruz, Nayarit, Guerrero, and Tamaulipas were also associated with development of aquiculture in freshwater bodies formed by newly constructed dams.\textsuperscript{13, 14} Freshwater fish were apparently introduced into Sinaloa from other lakes in Mexico, including Temascal, which has been shown to endemic for gnathostomiasis.\textsuperscript{12}

The clinical features of gnathostomiasis patients in Culiacan were characterized as recurrent migratory swellings or creeping lesions lasting for several years in upper or lower extremities and the head that lasted for several years. These features are similar to those described in patients from other localities in Mexico, and similar to those reported in patients infected with \textit{G. spinigerum} in Thailand and Japan, but different from individuals infected with \textit{G. hispidum}, \textit{G. doloresi}, or \textit{G. nipponicum}.\textsuperscript{2, 3, 9, 12, 14} In infections with the latter three species, typical signs are creeping eruptions on the trunk of relatively short duration (2–3 months), without relapses. In one patient, a \textit{Gnathostoma} larva was surgically removed from the posterior eye chamber. To our knowledge, this is the second case of ocular gnathostomiasis in Mexico since Ortiz and others reported the first case more than 10 years ago.\textsuperscript{18} Four patients in the present studypicked out the larvae emerging through the skin surface with needles or by scratching. All had been treated with albendazole. A similar observation has been reported in patients from Thailand after
Eosinophilia is a characteristic feature of helminthic infections, especially of larva migrans. In the present study, eosinophilia was noted in 69% of the patients, which was significantly higher than that of the controls (\(P < 0.00005\)). However, a fairly large proportion (46.1%) of control subjects also had eosinophilia > 5%. This may be due to a high prevalence of helminthic infections other than gnathostomiasis in Culiacan, Sinaloa. Up to 20% of the population in some communities of Sinaloa are infected with *Ascaris lumbricoides*, *Strongyloides stercoralis*, and hookworm as found by stool analysis (Diaz-Camacho S, unpublished data).

Although human gnathostomiasis is diagnosed best by detecting worms in the lesions, this is often difficult because of the migratory nature of the parasite and its location in diverse organs and tissues. Therefore, detection of antibodies by various immunologic methods such as skin tests, the Ouchterlony test, indirect hemagglutination, immunofluorescence, ELISA, and Western blot has been used for surveys and diagnosis in Thailand and Japan.\(^{19-23}\) Immunodiagnosis of gnathostomiasis in Mexico has been difficult due to insufficient adult or larval antigens because the definitive or intermediate/paratenic hosts with heavy infection have not been found in this country. In the present study, we used the crude somatic extract of adult *G. doloresi* worms in an ELISA with highly satisfactory results; 93% of the patients were seropositive. The test clearly discriminated between the patient and control groups, all of whom resided in the city of Culiacán. The highly significant differences between the serology of the largest number of controls (152 individuals) and patients with larva migrans (300) examined in an endemic area confirm the validity of this test. *Gnathostoma doloresi* adult worm antigen has also been used successfully for immunodiagnosis of gnathostomiasis in Ecuador.\(^{24}\)

In the present study, we were unable to identify *Gnathostoma* larvae in freshwater fish. We attribute this to not ex-
amining a sufficient number of samples (approximately 0.001% of the estimated annual production). Lamothe-Argumedo and others have also observed a low number of infected fish. However, ichthyophagous birds would seem to have a higher probability of acquiring $L_3$ from infected fish, since this is their main source of food. In this study, larvae were found in four different species of ichthyophagous birds ($E. alba$, $P. erythropychos$, $P. occidentalis$, and $A. herodias$). This is the first time that $P. occidentalis$ has been identified as a paratenic host of *Gnathostoma* in Mexico. This pelican is a marine bird that has recently been observed to feed in the vicinity of bodies of freshwater, possibly due to ecologic changes. The advanced $L_3$ have been found in Temascal, Oaxaca, (southeastern Mexico), an area endemic for human gnathostomiasis, in four genera of freshwater fish and seven species of ichthyophagous birds. In the same region, a few adult worms were found in wild cats and reported as *G. binucleatum*. The present results, in conjunction with those of previous publications, indicate that the life cycle of *Gnathostoma* has been established in Mexico.
The presence of stray domestic animals such as cats and dogs roaming and feeding freely on fish along the shores and banks suggests that these domestic animals may be definitive hosts for the adult worms of *Gnathostoma* in this geographic area.

The larvae recovered from birds in Culiacan were morphometrically identical with those reported previously for Mexican *Gnathostoma* and were indistinguishable from *G. spinigerum*. Thus, in addition to the clinical features, the morphologic properties of the recovered larvae are very similar to those of *G. spinigerum*. Because there is a possibility that *Gnathostoma* was introduced into Mexico through imported freshwater fish for populating lakes and reservoirs, further studies are required to establish whether Mexican *Gnathostoma* is in fact a new species. Work is ongoing in our laboratories for this purpose. Whatever the species, present results clearly show that gnathostomiasis is a serious health issue in Mexico.

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