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Original paper

Presence of *Contracaecum* sp. larvae in two characiform fish from northeastern Brazil

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ABSTRACT. *Pygocentrus nattereri* Kner, 1858 and *Serrasalmus rhombeus* (Linnaeus, 1766) are species of serrasalmids sold in open-air markets and market municipalities in Maranhão, Brazil. Fifty-two individuals of *P. nattereri* and 52 of *S. rhombeus* were acquired from a fish market in the Municipality of Pinheiro, Maranhão, Brazil, and transported to the laboratory of "Inmunohistoquímica" from "Universidade Estadual do Maranhão" (UEMA). The lateral musculature of the fish was analyzed by making fine cuts, internal organs were placed in Petri dishes with distilled water, and with the aid of tweezers and needles, the tissue was examined by making fine cuts that allowed observation inside them. The results of the study revealed the presence of nematode larvae L3 of *Contracaecum* sp. was found parasitizing the visceral cavity, intestines, pyloric cecum, and stomach of *P. nattereri* and *S. rhombeus*. This study alerts local authorities and the population to take the necessary measures to prevent the accidental ingestion of endoparasites with zoonotic potential.

Keywords: Anisakidae, endoparasites, Nematoda, redeye piranha, red piranha, Maranhão

Introduction

Artisanal fishing in Brazil has social importance, both in terms of local context, as well as regional, being a source of subsistence for riverside populations that depend on this activity directly or indirectly through activities related [1]. Furthermore, this activity has a significant contribution to the socioeconomic sphere for many communities, as, serves as a source of food, work, and cultural strengthening [2].

In the Northeast region of Brazil, as well as in Baixada Maranhense, the fish is sold mainly in open-air markets and market municipalities. The free market is considered one of the most traditional places of retail food sales, being a form of mobile commerce, with circulation within urbanized spaces. These places stand out for their marketing of fresh foods, a wide variety of products, and price diversity, thus bringing convenience to consumers [3]. Among the fish species commercialized in the fish markets, *Pygocentrus nattereri* Kner, 1858 and *Serrasalmus rhombeus* (Linnaeus, 1766) present marked acceptance by the population [3].

Pygocentrus nattereri is a species from the Characiformes: Serrasalmidae, widely distributed in South America (Peru, Argentina, Bolivia, Brazil, Colombia, Ecuador, Guyana, Paraguay, Uruguay), including outside the Amazon basin [4]. It is a fish-eating, aggressive species (dismembers their prey before feeding). Dwells both in the main channel of the water rivers white as well as in lagoons and areas of flood. It is a migratory species. It reproduces by attaching its eggs to the roots of floating plants [4].

Serrasalmus rhombeus is a species from the Characiformes: Serrasalmidae, widely distributed in South America (Peru, Bolivia, Brazil, Colombia, Ecuador, Guyana, Suriname, Venezuela), even outside the Amazon basin [4]. Is a carnivorous species, that consumes fish (mainly sardines), invertebrates, insects, and vegetal. It frequently lives in the main channel of rivers, streams, lagoons, and flood areas. Spawns more than once a year, with a peak in the flood period [4].

Fresh fish are vulnerable and exposed to a series of contamination hazards, such as parasitic contamination [5]. Parasitic zoonoses transmitted by fish increasingly attract the attention of researchers, due to the loss of quality of products from fishing and aquaculture, and the impact on the economy [6]. It is known that fish are responsible for most diseases associated with food consumption. However, the true incidence of such diseases is not known, either due to a lack of registration or a lack of knowledge among doctors and patients about the causes of these illnesses [6].

Countless parasites infect fish, but only some species of helminths are capable of causing zoonoses. These parasites can be found in various organs of the fish, including embedded in the muscles, which justifies the need for rigorous health inspection [7]. Among the nematodes of zoonotic potential, anisakids are prominent with reports in marine and freshwater fish [8]. Human anisakiosis is considered to be the most severe infection caused by consumption of contaminated fish harboring third-stage larvae of nematodes of the genera *Anisakis* (Dujardin, 1845), *Pseudoterranova* (Mozgovoy, 1950) and *Contracaecum* (Railliet & Henry, 1912) [9].

Due to the importance of *P. nattereri* and *S. rhombeus* as fish species for human consumption and the existence of parasites with hygienic-sanitary importance, the objective of this study was to provide information on the presence of *Contracaecum* sp. in two species of piranhas acquired in a fish market in the municipality of Pinheiro, Baixada Maranhense, Brazil.

Material and Methods

Fifty-two individuals of *P. nattereri* and 52 of *S. rhombeus* were acquired from a fish market in the Municipality of Pinheiro (3°12′00″ S, and 45°00′00″ W), Maranhão, Brazil. Fish were put into plastic bags with ice and transported to the

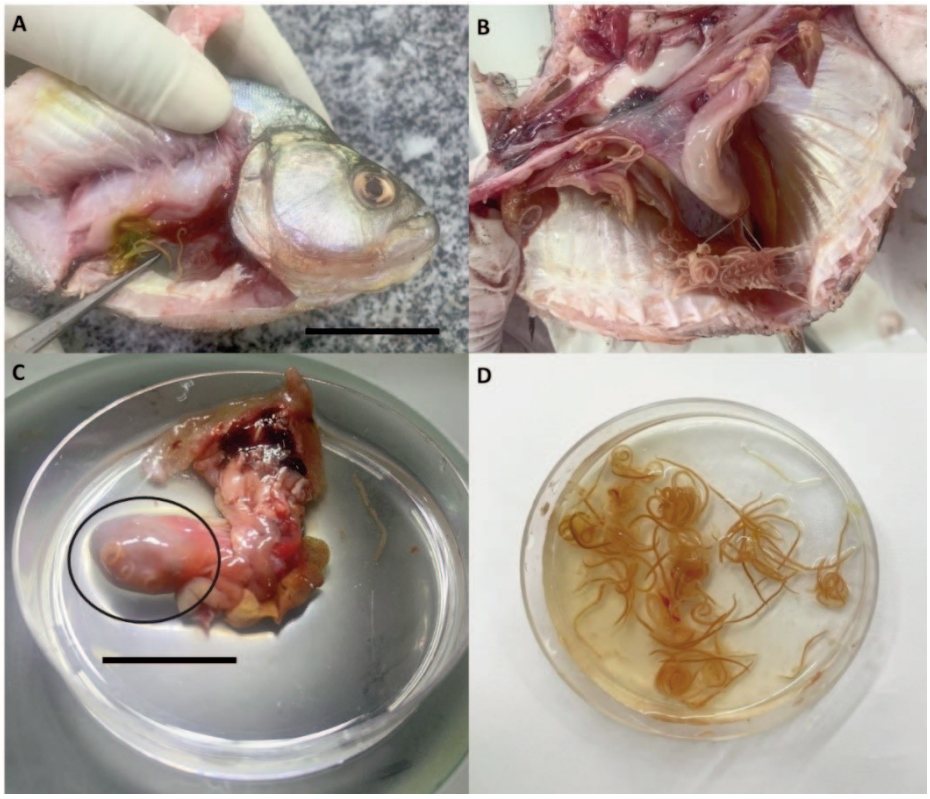

Figure 1. A. *Contracaecum* sp. in the visceral cavity of *Serrasalmus rhombeus*; B. *Contracaecum* sp. from *Pygocentrus nattereri*; C. *Contracaecum* sp. inside the stomach of *Serrasalmus rhombeus*; D. *Contracaecum* sp. from *Pygocentrus nattereri* in Petri dish

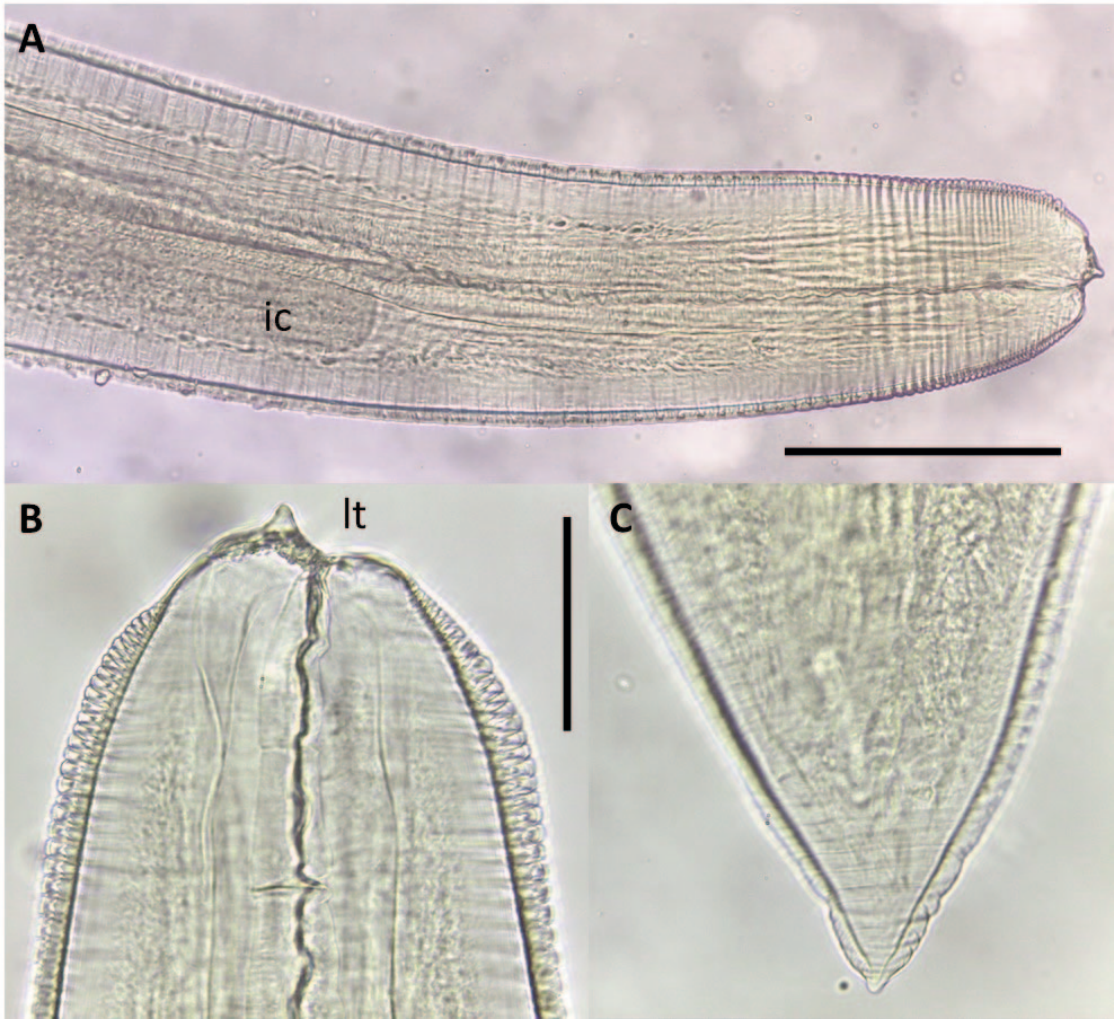

Figure 2. A. Ventral view of *Contracaecum* sp. ic = intestinal cecum; B. Anterior part of *Contracaecum* sp. lt = larval tooth; C. Tail of *Contracaecum* sp. Scale bar: A = 0.5 mm, B = 0.2 mm, C = 0.1 mm

laboratory of "Inmunohistoquímica" from "Universidade Estadual do Maranhão" (UEMA). The lateral musculature of the fish was analyzed by making fine cuts, which were carefully observed under stereoscope. Internal organs were analyzed using a stereoscope Leica EZ4. Internal organs were placed in Petri dishes with distilled water, and with the aid of tweezers and needles, the tissue was examined by making fine cuts that allowed to observe inside them. In the presence of any parasites, these were removed with tweezers and preserved in ethanol 70% for posterior analyses [10].

For taxonomic identification, parasites were placed into glass slides with lactic acid, which is used for the clarification of parasite tissue and consequent visualization of external structures and internal organs [10]. Parasites mounted in slides were observed under an optical microscope Leica DM750. Parasites were photographed with a digital camera (LEICA ICC50W) connected to the microscope, and the images were used to obtain the measurements of the length and width. Based on the morphological characteristics of the parasites, the identification was made using the information of [11] and [12] for nematodes. Parasitological indices followed those by [13].

The Shapiro-Wilk W statistic was used to test the normality of distributions. Spearman´s (*rs*) correlation test was used to evaluate the correlation between the total number of *Contracaecum* sp. and the fish's standard length.

Results

Nematode larvae L3 of *Contracaecum* sp. were found parasitizing the visceral cavity, intestines, pyloric cecum, and stomach of *P. nattereri* and *S. rhombeus* (Fig. 1). A total number of 844 parasites were found on *P. nattereri* while 186 in *S.*

Table 1. Parasitological indices of *Contracaecum* sp. from *Pygocentrus nattereri* Kner, 1858 and *Serrasalmus rhombeus* (Linnaeus, 1766) collected in northeastern Brazil. AF = analyzed fish, PF = parasitized fish. P% = prevalence, TNP = total number of parasites mI = mean intensity of infection, mA = mean abundance of infection

Parasite	AF	PF	P%	TNP	mI	mA
			Pygocentrus nattereri			
Contracaecum sp.	52	40	76.9	844	21.1	16.2
			Serrasalmus rhombeus			
	50	30	60	186	6.2	3.7

rhombeus. Parasitological indices are show in Table 1. The larval measurements corresponding to *Contracaecum* sp. found in *P. nattereri* presented 11.56 ± 4.42 µm length and 0.40 ± 3.8 µm width; *Contracaecum* sp. found in *S. rhombeus* 11.32 ± 5.37 µm length and 0.4 ± 3.8 µm width.

Contracaecum sp. presented the following characteristics: medium-sized nematodes, white when alive. Cephalic extremity rounded with a small cuticular tooth. The oesophageal muscle is narrow, the ventricle rounded and small, and the ventricular appendix is short. Presence of a long intestinal cecum that extends anteriorly to the nerve ring. The tail is conical without a mucron (Fig. 2).

The correlation coefficient analysis values revealed positive correlations between the size of *P. nattereri* and the total number of *Contracaecum* sp (rs = 0.2728; p = 0.049); likewise, a positive correlation was recorded between the size of *S. rhombeus* and the total number of *Contracaecum* sp. (rs = 0.5112; p = 0.0001).

In the life cycle of *Contracecum* sp. eggs are liberated to the water with the faeces of piscivorous birds that feed on *P. nattereri* and *S. rhombeus*; *Contracaecum* sp. larvae develop into larva L1 inside the egg, which is consumed by the first intermediate host (a copepod). Inside the copepod, larva L1 develops into L2 and L3. Small fish feed on the copepods infected with the larva L3. Small fish then are predated by *P. nattereri* and *S. rhombeus* acquiring the larva L3; in that way, piranhas act as paratenic hosts of *Contracaecum* sp. Finally, piscivorous birds (final host) feed on *P. nattereri* and *S. rhombeus* and inside the stomach of the birds, *Contracaecum* sp. larva develops into L4 and adult, completing its life cycle (Fig. 3).

Discussion

The diet of fish strongly influences the composition of their parasitic fauna. The occurrence of certain species of parasites in fish with little-known eating habits can provide good clues to understanding the composition of the host's diet. Parasite species present indirect life cycles and depend on the presence of more than one host and various predator-prey relationships to complete their life cycles. The occurrence of a parasitic species in a host organism not only reflects the presence of another organism that participates in the life cycle of the parasitic species but also the trophic pathways in the food chain [14].

The host's diet determines the acquisition, accumulation, and overall infection levels of parasites in a fish community. The structure and dynamics of interactions in trophic chains can be important determinants of helminth infections in fish definitive hosts [15]. Helminth fauna richness is highest in fish with a mixed carnivorous diet (invertebrates and fish). Followed by consumers of invertebrates and the smallest number of parasites occurs in fish with other eating habits. This is because carnivores have greater exposure to helminths when consuming prey such as invertebrates and smaller fish that are intermediate hosts for different groups of parasites [16].

Among the parasitic groups that are transmitted via the trophic route, nematodes stand out. The presence of this group of endoparasites can have negative implications for the marketing of fish, reducing its value in commercial markets. Humans acquire the infection by consuming undercooked fish [17]. Consumed nematodes cannot reach maturity in humans, but can remain in the third and fourth stages of larval development (L3 and L4). Symptoms that indicate infection include gastritis due to perforation of the intestines. The only possible means of cure is surgical removal or expulsion of the larvae through ingestion of medication [18].

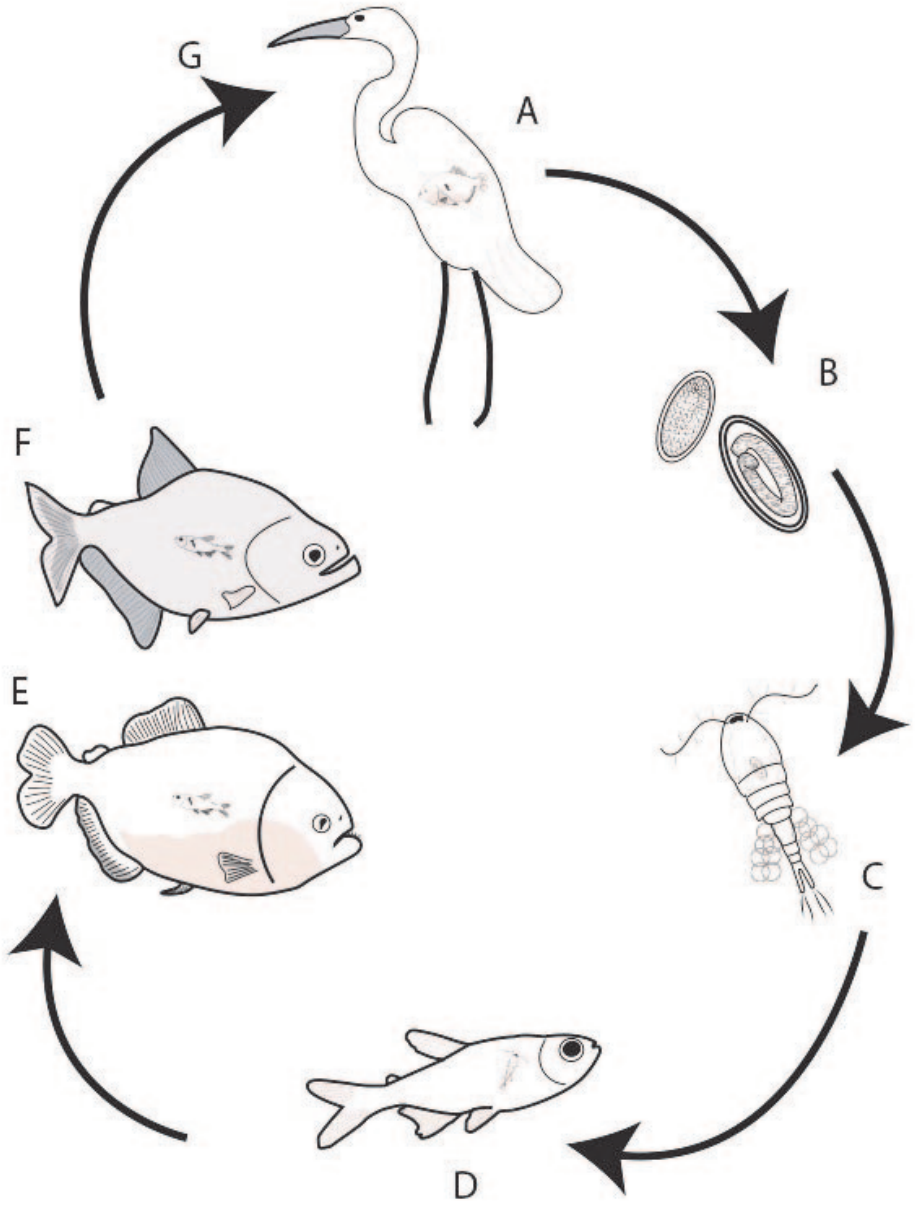

Figure 3. Life cycle of *Contracaecum* sp in the Municipality of Pinheiro, Maranhão, Brazil water bodies. A. Eggs are liberated to the water with the bird's (final host) faeces; B. *Contracaecum* sp. larvae develop into larva L1; C. Egg with larva L1 is consumed by a copepod (intermediate host). Inside the copepod, larva L1 develops into L2 and L3; D. Small fish feed on the copepod with the infective stage (L3). Fish participate as a paratenic host; E. *Pygocentrus nattereri* and/or *Serrasalmus rhombeus* (F) feed on small fish and acquire *Contracaecum* sp. L3 larvae; G. Piscivorous bird feed on the piranhas. Larvae L3 of *Contracaecum* sp. develop into L4 and adults inside the final host (piscivorous bird)

The type of cooking used to prepare fish can influence the risk of transmission of endoparasites to humans. In Brazil, the tradition of consuming raw or undercooked fish and the tendency to consume "sushi" of oriental origin increase the chances of acquiring an accidental zoonotic infection [10].

Contracaecum sp. was reported parasitizing *P. nattereri* from Arari Lake, Marajó Island, State of Pará, Brazil with a prevalence of 84% [19]; in Cuiabá, State of Mato Grosso with prevalences higher than 95% [9,20]. *Contracaecum* sp. was found in four fish species collected in the main channel of the Pericuma River, in Maranhão, Brazil: *Cichlasoma zarskei*, *Trachelyopterus galeatus*, *Hoplias malabaricus* and *Serrasalmus rhombeus* [21].

In the present study, *Pygocentrus nattereri* and *Serrasalmus rhombeus* presented parasitic variety.

This may have been influenced by the trophic level of this fish, as carnivorous fishes can become infected with different nematoid larvae when feeding on previously infected smaller fishes and are therefore more prone to infections by *Contracaecum* sp. [19, 22, 23].

Contracaecum larvae mostly occur in the visceral organs and mesentery of fish, thus limiting their zoonotic threat, which may explain why human cases are rare [9, 20]. In the present study, no case of infection in the skeletal muscles of the examined fish by *Contracaecum* sp. was recorded. confirming the visceral cavity and internal organs as common sites for the parasitism of this nematode.

The fact that the highest parasite prevalence and intensities occurred in the mesentery of both piranhas, was directly related to the marked tropism of *Contracaecum* sp. larvae, which high prevalence of specimens at this site. This characteristic behavior was also documented by [20]. Moreover, the absence of these larvae in the musculature of *P. nattereri* has already been reported by Barros et al. [24] and [20].

The variable length was observed to influence the intensity of infection occurring in specimens of *P. nattereri*. As it was suggested by [25] that bigger fish accumulate greater quantities of larvae since they frequently feed on smaller parasitized specimens and mostly escape from predation by the definitive hosts of these nematodes. The data obtained in the present study confirm the role that these fish play in the life cycle of nematodes of public health interest, with a high prevalence of the parasites and a higher number of nematodes in bigger fish specimens.

The two species were highly susceptible to infection, with heavy parasitism due to nematodes of *Contracaecum* sp. In the life cycle of *Contracaecum* sp. crustaceans act as intermediate hosts and piscivorous birds as final hosts. Fish can act as the second intermediate hosts by feeding on parasitized crustaceans or paratenic hosts, in the case of predatory fish. In both cases, fish may be a source of infection for piscivorous birds and other aquatic vertebrates such as reptiles or mammals [26]. As it was noticed in the entire Baixada Maranhense and especially in the municipality of Pinheiro, around the water bodies it can be noticed the presence of lots of piscivorous birds. This fact can explain the presence of *Contracaecum* sp. infection fish species that inhabit water bodies of the Baixada Maranhense.

Although in Brazil there are a few reports of parasitism in humans due to accidental consumption of endoparasites, this hypothesis cannot be ruled out, especially in the Amazon region, where it is common for children to consume small raw fish because they believe that consuming these animals will teach them to swim [12]. Similar cases may be occurring in Brazil, where stomach problems caused by accidental ingestion of parasites can be misdiagnosed, due to the lack of studies related to zoonotic parasites in fish, lack of specialists, and lack of interest on the part of the competent authorities to inspect farm meat. fish that is sold in the city's markets.

Currently, in Baixada Maranhense, Brazil, quality control mechanisms for fish meat that is sold in markets are deficient. There is no adequate quality and safety control. The implementation and adoption of stricter public control policies can be a long-term solution, as long as regulations related to health control are approved and enforced, and compliance with them is guaranteed.

However, the fish-consuming population can adopt some measures to avoid the accidental ingestion of parasites that, consequently, could cause some damage to their health. It is recommended to buy the fish meat and place it in the refrigerator at a temperature of approximately –20 to –30°C, for 12 to 24 hours, long enough to inactivate the parasites. Furthermore, preparations that use temperatures above 60ºC, such as frying, roasting, grilling, and smoking, are effective in eliminating parasites. This study alerts local authorities and the population to take the necessary measures to prevent the accidental ingestion of endoparasites with zoonotic potential.

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