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Eustrongylides spp. (Nematoda: Eustrongylidae) in *Polypterus endlicheri* in central Rio Grande do Sul, Brazil

Eustrongylides spp. (Nematoda: Eustrongylidae) em *Polypterus endlicheri* na região central do Rio Grande do Sul, Brasil

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Abstract
The demand for ornamental fish has increased in recent years in Brazil, however, the aquatic environment of artificial breeders facilitates the invasion of pathogens, including parasitic zoonoses, which are a limiting factor for production causing economic losses in creations. Knowledge of the distribution of agents causing parasitic diseases is important so that we can intervene in breeders, therefore, the objective of the present work is to report the occurrence of infection by *Eustrongylides* sp. (Nematoda: Eustrongylidae) in *Polypterus endlicherii* (Polypteriformes: Polypteridae) in central Rio Grande do Sul, Brazil. The nematode was identified after being released a cyst in the scales, of a fish of the species *P. endlicherii*. It can be concluded that this is the first record of the occurrence of *Eustrongylides* sp. larvae in fish in Rio Grande do Sul, showing that the parasite is present in the central region of the state. Therefore, further studies should be carried out in order to verify the presence of the parasites in fish intended for human consumption. It should also be noted the need to be more careful when feeding fish with oligochaetes, especially those intended for human consumption.
Keywords: aquarism; Saddled bichir; nematode; parasitism.

Resumo
A criação de peixes para ornamentação teve sua demanda aumentada nos últimos anos no Brasil, no entanto, o ambiente aquático dos criatórios artificiais facilita a invasão por agentes patogênicos, incluindo zoonoses parasitárias, que são um fator limitante para produção causando perdas econômicas nas criações. O conhecimento da distribuição de agentes causadores de enfermidades parasitárias é importante para que se possa intervir nos criatórios, portanto, o objetivo do presente trabalho é relatar a ocorrência da infecção por larva de *Eustrongylides* sp. (Nematoda: Eustrongylidae) em *Polypterus endlicherii* (Polypteriformes: Polypteridae) na região central do Rio Grande do Sul, Brasil. O nematódeo foi identificado após ser liberado de um cisto nas escamas, de um peixe da espécie *P. endlicherii.* Pode-se concluir que este é o primeiro registro da ocorrência de larvas de *Eustrongylides* sp. em peixes no Rio Grande do Sul, evidenciando que o parasito está presente na região central do estado. Portanto, mais estudos devem ser realizados a fim de verificar a presença dos parasitos em peixes destinados ao consumo humano. Salienta-se ainda, a necessidade de se ter maior cuidado ao alimentar peixes com oligoquetas, principalmente os destinados para alimentação humana.
Palavras-chave: aquarismo; Bichir selado; nematódeo; parasitismo.

1. Introduction

Polypterus are carnivorous freshwater aquarium fish, whose breeding for ornamentation has increased in recent years in our country [(1)]. Since the last century, new technologies have facilitated access and maintenance, making aquarism an accessible hobby and for many Brazilians, they are the most preferred animals, second only to dogs and ahead of cats [(2)]. The aquatic environment of the farms can facilitate the invasion by pathogenic agents. It is known that parasites become a limiting factor for production, as the environments in which animals are exposed to high animal density, favor situations of stress, which makes them weak and susceptible to being affected by parasites [(3)]. Still according to Schalch [(3)], parasites are the major causes of losses in fish farming.

Fish can act as intermediate, definitive or paratenic hosts in the life cycle of several helminths, including species with zoonotic potential. Highly parasitized animals do not grow, lose their commercial value, and do not please the eyes of consumers [(4)]. Several behavioral abnormalities are also described due to the presence of parasites. The most frequently observed signs in parasitized animals are: lethargy, apathy, anorexia, loss of balance, isolation from the rest of the school, tachypnea, excessive mucus production causing an opaque

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appearance, erosion of the skin and/or fins, inflamed or pale gills, inflamed abdomen with or without the presence of bloody fluid and even death [3].

Eustrongylides groups species that have piscivorous birds as definitive hosts, while their larvae infect oligochaetes, fish and occasionally amphibians and reptiles [5]. According to Barros et al. [6], humans can become accidental hosts after ingestion of raw or undercooked fish containing nematode larvae. The larvae can cause gastritis and intestinal perforation, resulting in severe abdominal pain [6]. Knowledge of the distribution of agents that cause parasitic diseases, as well as the relationship between environmental factors, hosts and parasites are important for intervening in the artificial breeding system. The use of adequate prophylactic techniques and the creation of preventive programs to control these diseases must be carried out in an attempt to avoid economic losses. Therefore, the aim of this study is to report the occurrence of larval infection of *Eustrongylides* spp. (Nematoda: Eustrongylidae) in an exotic fish species, *Polypterus endlicherii* Heckel, 1847, in the central region of Rio Grande do Sul, Brazil.

2. Material and methods

A reddish-colored nematode approximately five centimeters long was received at the Laboratory of Veterinary Parasitology (LAPAVET) of the Federal University of Santa Maria (UFSM) for identification. The parasite was released through an external cyst located between the scales of a specimen of *P. endlicherii* (Figure 1), about 15 days after its acquisition, in February 2021, at a commercial aquarium establishment in the city of Santa Maria (29º 41' 03" S; 53º 48' 25" W), in the center of the state of Rio Grande do Sul, in southern Brazil. According to the owner, after purchase, the fish was fed commercial feed.

The larva was placed in a Petri dish with a 0.65% NaCl solution, later fixed in AFA (ethanol, formalin and acetic acid) heated to 65ºC; preserved in 70% ethanol 5% glycerin; and clarified with Amann's Lactophenol, according to Knoff and Gomes [7], with modifications. The taxonomic classification of the nematode followed De Ley and Blaxter [8], while the morphological identification was performed according to Measures [9,10] and Moravec [11]. The measurements and the captured

Figure 1. Fourth-stage larva of *Eustrongylides* spp. emerging from the cyst in *Polypterus endlicherii* from an aquarium shop in central Rio Grande do Sul, Brazil

image of the specimen were performed using the ZEN 2 (Blue edition) ® Carl Zeiss Microscopy program, 2011.

3. Results and discussion

Since the larval forms cannot be identified to the species level, they are usually designated as *Eustrongylides* spp. [12]. The larva found in *P. endlicherii* showed a dark red color; conical anterior end with the presence of two rings and six labial papillae in each ring (Figure 2). At the rounded posterior end it was possible to observe the male's anus and genital primordium. In addition, it was possible to observe the three cuticle layers characteristic of fourth-stage larvae [10]. Furthermore, the larva of *Eustrongylides* spp. identified has a body length of 53.4 mm; Distance of the nervous ring in relation to the anterior end of 231 µm; Width at the nerve ring of 210 µm; Esophageal length 11.2 mm; Distance from the rectum to the posterior end of 203 µm and width at the posterior end of 216 µm.

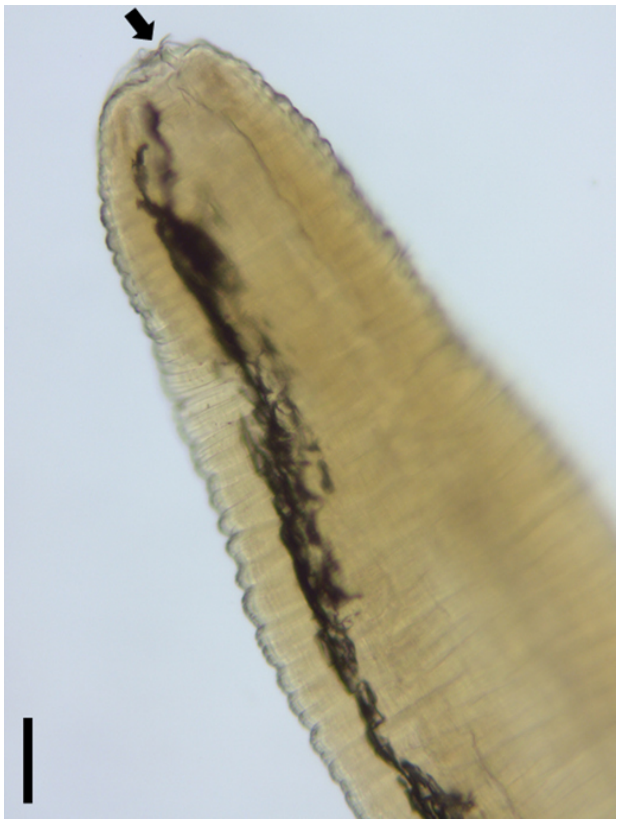

Figure 2. Anterior portion of the fourth instar larva of *Eustrongylides* spp. with indication of the buccal papillae by the arrow. Bar scale - 60µm.

The larvae of *Eustrongylides* spp. are parasites of carnivorous fish, such as the traíra (*Hoplias malabaricus*), the pintado (*Pseudoplatystoma coruscans*), the cachara (*Pseudoplatystoma fasciatum*), and the tucunaré (*Cichla ocellaris*), having been found in the skeletal musculature, in the mesentery, in the serosae that cover the viscera and in the general cavity of these fish [13]. In Rio Grande do Sul, only adult forms of *Eustrongylides* were recorded in piscivorous birds [14,15] and fourth-stage larvae in aquatic snakes [16]. Thus, this is the first report on a species of fish in the state, this being exotic and coming from an aquarium shop. Larval forms of Eustrongylids can cause a zoonotic infection known as human Eustrongylidosis. Transmission occurs through the consumption of raw or undercooked fish meat and presentations in humans can vary from stomach inflammation to intestinal perforation requiring surgical removal of helminths [17]. Thus, records of the occurrence of Eustrongylid larvae in the state are important, especially considering that Rio Grande do Sul has an extensive watershed and a large population that uses fish as its food source.

Fish belonging to *Polypterus* in captivity conditions are commonly fed with earthworms and authors show the potential of its use in the diet of fish species [18,19]. The production of earthworms is very cheap, in addition to having a high protein content and low fat value, which is advantageous as an alternative food to conventional food used in aquaculture [19]. However, annelids are intermediate hosts for parasites such as *Eustrongylides* spp. and can serve as a source of infection. Despite this, it is not possible to say how the fish became infected, as the species has carnivorous habits and may have fed on other infected fish. Also, birds as definitive hosts eliminate parasite eggs, which can contaminate the water in the captivity where the fish are raised, prior to sending them to commercial aquarium establishments.

Although there are no reports of this parasitosis in humans in Brazil [20,21], correct inspection and the use of safe techniques for preserving fish by freezing are the main preventive measures. Even if the parasitized fish is not bred for human consumption, its presence in aquatic environments with other fish commercialized for this purpose may favor the transmission of the parasite to species for consumption. The orientation of the consumer public, avoiding the ingestion of fish from risk areas is of fundamental importance for the prevention of the disease [21].

4. Conclusion

It can be concluded that this is the first record of the occurrence of *Eustrongylides* larvae in fish in Rio Grande do Sul, showing that the parasite is present in the central region of the state. Therefore, the report contributes to the knowledge of the host species and biogeographical distribution of this zoonotic parasite. However, more studies must be carried out in order to verify the presence of the parasites in fish intended for

human consumption. It is also emphasized the need to be more careful when feeding fish with oligochaetes, especially those intended for human consumption.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

Conceptualization: J.S. Lignon, T. M. Stainki and S.G. Monteiro. *Data curation*: J.S. Lignon, T. M. Stainki and S.G. Monteiro. *Research*: J.S. Lignon, T. M. Stainki and S.G. Monteiro. Methodology: J.S. Lignon, T. M. Stainki and S.G. Monteiro. *Supervision*: S.G. Monteiro. *Writing (original draft):* J.S. Lignon, T. M. Stainki and S.G. Monteiro. *Writing (proofreading and editing)*: J.S. Lignon, T. M. Stainki and S.G. Monteiro.

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