GNATHOSTOMA AND GNATHOSTOMIASIS IN ECUADOR

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Abstract. Three surveys were conducted during October 2000 and March 2002 in Guayaquil, Ecaudor, to determine the presence of *Gnathostoma* larvae in freshwater fish. Two hundred and twenty-two freshwater fish consisting of 5 species were purchased and examined for *Gnathostoma* larvae under a stereomicroscope. Two of 5 species of freshwater fish, *Hoplias microlepis* and *Rhamdia cinerascens*, were positive for the advanced third-stage larvae (L3) of *Gnathostoma*; the prevalence of *Gnathostoma* larvae in *Hoplias micolepis* were 61.4-76.9% and the intensily was (1-16) (mean 2.4-4.5). The prevalence and intensity in *Rhamdia cinerascens* were 16.9-80% and 1-13 (mean 5.2), respectively. Detailed investigations of 4 cases of gnathostomiasis at different lesions were also described.

INTRODUCTION

Gnathostomiasis has been recognized in Ecuador since 1979, with epidemic characteristics. Initially, distinguished dermatologists, such as Luis Carvajal, described it as 'migrating cutaneous inflammations'. In 1980, Wenceslao Ollague, described it as 'nodular migratory eosinophilic paniculitis'; in 1981, he discovered it was gnathostomiasis when he isolated the larvae from the tissue of a patient, and adult parasites in the stomachs of dogs and cats, in 1985.

The natural epicenter is located all over the hydrographic river basin of the Guayas, from its Daule-Babahoyo confluence to the north of Guayaquil City.

The natural habitats are the rice fields that abound in all these localities, where the intermediate hosts, copepods, and fishes, such as *Hoplias microlepis, Rhamdia cinerascens*, and *Tilapia mossambica* are found. In these localities, felines, and canines live in houses near the natural habits and feed on these fish, and when they defecate, the life cycle continues, because the stools are disseminated into river tributaries, where the copepods and fish are.

Fish may easily enter the freshwater tributaries of the rivers Daule and Babahoyo, and as an example, Corvidae infected with *Gnathostoma* larvae are transported by urban consumers.

In Japan, in May 2003, studies performed on tropical infectious diseases in Latin America were published, of which one was 'Etiological agents of Ecuadorian Gnathostomiasis in Guayas Province, Southwest Ecuador' following the proposed research protocol parameters of the international agreement between the University of Guayaquil, Ecuador, and Fukuoka University, Japan (Akahane, 2003).

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Clinical features of gnathostomiasis

In the year 1990, the cases of human gnathostomiasis exceeded 2,000. As the incidence was low, limited information was given to the population about this disease.

In 1995, Lazo *et al* reported a study of 63 patients with cutaneous gnathosthomiasis (nodular migratory eosinophilic paniculitis); most were superficial gnathostomiasis, in the age range 10-60 years. Localization was variable and the evolution of the disease was 1-3 years. An immunological study by Mimori (1987) in Ecuador was performed by skin test reaction with antigen elaborated from the parasite *G. spinigerum* from Japan; the reaction was variable, between 20-29 mm.

In order to find more information about intermediate hosts of *Gnathostoma* in Ecaudor, surveys of *Gnathostma* larvae in freshwater fish in a natural epicenter of gnathostomiasis were conducted.

Special cases of pleuro-pulmonary, ocular, cephalic, and cardiac gnathostomiasis, using epidemiological, immunological and eosinophilic diagnosis and micro-morphological study of the larvae extract by surgical intervention, were also presented.

MATERIALS AND METHODS

Surveys of *Gnathostoma* larvae in freshwater fish

In the period October 2000-March 2002, Akahane, the first author of the paper (Akahane *et al*, 2002),

Table 1
The prevalence and intensity of advanced third stage larvae of *Gnathostoma* in 122 freshwater fish.

Fish species	1st survey (Oct 2000)		2nd survey (Aug 2001)		3rd survey (Mar 2002)	
	Prevalence (%)	Intensity No. (mean)	Provalence (5)	Intensity No. (mean)	Prevalence (%)	Intensity No. (mean)
Hoplias microlepis (N=155)	61.4	1-6 (2.4)	70	1-16 (4.5)	76.9	1-6 (2.6)
Rhamdia cinerascens (N=54)	80	1-13 (5.2)	-	-	16.9	3.0
Colorada cichlasmajestae (N=5)	-	-	-	-	-	-
Isostitus remiser (N=5)	-	-	-	-	-	-
Tilapia mossambica (N=3)	-	-	-	-	-	-

visited Guayaquil three times to conduct three surveys with Ecuadorian coauthors, to determine the presence of *Gnathostoma* larvae in freshwater fish. The study was conducted in the natural epicenter, the hydrographic basin of the Guayas River.

A total of 222 freshwater fish (consisting of 5 species: 155 *Hoplias microlepis*, 54 *Rhamdia cinerascens,* 5 *Isostitus remiser*, 3 *Tilapia mossambica*, and 5 *Colorada cichlasmajestae*) were purchased at various markets in Guayaquil and neighboring towns. The muscles of the fish were sliced, pressed between two glass plates, and examined for the presence of *Gnathostoma* larvae under a stereomicroscope. The hooklet morphology of the larvae was observed without fixing under a light microscope. The head-bulbs were severed from the body and embedded in Faure's solution to count the number of hooklets.

RESULTS

Results of the surveys (Table 1)

In a survey of October 2000, advanced third-stage larvae of the genus *Gnathostoma* were detected in *Hoplias microlepis* and *Rhamdia cinerascens*, whereas no larvae were found in *Isostitus remiser, Tilapia mossambica*, or *Colorada cichlasmajestae*. The prevalence of *H. microlepis* with *Gnathostoma* larvae was 61.4%, and the intensity of infection was 1-16 (mean 2.4). On the other hand, the prevalence and intensity of *R. cinerascens* were 80% and 1-13 (mean 5.2), respectively. In the survey of August 2001, the prevalence and intensity of *H. microlepis* with *Gnathostoma* larval were 70% and 1-16 (mean 4.5), respectively, while in the survey done in March 2002, the prevalence of *H. microlepis* and *R. cinerascens* were 76.9 and 16.9%, respectively, and the intensities of *H. microlepis* and *R. cinerascens* varied from 1-6 (mean 2.6) and 3.0, respectively.

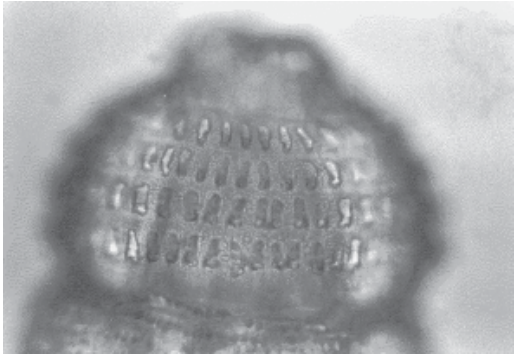

Fig 1- Third-stage larva of Ecuadorian *Gnathostoma*, the head-bulb.

In our 3 surveys, only 2 species of freshwater fish, *H. microlepis* and *R. cinerascens*, were positive for *Gnathostoma* larvae, showing a correlation between the prevalence or intensity and body sizes of *H. microlepis*. A high prevalence and large intensity were observed in large-sized fish; however these tendencies were not particularly strong. The third-stage larvae body length of Ecuadorian *Gnathostoma* from *H. microlepis* measured 3.0-5.0 mm, which was obviously the largest in those of the genus *Gnathostoma*. The hooklets were observed on the head-bulb and the base of each individual hooklet had a broad shape and was almost equal in size from the 1st to the 4th row (Fig 1). (Range: 34-42 μm in the first row; 42.3 (range 39-46) in the second; 44.0 (range 41-47) in the third; and 47.7 (range 42-52) in the fourth.

Investigations of four cases of gnathostomiasis

Pleuro-pulmonary gnathostomiasis. A 60-year-old mother, who presented a pleuro-pulmonary symptom, for which, after a wide differential diagnosis, there was no etiological definition. We associated his

case with both had ingested corvine ceviche 20 days earlier. On September 8th, 1989, a chest x-ray showed segmental congestion of the right pulmonary lobe. On September 16th, another chest x-ray showed shadowing of the costal frenic angle by pleural lesion. A blood test on September 7th showed an eosinophilic count of 8. On September 16th, the eosinophilic count was 32, and the skin test reaction with *Gnathostoma* antigen was positive at 10/45. After treatment with albendazole 15 mg/kg/ for 8 days the patient had a remarkable improvement in health.

Ocular gnathostomiasis (1995). A female 20-year-old student patient came from a rural population (Balzar) located 121 km north of the city of Guayaquil. She had signs hypertensive weitis for 3 months before undertaking surgery. In the left eye, a parasitic form being observed through it located in the internal superior quadrant (Fig 2).

Without previous consultation, it was intervened surgically and a specimen was extracted, which was sent to us with a presumption of *Onchocerca* microfilaria. We processed the material to practice the study.

Microscopic examination of the larva obtained from the left eye of the patient revealed a parasitic form, which, by it micro-morphology was a nematode larva, measuring 3.2 mm long x 0.30 mm wide. At the enlarged end was a cephalic bulb surrounded by 4 arrays of hooks. The esophagus was located in the first third of the body. The pharynx was continuous with the esophagus in the first third, with four tubular formations that ended in the bottom of the peri-esophagic sacks, belonging to the cervical sacks (Fig 3).

The hooklets of the cephalic bulb are not sharp. A lens objective No. 40 permitted visualization of the larval body, the cephalic bulb, and the rows of hooklets in a symmetric disposition.

Parasitological diagnosis: according to the micro-morphological description of the larva obtained by surgical intervention of the eye of the female student patient, corresponded to a third stage larva of *Gnathostoma* sp. The diagnosis of ocular gnathostomiasis, with localization of the parasite in the eye of this patient was the first communication of this clinical manifestation carried out in Ecuador.

From a parasitological viewpoint, it is important to point out that ocular localization has already been reported in other countries: Thailand, India, China, Israel, Burma, Pakistan, the Philippines, Malaysia, Bangladesh, and Mexico. Similarly, it is necessary to stress ocular localization, for differential diagnosis with cysticercosis, linguatuliasis, and onchocercosis, which have also been described in Ecuador.

In the last six months (December, 2003) in our Research Center CIDRALAS, we observed six new cases, among them one of cephalic dermal lesion and another suggestive of cardiac gnathostomiasis.

Cephalic gnathostomiasis. A female patient, (M.I.D.), presented with 1-month development of a dermal lesion in the cervical area, with characteristics of cutaneous gnathostomiasis. The lesion migrated to the head, to the intra-parietal area, and then emerged in the right frontal area, and then emerged in the inferior lid. Ivermectin was administered with clinical improvement (Fig 4).

The edema diminished, and the patient attended my consulting room. Serology by indirect immu-

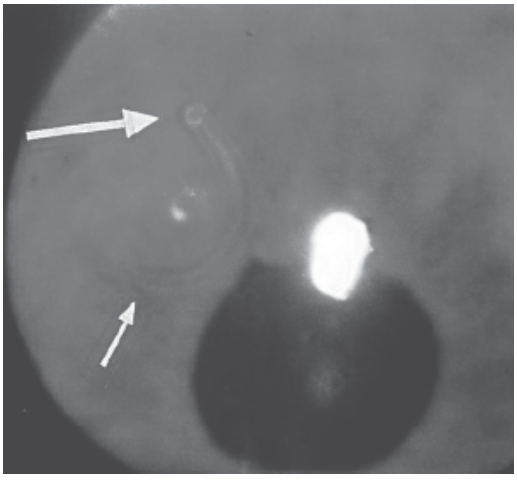

Fig 2- A gnathostoma larva located in the internal superior quadrant of the eye.

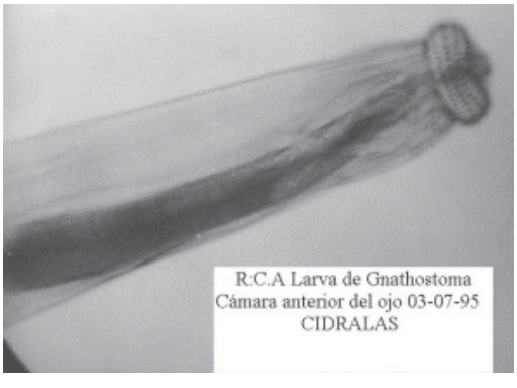

Fig 3- Larva obtained from the left eye of the patient.

nofluorescence was conducted with the following results: IgG 1:260 IgM 1:680. The skin test reaction was positive at 15 minutes 7/30 mm and at 24 hours it was 40/35 mm.

Cardiac gnathostomiasis. A 67-year-old man (D.R.L.), on March 19th 2003, was assisted to the Research Center of Parasite and Fungus Disease, having an erythematic plaque that began 8 days previously with a small erythematic nodule of 2 cm that started at the xiphoid appendix, and traveled to the epigastrium, developing a horizontal lesion 10 cm in length and 4 cm in width, with a very hard consistency. With a history of having eaten a ceviche of corvine fish, the case was diagnosed as nodular migratory eosinophilic paniculitis (cutaneous gnathostomiasis).

A laboratory test on March 19th found, by indirect immunofluorescence, IgG 1:640 and IgM 1:320 for antibodies to *Gnathostoma* sp. By skin test with antigen of *Gnathostoma* sp, after 15 minutes: macula/papule positive 22 x 14 with a pseudopodium of 7mm. We requested other biochemical and cytological blood tests to evaluate the picture and begin treatment. Some years ago, in 1986, the patient presented a similar picture with the same characteristics after having eaten a ceviche of corvine, with the same diagnosis of nodular migratory eosinophilic paniculitis and we treated him with albendazole 15 mg/kg, and he suffered hair loss from it. After that treatment, the patient never presented the dermatological picture and he recovered his hair. The patient informed me that on March 18th, before our study, after 2 hours' playing tennis he showed a normal clinical EKG examination. On March 20th, after a blood test, it was recommended he undergo another EKG and an ECG, and he was hospitalized with a diagnosis of acute myocardial infarct. He was assisted by an intern cardiologist, and he requested a cardiac catheterization. After hospitalization (D.R.L.), the course of disease improved very well, and the dermatological lesion was reduced to a small nodule without erythema. On March 29th, the IFI titer increased to IgG: 1:1280 and IgM 1:680. The skin test increased, with a relation of macula/papule 25 x 15 mm; after 24 hours, it increased to 70 x 50 mm. The evolution of his clinical picture was closely related to cardiac gnathostomiasis, so we asked for further cardiological controls, and anti-parasite treatment with ivermectin 200 μg/kg for 3 days. The patient traveled to a cardiology center in Houston, Texas. The patient related antecedent chest pain syndrome with an EKG and enzyme evidence of myocardial infarction, without any previous cardiac antecedent or associated symptoms. In Houston, the physicians took little note of his antecedent gnathostomiasis, and did not perform any test to verify it, or its association with cardiac gnathostomiasis. For this reason, our query is:

Fig 4- Cutaneous gnathostomiasis. The lesion migrated from the cervical area to the head to the inferior lid.

1) Is it possibly a cardiopathy produced by an unclearly-defined etiology? (cardiac gnathostomiasis).

2) Or, is it possibly a myocardiopathy caused by an immune allergic reaction, which, without the presence of the parasite in the heart, produced a reaction in this organ?

DISCUSSION

In Ecuador, according to Ollague (1985), the first case of human cutaneous gnathostomiasis was found in Guayaquil in 1979, and *Gnathostoma* larvae were first found in biopsied specimens from a patient in 1981. In 1985, the adults and the advanced third-stage larvae of Ecuadorian *Gnathostoma* were detected from dogs, cats, and from species of the freshwater fish *H. microlepis* and *R. cinerascens*. The prevalence of *H. microlepis* and *R. cinerascens* with *Gnathostoma* larvae was remarkably high, and those two species of freshwater fish were thus considered to be very important reservoir hosts in Ecuador. The prevalence or intensities were almost the same as those for snakehead fish (*Channa striata*) and swamp eels (*Fluta alba*), which are very important reservoir hosts in endemic areas of Thailand (Setasuban *et al*, 1991; Nuamtanong *et al*, 1998; Rojekitttikhun *et al*, 1998). We are concerned that the high prevalence of *H. microlepis* and *R. cinerascens* may thus be the cause of an epidemic of human gnathostomiasis in Guayas Province in the near feature. In Ecuador, one of the reservoir hosts, *H. microlepis*, is called 'guanchiche'

in Spanish and is a kind of catfish. Many Ecuadorians consume cooked *H. microlepis* and *R. cinerascens*, whereas they sometimes eat uncooked freshwater fish served in ceviche (a dish similar to Japanese sashimi), one of the typical Latin American dishes.

In the 1980s, the scientific name for Ecuadorian *Gnathostoma* had not yet been determined, but Ollague (1987) considered the species of *Gnathostoma* in Ecuador to actually be *G. spinigerum* and he used *G. spinigerum* as the scientific name for the Ecuadorian *Gnathostoma* in his textbook. In Ecuadorian *Gnathostoma*, the final hosts are carnivores, such as cats and dogs, and the number of hooklets resemble those of *G. spinigerum* (Ollague, 1987). However, the Ollague's conclusions were doutful. It was well known that *G. spinigerum* is only distributed in Asian countries, and Latin America is far from Asia. We thus decided to study the morphology and molecular biology of Ecuadorian *Gnathostoma*, including the causative agent of human cases in Ecuador.

However, just before the first survey in Ecuador, Almeyda-Artigas *et al* (2000) reported that the ribosomal DNA of Ecuadorian *Gnathostoma* was obviously different from that of *G. spinigerum* in Thailand, but the findings perfectly correlated with *G. binucleatum* in Mexico. As a result, he concluded that the Ecuadorian *Gnathostoma* was really *G. binucleatum*.

Moreover, the ribosomal DNA of Ecuadorian *Gnathostoma* larvae closely correlated with *G. binucleatum* from Mexico, whereas the sequence obtained from Ecuadorian larvae differed from that of *G. spinigerum* to some degree.

In our survey, the number of hooklets in each row of the head-bulb were very similar to those of Mexican *Gnathostoma* reported by Akahane *et al* (1994), but they were different from those of *G. spinigerum* and Ecuadorian *Gnathostoma* reported by Ollague (1987). On the other had, the ribosomal DNA of the Ecuadorian *Gnathostoma* closely related with *G. binudeatum* but was different from *G. spinigeum*.

Based on our findings, we therefore agree to the proposal of Almeyda-Artigas *et al* (2000), that the species of *Gnathostoma* in Ecuador should be identified as *G. binucleatum*.

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