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Research of *Ascocotyle (Phagicola) longa* in Heat Treated Fillets of Mullet (*Mugil platanus*)

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Abstract

Seafood can present many biological hazards, such as zoonotic parasites. Among these, *Ascocotyle* (*Phagicola*) *longa* trematode is generally found in mullets (*Mugil platanus*) and is the most common parasite involved in heterophyiosis outbreaks. This research aimed to detect viable metacercariae of *Ascocotyle* (*Phagicola*) *longa* after heating muscle of mullets. The method used was sedimentation followed by microscopy observation. It was found 100% (16/16) of inactivated metacercariae in the analyzed samples. This is the first study involving samples of mullets ready to eat sold directly to consumer. We conclude, consumers must be alerted to the risk of infection by raw mullet eating and proper heating or cooking kills this trematode.

Keywords: Parasite; Food safety; Public health

Introduction

The presence of parasites in marine and freshwater fishes is common and may carry risks, both economic and sanitary [1]. Most of the parasites are found in organs that are discarded during fish processing, some worms may be found in the muscle. In case of consumption of the seafood in an inadequate preparation, consumers may fell ill [2-4]. Among the parasites reported in mullets (*Mugil platanus*), *Ascocotyle* (*Phagicola*) Ransom, 1920 (*Digenea: Heterophyidae*) trematode is very common and can cause disease in human by consumption of parasitized raw seafood [5-7].

Adult *A.* (*Phagicola.*) live in the gut of birds and mammals. Metacercariae develop in mullets tissues [8]. According to Simões et al. [9], mollusks presence is essential for the occurrence of heterophyiosis. Depending on the region studied, particular specie of mollusk is involved with the biological cycle. Simões et al. [9] also reported the presence of the snake *Heleobia australis* as intermediate host for this parasite, increasing the risk of human infection. Even the elevated risk of infection present, this fishborne disease is underestimated due to absence of characteristic clinical signals [10,11]. Heating is the best method for inactivation of these parasites. Coelho [12] recommends heating at 100°C for 60 minutes. Antunes et al. [13] observed ionization with doses of 4.0 kGy gamma rays can also be efficient to kill metacercariae, but this method is not approved by sanitary authorities in some countries. Therefore, this study aimed to detects and identify viable *A. metacercariae* in mullets (*Mugil platanus*) fillet after heat treatment.

Materials and Methods

Sampling

Officers of sanitary police of the State of São Paulo (Brazil) sampled 16 baked fillet of mulltes (*Mugil platanus*) from "mullet festivities" between June and July of 2009 in the following cities: Bertioga, Praia Grande, Santos, and São Vicente in the State of São Paulo (South Eastern Brazil) (Table 1).

In this event, the fish is put in an oven to cook. Normally the product reaches 50-56°C in the centre for 2-3 minutes. The problem is when there are many people because they want the plate fast, and for that reason, the muscle doesn't reach the right temperature. Thereby, if seafood is parasitized by *Ascocotyle*, it can cause illness in the consumer.

Parasitological analysis

To guarantee correct identification, it was taken a piece of muscle of fresh fish and treated by heat of the same animal to equate results. This procedure is essential because heat can cause alteration in the morphology of the parasite, harming their identification.

After sampling, fish were put in plastic bags and destined to the parasitology laboratory of Reference Unit Laboratory Technology of Seafood of the Fishing Institute (*Instituto de Pesca*), in Santos, São Paulo, Brazil, for detection and identification of metacercariae. Five grams of muscle of each fish were taken and submitted to centrifugation with 300 mL of clean, tap water. The content was transferred to the

City	Number of fish sampled
Bertioga	4
Praia Grande	4
Santos	4
São Vicente	4
Total	**16**

Table 1: Number of mullets (*Mugil platanus*) sampled by city during June to July of 2009.

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conical glass jar remaining 20 minutes for sedimentation. Supernatant was discarded and more 300 mL of potable water were added. After 20 minutes wait, the sediment was collected and put in a slide using Pasteur pipette, to perform microscopic analysis of the sample [14]. Microscopic identification of parasites was performed according to Simões et al. [9].

Statistical analysis

We analyzed the prevalence of parasites. According to the results, samples were divided in two classes: "present" or "absent". The prevalence calculation was performed using R version 2.15.1 software [15].

Results and Discussion

In this study was observed in 100% of samples, the presence of parasites identified as Ascocotyle suggesting a contamination of mullets before cooking. Analysis of parasites suggested these were inactivated (Figure 1).

The high prevalence of metacercariae in mullets we observed in this study had already been described by Hutton [16], Armas de Conroy [17], Almeida-Dias e Woiciechovski [18], Antunes and Almeida Dias [19], Knoff et al. [20], Conceição et al. [21], Oliveira et al. [10], and Santos et al. [22]. The high quantity of studies showing high prevalence of Ascocotyle stresses the importance of the detection of this parasite in the world.

In Brazil, Chieffi et al. [23,24], Antunes and Almeida-Dias [19] illustrated cases of heterophyiosis in the state of São Paulo, probably caused by Ascocotyle (Phagicola). Based on these data, metacercariae detection in the muscle, adequate processing, and consumer awareness are crucial to prevent fishborne disease. Inactivation strategies must be realized to guarantee seafood security, since Santos et al. [22] demonstrated adequate heating importance for the safety to consumers.

This is the first study involving research of Ascocotyle mullets samples ready to eat. "Mullet festivities" attract many consumers and the time of fish cooking varies a lot according to demand. Most of the times, mullets are roasted quickly in high fire, causing external overcooking and internal undercooking. According to Huss et al. [25], inactivation temperature of trematodes is 55ºC for 1 minute inside the product.

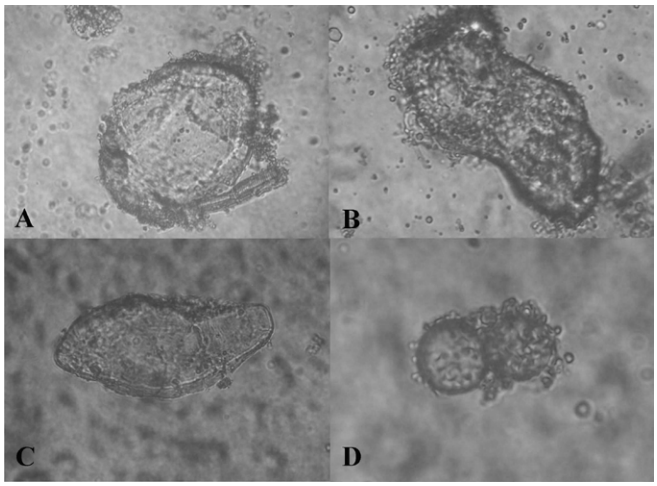

Figure 1: Ascocotyle longa metacercariae inactivated by heating observed in microscopy. 100X. A, B, and C: different larvae forms, D: cysts.

The presence of 100% of inactivated (dead) metacercariae in the samples observed in this study indicates, the temperature of roasting was adequate for parasite inactivation. All samples collected by officers of Sanitary Policy were too roasted, which is not common to observe during these parties, as it was observed by the authors. It is important to control both time and temperature to guarantee the inactivation of metacercariae as described by Huss et al. [25].

Oliveira et al. [10] report, fishes parasitized by Ascocotyle do not present any lesion suggesting any kind of parasitic infection. In 2010, this trematode was included in the Risk Classification of Biological Agents list of Brazil [26].

Sanitary inspection of seafood is not enough to guarantee safety for consumer, once this is based on visual analysis. It is necessary to explain to consumer that raw or undercooked fish eating may carry parasites, such as Ascocotyle which are dangerous to humans.

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