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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

Prevalence of anisakids in *sardinella aurita* and *mugil curema* from sucre state, Venezuela

Prevalencia de anisákidos en *sardinella aurita* y *mugil curema* del estado sucre, Venezuela

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ABSTRACT

Nematodes of the Anisakidae family were studied in *Sardinella aurita* Valenciennes, 1847 and *Mugil curema* Valenciennes, 1836, caught in Venezuelan coastal waters and sold in Cumaná's main market in Sucre state, Venezuela. Fifty fish of each species were analyzed, examined, dissected and their internal cavities, cephalic organs, abdominal trunk and caudal regions wereinspected. They were also debrided, and muscles dissected in search of nematodes in their third larval stage (L_3) through simple visual examination. Detected nematode parasites were mechanically extracted from the organs and tissues for identification and classification at the genus level. *M. curema* was the only fish found to be infected with parasites. Distinct parasitic species were observed in 48/50 (96%) of the *M. curema* specimens analyzed. The prevalence of helminths in these specimens included nematodes of the Anisakidae family, Acanthocephala and other unidentified parasites. Of the total specimens examined, the parasites were distributed as follows: Anisakidae family (66%) (33/50), *Pseudoterranova* sp. (44%) (22/50), *Anisakis* sp. (32%) (16/50) and *Contracaecum* sp. (12%) (6/50). These parasites were mainly located encysted in the caudal kidney (55.09%), the liver (28.81%), and the digestive viscera (inside the gut) (16.10%). This shows the existing human risk in acquiring anisakidosis larvae parasitism, depending mainly on the cultural habits of consuming raw or undercooked fish.

Keywords: Acanthocephalia – Anisakidae nematodes – Endoparasite – Saltwater fish – Public health – Venezuela

RESUMEN

Se estudiaron nematodos de la familia Anisakidae en *Sardinella aurita* Valenciennes, 1847 y *Mugil curema* Valenciennes, 1836, capturados en aguas costeras de Venezuela y vendidos en el mercado principal de Cumaná en el estado Sucre, Venezuela. Se analizaron cincuenta peces de cada especie, se examinaron, diseccionaron y se inspeccionaron sus cavidades internas, órganos cefálicos, tronco abdominal y regiones caudales. También se desollaron y se diseccionaron los músculos en busca de nematodos en su tercer estadio larvario (L_3) mediante simple examen visual. Los parásitos nematodos detectados se extrajeron mecánicamente de los órganos y tejidos para su identificación y clasificación a nivel de género. *M. curema* fue el único pez encontrado infectado con parásitos. Se observaron distintas especies parasitarias en 48/50 (96%) de los especímenes de *M. curema* analizados. La prevalencia de helmintos en estos especímenes incluyó nematodos de la familia Anisakidae, Acanthocephala y otros parásitos no identificados. Del total de especímenes examinados, los parásitos se distribuyeron de la siguiente manera: la familia Anisakidae (66%) (33/50), *Pseudoterranova* sp. (44%) (22/50), *Anisakis* sp. (32%) (16/50), y *Contracaecum* sp. (12%) (6/50). Estos parásitos se localizaron principalmente enquistados en el riñón caudal (55,09%), el hígado (28,81%) y las vísceras digestivas (dentro del intestino) (16,10%). Esto muestra el riesgo humano existente de adquirir larvas de anisakidosis, dependiendo principalmente de los hábitos culturales de consumir pescado crudo o poco cocido.

Palabras clave: Acanthocephalia – Anisakidae nematodos – Endopárasitos – Peces de agua salada – Salud pública – Venezuela

INTRODUCTION

In some countries, fish is prominent source of animal protein in the diet. Specialists recommend it for its high nutritional value, easily digestible proteins, and rich content of minerals, vitamins, and polyunsaturated fatty acids that benefit the body, However, despite its many benefits, fish carries a high risk of parasite transmission and cany (Gea, 2015; Rodríguez-Monje, 2019).

Certain parasites can infect fish and fishery products, affecting their muscles, skin, fins, and internal organs. However, only a few of these parasites pose health risks to humans. Sociocultural and behavioral factors, such as consuming raw or undercooked fish, contribute to human infection. This can lead to ichthyozoonosis, a disease transmitted to humans by bacteria, viruses, and parasites through the ingestion of contaminated fish and fishery products (Quijada *et al*., 2005; Tejada & López, 2012). The growing popularity of eating raw or uncooked seafood in western countries has resulted in the proliferation of certain parasitic infections in humans. One of the most severe fish-transmitted infections is anisakiasis, which occurs when third-stage larvae belonging to the family Anisakidae are accidentally ingested (Rosales *et al*., 1999).

In Latin America, the high consumption of fish and fishery products contributes to reported outbreaks of foodborne diseases (FBD), depending on the type of food involved (Balbachán *et al*., 2000). In Venezuela, during the period from 1996 to 2004, the ingestion of fish second and third leading cause of FBD (Maniscalchi *et al*., 2015). While most ichthyoparasitoses do not cause pathologies in humans, some species can lead to serious disorders when infected fish are consumed, posing a significant health risk that should not be underestimated (Puccio *et al*., 2008; Tejada & López, 2012; Castellanos-Garzón *et al*., 2019).

In 1974, Anderson *et al*. described anisakids as belonging to the phylum Nemathelmintes, class Nematoda. Among the different genera are *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* are notable (Anderson *et al.,* 1974). The species that stands out as the main infecting nematode due to its frequency and pathogenic action is *Anisakis simplex*, the etiological agent of the disease known as anisakiasis (Berruezo, 2000; Rello *et al*., 2004; Akbar & Ghosh, 2005; Tuemmers *et al*., 2014; Gea, 2015). Clinical signs and symptoms of anisakidosis include allergies and gastrointestinal symptoms. Gastric manifestations usually appear 12-48 hours after consuming fish infected with the L_3 larval stage, which can be mistaken for gastritis. Intestinal manifestations are characterized by intense pain in the lower abdomen, accompanied by nausea, vomiting and occult blood in the feces. Allergic manifestations, on the other hand, occur a few hours after consuming infected fish and present as pruritus, urticaria, asthma, and in some cases, anaphylaxis (Field-Cortazares & Calderón-Campos, 2009; Farias *et al*., 2021).

Human anisakidosis was first reported in Japan in 1965 (Oshima, 1972), and later in Spain (Arenal *et al*., 1991). The number of reported cases of this parasitosis has increased since then. However, it is important to note that it remains an under-diagnosed condition, as it is often associated with exotic countries and cuisines (Rello *et al*., 2004).

In Latin America, cases of this parasitosis have been documented in Chile, Peru and Brazil. In Peru, eight cases were described, of which five were confirmed to be caused by Anisakidae. In Chile, four cases were diagnosed, with two of them caused by the species *Pseudoterranova dicipiens*. Furher analysis revealed that the same species was responsible for seven cases in Chile. In Brazil, 85 fish were randomly sampled from Colares Island and Vigia, Pará, using net fishing. It was found that 80% and 76% of the sampled fish were were parasitized, particlularly the silver croacker (*Plagioscion squamosissimus* Heckel, 1840), kumakuma (*Brachyplatystoma filamentosum* Lichtenstein, 1819), and gilded catfish (*Brachyplatystoma rousseauxii* Castelnau, 1855) being the most parasitized species (Quijada *et al*., 2005; Rodrigues *et al*., 2015; da Silva-*et al*., 2021).

In Venezuela, it has been demonstrated that anisakids infest marine mammals such as manatees, otters and dolphins, as well as numerous piscivorous birds in the different marshes, inlets, lakes, lagoons and deltas along the Venezuelan coast (Windevohxel, 2003). Anisakid infections have also been confirmed in fish intended for mass consumption, in Caracas Capital District (Bandes *et al*., 2005), Nueva Esparta state (Puccio *et al*., 2008), the Médano Blanco coastal strip, Falcón state (Bracho-Espinoza *et al*., 2013), and the north-eastern and insular coastal region of Venezuela (Maniscalchi *et al*., 2015). These infections were found in fish species of the Mugilidae family. Furthermore, in the Gulf of Paria, on the eastern coast of Venezuela, has reported the presence of *Anisakis* spp L_3 larvae in different varieties of croaker (corvine) fish, specifically *Cynoscion virescens* Cuvier, 1830 and *Cynoscion microlepidotus* Cuvier, 1830 species (Botto, 1981).

The State of Sucre is a fishing region where fish such as sardines and mullet are consumed daily. In recent years, there has beena shift in the region's consumption habits, with an increased consumption of preparations such as anchovies, sushi, ceviche and raw or undercooked canned fish. Additionally, the sale of preferably uneviscerated fish is another factor to consider regarding sanitary risks that can contribute to the presence of parasites in fishery products. Anisakidosis could become a public health problem in this area, as the local economy and food industry revolve around fishing. This study aims to explore, document and analyze the presence of Anisakidae larvae and Acanthocephala in *Sardinella aurita* Valenciennes, 1847 and *Mugil curema* Valenciennes 1836, which are commonly consumed by the local population in Cumaná, Sucre State, Venezuela. The study also highlights the potential risk of human anisakidosis associated with the consumption of these species.

MATERIALS AND METHODS

The present study was carried out in the city of Cumaná, Sucre municipality, Sucre state, Venezuela. It involved the different fish outlets of the city's main market, where various varieties of marine species from artisanal shore and draft trawling are sold in the coastal area of the Gulf of Cariaco as shown in (Fig. 1). The Gulf of Cariaco is located south of the Araya Peninsula, in the state of Sucre.

Figure 1. Geographic location of the Gulf of Cariaco, Sucre municipality, Sucre state, Venezuela. Source: 10,51316° N, 63,97916° O, Google Maps.

In the period of June-September 2019, a total of 100 specimens of fish were processed: 50 of the *S aurita* (pilchard) and 50 of the *M curema* (mullet), which were acquired at different fish outlets in the main Cumaná market. The samples were selected considering the following parameters: clearness of the eyes, presence of gills, presence of viscera, weight, size and fishing time. They were preserved in ice and then transferred to the laboratory at the Instituto de Investigaciones em Biomedicina y Ciencias Aplicadas Dra. Susan Tai, Universidad de

Oriente (IIBCAUDO) to be evaluated using a previously established criterion (Cervigón, 1993; Osanz, 2001).

For the parasitological analysis of the different fish species, the methods described by the United States Food and Drug Administration (FDA, 2022), the Bacteriological Analytical Manual (BAM), the Center for Food Safety and Applied Nutrition (CFSAN) and the Canadian Health Canada OPFLP-02 laboratory procedures were used (Bier *et al*., 2001).

The fish were dissected and a simple visual examination of the organs was done to check for parasites. They were then placed into petri dishes with a 0.85% NaCl solution to be further examined under a stereoscopic microscope.

The collected nematodes underwent several processing steps including washing with a 0.85% NaCl solution, then fixation, and clarification followed by preservation in 70% glycerinated ethanol. The Acanthocephalans were fixed, clarified, stained then preserved. The taxonomic identification was done in accordance with the usual techniques in helminthology. Morphologic analysis was conducted using a Zeiss Axioscope light microscope and mounted samples were photographed using the Zeiss Axioscope light microscope.

To calculate the parasitic indices of prevalence, mean intensity in each type of helminth, mean abundance of each genus of the family Anisakidae and Acanthocephala mean intensity and mean abundance were done as described by Bush *et al*. (1997), and Morales & Pino (1995). The number of parasites found and the degree of parasitic infestation was determined as described by Verján *et al*. (2001).

Ethic aspects: The authors point out that the study material - Sardinella aurita and Mugil curema - were acquired in a municipal market (Cumaná market), Venezuela for direct sale to the local population, not alive and without any type of interference by the researchers regarding to the way of fishing, interference in the behavior or in vivo manipulation of the specimens. We highlight that the anonymity of the vendors and fish stalls in the Cumaná market was preserved.

RESULTS

Only the species *M. curema* were found to be parasitized with nematode larvae of the Anisakidae family and Acanthocephala, showing an overall prevalence of 88% and 58% respectively. The hosts were each parasitized by at least one species of parasite. A total of 118 Anisakidae L_3 larvae were collected, including 37 *Anisakis* sp., 74 *Pseudoterranova* sp. and 7 of *Contracaecum* sp. Additionally, 33 Acanthocephala specimens were collected. In total, 5915 third-stage nematode larvae (L_3) were collected, with 5,666 being Anisakidae larvae and 249 being Acanthocephala larvae in the processed *M. curema* specimens.

It is important to note that the simple visualization method does not for the observation of newly formed larvae that may be encysted within the muscles, so the data described here may be underestimated.

In this study, the Anisakidae family was found to parasitize the digestive viscera (*Anisakis* sp. 29.72%, *Pseudoterranova* sp. 8.10% and *Contracaecum* sp. 28.57%), kidneys (*Anisakis* sp. 32.43%, *Pseudoterranova* sp. 68.91% and *Contracaecum* sp. 28.57%) and liver. (*Anisakis* sp. 37.83%, *Pseudoterranova* sp. 22.97% *Contracaecum* sp. 42.86%). Acanthocephalans were found to parasitize the intestine (Fig. 2). Morphological characteristics of both Anisakidae and Acanthocephala were identified (Fig. 3). They were observed as immature larvae, whitish or pinkish in color (pearly), with rounded unsegmented bodies measuring approximately 2.5 mm in diameter and between 1 and 5 cm in length. These larvae were arranged in a spiral shape, and can be found in digestive viscera. This is the first study on the presence of Anisakidae in Sucre state, Sucre municipality, which is of great zoonotic importance. In the Anisakidae family, the main species of public health importance are *Anisakis simplex*, *Pseudoterranova decipiens* and *Contracaecum osculatum*.

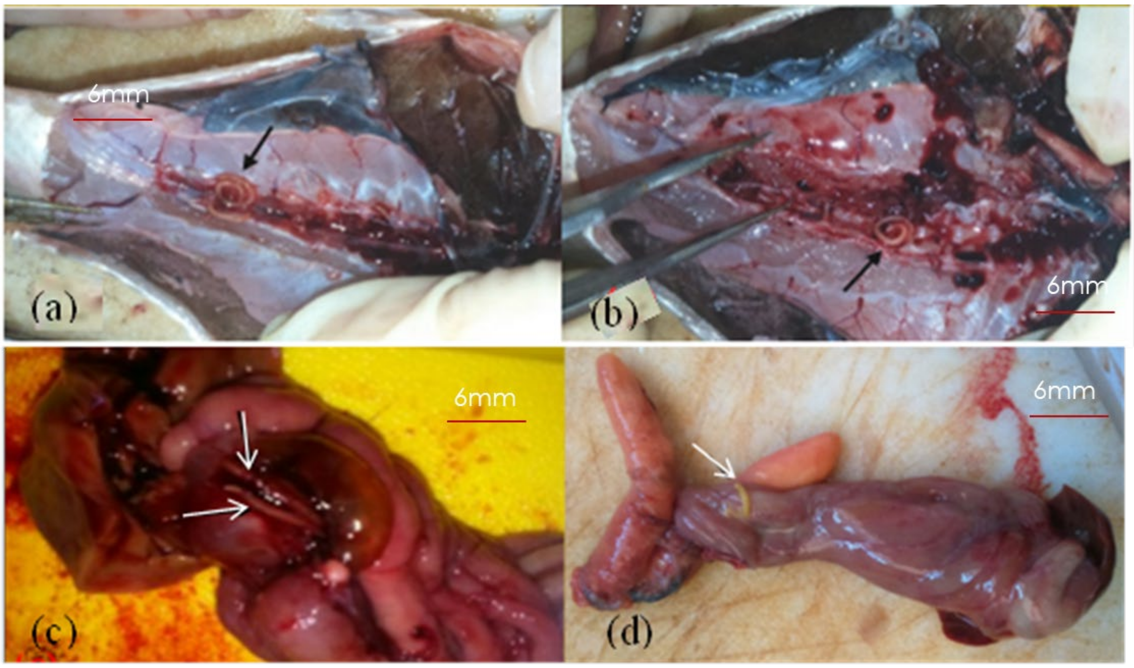

Figure 2. Specimens of Anisakidae L_3 larvae in caudal kidney (a, b), intestine (c) and Acanthocephalans in intestine (d) collected from *Mugil curema*.

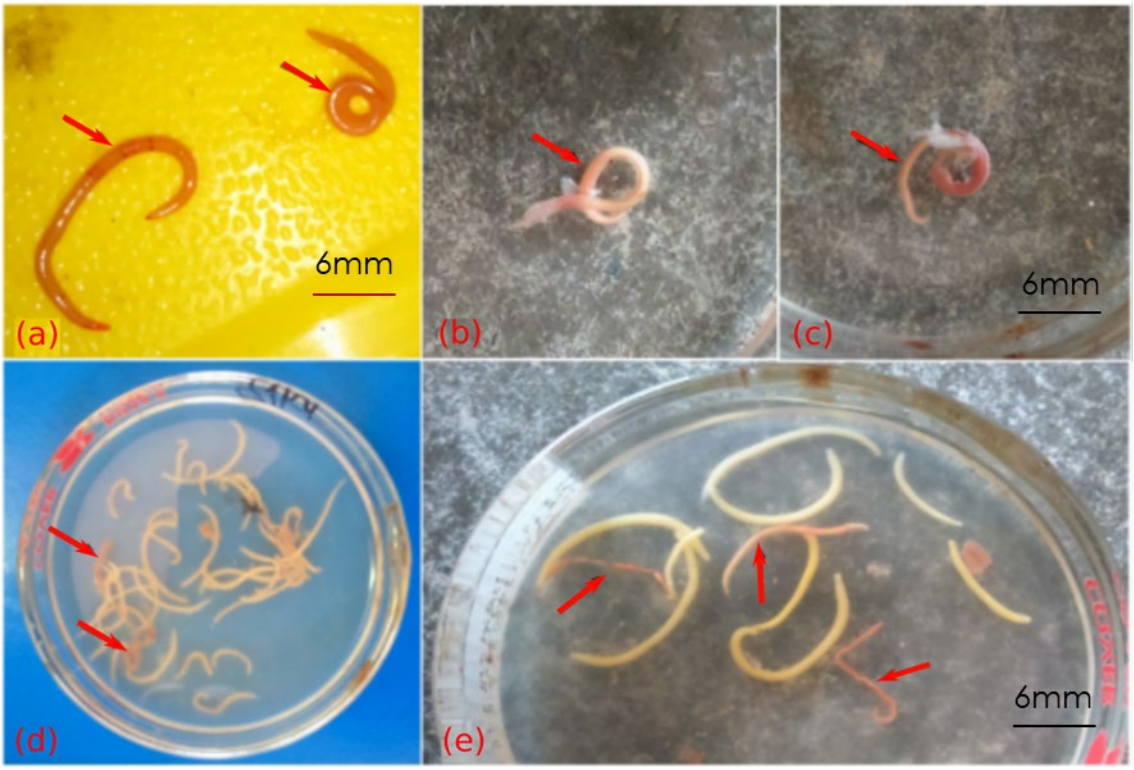

Figure 3. Helminths of the family Anisakidae mechanically extracted from digestive viscera, kidneys and liver and Acanthocephala extracted from digestive viscera, present in *Mugil*

curema. (a) L_3 larvae of the family Anisakidae, (b) and (c) L_3 larvae of the family Anisakidae with cyst membrane, (d) and (e) Petri dish with L_3 larvae of the family Anisakidae and Acanthocephalans. Parasites from the Aniakidae family highlighted with red arrows.

The Anisakidae family nematodes found in the *M. curema* species were identified at the genus level. These nematodes were examined a light microscope to determine their morphological characteristics such as size, color, presence or absence of a perforating tooth and mucron, location of the excretory pore opening, and characteristics of the digestive system as previously outlined. Among the Anisakidae nematodes, *Pseudoterranova* sp. showed the highest parasitic indices in terms of prevalence, mean intensity and mean abundance (Fig. 4). This was followed by *Anisakis* sp. (Fig. 5), while *Contracaecum* sp. exhibited the lowest parasitic indices (Fig. 6). Overall, this study determined a prevalence of *Anisakis* sp. in 32% (16/50), *Pseudoterranova* sp. in 44% (22/50), and *Contracaecum* sp. in 12% (6/50) of the fish examined (Table 1).

Table 1. Parasitic indices of prevalence, mean intensity (MI) and mean abundance (MA) of Anisakidae, Acanthocephala and unidentified helminths collected from *Mugil curema*, from the Gulf of Cariaco, Vanezuela.

Genus	Prevalence (%)	(MI)	(MA)
Anisakidae	66		
Anisakis	32	2.31	0.74
Pseudoterranova	44	3.36	1.48
Contracaecum	12	1.16	0.14
Acanthocephala	58	-	-
Unidentified helminths	22	-	-

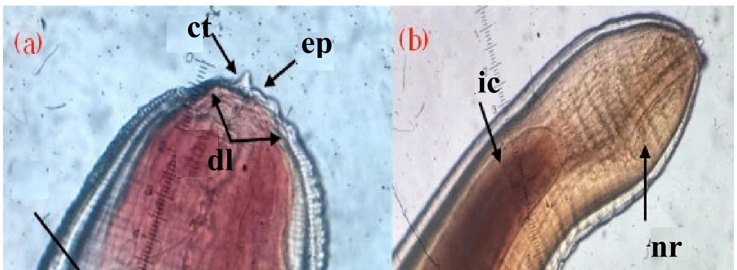

Figure 4. *Pseudoterranova* sp. L_3 larvae mechanically extracted from the digestive viscera, kidneys and liver, isolated from *Mugil curema* (a) ephalic end (ct: cuticular tooth, ep: excretory pore, dl: dorsal lip, nr: nerve ring) (b) ephalic end (nr: nerve ring, ic: intestinal cecum, es: esophagus) (c) Anterior end (es: esophagus, v: ventricle, ic: intestinal cecum) (d) Posterior end (a: anus, m: mucron).

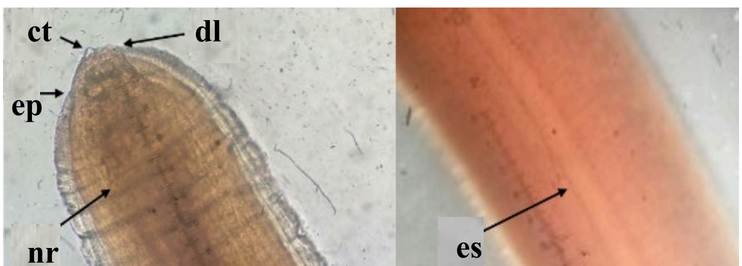

Figure 5. *Anisakis* sp. L_3 larvae extracted mechanically from the *Mugil curema* (a) ephalic end (ct: cuticular tooth, ep: excretory pore, dl: dorsal lip, nr: nerve ring) (b, c) Anterior end (es: esophagus) (d) Posterior end (a: anus, m: mucron).

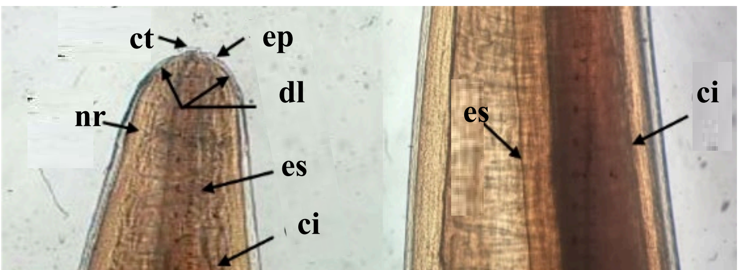

Figure 6. *Contracaecum* sp. L_3 larvae extracted mechanically from the collected from *Mugil curema* (a, b) ephalic end (ct: cuticular tooth, ep: excretory pore, dl: dorsal lip, nr: nerve ring, es: esophagus, ic: intestinal cecum) (c) Anterior end (es: esophagus, v: ventriculus, ic: intestinal cecum, va: ventricular appendage, i: intestine) (d) Posterior end (a: anus, ga: ganglia, m: mucron).

Adult Acanthocephala specimens were identified as having a coloration that varied between cream, yellow and brown. They have rounded bodies with a proboscis or invaginable tube at the cephalic end that is covered in characteristic spines. The size of these specimens ranged from 2mm to 1-meter-long (Fig. 7).

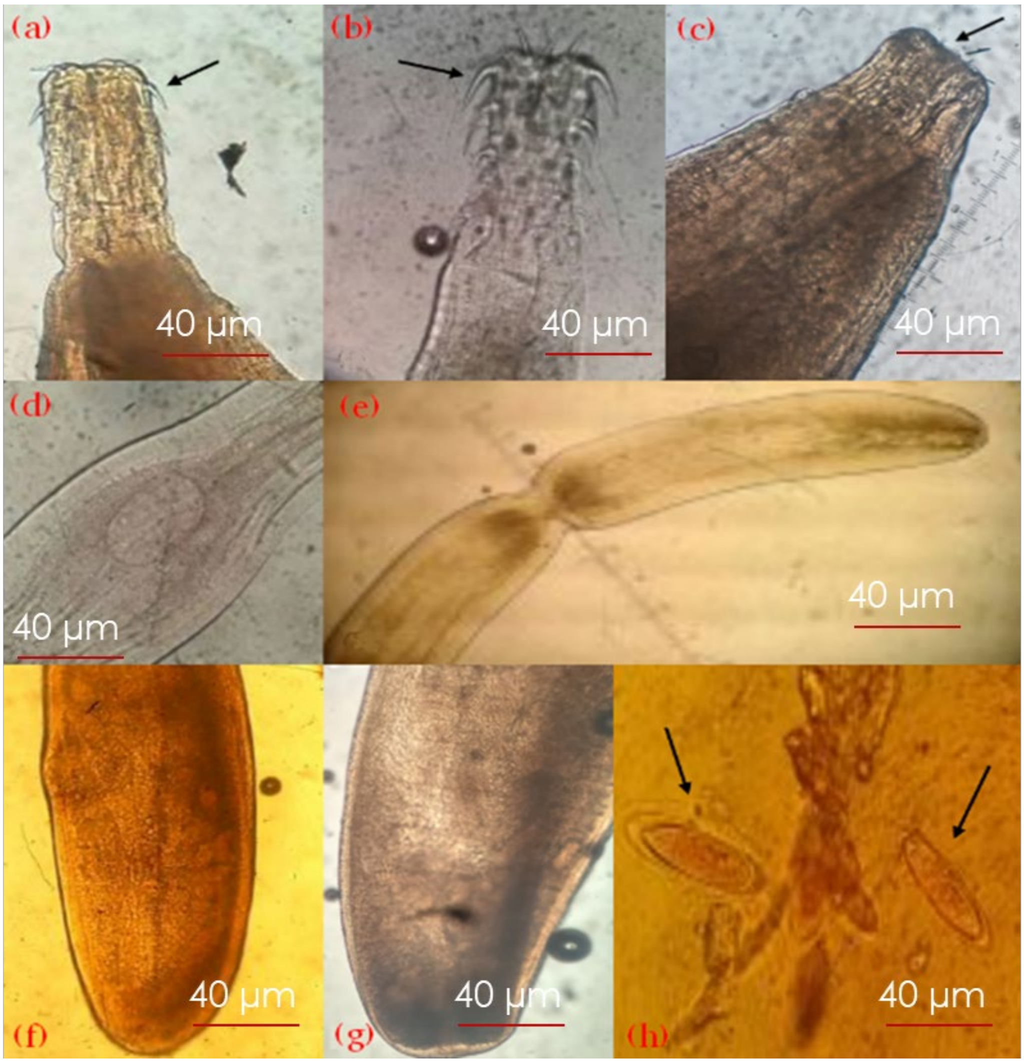

Figure 7. Acanthocephala specimens collected from *Mugil curema* (a-c) cephalic end showing proboscis and neck, the arrows show characteristics spines of the proboscis (d-e) Reproductive apparatus showing (f-g) Posterior end (h) Acanthocephalan eggs, arrows.

The tissue tropism of parasites in *M. curema* was found to be similar in all the specimens analyzed. The parasites were primarily located in the kidneys (caudal kidney), with a higher parasite load of 55.09%. They were also present in the liver (28.81%) and other digestive viscera, such as the mesentery, stomach, pancreas, gallbladder and spleen (16.10%). It is interesting to note that despite the assumption that larvae in the digestive viscera provide greater development and migration, the parasite load in the kidneys was higher.

The parasitic coinfections between nematodes of the Anisakidae family, demonstrated the variety of parasites that can simultaneously infect fish. We observed that 69.70% of the fish analyzed had a monoparasitic infection (*Pseudoterranova* sp.). Rates of biparasitism (*Anisakis* sp/*Contracaecom* sp) and triparasitism (*Pseudoterranova* sp. /*Anisakis* sp./*Contracaecom* sp.) were 27.27% and 3.03% respectively. Additionally, the parasitic coinfections between Anisakidae and Acanthocephala were 100%.

DISCUSSION

Only the species *M. curema* was found to be parasitized with nematode larvae from the Anisakidae family, with an overall prevalence of 96%. This result is of great zoonotic importance, as there have been several cases of Anisakiasis reported worldwide (Adams *et al*., 1997). In Europe, herring (*Clupea harengus* Linnaeus, 1758), anchovy (*Engraulis encrasicolus* Linnaeus, 1758) and sardines (*Sardina pilchardus* Walbaum, 1792) are the fish species most commonly associated with cases of anisakiosis (Audicana *et al*., 2002; Audicana & Kennedy, 2008; Klimpel & Palm, 2011). Likewise, adult Acanthocephala worms were identified and although they are not clinically significant for humans, they are of great veterinary importance in various fish species, and can also infect turtles, reptiles, and pigs (Drago, 2017).

These results are consistent with those obtained previous studies conducted in markets alongs the northeastern coastal and insular regions of Venezuela (Maniscalchi *et al*., 2015), the Médano Blanco coastal strip in Falcón State (Bracho-Espinoza *et al*., 2013), Nueva Esparta State (Puccio *et al*., 2008), and Caracas, the capital of Venezuela (Bandes *et al*., 2005). These studies analyzed different species of fish popular for consumption and found a predominance of Anisakidae in the Mugilidae family (genus *Mugil*). Unlike previous studies that showed a high infection rate of Anisakidae nematodes in *M. curema* species in the Gulf of Paria, in far eastern Venezuela. Botto (1981) also demonstrated the presence of *Anisakis* spp L_3 larvae in different varieties of corvine, specifically in the *Cynoscion virescens* Cuvier 1830, and *Cynoscion microlepidotus* Cuvier 1830 species, further highlighting the risk of this parasitic infection in other commonly consumed fish species.

Mugil curema is abundant, along all coasts of Venezuela and is capable of adapting and subsisting on a wide variety of foods by adjusting its feeding habits according to environmental conditions (Cervigón, 1993; Franco & Bashirullah, 1992; Guerra & Marín, 2002; Novoa, 2000). It is worth noting that the *M. curema* species was also found to be parasitized by Acanthocephala helminths, with an infection rate of 58% in the fish studied. Another study conducted in Lima, Peru, focusing on parasitesin marine fish intended for human consumption, observed that the *Mugil cephalus* had an 86.70% infection rate with Acanthocephala, but did not find Anisakidae larvae in the specimens analyzed (Serrano *et al*., 2017).

The processed *M. curema* species showed a parasitism rate of 66%. These results are similar to those reported by Maniscalchi *et al*. (2015) in a study conducted in the northeastern coastal and insular region of Venezuela, where 90 out of 143 (62.94%) specimens of the *M. curema* species tested positive for nematodes from the Anisakidae family.

Botto (1981) demonstrated in the Gulf of Paria that the genus *Anisakis* was prevalent in corvine species. Likewise, studies carried out along the Venezuelan coasts (Bandes *et al*., 2005), in Caracas (Puccio *et al*., 2008), in Nueva Esparta state (Maniscalchi *et al*., 2015), in the northeastern and insular Coastal Region of Venezuela, and the present study show a high prevalence of Anisakidae larvae in fish species of the Mugilidae family, with prevalence rates of 76%, 100%, 62, 94% and 66% respectively (Franco & Bashirullah, 1992; Novoa, 2000).

Live larvae has been found mainly in the viscera or encysted in the musculature of a number of commercially important fish species.

The infectious larval stages of *Anisakis simplex* and *Pseudoterranova decipiens* have been implicated in most cases of human anisakiasis. Several other species, such as *Contracaecum osculatum* Rudolphi, 1802 and *Phocascaris* spp., also pose a public health concern (Adams *et al*., 1997). Similar findings were made in this study, where *Anisakis* sp. and *Pseudoterranova* sp. were the most prevalent genera of nematodes. Consequently, they represent a possible source of infection of Anisakidae nematodes, constituting a public health risk to consumers. This risk can be preventable if the appropriate sanitary measures are applied. Moreover, it is important to mention that humans are paratenic hosts, as the larvae are unable to reach sexual maturity (López-Serrano *et al*., 2000). Puccio *et al*. (2008) and, Maniscalchi *et al*. (2015) on the coasts of the northeastern and insular region of Venezuela obtained similar results. In these studies, larvae of nematodes of the Anisakidae family were found in fish of the genus *Mugil*, with a higher prevalence for the genus *Anisakis* at 52% and 47.16%, respectively, followed by *Pseudoterranova* at 32% and 40.10%, and *Contracaecum* with 16% and 12.74%. Similarly, fish of the genus *Mugil* from the Venezuelan coast have been observed to be infected with Anisakidae family with *Pseudoterranova* spp having the highest prevalence (56%), followed by *Contracaecum* (48%) and *Anisakis* (16%) genera (Bandes *et al*., 2005).

Furthermore, a study carried out in Fálcon state revealed a high prevalence of anisakids, particularly of the genus *Contracaecum* (97%) with *Pseudoterranova* spp accounting for only 3%, but no *Anisakis* (Bracho-Espinoza *et al*., 2013). Shih & Jeng (2002) reported that in Golfete de Coro, specifically in the Médano Blanco sector, hosted numerous migratory birds and reptiles that are definitive hosts, of *Contracaecum* spp. parasite. This highlights that this area was conducive for the survival of nematodes from the Anisakidae family. Our results allow us to infer that if *Contracaecum* sp larvae are present in the fish evaluated, adult parasites are certainly present in species of birds or reptiles from the place where the study was carried out.

Globalization, cultural and gastronomy exchange have led to an increase in reported cases of anisakidosis worldwide, particularly in countries like Spain, Japan and Mexico. This poseses a potential risk to human health due to the rise in zoonotic parasitosis, which has become a significant public health concern in these countries (Chai *et al*., 2005; Hochberg *et al*., 2010). Venezuela is not immune to globalization, and the custom of consuming raw or seasoned fish such as ceviches has made the findings from our study a significant public health risk for the country.

Our findings on *Pseudoterranova* sp. mean intensity of infection and mean abundance were similar to those obtained by Bandes *et al*. (2005) and Maniscalchi *et al*. (2015), who reported 13.1 ± 5.2 and 2.26 ± 1.42 parasites per unit, respectively. These values are considered high when compared to the parameters set by the US FDA (2022). This highlights the risk of anisakid infection in humans in Sucre State, Venezuela if raw or undercooked fish is consumed.

The high mean intensity and prevalence of parasites demonstrate the colonization ability of these three genera of Anisakidae in the population of *M. curema.* This categorizes mullet as a potential intermediate host in the biological cycle of Anisakidae, particularly *Pseudoterranova* sp. which has the highest prevalence and mean abundance, followed by *Anisakis* sp. and *Contracaecum* sp. On the contrary, studies conducted in Colombia found that *Contracaecum* spp. was the most prevalent, occurring in 100% of the fish species analyzed, such as *Salminus affinis* Steindachner, 1880, *Hoplias malabaricus* Bloch, 1794, and *Pseudoplatystoma corruscans* Spix & Agassiz, 1829 (Pardo *et al*., 2007, 2008, 2009).

Anatomically, the kidneys in the Mugilidae family fish are attached to and run along vertebral column, covered by a conjunctival membrane. This suggests that the larvae have greater access to the vertebral column from the muscles adjacent to it, posing a higher risk of infection for humans due to possible larval migration towards the fish musculature. Alternatively, it is hypothesized that the larvae's tropism towards the kidney serves as a protective mechanism, preventing migration to the muscles. This may explain why no Anisakidae species were found in the musculature of the fish under study, which differs from other studies that found only one specimen of Anisakidae in the musculature of *M. curema* (Bandes *et al*., 2005; Maniscalchi *et al*., 2015).

Larval migrations in the mullet and blue grenadier fish species are not well documented, so understanding this is essential for assessing the potential risk of consuming marine fish and the need to quickly eviscerate the fish to prevent larvae from migrating to the muscle tissues (Bandes *et al*., 2005). Our findings confirm the presence Anisakidae species, their ability to migrate, and their tropism for fish muscle, as evidenced by the parasites found in close proximity to the muscles just a few hours after the fish was purchased at the local municipal market. Further studies could evaluate the the long-term preference or tropism of these parasites.

Parasites are consumers and therefore compete for resources. When multiple species of nematodes infect a host; there can be competition for nutrients, which is vital for parasite growth and reproduction. This is the case with Acanthocephala (intestinal helminths) where evidence suggests these species displace other worms (both adult and larval) from the best sites in the intestine, where nutrients are abundant (Silva-Melo *et al*., 2021). In this study, all *Acanthocephala* isolated from *M. curema* were found in the intestines.

This present describes the co-infection of nematodes from Anisakidae family, demonstrating the variety of parasites that simultaneously infect fish. Through our literature review, we found no published studies presenting similar findings on mixed parasitism. Thus, this study is the first to reveal the presence of multiple infections by these three nematodes within the same fish specimen, collected from the Gulf of Cariaco coast in Venezuela.

In the coastal areas of Venezuela's Sucre states, the Anisakidae L_3 larvae are present in *M. curema* fish, indicating a risk of infestation for residents who consume these fish. It is also possible thst other fish species inhabiting the Gulf of Cariaco may be similarly infested with these nematodes. We recommend conducting further research focusing on economically important fish and other fish consumed by humans to determine the extent of infestation by these helminths.

It is very important to note that identifying anisakid species solely on morphology is not reliable or efficient. Molecular techniques are currently used to clarify the genetic and ecological differences among anisakids, despite their morphological similarities (Mattiucci & Nascetti, 2008). Future studies should use molecular methods to identify and estimate the different Anisakid species in fish from the coastal region of Venezuela.

The state of Sucre is rich in fish, which is a primary protein source for the area and a major economic pillar for the municipal market in Cumaná and surrounding areas. The lack of sanitary control, combined with our findings, is a significant public health concern. Additionally, the customs in the region could contribute to the spread of anisakidosis in coastal settlements and markets in Sucre, Venezuela. This study provides data on a food-borne parasitic disease and offers insights to prevent human infections.

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