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Scientific Note

Clinostomum sp. (DIGENEA: CLINOSTOMIDAE) AND *Ascocotyle* sp. (DIGENEA: HETEROPHYIDAE): METACERCARIAE WITH ZOONOTIC POTENTIAL IN FISHES FROM TRAMANDAÍ RIVER BASIN, SOUTHERN BRAZIL

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ABSTRACT

The helminths belonging to the class Trematoda have great importance in ichthyoparasitology, and the subclass Digenea includes parasites with zoonotic potential. The family Heterophyidae is responsible for the heterophyiasis and, in Brazil, this disease is caused by the trematode genera *Ascocotyle* (*Phagicola*), commonly known as phagicolosis, both related to the fish-to-birds/mammals cycle. *Clinostomum* (belonging to the family Clinostomidae) has its larval stage in fish musculature and piscivorous birds as definitive hosts. It may parasitize humans accidentally, causing parasitic laryngopharyngitis and death by asphyxia. This study aims to quantify the presence of digenetic Heterophyidae and Clinostomidae parasites with zoonotic potential in fish from the Tramandaí River basin, in the state of Rio Grande do Sul, Brazil. The fish were purchased from fishermen from Terra de Areia/RS, from July 2016 to May 2017. The search for parasites was carried out by performing a necropsy on the fish. The digenetic parasites were preserved, stained and mounted on microscope slides for identification. Parasitological indices were calculated. *Astyanax* spp. were positive for *Ascocotyle* whereas *Geophagus brasiliensis* was infected by *Clinostomum* sp. Therefore, the results suggest a risk of infection to the consumer population in the region.

Key words: helminth; ichthyoparasites; zoonosis; freshwater fish; digenea.

Clinostomum sp. (DIGENEA: CLINOSTOMIDAE) E *Ascocotyle* sp. (DIGENEA: HETEROPHYIDAE): METACERCÁRIAS COM POTENCIAL ZOONÓTICO EM PEIXES DA BACIA DO RIO TRAMANDAÍ, SUL DO BRASIL

RESUMO

Os helmintos pertencentes à Classe Trematoda têm grande importância na ictioparasitologia, onde a Subclasse Digenea apresenta parasitos com potencial zoonótico. A família Heterophyidae é responsável pela heterofidíase e no Brasil essa doença é causada por trematódeos do gênero *Ascocotyle* (*Phagicola*), comumente conhecida como fagicolose, transmitida de peixes às aves e mamíferos. Já as larvas de *Clinostomum,* presentes na musculatura de peixes, tem aves piscívoras como seus hospedeiros definitivos, e podem parasitar seres humanos acidentalmente, causando laringofaringite parasitária e morte por asfixia. Este estudo busca quantificar a presença de parasitos digenéticos das famílias Heterophyidae e Clinostomidae com potencial zoonótico em peixes da bacia do rio Tramandaí/RS. Os peixes foram adquiridos de pescadores do município de Terra de Areia/RS, entre julho de 2016 e maio de 2017 e necropsiados à procura de parasitas. Os digenéticos foram fixados, corados e montados em lâminas para identificação. Índices parasitológicos foram calculados. *Astyanax* spp. foram positivos para *Ascocotyle* enquanto G*eophagus brasiliensis* apresentou-se infectado por *Clinostomum* sp. Sendo assim, os resultados obtidos sugerem risco de infecção para a população regional consumidora.

Palavras-chave: helmintos; ictioparasitas; zoonoses; peixe de água doce; digenea.

INTRODUCTION

Parasitic helminths represent a group of organisms responsible for parasitic diseases that are connected to socio-environmental conditions, which may lead to public health problems, including zoonoses (NEVES, 2009). Fish are considered an important food

resource due to their nutritional value and widely appreciated taste, being also the economic foundation of thousands of people worldwide (ALMEIDA FILHO *et al*., 2004).

Of the several helminths that parasitize fish used for human consumption, many are the cause of zoonoses. In the past, these parasitic diseases were restricted to underdeveloped countries or countries with unique feeding habits. However, in the last decades, such diseases have expanded because of globalization, which allowed a higher flow of food and people, spreading different cultures around the world (CHAI *et al*., 2005).

The class Trematoda is of great importance in ichthyoparasitology. The subclass Digenea includes most of the ichthyoparasitic helminths with zoonotic potential. Trematodes of the family Heterophyidae are responsible for heterophyiasis and in Brazil this disease is caused by trematodes of the genus *Ascocotyle* (*Phagicola*), commonly known as phagicolosis, which is transmitted by fish to other vertebrates such as birds and mammals, and to humans through the ingestion of raw or undercooked fish (BARROS and AMATO, 1995). Fish are the main form of transmission of this helminth to human beings and other animals since *Ascocotyle* does not show specificity toward its host (CHENG, 2012).

Among the symptoms of phagicolosis, one can mention flatulence, cramps and diarrhea (CHIEFFI *et al*., 1992). Several cases of infection by *Ascocotyle* (*Phagicola*) have been recorded in different American countries (CHIEFFI *et al*., 1990) and it was recently added to the list of Risk Rating of Biological Agents (BRASIL, 2010).

In the family Clinostomidae, species of the genus *Clinostomum* have also been the target of intense investigations because metacercariae of this digenean are found in the skin, muscles, fins and internal organs of fish (CARVALHO *et al*., 2008). *Clinostomum* spp. have fish-eating birds are definitive hosts, but can also accidentally parasitize humans, causing parasitic laryngopharyngitis and death by asphyxia (EIRAS, 1994). Events of human infection by this parasite have been reported by several researchers over the years (HIRAL *et al*., 1988; CHUNG *et al*., 1998; KITAGAWA *et al*., 2003). The reported symptoms included pain while swallowing, irritation of the pharynx, excessive saliva production, coughing, swelling of lymph nodes and mucous membranes, itching, among others (YOSHIMURA *et al*., 1991).

This study was conducted with the intention to quantify the presence of digenetic parasites with zoonotic potential in fish from the Tramandaí River basin, in the northern coastal region of Rio Grande do Sul.

METHODS

Between July 2016 and May 2017, in the district of Cornélios (29º 32' 18.7" S 50º14'45.5" W and 29º 32' 14.5" S 50º 14' 43.2" W), municipality of Terra de Areia, northern coastal region of Rio Grande do Sul, five different fish species (Table 1) from the Quadros Lagoon were bought from local fishermen. Later, in the Laboratory of Ichthyology of UNISINOS, the fish were identified following the guide of MALABARBA *et al*. (2013) and stored in a freezer until their necropsy. The parasitological examination followed the protocol of AMATO and AMATO (2010). First, we conducted a visual examination of the outer surface of the fish searching for ectoparasites. Soon after, the fish were eviscerated and their organs were separated in individual Petri dishes. Both the musculature and the internal organs were examined under a stereomicroscope.

The located parasites were collected and preserved in AFA solution (93% of 70°GL alcohol, 5% of formalin and 2% of glacial acetic acid) during 48h; after this procedure, they were stored in 70° alcohol. The helminths were cleared with clove oil, stained with Delafield's Hematoxylin and mounted on permanent microscope slides with Canada balsam. Morphometry was performed with the aid of the software AxioVision 4.8.2 (ZEISS, 2012), through microphotographs. The identification of the parasitic helminths followed the identification key of GIBSON *et al*. (2002). Quantitative descriptors (prevalence, mean intensity of the infection and abundance) follow the methodology proposed by BUSH *et al*. (1997).

RESULTS

Necropsy was conducted in 116 fish, 83 of the genus *Astyanax*, of which 27 were parasitized by cysts of *Ascocotyle* (Figure 1) and 33 of the species *Geophagus brasiliensis*, of which 24 were infected with cysts of *Clinostomum* sp. (Figure 2a and b). We collected 2534 metacercariae of the genus *Ascocotyle* from the heart of *Astyanax* spp. and 370 metacercariae of *Clinostomum* sp. from the musculature of *G. brasiliensis* (Table 2).

Table 1. Collected fish species.

Species	Common name	Collected individuals
Astyanax aff. fasciatus	Lambari	54
Astyanax laticeps	Lambari	16
Astyanax lacustris	Lambari	10
Astyanax eigenmanniorum	Lambari	03
Geophagus brasiliensis	Cará	33
	TOTAL	**116**

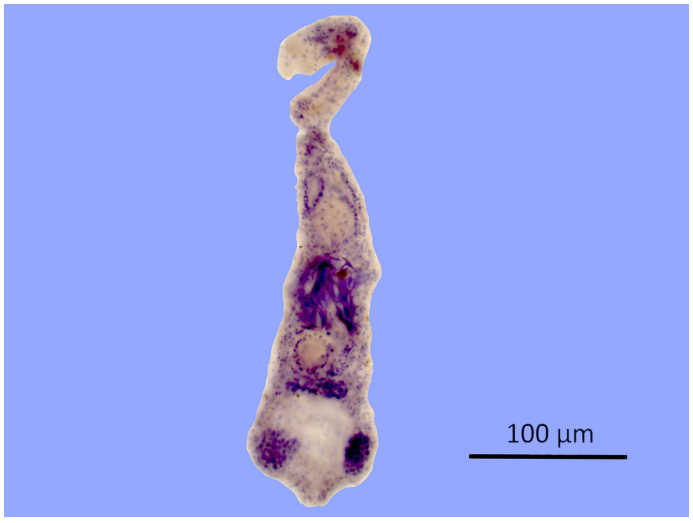

Figure 1. *Ascocotyle* sp. collected from *Astyanax* spp.

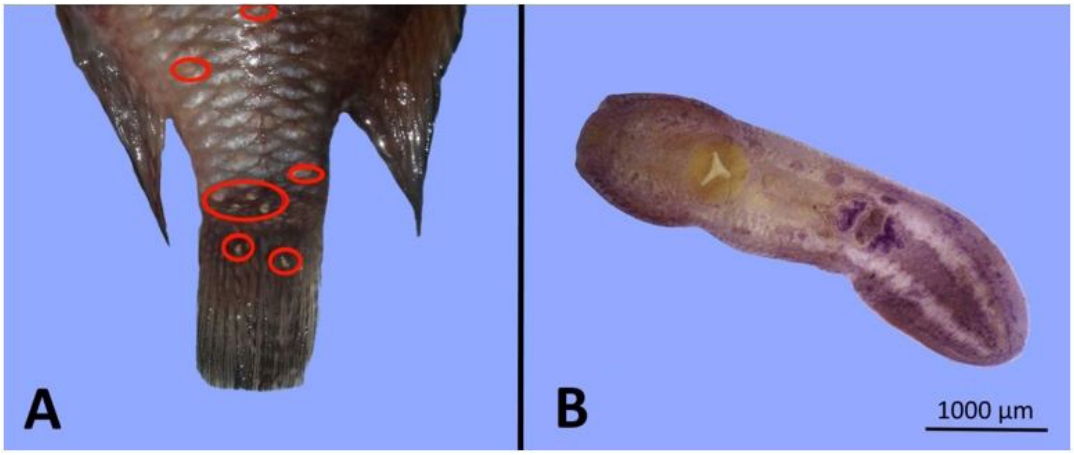

Figure 2. (A) *Clinostomum* sp. cyst in the musculature of *Geophagus brasiliensis.* (B) Metacercaria of *Clinostomum* sp. collected from *Geophagus brasiliensis.*

Table 2. Values of the parasitological indices for each studied species.

Helminth	Host	P (%)	MA Helminth Fish^{-1}	MII Helminth Fish^{-1}	Site of Infection
Ascocotyle sp.	*Astyanax aff. fasciatus*	31.48	32.61	103.58	Heart
	Astyanax laticeps	31.25	20.75	66.4	Heart
	Astyanax lacustris	50	44.1	88.2	Heart
	Total	**32.53**	**30.53**	**93.85**	------
Clinostomum sp.	*Geophagus brasiliensis*	72	8.5	11.7	Musculature

Legend: (P=Prevalence; AM=Mean Abundance and MMI= Mean Infection Intensity).

DISCUSSION

In this study, which is the first in the Tramandaí River basin, we found a high prevalence of the digenean *Clinostomum* sp. (72%). PARAGUASSU *et al*. (2005), in a study conducted with *G. brasiliensis* from the reservoir of Lajes/RJ, also recorded helminths of the genus *Clinostomum* but with a prevalence of only 3%. However, MADI (2005) reported prevalences of 16.6% and 85.09% in *Geophagus brasiliensis* collected in the reservoirs of Jaguari and Juqueri (SP). The differences between the values found by the authors are possibly related to the type of environment that was studied. Eutrophicated environments favor the growth of the ideal vegetation for the development of mollusk species, intermediate hosts of *Clinostomum* sp. (MADI, 2005).

Metacercariae of this digenean cause the yellow grub disease in freshwater fish (SILVA *et al*., 2008). In Brazil, larvae of *Clinostomum* spp. have been reported in different fish species, including *G. brasiliensis* (PARAGUASSÚ *et al*., 2005), *Rhamdia quelen* (SILVA *et al*., 2008; DIAS *et al*., 2016) and *Astyanax altiparanae* (TAKEMOTO *et al*., 2009).

When fish are ingested raw, the larvae in their flesh can lead to parasitic laryngopharyngitis and even death by asphyxia (EIRAS, 1994; KITAGAWA *et al*., 2003; PARK *et al*., 2009). Events of human infection by *C. complanatum* were diagnosed in Japan (YAMASHITA, 1938; HARA *et al*., 2014), Israel (WITENBERG, 1944), India (CAMERON, 1945), Thailand (TIEWCHALOERN *et al*., 1999) and Korea (PARK *et al*., 2009). In Brazil, so far, there are no records of human infection by *C. complanatum*, (SILVA *et al*., 2008; SUTILI *et al*., 2014).

The identification of this helminth in its larval stage was concluded only to genus level since there is a large interspecific morphological similarity. Over the last 200 years, the identification of *Clinostomum complanatum* and *Clinostomum marginatum* has been debated and only a molecular analysis could ensure a more precise species identification (CAFFARA *et al*. 2011).

In this study, metacercariae of *Ascocotyle* sp. were found in the heart of *Astyanax* spp., with a prevalence of 32.53%. In Brazil, *Ascocotyle* spp. were reported parasitizing the bulbus cordis of *Astyanax altiparanae* in the Parana River (LIZAMA *et al*., 2008), although with a lower prevalence (12.10%). *Ascocotyle longa*, whose metacercariae is found in all organs and in the musculature of *Mugil* spp., was found, in the state of São Paulo, in *Mugil platanus* (OLIVEIRA *et al*., 2007; CITTI, 2012), but it is in Mexico that this helminth is recorded in different species of *Cichlasoma*, as well as in *Astyanax fasciatus, Bramocharax caballeroi, Dormitator maculatus, Dorosoma petenense, Gobiomorus dormitory* and *Heterandria bimaculata* (SCHOLZ *et al*., 1997;

SCHOLZ *et al*. 2001; SALGADO-MALDONADO *et al*., 2005; VIOLANTE-GONZÁLES *et al*., 2007).

Among the zoonoses transmitted by fish, heterophyiasis deserves special attention, mainly in countries of Europe, Asia and Northern Africa, where trematodes of the family Heterophyidae are commonly diagnosed as agents that cause nausea, abdominal discomfort, chronical diarrhea and weight loss (BELIZARIO JUNIOR *et al.*, 2004). In Brazil, metacercariae of *Ascocotyle longa* have a great zoonotic potential due to the habit of using mullets in the preparation of Japanese dishes in which meat is ingested raw (LUQUE, 2004).

It was not possible to determine the number of rows and the number of spines in each row, this being a crucial characteristic for the determination of *Ascocotyle* species. However, according to SCHOLZ *et al*. (1995, 2001), characteristics such as site of infection (heart); thin-walled spherical cyst; pyriform body and prominent pre-oral lobe, which agree with what was found in this study, suggest that the metacercariae belong to the species *Ascocotyle tenuicollis*.

In the site where fish were collected, we observed that many fishermen eviscerate the animals and discard the organs in the environment. Additionally, we saw that the domestic animals living nearby feed on the discarded organs, contributing to the contamination of the environment and the spread of phagicolosis.

In our study, we observed that freezing the fish for over 24 hours makes the metacercariae inviable, which allows the adoption of this procedure as a prophylactic method. MYERS (1976) and JACKSON and BIER (1981), suggest freezing fish at -20°C for, at least, 60 hours, while MARQUES *et al*. (1995) states that the survival of the helminth larvae, after freezing, depends on factors such as parasite strain, freezing degree and type of freezer used.

CONCLUSION

According to the obtained results, considering the zoonotic potential of species of *Clinostomum* sp. and *Ascocotyle* sp. for humans and other animal species, we may conclude that there is a risk of infection for the local consumers. Therefore, we highlight the need of adopting local programs of prevention and health management in order to inform the communities about the potential risk of the zoonosis. Additionally, this work contributes to the knowledge on the geographic distribution of the genera *Ascocotyle* and *Clinostomum* in Rio Grande do Sul.

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