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UV-press method versus artificial digestion method to detect Anisakidae L3 in fish fillets: Comparative study and suitability for the industry

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ABSTRACT

To screen the presence of Anisakidae third stage larvae (L3) in fish, fast methods such as the visual inspection and candling have been widely used by the industry over the last 50 years, and they are regulated by the European Parliament and the Council. These methods are ineffective to detect L3 embedded in fish muscles, consequently alternative methods, such as the artificial digestion (AD) and the UV-Press (UVP) are increasingly applied, but their performance needs to be evaluated. The aims of the present work were: 1) to compare the performance of AD and UVP. methods by a Ring Trial (RT) involving highly experienced laboratories; and 2) to evaluate the potential transferability of the best performing method to the industry by a collaborative study involving industrial partners (β-testing). For RT, each participating laboratory (n = 5) received 6 samples of 100 g of fish fillets spiked with 1 L3 (1 sample), 3 L3 (2 samples), 7 L3 (2 samples), and a negative control sample (without L3). In each positive sample, there were live *Anisakis pegreffii* L3 collected from a naturally infected fish. The result evaluation was based on the agreement between the number of reported and the number of spiked L3. No false positive sample was detected. The L3 number detected by the UVP method showed higher (90%) level of agreement with the number of spiked L3 than the number of L3 detected by the AD method (83.3%); however, no significant difference in terms of accuracy (p = 0.32) was detected when the two methods were compared. Moreover, considering only the presence/absence of L3 in the samples, the UVP reached 100% of accuracy and 100% of sensitivity; whereas, AD showed 98% of accuracy and 96% of sensitivity. The variability of the UVP method was lower than that of the AD method, indicating a better reproducibility. On the basis of the RT results, the UVP method was selected for the β-testing. Each industrial partner (n = 3) received 15 samples of 100 g of fish fillet spiked with 1 L3 (2 samples), 2 L3 (2 samples), 3 L3 (2 samples), 4 L3 (2 samples), 5 L3 (2 samples) and 6 L3 (2 samples) and three negative control samples (without L3). The number of L3 counted in 34 out of 45 samples (75.6%) by the UVP method was in agreement with the number of spiked L3. One company reached 93.3% of agreement; whereas the other two companies reached an agreement of 66.7%. Two false negative results were found; whereas, no false positive results were obtained. Moreover, at the industrial level, considering only the presence/absence of larvae in the samples, the UVP reached 97% of accuracy, 94.4% of sensitivity, and 100% of specificity. However, the UVP method, in spite of its accuracy, needs further investigations to provide new time –temperature combinations that could allow a reduction of the testing time and its integration in the fishing deck.

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1. Introduction

The fish-borne parasitic zoonosis caused by nematodes of the genera *Anisakis* and *Pseudoterranova* known as anisakidosis, has been recognized as an important public health problem (Audicana and Kennedy, 2008; EFSA, 2010; Pozio, 2013). In addition to infections caused by ingestion of live larvae, allergic reactions to *Anisakis* proteins can also occur after ingestion of cooked or canned fish, although a prior exposure to live larvae seems to be necessary (Nieuwenhuizen and Lopata, 2014).

Currently, the geographical limits and human populations at risk of anisakidosis are expanding because of popularization of ethnic food (e.g. Oriental cuisine), human migration, growing international markets and improved transportation systems (Chai et al., 2005). Therefore, there is a change of the control responsibility in the fishery chain, which must be taken into account (D'amico et al., 2014). In the European Union, General Provisions of the Hygiene Package establish co-responsibility of the Food Business Operator (FBO) to produce safe fishery products (EC/178, 2002). Nevertheless, FBOs are not always properly trained in the use of techniques for the control of anisakid infections.

Over the last 50 years, non-invasive and fast methods, like visual inspection and candling, regulated by the European Parliament and the Council (EC/853, 2004; EC/854, 2004), have been widely used by industry for parasite screening in seafood. These methods are relatively ineffective and have the disadvantage that they cannot be applied for the analysis of processed products (Levsen et al., 2005). Even if the EU legislation (EC 2074/2005; EC 853/2004) recommends the visual inspection of fish gut for Anisakidae larvae, Llarena-Reino et al. (2012) evidenced the low efficiency of this method for predicting nematode larvae in fish fillets.

Other methods as the artificial digestion (Tejada et al., 2007) and UV-Press (UVP) of fish fillets for Anisakidae parasites (Karl and Leinemann 1993; Levsen and Lunestad 2010) are increasingly applied, especially in large-scale scientific surveys. The CODEX Alimentarius presents a digestion method aimed at assessing the viability of nematodes detected in fish, applicable only to salted Atlantic herring (*Clupea harengus*) and salted sprat (*Sprattus sprattus*) (CODEX STAN 244-2004). Llarena-Reino et al. (2013) performed an optimization of the pepsin digestion method for anisakid inspection in the fishing industry, which reduces the assay time and resulted to be more handy and efficient than the method reported by the CODEX. However, the performance in terms of accuracy, sensitivity and specificity of all these procedures are unknown. Therefore, within the 7th FP European project PArasite Risk ASsessment with Integrated Tools in EU fish production value chains (PARASITE, http://parasite-project.eu/), an inter-laboratory collaborative (Ring Trial, RT) study was organized. This study was carried out among experienced laboratories and was aimed to determining and comparing the performance of both the artificial digestion (AD) and UVP methods in detecting Anisakidae larvae (L3) in fish fillets. Moreover, to test the potential transfer of the best performing method to the industry, a β-testing was organized. Before the participation in the β-testing exercise, industrial partners were trained through the participation in specific training workshops.

2. Materials and methods

2.1. Ring trial (RT)

2.1.1. RT test material

The material forwarded to each laboratory consisted of a panel of 6 samples of ~100 g of fish fillet sandwiches from Anisakidae-free rainbow trout (*Oncorhynchus mykiss irideus*) farmed in freshwater. Each sandwich was spiked in the core with a different number of live L3 *Anisakis pegreffii* (Table 1) collected from the coelomic cavity of highly infected fish (silver scabbard fish). A known number of L3 live larvae free of their capsule, were carefully transferred by tweezers into an opened pocket in a fish fillet and a sandwich was prepared with another fish fillet. Single fish fillet sandwiches were individually preserved in vacuum-sealed plastic bags and stored at +4 °C, allowing the preservation of fish fillet freshness and assuring the L3 survival. Each sample was labelled with a unique code without any indication of the level of contamination or any information on the identity of the testing laboratory. Before shipping, each fillet sandwich was weighted and the value registered. Since samples for the detection of L3 were made by individually spiked samples, homogeneity was ensured by an accurate control of the number of L3 spiked into each sample by two operators.

Table 1
Ring trial samples and results. L3, *Anisakis pegreffii* larva.

Lab Code	Sample code	No. of L3 in the sample	No. of L3 detected by UVP*	No. of L3 detected by AD**
P1	P01	7	7	7
	P02	0	0	0
	P03	3	3	3
	P04	3	3	3
	P05	7	6#	7
	P06	1	1	1
P2	P07	7	7	7
	P08	1	1	0
	P09	7	7	7
	P10	3	3	3
	P11	0	0	0
	P12	3	3	2+1‡
P3	P13	3	3	3
	P14	7	7	7
	P15	7	5+2‡	5+2‡
	P16	1	1	1
	P17	0	0	0
	P18	3	2	2
P4	P19	3	3	3
	P20	0	0	0
	P21	7	7	7
	P22	1	1	1
	P23	7	7	7
	P24	3	3	2
P5	P25	0	0	0
	P26	7	7	7
	P27	1	1	1
	P28	3	3	3
	P29	3	3	3
	P30	7	7	7

*UVP: UV-Press method.
**AD: artificial digestion method.
#Boxed: L3 number found in disagreement with the L3 spiked number.
‡Entire L3 plus one or two L3 fragments.

For the shipping, each sample panel was further sealed under vacuum in a larger bag, which was inserted in a polystyrene box. Ice packs were placed into the box to maintain the temperature between 4 °C and 10 °C during transportation. The stability of the samples in this range of temperature and packing conditions had been previously evaluated by ad hoc experiments carried out at the European Union Laboratory for Parasites (Rome, Italy), in which L3 in the vacuum-sealed samples stored at 4 °C–10 °C were viable for at least 2 weeks after the preparation.

2.1.2. Participating laboratories

Five highly qualified experienced laboratories participated to the RT, namely: L'Agence Nationale de Sécurité Sanitaire de l'Alimentation, Boulogne sur Mer (France); Department of Public

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