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Research Note

Possible zoonotic implications of the discovery of the advanced third stage larva of *Gnathostoma turgidum* (Spirurida: Gnathostomatidae) in a Mexican fish species

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Summary

Gnathostomiasis in humans is acquired by consumption of any infected second intermediate host or paratenic host. This includes amphibians, snakes and poultry as well as fish. In this work we report for the first time in Mexico the presence of an $AdvL_3$ of *Gnathostoma turgidum* in the musculature of a wild fish (*Gobiomorus dormitor*, which also acts as intermediate host for the larvae of *G. binucleatum* and *G. lamothei*), from the Papaloapan River, Veracruz; previously, larvae of *G. turgidum* had only been recorded in amphibians in Mexico and in wild swamp eels from Tampa, Florida, USA. The larva found is extremely small (approximately 1,500 by 140 microns in length and width, respectively), and was obtained by artificial digestion with pepsin after examining the musculature against the light between two glass plates, a method by which it went unnoticed. Our finding of an $AdvL_3$ in this fish, together with a previous molecular phylogenetic analysis revealing that the five species involved in human infections do not nest in the same clade, suggest that all species in the genus are potentially zoonotic. In this context, we strongly recommend the identification of larvae extracted from human patients at specific level, in order to know the role played by the 3 species distributed in Mexico in human cases of gnathostomiasis.

Keywords: Zoonosis; Nematoda; Gnathostomiasis; Actynopterigii

Introduction

Human gnathostomiasis is a zoonotic disease with cosmopolitan distribution (Díaz-Camacho *et al*., 2020). Among the 13 valid *Gnathostoma* (Spirurida: Gnathostomatidae) species, advanced third-stage larvae ($AdvL_3$) of at least five species have been identified as etiological agents in this accidental human parasitosis: *Gnathostoma spinigerum*, *G. doloresi*, *G. hispidum*, *G. nipponicum* and *G. binucleatum* (Liu *et al*., 2020). According to these authors, humans can be infected through three routes: oral, trans-placental and through skin wounds. However, the most common via of human infection is through the accidental ingestion of $AdvL_3$ during the consumption of raw or undercooked fish meat.

This disease has been widely reported in the Mexican states of Veracruz, Tabasco, Sinaloa, Nayarit, Colima, Guerrero and Oaxaca, where local consumption of fish in the form of ceviche is very common (Ogata *et al*., 1998; Martínez-Salazar & León-Règagnon, 2005). Increased travel around the world, trade in fish from endemic areas, and its consumption in exotic dishes with raw or undercooked meat may promote gnathostomiasis in the Western Hemisphere (Bapat *et al*., 2022). In particular, three species of the genus *Gnathostoma* are distributed in Mexico: *G. turgidum*, *G. binucleatum* and *G. lamothei* (Díaz-Camacho *et al*., 2020). The $AdvL_3$ of two of these species infect the musculature of the second intermediate hosts in their life cycle, commonly fish (Herman & Chiodini, 2009; Nawa *et al*., 2015; Liu *et al*., 2020). In the case of

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G. turgidum, the first report of its larvae were made in the musculature of frogs (Mosqueda-Cabrera *et al*., 2009), and more recently in wild swamp eels (*Monopterus albus*) from Tampa, Florida (Cole *et al*., 2014). Among these species, until now only *G. binucleatum* has been shown to be the etiological agent of human infection (Díaz-Camacho *et al*., 2020). However, *G. lamothei* larvae have also been found in fish musculature (Hernández-Gómez *et al*., 2010), but to date there are no records that implicate this species as the causative agent of human infection.

The objective of this research is to document the first finding of the advanced third stage larva of *G. turgidum* in fish from Mexico and to discuss its possible role in human gnathostomiasis.

Material and Methods

Bigmouth sleeper (*Gobiomorus dormitor*: Actinopterygii: Eleotridae) were obtained from fishmongers in Tlacotalpan, Veracruz at the beginning of the rainy season (August, 2022). The musculature of each host was examined by making thin fillets, compressed between two thick glass plates against the light, in order to locate nematode larvae. After the revision, the musculature of each fish was subjected to a digestion process in artificial gastric juice (6 g of pepsin, 7 ml. HCl in 1 liter of water) with a volume ratio of 3:1, at room temperature. The beakers used were covered with aluminum foil for 3 to 4 hours under constant agitation. Subsequently, the macerated tissue was removed and the rest was decanted by successive washings with 0.6 % saline solution; after each washing, the sediment was recovered until a clear solution was obtained that facilitated its observation under a stereoscope. The recovered larva was fixed in hot 70 % ethyl alcohol and kept in cold 70 % alcohol until study. For its identification (made on the basis of Mosqueda-Cabrera *et al*., 2009), the larva was cleared with Amman´s lactophenol; measurements (presented in micrometers) and images were obtained with a digital camera mounted on a compound microscope. The specimen was deposited in the Colección Nacional de Helmintos [CNHE], Instituto de Biología de la Universidad Nacional Autónoma de México, Mexico City, with the catalogue number: CNHE (11733).

Ethical Approval and/or Informed Consent

The research related to animal use has been complied with all the relevant institutional policies for the care and use of animals in Mexico (DOF, 2001).

Results

During examination against the light of the entire musculature of 17 *G. dormitor* [170 – 225 mm (206.82 ± 17.2 standard length)], a *Gnathostoma* larva went unnoticed and was only detected by pepsin digestion. This larva measures 1,544 by 142.3 long and wide, respectively. Cephalic bulb 61.3 length by 107.9 width covered by four rows of transverse spines with a single point. Each row has 34, 36, 40 and 45, from the first to the fourth row, respectively. The spines of the fourth row are on average smaller (5.9 µm) than those of the first row (7.4 µm). Additionally, the bulb presented some scales without hooks after the fourth row (Fig. 1).Esophagus 624.8 long by 92.21 wide; occupies 40.4 % of the total length of the body. Four cervical sacs project from the base of the cephalic bulb (329.4, 344.4, 359.2, and 393.9 long), spanning 57.1 % of the length of the esophagus. The entire body is covered with one-point spines, arranged in 171 transverse rows. Two cervical papillae located laterally between 11th and 12th spine rows on the body; excretory pore in row 21. Genital primordium situated at 55.7 %. Two caudal papillae lateral to the body, both posterior to the genital primordium; the right at 56.5 %; the left to 70.4 %. Anus to 1.75 % of posterior end of body.

Additionally, the larval stages of the following species were found: Digenea: *Clinostomum marginatum* (Clinostomidae) and *Diplostomum* sp. (Diplostomidae), Nematoda: *Contracaecum* sp. (Anisakidae), *Spiroxys* sp., (Gnathostomatidae) and *Serpinema trispinosum* (Camallanidae), and Pentastomida: *Sebekia* sp. (Sebekidae).

Discussion

The morphometric study of the $AdvL_3$ collected from *G. dormitor* in the Papaloapan River, Veracruz, allowed its identification as *G. turgidum* according to the description presented by Mosqueda-Cabrera *et al*. (2009); this larva differs from those of *G. binucleatum* (Almeyda-Artigas, 1991; García-Márquez *et al*., 2009) and *G. lamothei* (Gaspar-Navarro *et al*., 2013), by the following combination of characters: 1) smaller body size, 1,544 (1,670) vs. (4,300) and (4,488), respectively]; position of the excretory pore in the row, 21 [(19.7) *vs.* (30.0) and (23.1)]; 3) less number of hooks per row on the cephalic bulb, 31.8, 35.0, 37.4, 41.4 *vs*. 38.7, 42.4, 44.7, 48.2 in *G. binucleatum* and 39.3, 43.3, 44.2, 47.3 in *G. lamothei*; and finally, 4) different dimensions of the spines in the rows of the cephalic bulb, being smaller in the fourth row of *G. turgidum* with respect to the 2 mentioned species, whose spines are of homogeneous size throughout the entire bulb.

The discovery of the larva of *G. turgidum* in this species of eleotrid represents the first for the nematode in a wild fish in Mexico and the second at global level (see Cole *et al*., 2014). In addition, Mosqueda-Cabrera *et al*. (2009) experimentally demonstrated that 2 other fish species (*Poecilia gracilis* and *Oreochromis niloticus*) are suitable hosts for *G. turgidum.* This is particularly relevant for the etiology of the gnathostomiasis, since several authors have suggested the possibility that any species of this genus present in Mexico could infect humans (Almeyda-Artigas *et al*., 2000; Hernández-Gómez *et al*., 2010; Díaz-Camacho *et al*., 2020), which seems to be supported by the molecular phylogeny carried out by Bertoni-Ruiz (2006). Based on the 28S gene of 7 of the 13 species that composed the genus, this author found that the species that have been recorded as parasites of man: *G. spinigerum, G. his-*

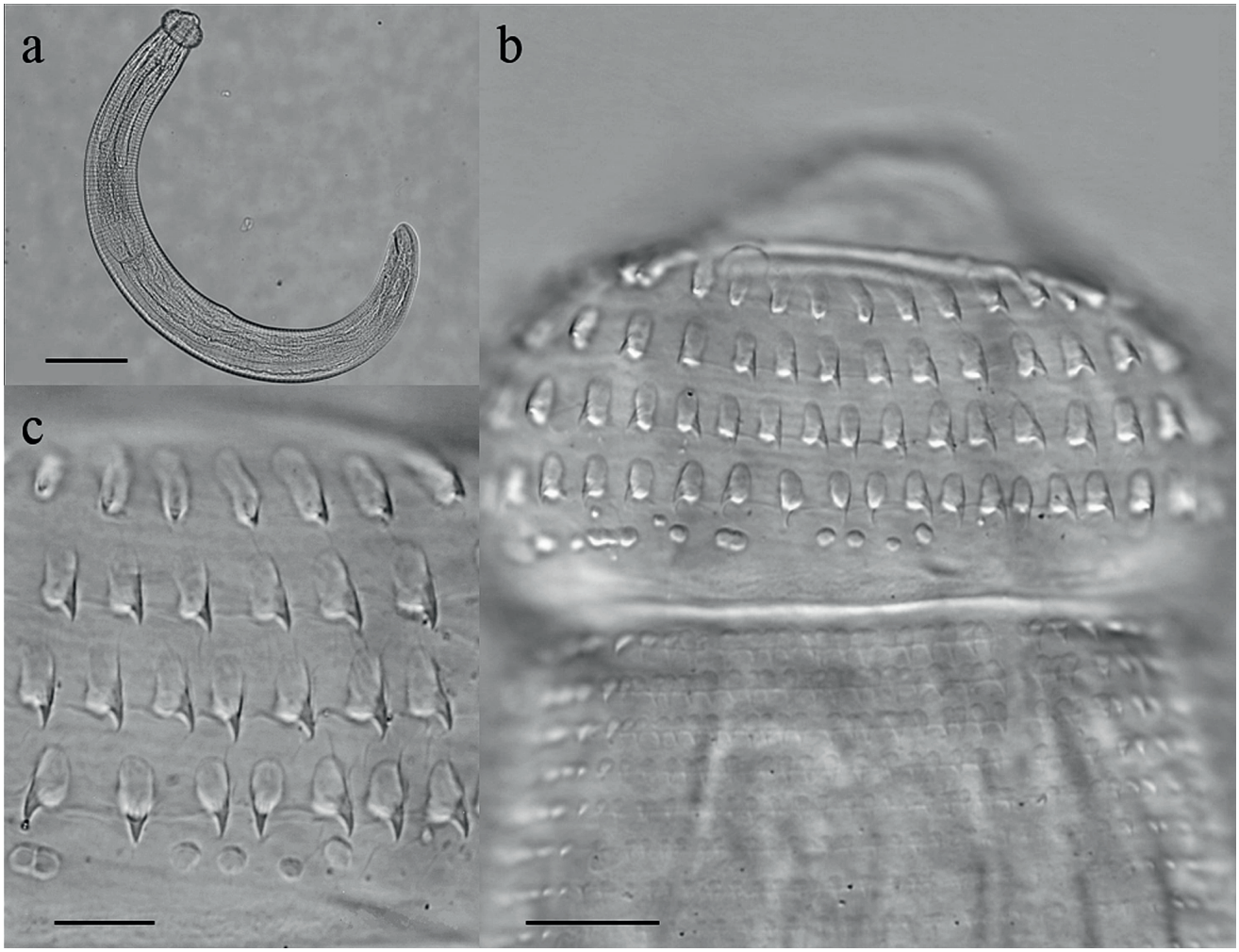

Fig. 1. Advanced third stage larva of *Gnathostoma turgidum*. a) Lateral view of the body; b) Cephalic bulb; c) Detail of the spines in the rows of the cephalic bulb. Scales: a= 200 µm; b=20 µm; c= 10 µm.

pidum, G. doloresi, G. nipponicum and *G. binucleatum* (Miyazaki, 1991; Almeyda-Artigas, 1991; León-Règagnon *et al*., 2002) do not group in the same clade. According to Bertoni-Ruiz (2006), these results could suggest that other species of the genus are potentially infectious for humans, provided they involve an intermediate or paratenic host in their food chain. However, this conclusion was obtained with only 50 % of the species represented, so it is necessary to deepen the phylogenetic analysis of the group.

The bigmouth sleeper has been also found be parasitized by *G. lamothei* and *G. binucleatum* larvae in Mexico (Hernández-Gómez *et al*., 2010). The above, added to our finding of $AdvL_3$ of *G. turgidum* in this species of fish (whose commercial value in its area of distribution establishes it as a host with a high risk of transmission), makes it necessary to highlight the importance of the methods of collection and identification of larvae of *Gnathostoma*, both in their natural hosts and in man.

In general, two methods have been used for the collection of *Gnathostoma* $AdvL_3$ in Mexico. The most widely used method is the examination of the musculature of fish by compressing it between two glass plates and its observation against the light using a 100-watt light source (Álvarez-Guerrero & Alba-Hurtado, 2007), or under a stereoscopic microscope (Hernández-Gómez *et al*., 2008; Hernández-Gómez *et al*., 2010). The second method (artificial digestion with gastric juice prepared with commercial pepsin and hydrochloric acid) has been implemented only in some cases (Lamothe-Argumedo & Osorio-Sarabia, 1998). For finding of larvae with the first method, the size of the larva is determinant; the observation with the naked eye of a well-defined red or orange point (appearance of the coiled or encysted larva) in the muscle tissue allows its location. With artificial digestion, the larvae are recovered from the bottom of the solution, observed under a stereoscope. In the particular case of the 3 species distributed in Mexico, the body size of the larvae (in millimeters) is different: 2.6 – 5.9 (4.3) for *G. binucleatum* (Almeyda-Artigas, 1991), 3.6 – 5.1 (4.5) for *G. lamothei* (Gaspar-Navarro *et al*., 2013) and 1.5 – 2.0 (1.7) for *G. turgidum* (Mosqueda-Cabrera *et al*., 2009). Therefore, the

probability that the larvae of the first two species will be detected using the backlight method is higher, while those of *G. turgidum* could go undetected.

Based on the findings of our study, we consider pertinent to continue the search for *G. turgidum* $AdvL_3$ in fish associated with water bodies where there is a record of adult forms, applying the artificial digestion method, as well as corroborating their specific identification using molecular markers. Additionally, it is highly recommended that the larvae extracted from human biopsies be identified at a specific level in order to know precisely the role of the three species of *Gnathostoma* in human infections in Mexico.

Conflict of Interest

Authors state no conflict of interest.

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