Rapid Method for Detection of Anisakidae Larvae in Marine Fishes, Based on UV Transillumination

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***Abstract*—A rapid and sensitive detection method is proposed to evaluate the presence and vitality degree of Anisakidae larvae in marine fishes by UV diaphanoscopy. 923 fish samples belonging to seven fish species (*Merluccius merluccius*, *Molva elongata*, *Sardina pilchardus*, *Scomber scomber*, *Triglia lucerna*, *Engraulis encrasicolus*, and *Trachurus trachurus*) were evaluated for the presence of Anisakidae larvae by using direct observation, peptic digestion, and UV transillumination. The results obtained indicate that UV transillumination can be considered a rapid and accurate tool for routine analysis of marine fishes even under field condition.**

***Index Terms*—Anisakidae, larvae, detection, UV, transillumination.**

I. Introduction

Anisakiasis, first reported in the Netherlands in the early 1960s, is a fish-borne parasitic disease caused by consumption of raw or undercooked fish or cephalopods contaminated by third larval stage of the Anisakidae family parasites, in particular *Anisakis simplex*, *A. pegreffii* and *Pseudoterranova decipiens* [1].

The prevalence and intensity of *Anisakis* infection varies with fish species, fishing area, and season [2]. Marine mammals or piscivorous birds are typically used as definitive hosts, while planktonic or benthic crustaceans act as intermediate hosts, and fish as principal transport hosts. Infection is caused by the larval stages carried by numerous fish species. Adult *A. simplex* usually live in the gastrointestinal tract of cetaceans, while the adults of *Pseudoterranova* spp. and *Phocascaris* spp. are mainly found in pinnipeds, although the definitive host range of many anisakid species is not well understood [3]. Alternative transmission routes have been suggested, such as direct infection of fish by larval stages ingestion, or the transfer of larvae from crustaceans [4]. Female worms lay eggs, which are shed in the faeces of infested marine mammals and embryonate in seawater to second-stage larvae that hatch out [5]. Then, the first intermediate hosts are eaten by fish and cephalopods, where the third-stage larvae encyst. Finally, the life cycle is completed when marine mammals eat infected fish or cephalopods.

Human infection may be considered accidental, as humans are not suitable hosts for anisakids.However, these parasites have been involved in foodborne infections caused by consumption of raw or undercooked seafood. Reports about detection of anisakids in different fish batches, in particular in the autumn-winter season, have drawn public attention to their potential impact on public health.

Several types of parasite detection methods have been proposed for anisakids, but they are all time-consuming and difficult. In fact, different DNA-based techniques are available [6]-[8], although their applicability in production environment is debatable.In the European Union, the official method for larvae detection (Council Directive 91/493/EEC) is direct observation (Reg. 2074/2005/EC), sometimes coupled with chlorine-peptic digestion. The aim of this study was to compare the official protocol with a new observation method, based on UV transillumination, in order to evaluate larvae location and degree of infestation in seven fish species.

II. Materials and Methods

923 fish samples belonging to seven fish species (*Merluccius merluccius*, *Molva elongata*, *Sardina pilchardus*, *Scomber scomber*, *Triglia lucerna*, *Engraulis encrasicolus*, and *Trachurus trachurus*) were analysed. Anisakidae larvae location and degree of infestation were evaluated by using direct observation, chlorine-peptic digestion and UV transillumination.

In all samples, the count was determined separately in the coelomic cavity, in the hypoaxial and epiaxial muscles, by careful dissection and direct observation of the various anatomical districts, according to the procedure described in Reg. 2074/2005/EC.

Peptic digestion was carried out by means of Trichomatic 35 (Foss Electric, Denmark). The samples were subjected to digestion with hydrochloric acid 9% (30 ml) and pepsin 1/10000 (5 g) for each cycle of 8 min. Digested samples were laid on membranes, and observed by optical microscopy at 10 x.

For UV transillumination, the method described by [9] was modified as follows: 1. saline solution (1:5 w/v) at 30 ℃ was added to fish samples, which were then homogenized in stomacher for 1-2 min; 2.the homogenate was pressed to a thin layer of 5 mm and examined under UV light (TransilluminatorFotodine 3-3102, Celbio, Italy) at 366 nm in a dark room. In such operating conditions, the larvae

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remain intact, andAnisakis spp. shows bluish-white fluorescence [10].

III. Results and Discussion

The results obtained by comparing direct observation, chlorine peptic digestion, and UV transillumination, are shown in Table I. Our data indicate that peptic digestion was not suitable for anisakids detection, because only fragments of larvae were recovered.

TABLE I: Distribution of Anisakidae Larvae in Different Anatomical Regions of Seven Fish Species

Species	Samples (total number)	Method of analysis											
		Direct observation				Chlorine peptic digestion				UV light observation			
		Total number of larvae	Coelomic cavity	Hypoaxial muscle	Epiaxial muscle	Total number of larvae	Coelomic cavity	Hypoaxial muscle	Epiaxial muscle	Total number of larvae	Coelomic cavity	Hypoaxial muscle	Epiaxial muscle
Engraulisencrasicolus	95	-	-	-	-	N.D.	N.D.	N.D.	N.D.	11	6	2	3
Sardinapilchardus	106	2	2	-	-	N.D.	N.D.	N.D.	N.D.	14	9	3	2
Triglialucerna	54	6	6	-	-	N.D.	N.D.	N.D.	N.D.	27	22	5	-
Molvaelongata	96	7	7	-	-	N.D.	N.D.	N.D.	N.D.	49	39	11	-
Trachurustrachurus	103	132	130	2	-	N.D.	N.D.	N.D.	N.D.	243	275	18	-
Scomberscomber	207	312	273	33	6	N.D.	N.D.	N.D.	N.D.	462	358	85	19
Merlucciusmerluccius	262	632	581	48	3	N.D.	N.D.	N.D.	N.D.	937	465	378	74

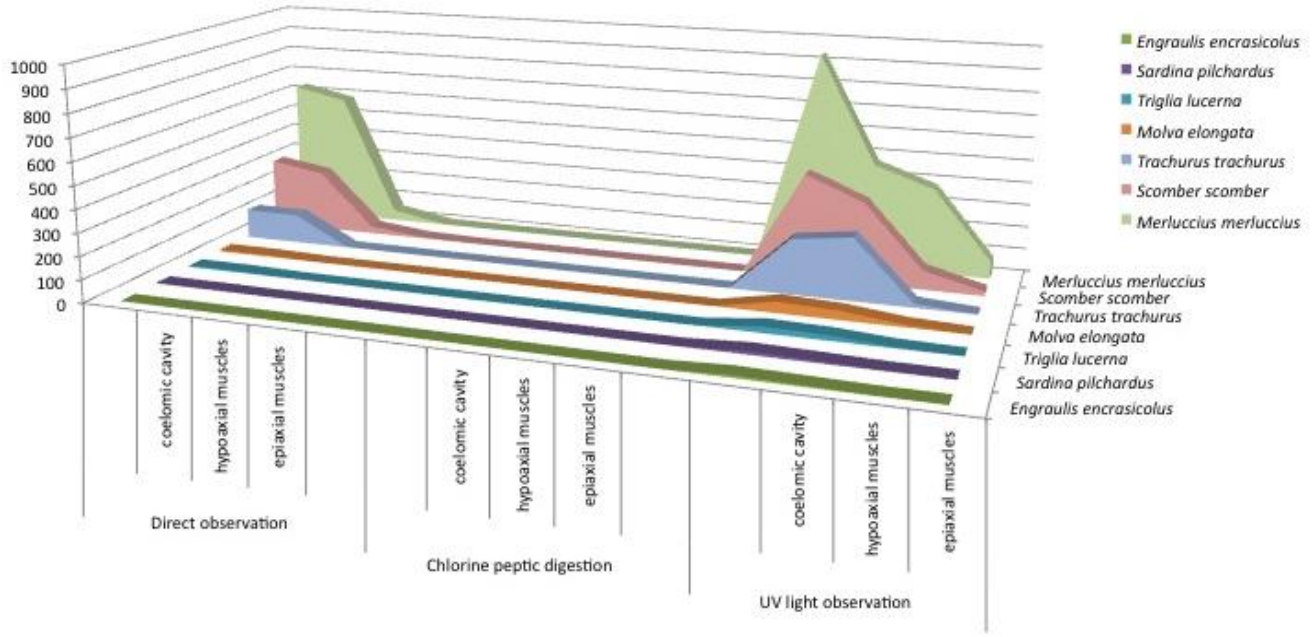

Fig. 1. Effect of fish species on the number of recovered larvae.

By comparing data obtained with visual inspection and UV transillumination, it is clearly evident that visual inspection was less effective in larvae detection. In UV transillumination, the larvae with a size from 1 to 3 cm were white fluorescentand could be easily differentiated from muscle fibres.In a large number of samples (data not shown), the nematodes were moving actively and were clearly visible under UV transillumination.All positive samples were identified by light microscopy as Anisakis simplex, following the morphological criteria proposed by [11].

Fig. 1 shows the distribution of positive samples in the different anatomical regions of the seven fish species, analysed by the three methods used in this study. Our data suggest that the degree of infestation of the coelomic cavity is not in correlation with the degree of infestation and localization in the muscle, but may be related to fish species.

In particular, the nematodes detected in the coelomic cavity were 93.9% of total larvae in Trachurustrachurus, 81.5% in Triglialucerna, 78% in Molvamacrophtalma, 77.5% in Scomberscombrus, 64.3% in Sardinapilchardus, 54.5% in Engraulisencrasicolus, and 50.7% in Merlucciusmerluccius. Conversely, in Oncorhynchus salmon from Alaska, more than 90% of Anisakis larvae were found in the flesh, mainly in the hypaxial muscle [12].

In our study, the recovery from muscle tissues wasremarkably higher in the samples analysed by UV transillumination. The same method can also be used to distinguish viable from dead larvae by colouring the larvae with several dyes or adding tetrazolium chloride [13].

According to our data, visual inspection of the coelomic cavity is not a suitable method to predict the presence of nematodes in the edible tissues. Our results are in agreement with [14], who have recently found a poor statistical significance between the number of Anisakis larvae in the abdominal cavity and the number of parasites in the edible part of Micromesistiuspoutassou and Scomberscombrus.

IV. Conclusions

Our findings indicate that the official method used in the European Union for anisakids detection in fish samples, based on visual inspection of the whole fish abdominal cavity and gut, does not provide a sufficient guarantee of larvae recovery, particularly in epaxial and hypaxial muscles.

To control the risk of viable parasites, accurate inspection of fish muscles appears the only scientific criterion to estimate infestation in edible tissues. In this respect, UV transillumination can be considered a rapid alternative tomolecular methodswhen large sample amounts need to be examinedquantitatively. In future studies, we will evaluate the effectiveness of UV transillumination in processed fish samples. Moreover, investigating how this method applies to industrial environment would be of great interest.

In conclusion, we believe that the method proposed in this studycan be applied to upgrade detection of Anisakis spp. in the edible part of the fish.Furthermore, theproceduredescribed in this paper is rapid and accurate, does not modify parasite morphology, and does not seem to affect larvae viability. This method can be considered a valuable tool for routine analysis of marine fishes even under field condition.

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