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Anisakis spp. in European hake, *Merluccius merluccius* (L.) from the Atlantic off north-west Africa and the Mediterranean off southern Spain[1]

Adela Valero*, María del Mar López-Cuello, Rocío Benítez and Francisco J. Adroher

Departamento de Parasitología, Facultad de Farmacia, Universidad de Granada, 18071-Granada, Spain

Abstract

We studied the parasitization by *Anisakis* spp. in European hake (*Merluccius merluccius*) of 40–52 cm from the Atlantic off north-west Africa and the Mediterranean off southern Spain. Infection parameters differed: The fish from the Atlantic showing a prevalence of 87.97% and a mean intensity of 4.69, while, in those from the Mediterranean, these were 41.27% and 1.73, respectively. In both samples the two-third larval stage types were isolated: *Anisakis simplex* sensu lato and *Anisakis* larvae type II, with prevalence of 85.71% and 30.83% in fish from the Atlantic and 41.27% and 1.59% for those from the Mediterranean, respectively. In both samples, the prevalence of larvae in viscera was clearly higher than in the muscle tissue. We also observed an increase in parasitization with increasing host length, those ≥46 cm having the highest prevalences (94.87% for those from the Atlantic and 58.33% for those from the Mediterranean; $p<0.03$).

Key words

Epidemiology, *Anisakis*, *Merluccius merluccius*, Atlantic and Mediterranean fishes

Introduction

Two decades ago, anisakidosis was still relatively unknown in Western Europe (Lucas *et al*. 1985, Mudry *et al*. 1986), except in Holland, and protection against parasites in fish for human consumption was not a priority. Nowadays, however, health authorities and the fishing industry are increasingly becoming aware of the problems caused by these parasites, which affect both human health and the commercial value of the product. Species of the family Anisakidae have acquired importance due to the traumatic effect produced by the third larval stage when it becomes attached to the wall of the human digestive tract (Ooiwa *et al*. 1989, Kikuchi *et al*. 1990, Ishikura *et al*. 1992, Louredo Méndez *et al*. 1997) and to allergic episodes caused by their metabolic antigens following ingestion of the parasitized fish (Mendizábal-Basagoiti 1999, López-Serrano *et al*. 2000, Foti *et al*. 2002). Over the last decade a number of cases of anisakidosis in man have been reported in Europe. However, in spite of these nematodes being relatively frequent in fish, measures to prevent infection are obviously difficult to apply, since, in marine animals this parasitosis is determined by multiple ecological factors, such as parasite burden of final hosts, occurrence of intermediate hosts, fish stocks and fishing ground (Angot and Brasseur 1995). The measures taken after the capture and processing of the product (evisceration, freezing of fish immediately after catching and incineration of their viscera) are important in the prevention of human illness. Also of interest are epidemiological studies carried out to determine which commercial species are most parasitized and the geographical zone of fish catching. We thus decided to study the parasitization by *Anisakis* spp. in *M. merluccius*, especially in view of its commercial value and popularity.

This survey shows that the hake are parasitized with the third larval stage of the anisakids *A. simplex* sensu lato and *Anisakis* larvae type II, those from the Atlantic off north-west Africa showing a higher prevalence than those from the Mediterranean off southern Spain.

Materials and methods

We studied 133 hake (*M. merluccius*) from the Atlantic off north-western Africa and 63 from the Mediterranean off southern Spain acquired from two fishmarkets in Granada during the years 1998–1999. The fish were chosen at random with a length of 40–52 cm. Host identification was carried out according to Lloris *et al*. (2003) and the fish measured to the nearest 0.1 cm. After dissection and isolation of the free larvae

*Corresponding author: avalero@ugr.es

[1]This paper is dedicated to the memory of Prof. Dr Ignacio Navarrete López-Cózar, DVM, recently deceased.

Table I. *Anisakis* infection parameters of European hake from different fishing zones

Fishing zones		*Anisakis* spp. total	*Anisakis* spp. viscera	*Anisakis* spp. musculature	*A. simplex* s.l.	*Anisakis* larvae type II
Atlantic off north-west Africa	prevalence (F = 133)	87.97	84.96	19.55	85.71	30.83
	mean intensity	4.69	4.58	1.19	4.11	1.54
	(range)	(1–29)	(1–24)	(1–4)	(1–24)	(1–5)
	mean abundance	4.13	3.89	0.23	3.65	0.47
Mediterranean off southern Spain	prevalence (F = 63)	41.27	41.27	6.35	41.27	1.59
	mean intensity	1.73	1.58	1	1.69	1
	(range)	(1–4)	(1–4)	(1)	(1–4)	(1)
	mean abundance	0.71	0.65	0.06	0.70	0.02

Prevalence = 100·N/F, mean intensity = P/N, mean abundance = P/F; F = number of examined fish, N = number of infected fish, P = number of parasites.

found in the body cavity, the viscera and hypoaxial and epiaxial muscles were subjected to digestion by immersion in pepsin and HCl solution as described by McGladdery (1986), at pH 2–2.3 and 36°C for four hours. The larvae were then isolated and washed by immersion in NaCl solution at 0.9% w/v. Subsequently, after preserving in 70% ethanol, they were cleared in lactophenol. For morphological identification we followed the work of Grabda (1976) and Petter and Maillard (1988) for *A. simplex* s.l. (including *A. simplex* sensu stricto, *A. pegreffii* and *A. simplex* C) and Petter and Maillard (1988) for *A. physeteris*. No electrophoretic diagnosis was carried out. Recently, Mattiucci *et al*. (2005) carried out genetical studies of *Anisakis* by multilocus allozyme electrophoresis, showing that *A. physeteris*, *A. brevispiculata* and *A. paggiae* form a first clade, and the other species of the genus *Anisakis* form a second clade (*A. simplex* s.l., *A. typica* and *A. ziphidarum*). The L3 larval morphology is type II (sensu Berland 1961) for the species of the first clade but type I (sensu Berland 1961) for the species of the second clade. In this sense, we must reconsider our provisional identification of *A. physeteris* L3 as *Anisakis* L3 type II, until genetical studies can be carried out. To analyze the data, we used Fisher's exact test for comparing prevalences and bootstrap 2-sample t-test for comparing mean intensities and mean abundances. For these analyses, we used the free QP 3.0 software designed to deal with the notoriously left-biased frequency distributions of parasites and performed by Reiczigel and Rózsa (2005, Quantitative Parasitology 3.0, Budapest; http://bio.univet.hu/QP/) based on the theoretical background published by Rózsa *et al*. (2000).

Results

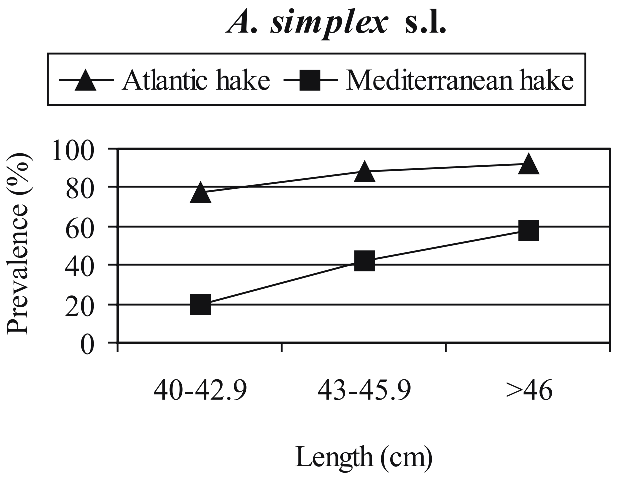
A total of 549 *Anisakis* larvae, all in the third stage (L3), were taken from the hake captured in the Atlantic, with a prevalence of 87.97%, mean intensity of 4.69 and mean abundance of 4.13. Two species were found: 486 larvae of *A. simplex* s.l. and 63 *Anisakis* larvae type II (sensu Berland 1961) with prevalences of 85.71% and 30.83%, respectively (Table I). Larvae were found in the muscle tissue of 26 hosts; 29 *A. simplex* s.l. and 2 *Anisakis* larvae type II. Prevalence was higher in the hypoaxial muscle (16.24%) than in the epiaxial (5.98%), the differences being significant ($p = 0.002$).

From the Mediterranean hake, 45 larvae were isolated (all L3), only one of which was *Anisakis* larvae type II. Total prevalence was 41.27% (Table I). Four larvae of *A. simplex* s.l. were isolated from the hypoaxial muscle and none from the epiaxial.

In the study of infection parameters according to host length, anisakid prevalence in both samples was lower for fish of 40–42.9 cm, with values of 77.27% for the Atlantic (n = 44) and 20% for the Mediterranean (n = 20), increasing to 94.87% (n = 39) and 58.33% (n = 24) in those of ≥46 cm, respectively ($p<0.03$ and $p<0.02$). The Figure 1 shows these data separately for *A. simplex* s.l. and *Anisakis* larvae type II.

Discussion

The data obtained (Table I) show that the two hake samples examined hosted L3 of *Anisakis* spp., although the prevalences were different ($p<0.0001$). The data provided by Huang (1988) for hake from different markets in Paris showed prevalence for *A. simplex* of 88.57% and an intensity range of 2–254 larvae. Our data for prevalence of *A. simplex* s.l. (85.71%) in Atlantic hake are very close to those of Huang, although they differ with regard to the range of larvae found, since, in our case, none of the examined fish contained more than 24 larvae. Pereira Bueno (1992) studied the parasitization by anisakids in hake from the markets of Bilbao (northern Spain), proceeding from several Spanish ports, finding preva-



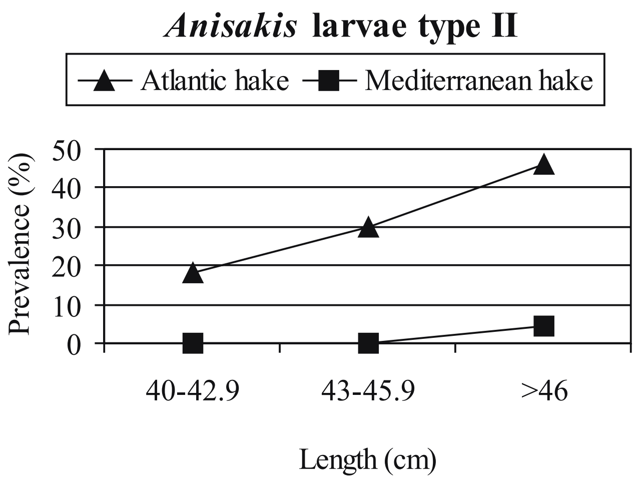


Fig. 1. Prevalence of *Anisakis simplex* s.l. (top) and *Anisakis* larvae type II (bottom) in European hake from the Atlantic off north-west Africa and the Mediterranean off southern Spain according to fish length. The number of examined hosts per length class was as follows: 44 (40–42.9 cm), 50 (43–45.9 cm) and 39 (≥46 cm) for Atlantic fish and 20 (40–42.9 cm), 19 (43–45.9 cm) and 24 (≥46 cm) for Mediterranean fish. Prevalence is significantly associated to host length for both anisakids in Atlantic and Mediterranean fish (Fisher's exact test for comparing prevalences, $p<0.04$)

lence of anisakids of 45.45% and a mean intensity of 63.60. Although the prevalence found by this author is similar to ours for the Mediterranean hake (41.27%), it is very different for the Atlantic hake (87.97%) as well as for the mean intensities (Table I). These differences may be due to the different geographical origins of the fish. The infection parameters of different fish hosts vary from one geographical zone to another (Adroher *et al*. 1996, Valero *et al*. 2000, Rello 2003). According to Angot and Brasseur (1995), these fluctuations are the result of diverse biological factors such as: (a) the level of infection of marine mammal final hosts; (b) the abundance of obligatory intermediate hosts; and (c) the importance of each fish stock in the trophic chain since it includes all natural hosts which host the stages of the life cycle of the parasites and the trophic relationship among the all hosts. With regard to *Anisakis* larvae type II, prevalence and mean intensity were low in both our samples (Table I). Mattiucci *et al*. (2004) reported the occurrence of larvae of seven *Anisakis* species, which can be used as biological tags for hake, suggesting the existence of different stocks of *M. merluccius* since the migration of hake from Atlantic to Mediterranean waters appears not to occur. These authors showed only *A. pegreffii* and *A. physeteris* in hake from western Mediterranean Sea (prevalence: 16–30% and 7–22%, respectively) and six species of *Anisakis* off Morocco, the most prevalent being *A. pegreffii* (68.8%) and *A. physeteris* (17.7%). Other species of *Anisakis* had prevalences <10% (*A. simplex* s.s., *A. typica*, *A. ziphidarum* and *A. brevispiculata*). According to these data from Mattiucci *et al*. (2004), probably the larvae of *Anisakis* from the Mediterranean off southern Spain collected in this survey are *A. pegreffii* and *A. physeteris*. Likewise, the larvae from the Atlantic off north-west Africa probably are *A. pegreffii* and *A. physeteris*, although in this case larvae from other species, such as *A. simplex* s.s. and *A. brevispiculata*, respectively, can occur.

In our two samples the prevalence of larvae in viscera was higher than in the muscle tissue (Table I), these differences being statistically significant ($p<0.0001$). The data reported by Pereira Bueno (1992) for anisakids (probably *Anisakis*) in the muscle tissue of hake showed prevalence of 33.33% and mean intensity of 73.64. These figures are much higher than those we obtained, both for fish from the Atlantic and those from the Mediterranean (Table I). Although it is difficult to determine the factors responsible for the distribution of anisakid larvae in fish, Smith and Wootten (1975) and McGladdery (1986) observed in herring that parasitization by *Anisakis* in muscle tissue was greater in those geographical areas where parasitization of the body cavity was more frequent and intense. On the other hand, we observed that total prevalence was higher in the hypoaxial than in the epiaxial muscles ($p = 0.002$), these findings agreeing with those of Herreras *et al*. (2000) in *M. hubbsi*. Smith and Wootten (1975) suggested that this may be due to the fact that the hypoaxial muscles, which surround the body cavity, are the nearest to migrating larvae. However, Strømnes and Andersen (1998) suggest that L3 *Anisakis* distributions are governed by the conditions encountered within host tissues such as the fat content. When infection parameters are studied in relation to fish length it can be seen that these increase significantly in both samples with increasing length (Fig. 1, $p<0.04$). Several studies confirm this relationship between length and parasitization in other hosts (Takao 1990; Hemmingsen *et al*. 2000; Valero *et al*. 2000, 2006).

Further work is necessary to clarify *Anisakis* taxonomy and anisakidosis pathogeny in order to add new information in epidemiological studies and to evaluate the real risk of anisakidosis by hake and other fish consumption, although the report of hybrid specimens (Abollo *et al*. 2003, Martín-Sánchez *et al*. 2005) constrains this research.

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