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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

ASCOCOTYLE SP. METACERCARIAE (DIGENEA: HETEROPHYIDAE) IN TISSUES OF MULLETS *MUGIL LIZA* AND *MUGIL CUREMA* (OSTEICHTHYES: MUGILIDAE) COLLECTED IN THE FISH TRADE OF THE IGUAPE CITY, SAO PAULO, BRAZIL

METACERCARIA DE *ASCOCOTYLE* SP. (DIGENEA: HETEROPHYIDAE) EN TEJIDOS DE LISAS *MUGIL LIZA* Y *MUGIL CUREMA* (OSTEICHTHYES: MUGILIDAE) COLECTADAS EN PECES COMERCIALES DE LA CIUDAD DE IGUAPE, SAO PAULO, BRASIL

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Abstract

The world distribution of oriental culture and the common use of fish which is not cooked manifested problems related to parasites. There is a great incidence of *Ascocotyle* sp. parasites in mullet fishes, which are part of human culinary spread all over world. The aim of the present study is to estimate the presence of trematode Heterophyidae metacercariae among mullet fishes commercialized at Iguape city Sao Paulo, Brazil. *Ascocotyle* sp. metacercarie (Digenea: Heterophyidae) were observed and analyzed in viscera and muscles of 60 mullets *Mugil liza* and 60 mullets *Mugil curema* (Osteichthyes: Mugilidae) purchased in the fish market of the Iguape city. The extraction of metacercariae of Ascocotyle sp. from viscera and muscles was performed by homogenization in homemade processor. All mullets samples were infected. *M. liza* and *M. curema* marketed in city constitute in a potential risk to the population, because of high prevalence of infection (100%) and large numbers of metacercarie in their tissues (up to 939 to *M. liza* and 92 to *M. curema*).

Keywords: *Ascocotyle* - fish trade - metacercariae - *Mugil curema* - *Mugil liza* - mullets.

Resumen

La expansión de la cultura oriental y el aumento del consumo de pescados crudos presenta algunos problemas, tales como enfermedades causadas por parásitos. Hay una gran incidencia de *Ascocotyle* sp. en peces lisas, muy consumidos en diversas partes del mundo. El estudio tuvo como objetivo verificar la presencia de metacercarias de trematodos Heterophyidae en peces lisas comercializados en el municipio de Iguape, Sao Paulo, Brasil. Se observaron y analizaron metacercarias de *Ascocotyle* sp. (Digenea: Heterophyidae) en vísceras y musculatura de 60 *Mugil liza* y 60 *Mugil curema* (Osteichthyes*:* Mugilidae) adquiridas en el comercio de pescados del municipio de Iguape. La extracción de metacercarias de los tejidos de los peces se realizó por homogeneización un procesador casero. Todas las muestras de peces estuvieron infectadas. *M. liza* y *M. curema* comercializados en la ciudad son un riesgo potencial para la población ante la alta prevalencia de infección en los peces (100%) y el gran número de metacercarias en sus tejidos (hasta 939 en *M. liza* y 92 en *M. curema*).

Palabras-clave: *Ascocotyle* - metacercarias - *Mugil curema* - *Mugil Liza* - peces comerciales - peces lisas.

INTRODUCTION

Fish known as mullets *Mugil liza* Valenciennes, 1836 and *Mugil curema* Valenciennes, 1836 (Osteichthyes: Mugilidae), migratory, live in large shoals, reaching 1m in length and six kg of weight. They have high commercial value and it is commonly found in various regions of the world (Miranda & Carneiro, 2007; Seckendorff & Azevedo, 2007).

Generally fish are in parasitological fauna of protozoa and helminthes hosts (Luque, 2004; Poulin & Morand, 2000), and several of these representatives are able to infect humans by eating raw or undercooked fish. Several studies have presence reported parasites in various mullet tissues (Knoff & Serra-Freire, 1993; Merella & Garippa, 2001; Ranzani-Paiva & Silva-Souza, 2004; Cavalcanti *et al*., 2005).

Digenetic trematode *Ascocotyle* sp. (Digenea: Heterophyidae) has often been reported in mullet tissues (Almeida Dias & Woiciechovski, 1994; Okumura *et al.*, 1999; Oliveira *et al*., 2007). Chieffi *et al*. (1990) described an infection case of *Ascocotyle* (= *Phagicola* sp.) in a woman who eating mullet raw flesh in Cananéia City, Sao Paulo, Brazil.

Therefore, the Eastern culture expansion, among other factors, has contributed to changes in men dietary habits and increased raw fish meat consumption. With the human infection occurrences cases by *Ascocotyle* sp. in Brazil, further studies are needed for parasite general knowledge.

The present study aimed to analyze metacercariae of *Ascocotyle* sp. presence (Digenea: Heterophyidae) in viscera and muscle of mullets *M. liza* and *M. curema* (Osteichthyes: Mugilidae) acquired in the fish trade of the Iguape city, Sao Paulo, Brazil.

MATERIAL AND METHODS

All samples were purchased in the fish trade of the Iguape city (24^0 41'51"S, 47^0 34'16" W), between January 2009 and February 2010. In all, 60 *M. liza* and 60 *M. curema* were acquired, been 30 *M. liza* and 30 *M. curema* caught in beach, and 30 *M. liza* and 30 *M. curema* caught in river. The beach environment referred to Iguape coast, Sao Paulo, Barra do Ribeira district and Ilha Comprida city, São Paulo. The river referred to the estuarine environment, known as the Small Sea. The entire region is known as Estuarine Lagoon Complex Cananéia-Iguape-Ilha Comprida.

The metacercariae *Ascocotyle* sp. extraction, muscles and viscera were performed by homogenization (Castro, 1994). Liver, spleen, kidney and heart were removed from each specimen and separated partially fragmented 5 g of this "viscera pool". About 300 mL of purified water was added to 5 g of "viscera pool" in a household blender, and for about 10 s, the sample was processed. Then the mixture was screened to 0.07 mm mesh and placed in sedimentation cup for 5 min. Then, the supernatant was removed and have added 300 mL of water again to the pellet. The process was repeated to obtain the supernatant clear water. The same procedure was performed with a muscle tissue sample, consisting about 5x5 cm quadrant, taken near the left pectoral fin of each specimen. The samples muscles settling time in the cup was 15 min. All pellet obtained was observed by light microscopy to identify and count metacercariae.

Identification was based on metacercariae Scholz (1999) and Scholz *et al*. (2001). Parasites prevalence and density were calculated according to Bush *et al.* (1997). Tests were used for Duncan multiple comparisons and Pearson tests linear correlation. Statistical tests were performed using SAS (SAS, 1996), with interval confidence equal to 5%.

RESULTS

By morphological characteristics, were identified as *Ascocotyle* sp. metacercariae. Confirmed metacercariae presence in 100% of mullet specimens *M. liza* and *M. curema* and all showed metacercariae greater density in viscera than in muscle tissue (Table 1)

In *M. liza*, the metacercariae number was greater than *M. curema* captured in the same environment, beaches ($p = 0.004$), and rivers ($p = 0.009$). The metacercariae muscle were more numerous in *M.*

liza (p = 0.0001), and no significant difference between the sexes of both fish species (p = 0.44, p = 0.69). In both species, density was higher in viscera than in muscle, and higher in viscera of *M. liza* than *M. curema*, regardless origin place (p = 0.0001).

However, *M. curema*, density was significantly higher in fish viscera caught in river than in beach. The metacercariae density in musculature, *M. liza* had significantly higher than *M. curema*, regardless site collection (p = 0.02 in beach and p = 0.003 in river).

There were no differences metacercariae density muscles in relation to sex of two fish species. *M. liza* caught in river showed low correlation between metacercariae frequency in the muscles and biometric parameters (r = 0.37; p = 0.04 for length and r = 0.41; p = 0.02 for weight) and metacercariae density in viscera and weight (r = 0.39; p = 0.03). There was also no correlation between metacercariae and biometric parameters (length and weight) for *M. liza* collected from the beach (r = 0.15, p = 0.40; r = 0.04, p = 0.81) and in *M. curema* in both (r = 0.01, p = 0.94; r = 0.14, p = 0.44 for beach and r = 0.21, p = 0.25; r = 0.24, p = 0.18 for river)

DISCUSSION

The metacercariae presence in 100% of units of *M. liza* and *M. curema* is in agreement with shown by Almeida Dias & Woiciechovski (1994). Oliveira *et al.* (2007) also showed parasite presence in all mullets samples studied in Cananéia City, Sao Paulo, Brazil.

The efficiency of "viscera pool" for metacercariae recovery was quoted by other authors (Almeida Dias & Woiciechovski 1994; Antunes & Almeida Dias 1994; Oliveira *et al.*, 2007). The viscera diverse attention because they can be discarded on public roads or given to animals, which contributes to the parasite cycle, since dogs and wildlife such as *Procyon lotor* Linnaeus, 1758 and *Vulpes vulpes* Linnaeus, 1758 have been found parasitized by *Ascocotyle* sp. (Snyder *et al*., Chieffi *et al*., 1992; Eira *et al*., 2006; 1989).

In this work, the muscle tissue was removed from the left side of each specimen, near the pectoral fin. But, according to Oliveira *et al*. (2007), the cranial and caudal muscles also proved efficient for metacercariae collecting.

In the present study were found up to 38 metacercariae in 5g sample of mullet sample, and up to 14 in parati muscle. Barros & Amato (1996) found that 300 metacercariae are sufficient to infect dogs. Because all fish examined were infected, the risk of infection to local population that consumes fish is high. But in the survey, a small population, revealed that only 3.2% eat raw fish, and others, roasted, baked or fried. This habit of eating fish greatly decreases the possibility of

Table 1. Mean number of *Ascocotyle* sp. metacercariae (Digenea: Heterophyidae) obtained in samples of 5g mullet tissue and biometric parameters of *Mugil liza* and *Mugil curema* (Osteichthyes: Mugilidae), acquired in the fish trade of the Iguape city, São Paulo, Brazil.

	Mugil liza		*Mugil curema*	
	Beach (n=30)	River (n=30)	(Beach n=30)	River (n=30)
Fish length (cm)	46.3 ± 4.9	44.8 ± 4.8	36.0 ± 2.8	36.5 ± 2.0
Fish weigth(g)	906.6 ± 286.0	814.6 ± 141.4	441.9 ± 44.2	455.0 ± 101.1
Metacercariae - viscera	252.9 ± 256.0	232.5 ± 202.2	21.6 ± 9.8	31.4 ± 19.6
Metacercariae - muscle	8.7 ± 8.6	7.4 ± 6.7	3.1 ± 4.5	2.5 ± 3.1

infection as Coelho *et al.* (1997) showed that preventing *Ascocotyle* sp. can be effective by cooking fish at 100°C and freezing at -20°C.

The significantly greater density of *Ascocotyle* sp. metacercariae in the *M. curema* viscera caught in river can be explained by known the access to the river for this species, showing potential proximity to intermediate hosts and infective parasite forms.

Despite significant correlation between metacercariae density and *M. liza* biometric parameters, the coefficient values were very low, indicating that the metacercariae presence is not dependent of fish species length and weight. It is worth mentioning Almeida Dias & Woiciechovski (1994) and Oliveira *et al.*, (2007), which had infected fish from specimens with four cm fingerlings, not observing infection in juvenile smaller. Although the metacercariae density in *M. liza* viscera and muscles had been greater than in *M. curema*, the study results showed no clear biometrics and density correlation of biometric and density of *M. liza* metacercariae. However, other authors found a greater number of metacercariae in larger fish (Coelho *et al.*, 1997). Vianna *et al.* (2005) studied metacercariae of *Clinostomum complanatum* (Rudolphi, 1819) and *Rhamdia quelen* (Quoy & Gaimard, 1824) showed that fish parasites density is greater than 30 cm. Literature data suggest gradual metacercariae acquisition, which minor variations, all in accordance with the size set by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováeis) extractive fishing.

Ascocotyle sp. metacercariae were found in 100% fish mullets obtained in the fish trade of the Iguape city, Sao Paulo, Brazil, between January 2009 and February 2010. The *M. liza* and *M. curema* consumption generates a zoonotic risk potential to population for high infection prevalence and large metacercariae numbers in their tissues.

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