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ORIGINAL ARTICLE

HIGH PREVALENCE AND INTENSITY OF FISH NEMATODES WITH ZOONOTIC POTENTIAL IN THE BRAZILIAN AMAZON, INCLUDING A BRIEF REFLECTION ON THE ABSENCE OF HUMAN INFECTIONS

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ABSTRACT

This study aimed to characterize the prevalence, mean abundance, and mean intensity of potential fish-borne zoonotic nematode larvae infecting the predator fish *Hoplias* aff. *malabaricus* from the Tapajós River, in the municipality of Santarém, in the Brazilian Amazon. After capture, the specimens of *H*. aff. *malabaricus* were analyzed for infection by *Contracaecum* sp. and *Eustrongylides* sp. third-stage larvae, and the prevalence, mean abundance, and mean intensity were calculated. A literature search was carried out to clarify the relationship between these indicators and eventual human cases of infection in the Amazon region. Third-stage larvae of nematodes of the *Contracaecum* and *Eustrongylides* genera were found in the specimens of *H*. aff. *malabaricus* sampled from the Tapajós River. The prevalence of *Contracaecum* larvae was 100%, while its mean abundance and mean intensity were both 54.8 larvae/fish. The prevalence of *Eustrongylides* larvae was 62.9%, and its mean abundance and mean intensity were 1.8 and 2.8 larvae/fish, respectively. Despite the high prevalence and intensity values, there are no cases of human infection by these nematode larvae in the Brazilian Amazon reported. The absence of human infections by these nematode larvae in the Brazilian Amazon despite the high prevalence/intensity of *Contracaecum* and *Eustrongylides* larvae and the high consumption of fish in the region, is most probably due to the fact that the local populations do not eat raw or undercooked fish. However, it is noteworthy that the absence of zoonotic cases in the region is based only on the examination of the available published papers. A better knowledge of the situation would require surveying hospitals and clinics, and data from the region's medical treatment facilities. However, the authors consider that cases of human infection, if any, are extremely rare, mainly due to the eating habits of the local population.

KEY WORDS: Amazon basin; freshwater fish; public health; parasitic infection; zoonosis.

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INTRODUCTION

The fish species *Hoplias malabaricus* (Bloch, 1794), is a characiform of the Erythrynidae family, which includes the genera *Erythrinus* Scopoli, 1777, *Hoplerythrinus* Gill, 1896, and *Hoplias* Gill, 1903 (Nelson, 2006). The genus *Hoplias* comprises two groups of species: i) the group *Hoplias lacerdae*, with nine species – *H. lacerdae* (Miranda Ribeiro, 1908), *H. brasiliensis* (Spix & Agassiz, 1829), *H. aimar*a (Valenciennes, 1847), *H. patana* (Valenciennes, 1847), *H. teres* (Valenciennes, 1847), *H. intermedius* (Günther, 1864), *H. microlepis* (Günther, 1864), *H. macrophthalmus* (Pellegrin, 1907), *H. australis* (Oyakawa & Mattox, 2009) and *H. curupira* (Oyakawa, 2009), ii) the group *H. malabaricus*, which, based on cytogenetic and molecular biology studies, has also been considered a complex species (Jacobina et al., 2011; Marques et al., 2013; Santos et al., 2009; Vitorino et al., 2011).

Fish parasites are found in larval and/or adult stages, and usually occur in the digestive tract, musculature, gonads, liver, heart, mesentery or abdominal cavity (Barros et al., 2007; Virgilio et al., 2020). Some are zoonotic, may infect humans when fish are ingested raw or undercooked, and can present serious health consequences (Eiras et al., 2018a). In general, fish-borne parasitic zoonoses are a major and important part of food-borne parasitic zoonosis (Eiras et al., 2018b). Although in the past fish-borne parasites were for the most part limited to populations living in low- and middle-income countries, the growth in international markets, improved transportation systems, and population movement (migration) have been responsible for expanding the geographical limits and populations at risk (Chai et al., 2005).

With regard to zoonotic fish-borne nematodes, human infection is caused by the third-stage larvae present in fish, mainly anisakids, gnathostomids, dioctophymids (Amer, 2014; Argumedo, 2003; Audicana & Kennedy, 2008; Bunyaratavej et al., 2008; Choi et al., 2009), and capillariid adults and larvae (Belizario et al., 2000). Several cases of nematodiasis have been reported in various regions of the world (Adams et al., 1997; Bao et al., 2017; Chai et al., 2005; Nawa & Nakamura-Uchiyama, 2004; Pal et al., 2013).

However, despite the fact that the Amazonian population has a high rate of fish consumption, and that fish native to the region infected with potential zoonotic nematodes have been reported, namely *Contracaecum* spp. (Anisakidae) and *Eustrongylides* spp. (Dioctophymidae) (Barros et al., 2007; Eiras et al., 2016; Oliveira et al., 2019) there are no reports of human infections with these nematodes in the Brazilian Amazon.

The present study presents data about the prevalence, mean abundance, and mean intensity of third-stage larvae of the nematodes *Contracaecum* sp. Railliet & Henry, 1912 and *Eustrongylides* sp. Jägerskiöld, 1909. The larvae were recovered from *Hoplias* aff. *malabaricus* sampled in the Tapajós River, in the municipality of Santarém, in the Brazilian Amazon. This fish species occurs in several South American watersheds and is widely consumed in various regions of Brazil (Lima et al., 2019). The reasons for no reports of zoonotic infections transmitted by fish in humans are also discussed, their aspects and relevance focusing on pathology and public health in the Brazilian Amazon.

MATERIALS AND METHODS

The *H*. aff. *malabaricus* specimens were captured/recovered in marginal lakes of the Tapajós River (2°17'42.42"S 54°47'9.50"W - Quantum GIS version 3.14.15), in Santarém municipality, in the state of Pará, in the Brazilian Amazon (Figure 1). The map design was necessary, in order to visualize the collection point with geo-references in the Brazilian Amazon specifically in the northern region of Brazil. After fish being euthanized with analgesic (Clove oil) - 150 mg/L diluted in water - according to Fernandes et al. (2017), the internal organs were examined with the aid of a stereomicroscope, and the parasites recovered were fixed in 5% formaldehyde in water or 70% ethanol.

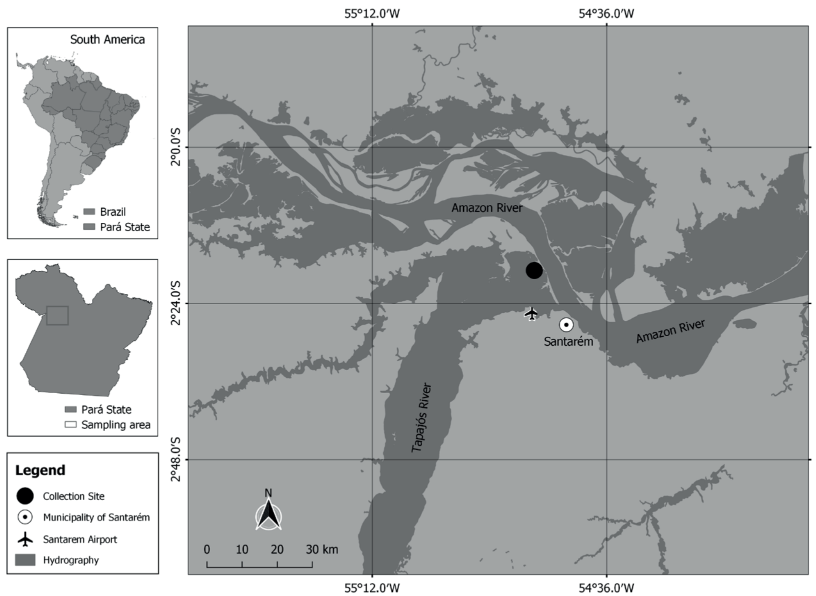

Figure 1. Geographic location of the collection points of *Hoplias* aff. *malabaricus* in the Tapajós River, State of Pará, in the Brazilian Amazon.

For the morphological observation, the third-stage larvae were prepared according to Lacerda et al., (2009), analyzed and photographed using a light microscopy Zeiss Axioplan equipped with an Axiocam ERc 5s Cam and AxioVision AxioVs 40V4.8.2, and the morphometric data were analyzed following Thatcher & Brites-Neto (1994). The morphometric data and features of the larvae were compared with data available from Benigno et al., (2012), for genus identification.

The ecological terms of prevalence, mean abundance, and mean intensity were used according to Bush et al., (1997). Using Quantitative Parasitology 3.0 software, in accordance with Reiczigel et al., (2019).

For scanning electron microscopy (SEM), the *Eustrongylides* larvae were fixed in 70% ethanol, washed in PBS, post fixed in 1% osmium tetroxide (O_sO_4) solution for 2 h, and washed in PBS. The dehydration was initially carried out in an ascending ethanol series, and finalized in hexamethyldisilane (HMDS) modified from Bray et al., (1993). The larvae were placed on a Stub, coated with gold-palladium, and analyzed under a SEM Leica/Cambridge Leo Stereoscan S-440 at the São Paulo Federal University.

Ethical approval: Sampling and access to genetic heritage was authorized by the Environment Ministery from Brazil (SISBIO authorizations # 44268-4 and SisGen # A0A0058). The methodology was approved by the Research Ethics Committee from Federal University of São Paulo (CEUA N 2828270219), in accordance with Brazilian Legislation (Federal law No. 11794, dated 8 October 2008).

RESULTS

All 35 *H*. aff. *malabaricus* specimens (18 females, 12 males and 5 immature fish, with an average length of 22.6 ± 2 cm and an average weight of 133.3 ± 40 g) were recovered infected naturally with third-stage nematode larvae. The morphological analysis, based on light and scanning electron microscopy, showed that these specimens belong to the genera *Contracaecum* and *Eustrongylides* (Figure 2A-C; Table). 1,979 larvae were recovered, 1,917 identified as *Contracaecum* sp. and 62 as *Eustrongylides* sp. (Figure 2E-G). The larvae of *Contracaecum* sp. were encysted in the mesentery, had a prevalence of 100% and a mean abundance and mean intensity of 54.8 larvae/fish. The larvae of *Eustrongylides* sp. were found encysted in musculature and had a prevalence of 62.9%, a mean abundance of 1.8, and a mean intensity of 2.8 larvae/fish.

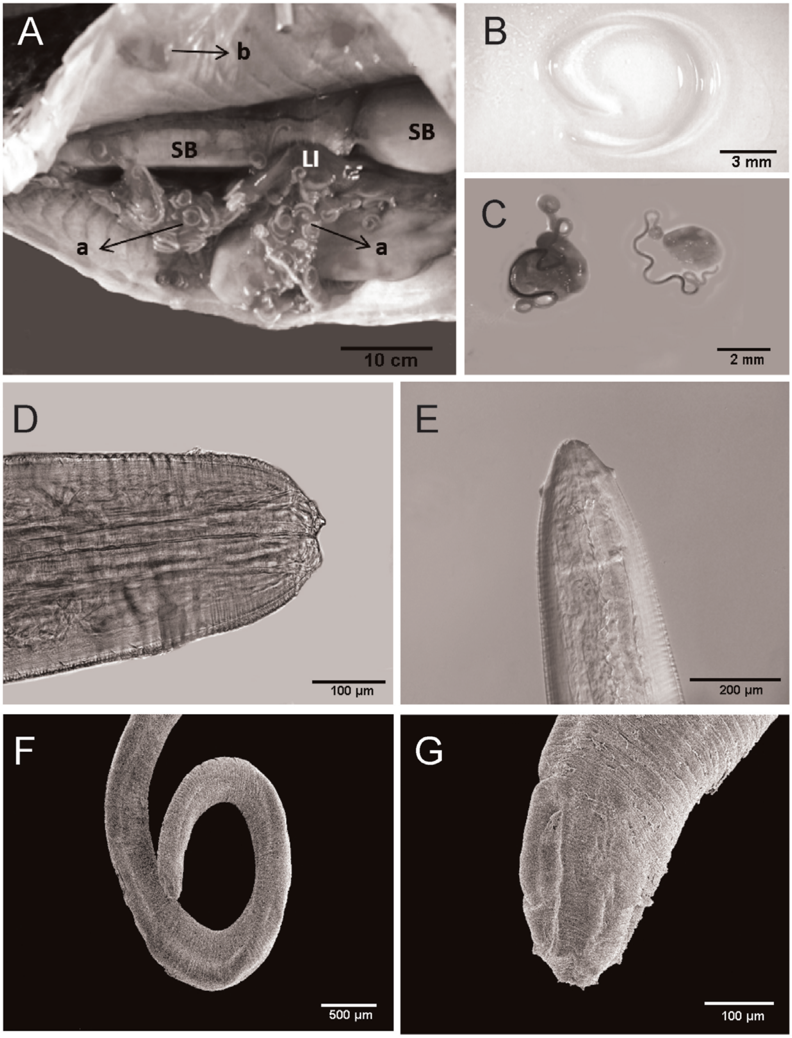

Figure 2. A- Open general cavity of *Hoplias* aff. *malabaricus* showing swim bladder (SB) and liver (LI). B- *Contracaecum* sp. collected from mesentery region and C- *Eustrongylides* sp. collected in musculature. D- Light microscopy of *Contracaecum* sp. collected from *H*. aff. *malabaricus* anterior region. E- Light Microscopy of anterior end of *Eustrongylides* sp. F- Scanning electron microscopy of anterior region of *Eustrongylides* sp. G- Scanning electron microscopy showing in detail the anterior end of *Eustrongylides* sp.

Table. Morphometric comparison between third stage larvae of *Contracaecum* sp. and *Eustrongylides* sp. sampled from *Hoplias* aff. *malabaricus*.

Characteristics	*Contracaecum* sp.		*Eustrongylides* sp.	
	This study (n=10)	Moravec et al., (1993)	This study (n=10)	Benigno et al., (2012)
Length	11-32.5	3.89-4.80	60.17-126.20	76.37-153.57
Width	0.320-0.410	0.150-0.313	0.650-1.39	-
Nervous ring	181-350	205-225	230-382	250-405
Esophagus^{L}	370-615	537-693	12.53-20.15	16.10-22.5
Esophagus^{W}	30-90	-	270-680	300-750
Intestinal caecum^{L}	410-700	310-476	240-255	220-260
Intestinal caecuml^{W}	180-210	-	-	-
Ventricle^{L}	40-90	24-33	-	-
Ventricle^{W}	40-120	33-36	-	-
Ventricular appendix^{L}	400-720	510-721	-	-
Ventricular appendix^{W}	60-150	-	-	-

n: number of specimens observed; all measurements are in micrometers, except length and width (mm). Measurements in millimeters; L = length; W = width; n = number of specimens measured.

DISCUSSION

The present study showed a high prevalence rate of *Contracaecum* sp. and *Eustrongylides* third-stage larvae in *H*. aff. *malabaricus* from the Brazilian Amazon. In Australia, four fish species were reported infected by *Contracaecum* larvae, showing a prevalence and mean intensity of 100% and 12.7 larvae/fish for *Aldrichetta forsteri*, 81% and 9.8 larvae/fish for *Mugil cephalus*, 6% and 1.5 larvae/fish for *Sillaginodes puncta*, and 4% and 1 larva/fish for *Acanthopagrus butcheri*, respectively (Chai et al., 2005; Lymbery et al., 2002). Our results showed variable prevalence values of *Contracaecum* sp. between fish specimens, high in some fish and lower in others, while the mean intensity was much higher in *H*. aff. *malabaricus* specimens. Concerning *Eustrongylides* sp. larvae, the data of the present study are much higher than those observed for *Lepomis gibbosus, Micropterus salmoides*, and *Perca* *fluviatilis* from northwest Italy, for which prevalences of 18.3%, 16.7%, and 10%, respectively were recorded (Menconi et al., 2020). The mean intensity of *Eustrongylides* sp. larvae reported by these authors was also lower, with 1.15, 1 and 1 larvae/fish, respectively, in comparison with 2.8 larvae observed in *H*. aff. *malabaricus*.

There are several reports of *Contracaecum* sp. and *Eustrongylides* sp. larvae infecting specimens of *H*. aff. *malabaricus* in Brazil and distinct levels of prevalence and intensity have been observed (Reis et al., 2017; Rodrigues et al., 2017; Gião et al., 2020; Gueretz et al., 2020). For the *Contracaecum* genus, a prevalence of 100% was reported in São Paulo state, in southeastern Brazil, but the mean intensity was low, with only 15.5 larvae/fish (Madi & da Silva, 2005), when compared with the 54.8 larvae/fish of the present study. A prevalence of 90% and a mean intensity of 5.5 larvae/fish were reported from Amapá State, in northern Brazil (Oliveira et al., 2018); and a prevalence of 100% and a mean intensity of 77.8 parasites/host were reported in Colombian fish (Olivero-Verbel et al., 2006). The prevalence of *Contracaecum* sp. is not very different from the results obtained in this research concerning the infection of *H*. aff. *malabaricus*. For *Eustrongylides* sp. larvae, a prevalence of 44.4% and a mean intensity of 17.3 nematode/fish were reported in *H*. aff. *malabaricus* examined in Rio de Janeiro State, southeastern Brazil (Kuraiem et al., 2020); a prevalence of 53.8% was found in Marajó Island, in northern Brazil (Benigno et al., 2012), and a prevalence of 33% was found in Mato Grosso State, in the Brazilian midwest (Barros et al., 2007).

Despite common occurrence and high prevalence, there are few reports of human infections caused by larvae of *Contracaecum* sp. and *Eustrongylides* sp. around the world (Adams et al., 1997; Benigno et al., 2012; Eberhard & Ruiz-Tiben, 2014; Narr et al., 1996; Shamsi, 2014; Wittner et al., 1989). In the Brazilian Amazon, there is currently no medical/scientific literature reporting human infections by these nematode larvae, although this region has high fish consumption *per-capita* values (Begossi et al., 2019). Human infection with nematodes is known to occur when third-stage larvae are eaten together with raw, undercooked, or inadequately salted or smoked meat from infected fish. In such cases, the larvae can penetrate the digestive tract and may invade several abdominal organs, resulting in various pathological effects, but usually do not complete their development, meaning that humans represent in most of the cases an accidental host (Eiras et al., 2016; Magalhães et al., 2012).

In the case of *Contracaecum* sp. larvae, the site of infection in the host fish seems to represent an important biologic barrier for human infection, as their encapsulation occurs mainly in the viscera and/or mesentery (Corrêa et al., 2015). This makes human ingestion unlikely, and means infection occurs only accidentally, as these organs are removed before retail/consumption. One possible form of human infection in such cases would be eating the fish alive, as traditionally occurs in some regions of the world (Akahane et al., 1998).

However, larvae ingestion cannot be totally ruled out, as a small number of *Contracaecum* sp. larvae may be found in muscles of the host fish. In relation to *Eustrongylides* sp., the main larvae infection site is the musculature of the fish (Barros et al., 2004; Branciari et al., 2016; Melo et al., 2016; Zhokhov & Pugacheva, 2019) This is the perfect location for a fish-borne zoonotic nematode, which fundamentally depends on the localization of the infective larvae (i.e., it should be in edible sites) but also, again, on the habit of eating raw or undercooked fish (Cavallero et al., 2018; Eiras et al., 2018a).

The direct relationship between the ingestion of raw fish infected with different nematode larvae of marine and freshwater species and clinical symptoms has been reported in South America countries for *Gnathostoma binucleatum* (Eiras et al., 2018a), *Gnathostoma spinigerum* (Eiras et al., 2018a), *Anisakis* sp. (Eiras et al., 2016), (although it is suggested that this could be *Gnathostoma* sp.), *Anisakis* sp. (Knoff et al., 2007) and *Dioctophyme renale* (Lisbôa, 1945) as well as *Contracaecum* sp. and *Eustrongylides* sp. worldwide (Adams et al., 1997; Eberhard & Ruiz-Tiben, 2014; Narr et al., 1996; Shamsi & Suthar, 2016; Sonko et al., 2019; Wittner et al., 1989). These infections are particularly abundant in countries like Peru (Costa et al., 2001) and Chile (Jofré et al., 2008) where ingesting raw fish is a common practice. It is interesting to note that for *D. renale,* a dangerous mammal kidney parasite, including human, there are a number of fish intermediate hosts described in Brazil (Abdallah et al., 2012, Mascarenhas et al., 2016), and at least 1,145 cases of reported mammals infected by 14 different host species which act at definitive hosts in the same country, and a sole case of human infection described so far (Eiras et al., 2021).

Even with recent cultural changes, which have led to the increased consumption of raw fish such as sushi, sashimi and ceviche, mainly in large urban centers in the states of the Brazilian Amazon, there are no reports of human infections in this region. It is important to stress that in such places, fish dishes are prepared in accordance with sanitary patterns that prevent/restrict larvae viability (Barros et al., 2008). Another important point is that, especially in the case of *Eustrongylides* sp., the larvae are large and visible during the preparation of the dishes. In contrast, far from the large cities, where people usually eat fresh fish, the habit of consuming raw fish is not observed, which is an important factor in preventing fish-borne zoonotic parasites (Murrieta et al., 2008).

Despite the high prevalence and intensity of third-stage larvae of *Contracaecum* sp. and *Eustrongylides* sp. found in *H*. aff. *malabaricus* and other fish species in the Brazilian Amazon (Benigno et al., 2012; Melo et al., 2016; Oliveira et al., 2018; Pinheiro et al., 2019; Virgilio et al., 2020) there are no reported cases of human infection by nematodes of these genera in this region.

The absence of reported human cases of these nematode larvae in the Brazilian Amazon region, despite the high prevalence/intensity of *Contracaecum* sp. and *Eustrongylides* sp. larvae and the high levels of fish consumption in the region may be linked to local cultural habits, which do not include eating raw or undercooked fish. Notwithstanding, this conclusion is based on the scientific literature. Better knowledge of the situation is possible with data collection from hospitals, clinics, and medical treatment facilities in the region. Nevertheless, the authors consider that human cases, if any, are extremely rare, a finding that seems to be due to the eating habits of the local population.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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