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Original Article

Species of Anisakidae nematodes and *Clinostomum* spp. infecting lisa *Mugil curema* (Mugilidae) intended for human consumption in Mexico

Espécies de nematoides Anisakidae e *Clinostomum* spp. infectando tainhas *Mugil curema* (Mugilidae) destinadas ao consumo humano no México

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Abstract

Anisakis spp. nematodes are potentially zoonotic parasites; that infects a wide variety of aquatic species worldwide, with marine fish being the paratenic hosts. The aim of study was identify the presence of Anisakidae nematodes, and other parasites in *Mugil curema*. A total of 96 *M. curema* obtained from local markets in Tulancingo, Hidalgo, Mexico, were analyzed by necropsy. Only five *M. curema* present nematode collection in epaxial muscle. The tissues with the highest prevalence of parasites were identified, and samples of epaxial muscle with larval migration analyzed by histopathology. Visible parasites in necropsy tissues were classified according to their morphology. Nematode found in the liver were *Contracaecum* spp. (41.17%) and *Pseudoterranova* spp. third stage (7.36%); in the caudal part of the kidney were *Anisakis* spp. (13.23%), *Pseudoterranova* spp. third stage (11.77%) and *Contracaecum* spp. (5.88%); and in epaxial muscle were *Anisakis* spp. Larva I (5.88%) and *Pseudoterranova* spp (4.42%). In one fish, *Clinostomum* spp. was detected in epaxial caudal muscle. The present work reports for the first time the presence of nematodes of the family Anisakidae and *Clinostonum* spp. metacercariae, with zoonotic potential, in *M. curema* intended for human consumption in Tulancingo, Hidalgo, Mexico.

Keywords: Parasite, zoonotic, fish, epaxial muscle, histopathology.

Resumo

Anisakis spp. são parasitas potencialmente zoonóticos que infectam uma grande variedade de espécies aquáticas em todo o mundo, sendo os peixes marinhos hospedeiros paratênicos. O objetivo deste estudo foi identificar a presença de nematóides da família Anisakidae e de outros parasitas em peixes *Mugil curema*. Um total de 96 *M. curema*, obtidos em mercados locais em Tulancingo, Hidalgo, México, foram submetidos a necropsia. Apenas cinco *M. curema* apresentaram coleção de nematóides no músculo epaxial. Os tecidos com maior prevalência de parasitas foram identificados e amostras do músculo epaxial com migração larval foram analisadas por histopatologia. Os nematóides encontrados no fígado foram *Contracaecum* spp. (41,17%) e *Pseudoterranova* spp. terceira etapa (7,36%); na parte caudal do rim *Anisakis* spp. (13,23%), *Pseudoterranova* spp. terceira etapa (11,77%) e *Contracaecum* spp. (5,88%); e no músculo epaxial

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Anisakis spp. larva I (5,88%) e *Pseudoterranova* spp. (4,42%). Em um peixe, *Clinostomum* spp. foi detectado no músculo caudal epaxial. O presente trabalho relata pela primeira vez a presença de nematóides da família Anisakidae e *Clinostonum* spp. metacercárias, com potencial zoonótico, em *M. curema* destinado ao consumo humano em Tulancingo, Hidalgo, México.

Palavras-chave: Parasita, zoonótica, peixe, músculo epaxial, histopatologia.

Introduction

Anisakis spp. are a nematode that infects a wide variety of aquatic organisms during the development of the parasite larval stages. Marine mammals are the primary hosts, with the adult stage developing in the stomach and intestine (Laffon-Leal et al., 2000). The participation of cephalopods, shrimp, and crustaceans as intermediate hosts is important for the development of the L2 larvae (Pekmezci & Yardimci, 2019). Subsequently, marine fish participate as paratenic host carriers of the L3, which are located in the gastrointestinal tract before finally migrating to the celomic cavity. The L3 can be free, cystic, or penetrate the internal tissues and epaxial muscle. Finally, the life cycle is completed when the infested fish are ingested by marine mammals, allowing the L4 stages to develop (Castellanos et al., 2017).

Anisakidosis is a globally distributed disease caused by the consumption of marine animals, mainly poorly cooked or raw fish due to the acquisition of new gastronomic habits from different cultures (Tokiwa et al., 2018). When the parasite is ingested alive it has the ability to survive for a short period of time in the human body and can adhere to various tissues, such as the esophagus, stomach, duodenum, jejunum, ileum, or colon, generating a primary inflammatory response (Amir et al., 2016). Clinical manifestations include moderate or severe allergies, angioedema, hypotension, bronco spasms, anaphylactic shock, and asthma (Nieuwenhuizen & Lopata, 2014). Clinical symptoms are nonspecific and include epigastralgia, nausea, vomiting, bloating with intense pain, and, in chronic stages of infection, eosinophilic granulomas and edema at the site of the lesion (Amir et al., 2016). The objective of this work was to perform a systematic necropsy of *Mugil curema* from fish markets in Tulancingo, Hidalgo, to determine the presence of Anisakidae nematodes and other parasitic genera.

Materials and Methods

A total of 96 fish were purchased from different commercial establishments in Tulancingo, Hidalgo. Each fish was individually placed in a plastic bag, and the samples were transported together at 4 °C (Torres-Frenzel & Torres, 2014). Fish were processed at the bacteriology laboratory of the Academic Area of Veterinary Medicine and Zootechnics of ICAp in the Autonomous University of the State of Hidalgo. Each fish was measured and visually examined. Necropsy was performed based on the Noga (2010). Internal organs (heart, esophagus, liver, stomach, pyloric sacs, spleen, intestine, gonads, and swim bladder) were removed to be dissected, the celomic cavity was examined, kidney and epaxial muscle dissection was performed (Torres-Frenzel & Torres, 2014), and macroscopic findings were described. Epaxial muscle samples with larval migration were fixed in 10% formaldehyde buffer at a pH of 7.2 for 24–48 h. The tissue was placed in histological cassettes, dehydrated, embedded in paraffin, and stained with hematoxylin-eosin (Zepeda-Velázquez et al., 2017). Each histological section was examined under a Leica DM 750 P compound microscope, and the lesions were described and photographed. Parasites were collected in Eppendorf tubes with 1.5 ml of 70% ethyl alcohol (Castellanos et al., 2017). The morphological criteria of Moravec et al. (1997) and Moravec (1998) were used to identify the parasitic genera.

Results

Larval migration in epaxial muscle of nematodes of the family Anisakidae was observed in 5 out of 96 fish. For the morphological identification of nematode Anisakidae, is important the presence or absence of different structures, based on the morphological criteria, *Anisakis* spp. larva I (Figure 1a), *Contracaecum* spp. (Figure 1b) and *Pseudoterranova* spp. third stage (Figure 1c) genus were identified. A total of 68 nematodes were collected in 48.52% of livers, representing the genera *Contracaecum* spp. (41.17%) and *Pseudoterranova* spp. (7.36%). In 30.88% fish, the caudal part of the kidney was infested with *Anisakis* spp. larva I (13.23%), *Pseudoterranova* spp. (11.77%), and *Contracaecum* spp. (5.88%). Parasites were seen in 10.29% of epaxial muscle, and included *Anisakis* spp. larva I (5.88%) and *Pseudoterranova* spp. third-stage (4.42%). The medial part of the kidney and viscera were infested in 4.41% of fish by *Pseudoterraova* spp. third-stage (5.88%) and *Contracaecum* spp. (2.94%). The cranial part of the kidney in 1.47% fish contained the genus *Contracaecum* spp. In five fish showed migration in the epaxial muscle, nematodes were observed in the caudal part of the kidney, specifically in the hemal canal (Figure 2a) and in the caudal vein (Figure 2b). Similarly, nematodes in the epaxial muscle were identified (Figure 2c and 2d).

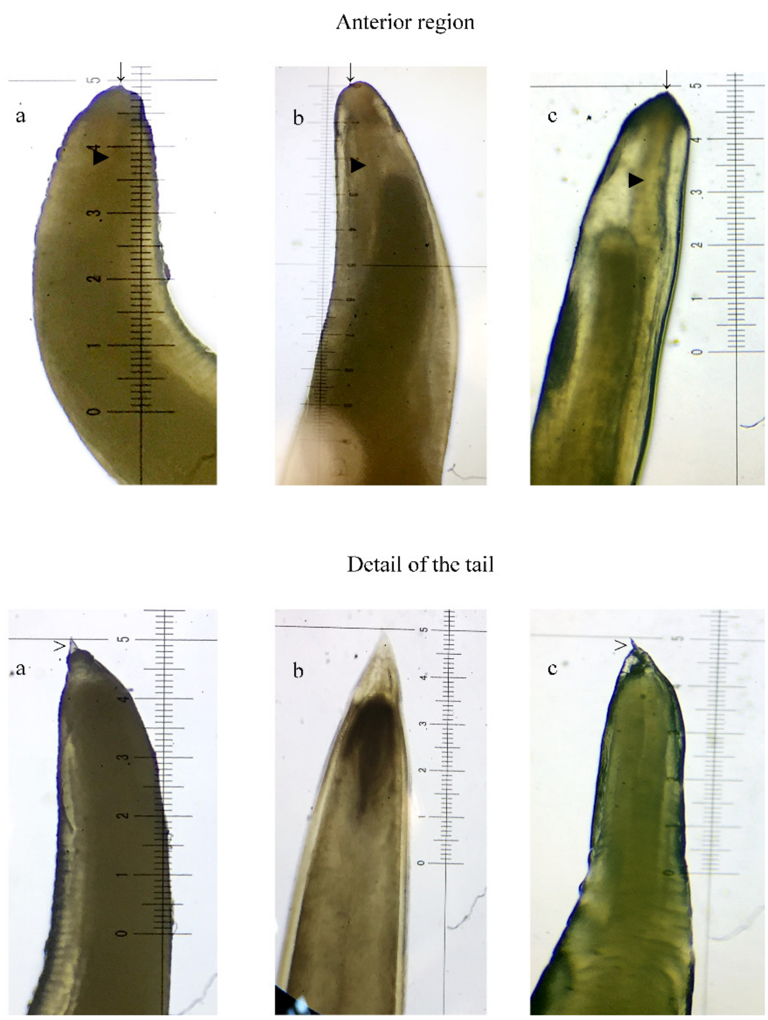

Figure 1. Nematode Anisakidae collected from *Mugil curema*. (a) *Anisakis* spp., (b) *Contracaecum* spp. and (c) *Pseudoterranova* spp. Larval structures for larval identification: larval tooth (→), esophagus (►) mucron (>).

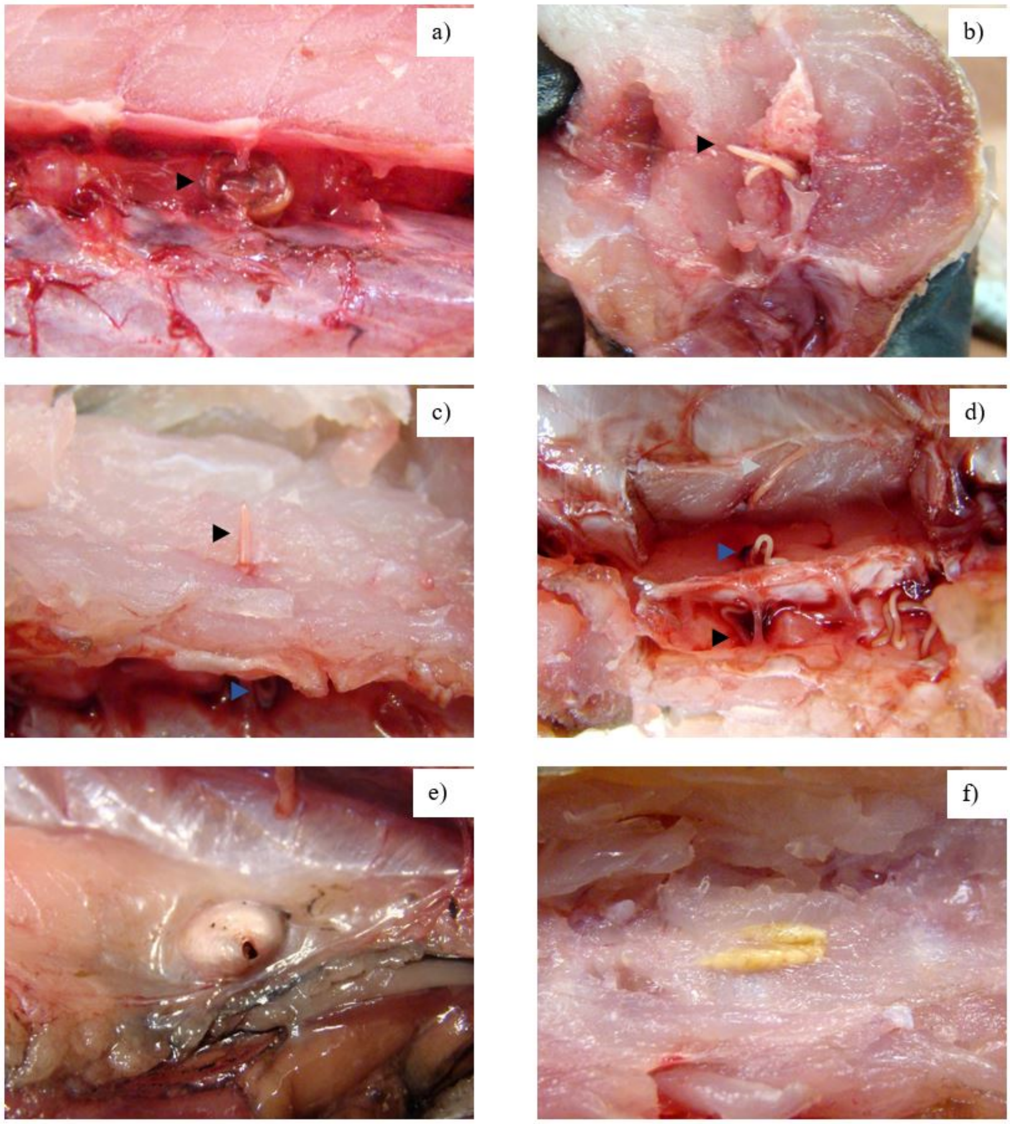

Figure 2. *Mugil curema*. (a) Parasitic cyst located within the hemal arch (►). (b) Nematodes inside the caudal vein; the caudal portion of the kidney has been removed. (c) Larval migration in epaxial muscle (►); note the presence of a second nematode located below and within the hemal arch (►). (d) Free parasites within the hemal arch (►); parasitic migration to epaxial muscle (►); and located within the epaxial muscle (►). (e) Granuloma in hypaxial muscle (celomic cavity); the structure has been perforated. (f) *Clinostomum* spp. metacercariae.

In one of the five fish with parasites in the epaxial muscle, the presence of a granuloma (approximately 0.9 mm x 0.6 mm in the lateral hypaxial muscle was observed inside the celomic cavity and at the height of the last portion of the intestine (Figure 2e); and in another fish the presence of a straw yellow structure was observed (0.25 to 0.5 ml), embedded in the caudal epaxial musculature with morphology similar to *Clinostomum* spp. metacercariae (Figure 2f). Of the five fish, eosinophilic infiltrates of moderate grade where observed in two and moderate to severe in three fish (Figure 3a). Necrosis of muscle fibers and presence of fibrin around the parasite was seen in all five fish (Figure 3b). The granuloma was accompanied a severe leukocyte infiltration, consisting of eosinophils and macrophages, melanomacrophage centers and diffuse free melanin. In the center of the lesion, tissue necrosis with cellular detritus, non-identifiable exogenous material, and basophilic aggregates formed by bacillary shapes were observed. Histopathology of muscle tissue was also performed where the presence of *Clinostonum* spp. metacercariae, however at histopathology it is possible to appreciate the oval form of the parasite (Figure 4).

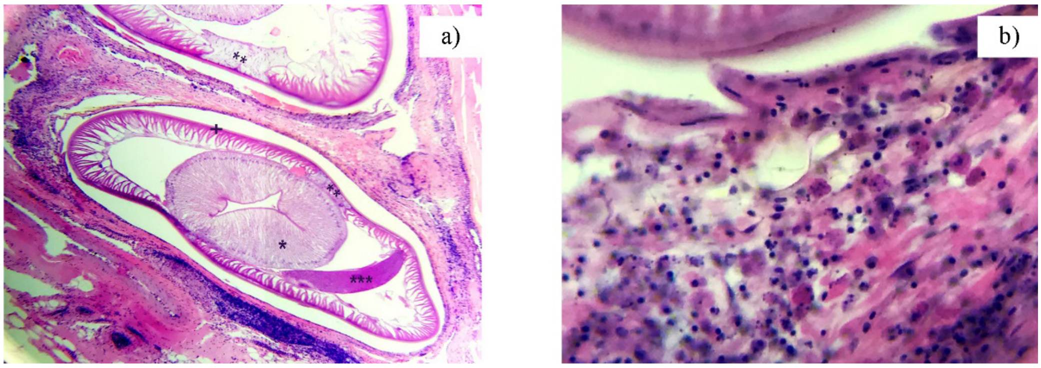

Figure 3. *Mugil curema* **muscle tissue.** (a) Parasitic myositis due to Anisakidae nematodes. At the center, the parasite and its structures, the digestive tract (*), lateral epidermal cord in the form of "T" (**), the excretory gland (***) and the parasitic cuticle (+) (hematoxylin-eosin) (10x magnification). (b) Eosinophilic infiltration, edema and myocyte necrosis, severe focal around the parasite cuticle (+) (hematoxylin-eosin; 40x magnification).

Figure 4. *Mugil curema* **muscle tissue.** Panoramic reconstruction of *Clinostonum* spp. metacercariae, in epiaxial muscle (hematoxylin-eosin; 10x magnification).

Discussion

The present work reports, for the first time, the presence of *Anisakis* spp. larva I, *Contracaecum* spp., and *Pseudoterranova* spp., as well as *Clinostomum* spp. metacercariae, in *M. curema* marketed for human consumption in the state of Tulancingo, Hidalgo. With regards to Mexico, infestation by nematode Anisakidae family in the Mugilidae has been reported, in Lisa cabezona (*M. cephalus*) in the Colorado River, Baja California, Mexico (Valles-Ríos et al., 2000) and in Lisa (*M. cephalus*) in Topolobampo, Sinaloa, Mexico (Juárez-Arroyo & Salgado-Maldonado, 1989). In this study we identify the presence of the tooth and mucron in the case of *Anisakis* spp. larva I (Berland, 1961). For *Contracaecum* spp. booring tooth presence near to oral apperture, tail conical and mucron absent, were the structures employed for identification (Fonseca et al., 2016), and *Pseudoterranova* spp. third stage nematode was described for triangular mouth with a prominent boring tooth, and the presence of mucron in the body ended (Al Quraishy et al., 2019).

Due to the zoonotic importance of the parasite, only five fish were reported in this paper. Liver having the highest parasitic infection by *Anisakis* spp., *Pseudoterranova* spp. and *Contracaecum* spp. (48.52%). This is consistent with the study conducted by Valle-Ríos et al. 2000, who identified an 84.5% incidence of *Contracaecum multipapillatum* in *M. cephalus* in the Colorado River in Baja California, Mexico. A study by Maniscalchi et al. (Maniscalchi et al., 2015) on *M. curema* from Venezuela, found *Contracaecum* spp., *Pseudoterranova* spp., and *Anisakis* spp., in 18% of digestive viscera, 36% of kidney, and 1.8% of muscle. However, in this work, the prevalence was slightly lower in caudal kidney (30.88%), middle kidney and viscera (4.41%), and cranial kidney (1.47%); in epaxial muscle (10.29%), the percentage parasite infestation was higher compared to that reported by the same author, and represented a risk factor for consumers.

The parasite collection sites reported in this work have been previously reported in other fish species (Castellanos et al., 2017; Olivero-Verbel et al., 2005). The prevalence of *Clinostomum* spp. metacercariae, which has zoonotic potential (Sohn, 2009), was 0.01%, compared to other types of parasites, such as the copepod *Ergasilus versicolor,* that can present a prevalence of 74% in *M. cephalus* (Valles-Ríos et al., 2000). Identification by morphology has been widely used (Castellanos et al., 2017); however, molecular tests are necessary for a more precise identification, and they can facilitate the identification of the species that infests *M. curema*, which will be the next step of the investigation.

Identification of a granuloma in hypaxial muscle, and the presence of the exogenous material inside, suggests that a parasite was encysted and that the immune system was involved in its destruction. The structural elements described in this work are consistent with those reported by Murata et al. (2018) for a case of hepatic anisakiasis; however, in this work, elongated bacillary structures were observed in the center of the lesion, suggesting the presence of nematode-like bacteria, such as *Flavobacterium* spp., *Pseudomonas* spp., or *Bacillus cereus* (Svanevik & Lunestad, 2017). The histopathology of the nematode revealed the lateral epidermal cord in the form of a thick "T"; this is probably because the tissue was squashed and the structure changed; however, there is a possibility that the shape of the cord may be related to the parasitic genus, although more studies are needed to test this hypothesis. In humans, the lateral epidermal cord and the intestinal lumen are used to determine the genus of the parasite, by using the "Y" shape in *Anisakis* spp. and butterfly wing shape for *Pseudoterranova* spp. (Lauwers et al., 2017); the specification of this structure in human medicine has been reported for hepatic anisakiasis due to *Pseudoterranova decipiens* (Murata et al., 2018), *A. pegreffii,* and *A. simplex* in cases of dysentery (Amir et al., 2016). In fish, only Laffon-Leal et al. (2000) reported the genus *Contracaecum* spp. In conclusion, this work reports, for the first time, the occurrence of Anisakid nematodes and the presence of *Clinostomum* spp. metacercariae in *Mugil curema* intended for human consumption, which represent a risk to human health in Tulancingo, Hidalgo, Mexico.

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