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Short communication

Survey of anisakids in commercial teleosts from the western Mediterranean Sea: Infection rates and possible effects of environmental and ecological factors

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ABSTRACT

This study aims to investigate the occurrence of Anisakidae larvae in fishes of commercial value and largely used for human consumption, from the Spanish Mediterranean coasts. The influence of environmental (geographical location, depth, temperature and salinity of the fishing grounds) and biological (weight, length and corporal condition) variables were evaluated. A total of 290 fishes belonging to 10 different species sampled from 36 geographical sectors were analyzed using enzymatic digestion method. The total prevalence of Anisakidae was 13.1%, with the prevalence of *Anisakis* sp., *Hysterothylacium* sp. and *Contracaecum* sp. being 6.21%, 6.21%, and 2.41%, respectively. The highest anisakid larvae prevalence, was observed in surmullet (*Mullus surmuletus*) and common pandora (*Pagellus erythrinus*). Total Anisakidae prevalence was positively correlated with length, weight and condition factor of the host and with the depth of the capture site. *Anisakis* sp. total prevalence was positively correlated to fishing ground depth. No significant correlation was observed between anisakid prevalence and geographical sector of capture.

1. Introduction

Anisakidosis is an important fish-borne zoonosis caused by the larval stages of nematodes of the family Anisakidae or raphidsacaridae, which are commonly referred to as anisakids. Over the last 30 years, there has been a marked increase in the reported prevalence of this zoonosis throughout the world (EFSA Panel on Biological Hazards, 2010). The accidental intake of these parasites, generally after the consumption of raw or inadequatelycooked parasitized fishery products, can cause digestive disorders and/or allergies in humans (Audicana, Ansotegui, Fernández de Corres, & Kennedy, 2002; Butt, Aldridge, & Sanders, 2004). Clinical signs and symptoms of anisakidosis include edema in the gastric mucosa, epigastric pain, vascular occlusion, diffuse abdominal pain that mimics a gastric ulcer, and pseudo-tumoral formation (Bouree, Paugam, & Petithory, 1995; Takabe et al., 1998). Hypersensitivity reactions to parasite antigen can occur after ingestion of fresh, but also previously frozen, or cooked parasitized fish products (Fernández de Corres et al., 1996; Pozio, 2013). In rare instances, an acute allergic response associated with ingestion of larvae or physical contact with an infected fish can cause a fatal anaphylactic reaction (Audicana et al., 2002; Fernández de Corres et al., 1996).

It is believed that most species of fish and cephalopods can harbor these parasites (Abollo, Gestal, & Pascual, 2001; McClelland, Misra, & Martell, 1990). Despite the commercial and zoonotic importance of anisakid infections, there is a lack of adequate data on the geographical distribution, prevalence, intensity, and anatomical distribution of parasites of public health importance in fishery products from the Mediterranean Sea. For this reason, the European Food Safety Authority recommended that research be encouraged to clarify anisakid geographical and seasonal distribution, prevalence, intensity, and anatomical location in wild fishery products (EFSA, 2010). To assess the food safety concerns of possible anisakidosis caused by parasites in fishery products, we studied the prevalence and distribution of anisakids in commercial

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wild fish from the Spanish Mediterranean coasts and identified biotic and abiotic factors related to infection such as fish size and anatomical sites of infection, along with fishing ground location, fishing water depth and final host abundance.

2. Materials and methods

2.1. Study area and sample collection

This study was carried out within the framework of the Mediterranean International Trawl Survey (MEDITS financed by DG MARE and EU members Council Regulation (EC) N° 199/2008) and was conducted along the Spanish Mediterranean coast by the Spanish Institute of Oceanography. A total of 290 fish belonging to 10 species (Table 1) were randomly collected in May 2012 from 36 geographic sectors (Fig. 1 and Supplementary material) using a bottom trawl (model GOC-73) with a 4 m vertical opening and a 20 mm cod end mesh size. Depth, temperature and salinity were recorded by means of a CTD SBE-37 probe located in the opening of the trawl. Further information on the sampling design and on the characteristics of the sampling gear is available in the MEDITS Handbook (2012). Each fish was identified to the species level following the descriptions from Fisher, Bauchaud, and Shneider (1987) and immediately frozen and stored at −20 °C.

2.2. Anisakid detection

Once thawed at the laboratory (Murcia University), fish were weighed and measured, and the whole fish except the head, the backbone, skin and tail was analyzed for the presence of nematodes. The viscera and muscles of 185 fish were analyzed separately to establish whether anisakids were present at the muscular level. For anisakid detection, samples were homogenized and flattened in a stomacher (Stomacher IUL Instrument, Germany) as previously reported by Llarena-Reino et al. (2013) for 90 s previous to enzymatic digestion. Digestion was carried out at 37 °C for 15 min in a fresh pepsin solution (0.1% (w/v) pepsin (2000 FIP-U/g) and 0.063 M hydrochloric acid) in distilled water at a weight/volume ratio of 1:10. Digested tissue was filtered through a sieve with a mesh size of 400 μm, flushed carefully with tap water and then observed on a Petri dish with a stereomicroscope. Individual parasites were fixed in 70% ethanol until identification. Anisakid larvae obtained from wild mackerel (*Scomber scombrus*) were flattened in the stomacher and incubated in the same conditions as controls of parasite resistance to the homogenization and enzymatic process.

For morphological analysis, parasites were clarified in glycerol, observed under a microscope and identified to the genus level based on the morphological characters of the digestive tract, the shape and presence of the boring tooth or the lips at the anterior end (Koyama et al., 1969; Quiazon, Yoshinaga, & Ogawa, 2011; Shih, 2004; Zhang et al., 2007).

Condition factor (K) was calculated following Fulton's index as $K = W/L^3 \times 100$ (Ricker, 1975), where W was the fish weight and L the fish total length.

2.3. Statistical analysis

The terms prevalence (percentage of infested individuals), mean intensity (mean number of parasites in infested hosts) and mean abundance (mean number of parasites per host, including uninfected individuals) were used according to Bush, Lafferty, Lotz, and ShoslaK (1997). The odds ratio and 95% confidence intervals were obtained.

Data on anisakid prevalence were normal according to the Shapiro–Wilk test. Thus, a Pearson test was applied to explore the association between anisakid prevalence in sampled teleosts and different environmental and biological variables. Data on Atlantic mackerel and blackbelly rosefish were not included due to the small sample sizes. Environmental variables included geographical location, depth, temperature and salinity of the fishing ground, while biological variables were weight, length and corporal condition. A non-parametric ANOVA test (Kruskal–Wallis) was used to explore the association between *Anisakis* sp., *Contracaecum* sp. and *Hysterothylacium* sp. total prevalence and fishing ground depth. For this purpose, depth was stratified into three groups: <70 m, 70–200 m and >200 m. The Kruskal–Wallis test was also

Table 1
Parameters of parasitization by anisakids in fish from West Mediterranean Sea.

Host species	No	Prevalence 95% CI[a]	No. of collected larvae	Mean intensity (range)	Prevalence in fish muscle 95% CI (no.)	Mean intensity in fish muscle (range)	Geographical sectors with infected fish
Sardina pilchardus (sardine)	85	14.12 (7.82–23.8)	18	1.5 (1–3)	1.18 (0.06–7.29)	1 (0–1)	11, 12, 16, 23, 31, 35
Engraulis encrasicolus (anchovy)	67	4.48 (1.16–13.37)	3	1 (1–1)	0	0	23, 28
Lophius budegassa (black anglerfish)	36	5.56 (0.97–20.01)	4	2 (2–2)	0	0	13, 35
Trisopterus minutus (poor cod)	29	13.79 (4.51–32.57)	6	1.5 (1–2)	0	0	2, 3, 36
Mullus surmuletus (surmullet)	21	33.3 (15.48–56.89)	7	1 (1–1)	9.5 (1.67–31.83)	1 (0–1)	5, 16, 17, 26, 27, 33
Micromesistius poutassou (blue whiting)	18	16.67 (4.41–42.26)	3	1 (1–1)	5.56 (0.29–29.37)	1 (0–1)	1, 14, 25
Phycis blennoides (greater forkbeard)	16	25 (8.33–52.59)	12	3 (1–6)	6.25 (0.33–32.29)	1 (0–1)	24, 32
Pagellus erythrinus (common Pandora)	9	33.3 (9.04–69.08)	4	1.33 (1–2)	0	0	26, 29
Scomber scombrus (Atlantic mackerel)	6	0	0	0	0	0	–
Helicolenus dactylopterus(blackbelly rosefish	3	0	0	0	0	0	–
Total	290	13.1 (9.55–17.67)	57	1.5	1.72 (0.63–4.21)	1 (0–1)	1, 2, 3, 5, 6, 11–14, 16, 17, 23–29, 31–33, 35, 36

[a] CI: Confidence interval.