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Short Communication

Distribution of cysts holding *Ascocotyle* (*Phagicola*) *longa* metacercariae in tissues and organs of mugilid

Distribuição de cistos contendo metacercárias de *Ascocotyle* (*Phagicola*) *longa* em tecidos e órgãos de mugilídeos

Juliano Santos Gueretz[1]*; Maiara Boeing[1]; Juliana Murasaki[1]; Elizabeth Schwegler[1]; Anderson Barbosa de Moura[2]; Maurício Laterça Martins[3]

[1]Instituto Federal Catarinense – IFC Araquari, Campus Araquari, Araquari, SC, Brasil
[2]Programa de Pós-graduação em Ciência Animal, Universidade do Estado de Santa Catarina – UDESC, Lages, SC, Brasil
[3]Laboratório AQUOS, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil

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Abstract

Ascocotyle (*Phagicola*) *longa* is an etiological agent of human phagicolosis. Mugilids are the second intermediate host, the first being *Heleobia australis*, and mugilids predatory birds and mammals are its definitive hosts. The occurrence of cysts holding *A. longa* metacercariae is described in mugilids with a prevalence of up to 100%. The wide geographical distribution of *A. longa* and its intermediate hosts coupled with the rise in the consumption of raw or poorly cooked fish may elevate the risk of human infection. Therefore, in this study, we aimed to verify the distribution pattern of cysts holding *A. longa* in mugilids. The tissue and organ samples of these fish were processed in a domestic blender and examined under a stereoscopic microscope to identify the cysts holding the digenetic metacercariae. Of the 24 (100%) fish samples that were analyzed, 12 of *Mugil curema* and 12 of *Mugil liza* possessed cysts holding *A. longa* metacercariae. Digenetic cysts were identified to be present in the gills, heart, stomach, liver, intestines, mesentery, and muscular tissues collected from *M. curema* and *M. liza*. Conclusively, in *M. curema*, the cysts holding *A. longa* metacercariae were found to be distributed randomly throughout the fish body in almost every tissue and organ that was examined.

Keywords: Digenetic, phagicolosis, mullet, parati, zoonosis.

Resumo

Ascocotyle (*Phagicola*) *longa* é o agente etiológico da fagicolose humana. Os mugilídeos são os segundos hospedeiros intermediários. O primeiro é *Heleobia australis* e pássaros e mamíferos predadores de mugilídeos, os hospedeiros definitivos. A ocorrência de cistos contendo metacercárias de *A. longa* é descrita em mugilídeos, com até 100% de prevalência. A ampla distribuição geográfica de *A. longa* e seus hospedeiros intermediários, acompanhado do aumento do consumo de peixe cru ou mal cozido, pode aumentar o risco de infecção humana, portanto o objetivo do presente estudo foi verificar o padrão de distribuição de cistos, contendo metacercárias de *A. longa,* em mugilídeos. Amostras de tecidos e órgãos desses peixes foram processadas em liquidificador doméstico e observadas em microscópio estereoscópico à procura de cistos contendo metacercárias do digenético. Todos os 24 (100%) mugilídeos, 12 *Mugil curema* e 12 *Mugil liza* examinados apresentaram cistos contendo metacercária de *A. longa*. Foram observados cistos do digenético nas brânquias, coração, estômago, fígado, intestino, mesentério e tecido muscular de *M. curema* e de *M. liza*. Em *M. curema*, os cistos contendo metacercárias de *A. longa* estão distribuídos de forma aleatória por praticamente todos os tecidos e órgãos dos mugilídeos examinados.

Palavras-chave: Digenéticos, fagicolose, parati, tainha, zoonose.

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*Corresponding author: Juliano Santos Gueretz. E-mail: juliano.gueretz@ifc.edu.br

The digenetic trematode *Ascocotyle* (*Phagicola*) *longa*, Ransom, 1920 (Digenea: Heterophyidae), which is the etiological agent of human phagicolosis, exhibits a complex life cycle. There are two intermediate hosts, the first is the snail belonging to the family Cochliopidae, *Heleobia australis*, and the second is the mugilid fish. Its definitive hosts are the mugilid predators, such as birds and mammals. Its epidemiology and life cycle are related to the estuaries and offshore lagoons, where the juvenile mugilids and the above-mentioned snails inhabit together (Simões et al., 2010).

The wide geographical distribution of *A. longa* and its intermediate hosts coupled with the rise in the consumption of raw or poorly cooked fish may elevate the risk of human infection. However, this is an underestimated zoonosis, mainly due to the absence of clinical signs and characteristic symptoms (Montejo et al., 2008; Santos et al., 2013; Tavares et al., 2018). Chieffi et al. (1990) in the region of Cananéia, Chieffi et al. (1992) in Registro and Almeida Dias & Woiciechovski (1994) in Cananéia and Registro, all in the São Paulo State, Brazil, highlighted and discussed phagicolosis in humans who ingested mugilids under inadequate conditions. The parasite keeps the capacity to infect humans if not treated by thermal process. Santos (2010) discussed diseases transmissible by sins, including phagicolosis and concluded that these diseases deserve more attention, confirming their importance in public health.

In similar studies, the trematode metacercariae were identified to be present in the spleen, heart, stomach, liver, gonads, mesentery, muscles, and kidneys of mullets (*Mugil liza* Valenciennes, 1836) and paratis (*Mugil curema* Valenciennes, 1836) from the Rodrigo de Freitas lagoon, Rio de Janeiro, Brazil (Simões et al., 2010). Ranzani-Paiva & Tavares-Dias (2002) found 99.0% of mullet livers, *Mugil platanus* (currently *M. liza*), with round, gray, and granular cysts diagnosed as *Phagicola* sp., currently *A. longa* in Cananéia SP, Brazil. Using histological techniques, Oliveira et al. (2007) found a 100% prevalence of *A. longa* metacercariae in *M. platanus* in Cananéia SP, Brazil estuary. Namba et al. (2012) found a 100% prevalence of *A. longa* metacercariae in *M. liza* and *M. curema* in Iguape SP, Brazil. Santos et al. (2013) verified the prevalence of *A. longa* metacercariae in *M. liza* in the Rodrigo de Freitas lagoon, Rio de Janeiro, Brazil, and identified 100% in the spleen, 98% in the heart, 97% in the bowel wall, 97% in the liver, 87% in the muscles, 77% in the stomach wall, 47% in the brain, 30% in the gonads, and 30% in the gallbladder. Citti et al. (2015) observed the presence of *A. longa* in 100% (92/92) of muscle samples and 80.43% (74/92) of a "guts pool" of mullets in São Paulo SP, Brazil. Rodrigues et al. (2015) confirmed *A. longa* metacercariae viability in mullet filets (*M. platanus*) after heat treatment at 50-56 °C for 2-3 min.

Ferraz et al. (2014) demonstrated the efficiency of homogenization using a blender or a mixer to extract *Ascocotyle* sp. metecercariae from the muscular tissue and guts of *M. liza* in Iguape SP, Brazil.

Considering the importance and zoonotic nature of this parasitosis, in this study, we aimed to elucidate the distribution pattern of cysts holding *A. longa* in different tissues of mugilids, particularly *M. liza* and *M. curema*, as well as a simple and practical methodological proposal for its diagnosis in fish.

This study was conducted with 24 mugilids, 12 of *M. curema* and 12 of *M. liza*, during 2018. All mugilids were acquired from a commercial establishment in São Francisco do Sul SC, Brazil. They were collected from Baía da Babitonga (26°13'44" S, 48°40'40" W). All adult fish, with *M. liza*, in reproductive migration and in the proportion of 50% females and 50% males, and in *M. curema* the specimens were of undetermined sex. The gills, heart, stomach, liver, bowel, mesentery, and muscle tissue samples were collected after necropsy. The gill tissue samples consisted of four slides or one organ hemilate. The muscular tissue was collected from both sides of the fish, a 2×1×15 cm (width×thickness×length) filament sample using which three fragments (5.21 ± 1.14 g) were deducted, with one central and two from the ends of the filament sample, resulting in six muscular tissue samples. The muscular tissue fragments and the cyst count were standardized to 5.0 g. The bowel samples consisted of a 5 cm length fragment. From the mesenteries, a 3×3 cm sample was collected. The heart, stomach, and liver organs were considered as the tissue samples.

According to the technique described by Namba et al. (2012), Ferraz et al. (2014) and Citti et al. (2015), the organ fragments were processed using a domestic blender with 240 mL of water at 224 G-force for 20 s. The processed content was strained using a 600 µm sieve to a decantation cup in which the water was added to achieve a capacity of 390 mL. About 20 min later, the supernatant was despised and fractions of 9 mL of the decanted content were added to a Petri plate for analysis using a binocular stereoscopic microscope to search for cysts holding *A. longa* metacercariae that were detected based on its morphological characteristics.

The cysts holding *A. longa* metacercariae were identified according to the characteristics described by Scholz (1999), Scholz et al. (2001), Simões et al. (2010), Martorelli et al. (2012), and Santos et al. (2013). The mugilidae, mullets (*M. liza*), and paratis (*M. curema*), were identified according to the characteristics described by Menezes (1983) and Menezes et al. (2010).

The average cyst count, standard error, and coefficient of variation were calculated. The set of data considered normal, in their distribution, were identified in the analysis of variation, in case this showed a difference, the Tukey test was applied, to verify which or which sets were different. To compare sets for normality standards, the Kruskal-Wallis and Mann-Whitney test was applied.

The 24 mugilids, 12 of *M. curema*, and 12 of *M. liza*, possessed cysts holding *A. longa* metacercariae. Digenetic cysts were identified to be present in the gills, heart, stomach, liver, bowel, mesentery, and muscular tissues of *M. curema* and *M. liza*. In the *M. curema* samples, the occurrence of cysts was higher in the muscular tissue than in the guts, and in the *M. liza* samples, it was higher in the guts than in the muscular tissue. However, no correlation was observed between the tissues of any organs (Tables 1, 2 and 3).

The sampling and parasite diversity might lead to the establishment of a risk gradient, which might put the batches, species, and fish varieties or their by-products under the vigilance of the competent sanitary authorities. For these issues, the use of field or laboratory applicable methods with high levels of diagnostic ability might assist in standardizing the methodologies and establishing the techniques and protocols that could increase the determination precision (Ferraz et al., 2014). Based on these findings, this study unveiled the distribution pattern of cysts holding *A. longa* metacercariae in different tissues and organs to establish an identification method for the

Table 1. Distribution, average, standard error, and coefficient of variation of cysts holding *Ascocotyle* (*Phagicola*) *longa* metacercariae in the tissues and organs of *Mugil curema* and *Mugil liza*.

Organs	*Mugil curema*			*Mugil liza*		
	$\bar{x}$	$\delta\bar{x}$	CV (%)	$\bar{x}$	$\delta\bar{x}$	CV (%)
Gills	15.50	9.50	86.65	33.45	10.68	105.95
Heart	1.00	0.70	141.00	84.92	26.37	107.57
Stomach	2.00	1.225	122.50	46.50	9.06	67.52
Bowel	0.00	-	-	131.73	32.61	82.09
Mesentery	0.00	-	-	86.45	57.98	222.42
Muscle[1]	15.27	2.98	67.70	3.87	2.15	200.46
Liver	5.83	4.14	246.31	59.00	85.70	145.25

[1]standardized to five grams; $\bar{x}$: average; $\delta\bar{x}$: standard error; CV: coefficient of variation.

Table 2. Distribution, average, standard error, and coefficient of variation of cysts holding *Ascocotyle* (*Phagicola*) *longa* metacercariae in the muscular tissue samples of *Mugil curema*.

Samples	n											
	1	2	3	4	5	6	7	8	9	10	11	12
1	18	7	7	6	10	16	16	5	9	2	1	0
2	18	7	14	6	11	22	31	6	9	4	2	0
3	21	11	15	8	14	23	36	11	12	8	4	0
4	25	13	19	9	15	31	39	14	21	12	6	0
5	27	15	20	12	15	42	43	15	21	15	7	0
6	29	15	21	14	25	42	59	17	26	18	10	1
$\bar{x}$	23.00	11.30	16.00	9.17	15.00	29.30	37.20	11.00	16.00	10.00	5.00	0.17
$\delta\bar{x}$	1.91	1.50	2.13	1.33	2.18	4.45	5.78	2.01	2.96	2.56	1.37	0.17
CV (%)	20.39	32.48	32.62	35.45	35.53	37.20	37.93	44.78	45.36	62.74	66.93	241.18

n: number of fishes; $\bar{x}$: average; $\delta\bar{x}$: standard error; CV: coefficient of variation.

Table 3. Distribution, average, standard error, and coefficient of variation of cysts holding *Ascocotyle* (*Phagicola*) *longa* metacercariae in the muscular tissue samples of *Mugil liza*.

Samples	n											
	1	2	3	4	5	6	7	8	9	10	11	12
1	0	0	11	3	2	2	1	0	0	0	0	0
2	0	0	41	4	2	0	0	0	0	0	0	0
3	0	0	44	6	5	0	0	0	0	0	0	0
4	0	0	17	7	8	2	1	1	0	0	0	0
5	0	0	33	12	8	0	2	1	2	1	1	0
6	0	0	25	12	11	2	4	2	2	1	2	1
$\bar{x}$	0	0	28.50	7.33	6.00	1	2.17	0.67	0.67	0.33	0.50	0.17
$\delta\bar{x}$	0	0	5.38	1.58	1.48	0.45	0.98	0.33	0.42	0.21	0.34	0.17
CV (%)	0	0	46.20	52.93	60.55	100.00	111.00	122.47	154.47	154.91	167.33	244.95

n: number of fishes; $\bar{x}$: average; $\delta\bar{x}$: standard error; CV: coefficient of variation.

digenetic presence in mugilid. However, it was not possible due to the random distribution of cysts among tissues and organs without a definite pattern.

Based on their results [100% (92/92) *A. longa* cysts in muscle samples and 80.43% (74/92) in "guts pool"], Citti et al. (2015) advised not to consume raw or undercooked mullets. The results of this study support their hypothesis, because the presence of cysts holding metacercariae of the above-mentioned digenetic was confirmed in every fish tissue and organ examined, and was highly prevalent in edible tissues.

Citti et al. (2015) confirmed a higher concentration of cysts holding *A. longa* metacercariae in the muscular samples (100%) than in the "guts pool" (80.73%). In contrast to the findings of this study, a higher concentration of cysts was found to be present in the guts than in the muscular tissue. However, a gut mix was not used in this study.

A high prevalence (100%) of cysts holding *A. longa* mertacercariae was found to be present in the guts and muscles of mullets from Rio de Janeiro, Brazil, which indicated a potential impact on public health upon consumption of these fish (Simões et al., 2010). The authors inference is in accordance with that of this study, since there is a population under the same risk residing around the origin sites of the mugilids that were studied herein.

Santos et al. (2013) identified this parasite in 100% of the examined fish, and a seasonal variation of the cysts holding *A. longa* metacercariae was not observed. The prevalence varied greatly among the organs, with 100% in the spleen, 98% in the heart, 97% in the bowel wall, 97% in the liver, 87% in the muscle, 77% in the stomach wall, 47% in the brain, 30% in the gonads, and 30% in the gallbladder. These cysts were not identified in the eyes or gills. In this study, seasonal evaluation was not conducted; it was a punctual study, and the authors mentioned that there was a significant variation in the number of cysts among the tissues and guts studied. The coefficient of variation observed varied from zero to 246.31 in the muscular tissue of *M. curema* and from 46.20 to 244.95 in *M. liza* (Table 1).

A significant variation in the presence of cysts between the muscular tissue samples was also observed (Tables 2 and 3). A variation between the fish was expected, however, a variation among the six samples of the same fish was not expected.

Additionally, it is important to infer the care that needs to be taken during the food preparation using these fish, as the digenetic may potentially cause zoonosis.

The cysts holding *A. longa* were found to be distributed randomly throughout the fish body in almost all the tissues and organs examined. Therefore, it was not possible to define that only one sample of muscular tissue or organ is accurate in determining the affected specimens, as well as defining the digenetic cyst concentration in these fish.

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