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Validation of a method for trematode metacercariae detection in freshwater fish through the helminthological analysis of striped catfish, *Pangasius hypophthalmus*, fillets
Originals

VALIDATION OF A METHOD FOR TREMATODE METACERCARIAE DETECTION IN FRESHWATER FISH THROUGH THE HELMINTHOLOGICAL ANALYSIS OF STRIPED CATFISH, *PANGASIUS HYPOPHTHALMUS*, FILLETS

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Abstract

Fish-borne zoonotic trematodes are recognized as a serious health problem for humans. These parasites are particularly prevalent in South East Asia, but the high demand for fish on a global scale together with increasing aquaculture in Asian countries increase the risk of infection for exporter and importer countries. Spain is the main importer of freshwater fish, potential hosts for these parasites. The aim of this study is to validate an artificial pepsic digestion method for trematode metacercariae detection in freshwater fish fillets, based on WHO and the European Union Reference Laboratory for Parasites procedures. Deep frozen striped catfish, *Pangasius hypophthalmus*, fillets acquired at various supermarket chains were used for the study and helminthologically analysed. Artificial infection with 50 trematode metacercariae was carried out in 5 fish fillet samples, previously shown to be negative for other metacercariae. All metacercariae were recovered after studying the total decantation volume, obtaining a sensitivity of 100%, and minimizing the sample (20 g) and reagents (1 g pepsin powder, 2 ml HCl 37% and 100 ml water) with respect to the reference methods. Therefore, this method is proposed to be used routinely for epidemiological studies on trematode metacercariae detection in freshwater fish. Moreover, none of the striped catfish fillets was found to be parasitized by any kind of helminth. Considering this negative result, as well as the deep frozen conservation of fish for many months, it can be ascertained that the striped catfish does not pose a helminthological risk for consumers in Spain.

Resumen

Las trematodiasis de transmisión alimentaria están reconocidas como un serio problema de salud humana. Estos parásitos son particularmente relevantes en el sudeste asiático, pero la elevada demanda de pescado a escala global junto con el incremento de la acuicultura en países asiáticos aumenta el riesgo de infestación para los países exportadores e importadores. España es uno de los más importantes importadores de pescado de agua dulce, potenciales hospedadores de estos parásitos. El objetivo fundamental de este estudio es validar un método de digestión pépsica artificial para la detección de metacercarias en filetes de pescado de agua dulce, basado en los procedimientos de la OMS y del Laboratorio de Referencia para Parásitos de la Unión Europea. Filetes ultracongelados de panga, *Pangasius hypophthalmus*, adquiridos en varias cadenas de supermercados fueron utilizados para el estudio y el análisis helmintológico. 5 filetes, negativos en el análisis helmintológico previo, fueron infestados artificialmente con 50 metacercarias. Todas las metacercarias fueron recuperadas tras el estudio del total del volumen de decantación, obteniéndose una sensibilidad del 100%, y con la minimización de la muestra (20 g) y de los reactivos (1 g de pepsina en polvo, 2 ml de HCl 37% y 100 ml de agua) con respecto a los métodos de referencia. Por tanto, se propone este método para ser usado de forma rutinaria en estudios epidemiológicos sobre la detección de metacercarias en peces de agua dulce. Además, ninguno de los filetes de panga fue hallado parasitado por helminto alguno. Considerando este resultado negativo, así como la ultracongelación del pescado durante varios meses, puede cerciorarse que la panga no supone un riesgo helmintológico para los consumidores en España.

Keywords / Palabras clave

fish-borne trematodes / trematodiasis transmitidas por peces, metacercariae detection / detección de metacercarias, artificial pepsic digestion / digestión pépsica artificial, method validation / validación de método, freshwater fish / pescado de agua dulce, striped catfish / panga

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1. Introduction

Fish borne-trematodes are considered a particularly harmful group of human pathogens [1-4] as they are able to cause a wide range of liver- and intestinal diseases in humans, including cancer or even death [5-7]. According to the World Health Organisation estimates, more than 18 million people are infected, and more than half a billion people worldwide are at risk of infection [2]. These zoonotic parasites are particularly prevalent in South East Asia, posing a serious public health problem in some countries, such as Vietnam, although epidemiological information is quite limited [8]. Moreover, the high demand for fish on a global scale together with increasing aquaculture in Asian countries increase the risk of infection for exporter and importer countries. Given that Spain is one of the biggest importers of freshwater fish from Vietnam, strict control measures are imperative.

The main aim of the present article is to validate a method for trematode metacercariae detection in freshwater fish fillets, based on a modification of the procedures of the reference methods of WHO and the European Union Reference Laboratory for Parasites [1], [9]. In recent years, the striped catfish, *Pangasius hypophthalmus*, has become very popular among Spanish consumers, mainly thanks to its versatility in cooking and, particularly since it is inexpensive. In spite of the fact that away from Vietnam, and the other countries of origin, this fish can only be acquired as frozen, skin- and boneless fillets and never as a fresh product, pathogenic helminths are expected not to be viable [10]. Yet, the presence of trematode metacercariae in deep frozen striped catfish fillets was analysed to shed light on the potential risk of infection associated to the consumption of this fish species.

2. Materials and Methods

A total of 50 deep frozen, skin- and boneless fillets of *P. hypophthalmus*, originating from Vietnam, were purchased in branches of various nationwide Spanish supermarket chains located in València in November 2014. In the laboratory, the fillets were thawed at room temperature, weighed and their total length was measured.

The analysis of the fillets was carried out using two diagnostic techniques: visual observation using a conventional trichinoscope, according to the standard methodology [11], and artificial pepsic digestion. Two small pieces of fillet of about 2 mm x 10 mm, with a total weight of about 0.5 g, were taken from every specimen and then compressed between two glass plates until they become translucent. Finally, the trichinoscope with the samples was examined under a stereomicroscope at 50x magnification. Concerning artificial digestion, a modification of the WHO and the European Union Reference Laboratory for Parasites reference methods [1], [9] was designed. To validate the new method, 5 striped catfish fillets, which resulted negative for metaercariae following the reference diagnostic methods, were artificially infected with a total of 50 trematode metacercariae, inserted into the flesh of each specimen using an intradermal syringe. The artificially infected fish were then digested according to the following procedure (Figure 1): 1) homogenize 20 g of the fillet with the artificial digestion solution, prepared with 1 g pepsin powder, 2 ml HCl 37% and 100 ml water; 2) pour the mixture into a beaker together with additional digestion solution until reaching a total volume of 200 ml; 3) incubate at 37°C for 30 min; 4) filter the solution through a stainless steel sieve, of a mesh size of 1000 µm, and leave it to settle for 30 min; 5) recover 40 ml of the solution, leave it to settle for 5 min and aspirate 30 ml of the supernatant; 6) pour the pellet into a Petri dish and analyse it under a stereomicroscope at 50x magnification. Some small pieces of the infected samples used for the artificial digestion were also examined by trichinoscope using the methodology previously described.

3. Results

Regarding the validation of the new method, metacercariae in 5 artificially infected pieces of fillet were visualized through a trichinoscope. After the artificial digestion procedure, all parasitic forms used in the artificial infection of the samples were recovered in the 10 ml of the final decantation volume (Figure 2). Thus, the sensitivity shown by the method was 100%, having been validated to analyse the rest of the samples.

The 50 striped catfish fillets examined helminthologically, by trichinoscopy and artificial digestion, were negative for trematode metacercarie and other helminth pathogens.

Figure 1. Artificial digestion method proposed: A) homogenization of samples with artificial digestion solution; B) pour the mixture into a beaker together with additional digestion solution until reaching a total volume of 200 ml; C) incubation at 37°C for 30 min; D) filter the solution and leave it to settle for 30 min; E) recover 40 ml of the solution, leave it to settle for 5 min and aspirate 30 ml of the supernatant; F) pour the pellet into a Petri dish and analyse it under stereomicroscope at 50x magnification.

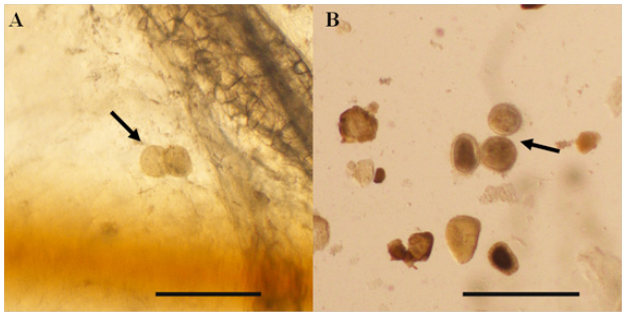

Figure 2. Views of trematode metacercariae (arrows) observed in the artificially infected samples: A) in the fish flesh under a trichinoscope; B) in the decantation volume of the artificial digestion under a stereomicroscope. Scale bar: 500 µm.

4. Discussion

A wide range of freshwater fish species has been shown to be naturally infected with zoonotic liver and intestinal trematodes [3], [12], acting as their second intermediate host in their life cycle. The definitive host (humans among other mammals) becomes infected when ingesting raw or undercooked fish parasitized by the infective metacercariae [13], being able to generate several serious clinical problems or even a person's death [14]. The transmission of fish-borne trematodiases is restricted to areas where the first and the second intermediate hosts coexist and where humans have the habit of eating raw, pickled, or insufficiently cooked fish [15], turning these zoonoses into serious public health concerns, especially in Asian countries. Nevertheless, the growth in international trade in fish and fish products as well as the spectacular boom in aquaculture, among other reasons, have lead to a significant increase of these diseases in recent years worldwide [4].

Spain is one of the main importer countries of the striped catfish from Vietnam [16], and the Spanish Consumer Agency affirms that this product is commercialized only once it has passed all safety checks, including appropriate laboratory analyses [17]. Some studies show, in fact, a low metal concentration in this fish [18], but there is no public data about other pathogens that are also of concern for humans, such as fish-borne trematodes. However, the striped catfish has proven to be a potential host of zoonotic trematodiases, since the detection of metacercariae of *Clonorchis sinensis* and *Haplorchis* spp. [19-20] in this fish. So far, there have not been any human cases reported associated with the consumption of this product, but the detection of the infective forms should not be overlooked, since the risk of infection does in fact exist. The present article is, as far as we know, the first helminthological survey in striped catfish carried out in Spain, showing that none of the analysed fish was parasitized by trematode metacercarie or other helminth species. Thus, taking into account the present results, as well as the fact that the fish is deep frozen, sometimes for many months, from being packed at its origin, it can be ascertained that the imported striped catfish does not represent a source of helminth parasitic forms. Consequently, although the results are preliminary and need to be confirmed after the analysis of a larger number of fish specimens, it may be concluded that the consumption of this product does not involve a risk for consumers.

The presence of trematode metacercariae in fish fillets is checked by means of artificial digestion, which allows the collection of live parasites and enables the morphological and/or molecular identification at species/genotype level as well as other laboratory assays such as in vitro culturing or artificial infection of laboratory animals [9]. However, although the technique is sensitive and allows analysing the viability of the parasitic forms detected, the standard procedures are not considered suitable for industrial inspections, and it is only recommended for the examination of a small number of samples or an accurate analysis as this technique is laborious and time-consuming. Other methods for the detection of metacercariae in fish, which are, unfortunately, also time-consuming and can only be applied in small specimens, have been proposed [21]. Therefore, optimizing current fish inspection techniques is imperative in order to guarantee the parasitological safety of these products. In the present article a modification of the reference methods [1], [9] has been validated, obtaining a sensitivity of 100%. Additionally, a smaller amount of the sample as well as reagents, in comparison to the original methods, were used. Consequently, this optimized method is proposed to be used routinely in epidemiological studies on trematode metacercariae detection in freshwater fish.

References

1. Worl Health Organization (WHO). Control of foodborne trematode infections. WHO Tech Rep Ser. 1995; 849:1-157.

2. World Health Organization (WHO). Report of Joint WHO/FAO Workshop on foodborne trematode infections in Asia, Hanoi, Vietnam, 26-28 November, 2002. Geneva: World Health Organization; 2004.

3. Chai JY, Murrell KD, Lymbery AJ. Fish-borne parasitic zoonoses: status and issues. Int J Parasitol. 2005; 35(11-12):1233-1254.

4. Lima dos Santos CAM, Howgate P. Fishborne zoonotic parasites and aquaculture: A review. Aquaculture. 2011; 318:253-261.

5. Lun ZR, Gasser RB, Lai DH, Li, AX, Zhu XQ, Yu XB, Fang YY. Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect. Dis. 2005; 5(1):31-41.

6. Chai JY. Intestinal flukes. In: Murrell, K.D., Fried B, editor. Food-borne parasitic zoonoses: fish and plant-borne parasites. New York: Springer Science; 2007. p. 53-116.

7. Kaewipitoon N, Kaewipitoon SJ, Pengsa AP, Sripa B. *Opisthorchis viverrini*: the carcinogenic human liver fluke. World J Gastroenterol. 2008; 14:666-674.

8. Thu ND, Dalsgaard A, Loan LT, Murrell K. Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang Province, Vietnam. Korean J Parasitol. 2007; 45(1):45-54.

9. Instituto Superiore Di Sanità (ISS). European Union Reference Laboratory for Parasites. Artificial digestion of fish fillets for the isolation of Anisakidae and Opisthorchidae larval stages. 2006 [cited 2015 Oct 15]. Available from: http://www.iss.it/binary/crlp/cont/SOP_Artificial_digestion_of_fish_fillet.pdf.

10. New Zealand Food Safety Authority (NZFSA). Import risk analysis: frozen, skinless and boneless fillet meat of *Pan-*

gasius spp. fish from Vietnam for human consumption. 2008 [cited 2015 Oct 15]. Available from: http://www.biosecurity.govt.nz/files/regs/imports/risk/*Pangasius*-risk-analysis.pdf

11. Nöckler K, Pozio E, Voigt WP, Heidrich J. Detection of *Trichinella* infection in food animals. Vet Parasitol. 2000; 93(3-4): 335-350.

12. Adams AM, Murrell KD, Cross JH. Parasites of fish and risks to public health. Rev Sci Tech Oie. 1997; 16:652-660.

13. Hung NM, Madsen H, Fried B. Global status of fish-borne zoonotic trematodiasis in humans. Acta Parasitol. 2013; 58:231-258.

14. Fürst T, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis. 2012; 12(3):210-221

15. Keiser J, Utzinger J. Food-borne trematodiases. Clin Microbiol Rev. 2009; 22:466-483.

16. Halls AS, Johns M. Assessment of the vulnerability of the Mekong Delta *Pangasius* catfish industry to development and climate change in the Lower Mekong Basin. Report prepared for the Sustainable Fisheries Partnership. 2013 [cited 2015 Oct 15]. Available from: http://cmsdevelopment.sustainablefish.org.s3.amazonaws.com/2013/01/22/*Pangasius*%20Mekong%20Delta-4b2036ad.pdf

17. Agencia Española de Consumo, Seguridad Alimentaria y Nutrición [Internet]. Spain: Agencia Española de Consumo, Seguridad Alimentaria y Nutrición; 2014 [cited 2015 Oct 15]. Available from: http://www.aesan.msssi.gob.es/SIAC-WEB/pregunta.do?reqCode=retrieve&bean.id=3118

18. Olmedo P, Pla A, Hernández AF, Barbier F, Ayouni L, Gil F. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ Int. 2013; 59:63-72.

19. Nguyen DT, Dalsgaard A, Ly TTL, Murrell KD. Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang Province, Vietnam. Korean J Parasitol. 2007; 45(1):45-54.

20. Bình ĐT, Hạ-Quyên VĐ, Thu-Hà LT, Sáng TQ, Kiên ND. Parasitic metacercariae infected in fish species based on morphological and genetic characters. Tạp chí Khoa học Trường Đại học Cần Thơ. 2014; 2:15-23.

21. Majoros G. A simple staining method for the visualisation of metacercariae in small fish and tadpoles. Folia Parasit. 2001; 48:73-75.