Occurrence of *Anisakis* spp. in horse mackerel (*Trachurus trachurus* L.) from the North Atlantic Moroccan coasts

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Abstract

The *Anisakidae* (*Nematoda*) have a global distribution among a wide variety of marine fish species that serve as intermediate or paratenic hosts. The aim of this work was to study the occurrence of *Anisakis* spp. in the horse mackerel (*Trachurus trachurus* L.) from the North Atlantic Moroccan coasts. A fish sampling was conducted monthly during the August 2010 - July 2011 period. Samples were collected from the zone between Tangier and Larache. Morphological identification showed that the larvae found in this fish species correspond to *Anisakis* spp. Most third-stage (L3) larvae observed were found in the abdominal cavity and rarely in the muscle. The infection prevalence and intensity vary with the sampling period and fish size. A positive but non-significant correlation was found between infestation intensity and the fish host length ($R^2 = 0.25$). The highest mean intensities were observed for sizes > 28 cm during the March - June period.

Key words: *Anisakis* spp., *Trachurus trachurus*, Moroccan coasts.

Introduction

The *Anisakidae* belong to the Order of *Ascaridida*, parasitizing fish, mammals, birds and rarely reptiles (Hartwich, 1974). They have a global distribution among a wide variety of marine fish species that serve as intermediate or paratenic hosts (Koie *et al.*, 1995).

Anisakis spp. is one of the most damaging nematodes in terms of public health. Indeed, it is responsible of anisakiasis (zoonosis). After the consumption of raw or undercooked parasitized fish (Ishikura, 1989), the third-stage (L3) larvae invade the gastrointestinal mucosa and secrete proteins implicated in the anisakiasis pathogenesis (Matthews, 1982; Hotez *et al.*, 1994; Dziekonska-Rynko *et al.*, 2004). The penetration of this parasite in the tissue can induce allergic reactions with several clinical manifestations ranging from urticaria or angioedema to anaphylaxis (Kasuya *et al.*, 1990; Audicana *et al.*, 1995; Gonzalez-Quijada *et al.*, 2005). Although they could not continue its development in humans, the larvae can become attached to the stomach or duodenum, causing digestive problems (pain, nausea, vomiting) which can be severe (Hartwich, 1974).

The horse mackerel [*Trachurus trachurus* Linné, 1758 (Quéro *et al.*, 2007) is among the principal hosts of L3 larvae of *Anisakis* spp., with a prevalence of 80 - 100% (Koie *et al.*, 1995). In the North of Morocco, horse mackerel (*T. trachurus*) presents one of

the fish species that are most commonly consumed. Horse mackerel acquires maturity at 3 years for males (20-22 cm) and around 4 or 5 years for females (26-30 cm) (Eaton, 1983; Eaton, 1989; Iversen *et al.*, 1989). The juvenile ones feed mainly on copepods but also ostracods, mysids and fishes (gobies, run). In the adult stage, their diet consists mainly of fishes (anchovy, sprat, sardine, herring and sand lance), cephalopods and crustaceans (Quéro and Vayne, 1997). The analysis of stomach content of horse mackerel showed that it contains *Crustacea* (*Euphausiacea, Mysidacea, Decapoda*), *Teleostei*, *Cephalopoda* and *Polychaeta*. *Euphausiacea* were the most ingested (Santić *et al.*, 2003). *Euphausiids* are intermediate hosts of *Anisakis* spp. (Adroher *et al.*, 1996).

Despite the commercial and zoonotic risk of larval anisakid infections of fishes, their distribution among the Moroccan marine resources (teleosts) was not studied to our knowledge. The monitoring of *Anisakis* species prevalence to identify fishing areas most infected and the risked periods during the year would contribute to establish effective control measures, useful for operators and consumers.

The present study focuses on surveying *Anisakis* spp., a nematode parasite, in *T. trachurus* caught in the Tangier-Larache coasts (NW of Morocco).

Materials and methods

A total of 205 specimens of *T. trachurus* were captured in the Tangier - Larache coasts (NW of Morocco), from August 2010 to July 2011.The sampling site was the wholesale fish market of Tangier. The individuals were taken randomly and represent various sizes, ranging from 18 to 38 cm.

Fish samples were measured, weighed and dissected by making an incision along the ventral line from the anus to the buccal aperture. The visceral cavity and digestive tract were examined. The muscle was removed from the spine and immersed in warm water for several minutes to facilitate the migration of the parasite to the outside of the fish.

After dissection of the individuals, encapsulated or free nematode larvae were collected from the visceral cavity, internal organs and musculature. Parasites were counted and, after being washed in a 0.9% NaCl, fixed in a 70% ethanol/glycerin solution (9/1), and cleared in glycerin solution 99% (Neveu-Lemaire, 1936). Then, they were observed in microscope. Larvae were identified according to previous works namely the structural features (Hartwich, 1974; Yoshinaga *et al.*, 1987; Petter and Maillard, 1988; Ruiz-Valero *et al.*, 1992; Quiazon *et al.*, 2008).

Parasitological indexes related to prevalence (P), intensity (I) and abundance (A) are calculated in accordance with Bush *et al.* (1997): P = [(number of infected fiches/number of fiches examined)*100]; I = (total number of parasites/total number of infected fish); A = (total number of parasites/total number of examined fish).

To compare mean prevalence, we used the Fisher's exact test. The bootstrapping 2-sample t- test is used to compare mean intensities and mean abundances. Comparisons based on normal theory such as Student's *t*-test or ANOVA are usually not applicable for parasites because of the skewness of their distribution. The statistical analysis was based on SPSS (Statistical

Package for the Social Sciences) version 14.0 **(**Rózsa *et al.*, 2000).

Results

Localization of *Anisakis* spp. in *T. Trachurus* tissue

The parasite was found free or encysted in spiral form in different organs of mackerel: gonads, liver, intestine, viscera, mesentery and muscle. Larvae were frequently found in the abdominal cavity in visceral organs. Only a few ones were observed in the muscle (Figure 1).

Morphological identification

Anisakis spp. were identified on the basis of the morphological features. L3 larvae of *Anisakis* spp. are characterized by its whitish color. They have an oesophageal ventricle more or less developed. They are devoid of ventricular appendix and intestinal caecum. In the majority of *Anisakis* spp. larvae observed, the plan of ventricular-intestinal union is oblique and the posterior end is conical and ends with a mucro (Figure 2).

Effect of season variation on infection levels

The *Anisakis* spp. larvae were found with a prevalence of 100% in September-October 2010 and April-July 2011 periods. However in August 2010, the prevalence was relatively low (57.7%). This is due to the small size of individuals in the sample (23.63 cm, 108.1 g). The intensity of infection varies throughout the study period. It is more important in March and June 2011 and less important in August 2010 (Figure 3).

There was a high significant increase (p=0.01) in prevalence and intensity of infection with the increase

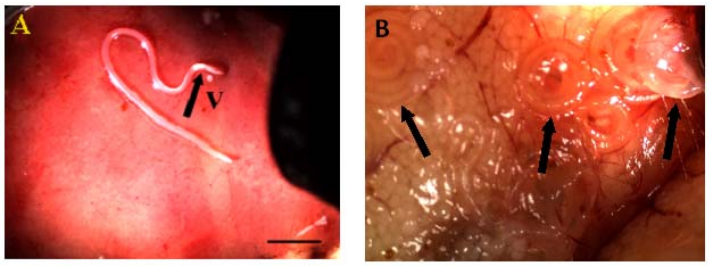

Figure 1. *Anisakis* spp. larvae in horse mackerel. A: L3 larvae showing esophageal ventricle. B: L3 larvae encapsulated on gonad. Legend: v (ventricle), ➔ (L3 Larvae).

Scale bar = 1 mm.

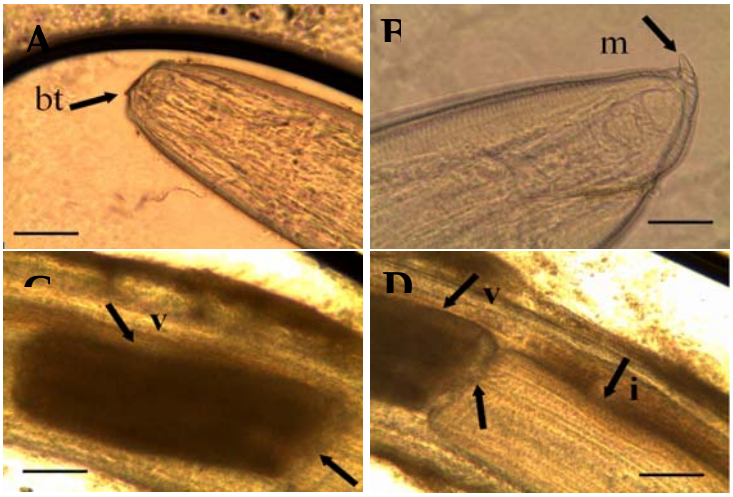

Figure 2. Morphological characteristics of L3 larvae of *Anisakis* spp. observed. A: Anterior end of *Anisakis* spp. L3 larvae. B: Tail of *Anisakis* spp. L3 Larvae. C: Ventricular part of *Anisakis* spp. (V). D: L3 larvae showing oblique junction between ventricle and intestine (arrowed). Legend: bt: boring tooth; i: intestine; m: mucro; v: ventricle.

Scale bar: 200µm.

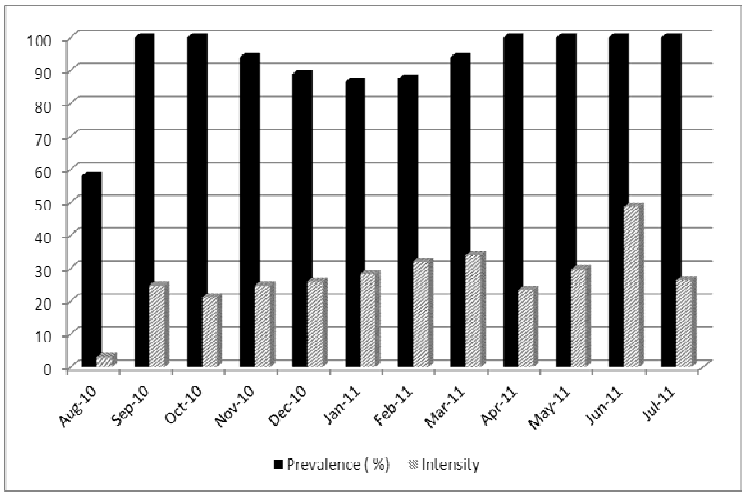

Figure 3. Variation in prevalence and intensity of infestation by L3 larvae of *Anisakis* spp. in horse mackerel.

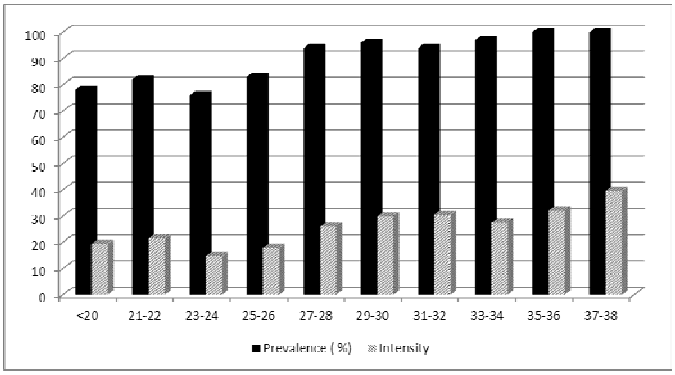

Figure 4. Variation prevalence and intensity of infestation by L3 larvae of *Anisakis* spp. according to the fish length in horse mackerel.

of the total length of fish. The mean intensity of *Anisakis* spp. larvae was high in 31-38 cm fishes and low when the length fish is inferior to 26 cm. The mean prevalence was highest in fish exceeding 28 cm of size (96% for 29-30 cm class) and lowest in fish measuring less than 23 cm (Figure 4).

Discussion

The larvae and adults of *Anisakis* spp. are common parasites of a wide range of marine fish species (Smith and Wootten, 1978; Anderson, 1992; Williams and Jones 1994). *Anisakis* spp. occurs in fish at the larval stage and its infection of *T. trachurus* was previously reported in the Mediterranean and North Atlantic costs (Adroher *et al.*, 1996; Farjallah *et al.*, 2008; Mattiucci *et al.*, 2008). Horse mackerel may become infected with *Anisakis* larvae by consuming euphausiids, which are intermediate hosts of these nematodes. It is a pelagic specie and eats small fishes and planktonic crustaceans (Muus and Dahlstrom, 1966; Smith, 1983).

When observed under the stereomicroscope and light microscope, L3 larvae of the nematode *Anisakis* spp. showed morphological characteristics similar to those mentioned by Rello-Yubero *et al.* (2004). These larvae are characterized by a cuticle with thin transversal striation. Anterior extremity is with a dorsal and two poorly developed ventro-lateral lips. We note the presence of six cephalic papillae, one pair in the dorsal lip and a pair in each ventro-lateral lip. Then, boring tooth is below the oral aperture, between the two ventro-lateral lips. The ventricle oesophagien is present, the ventricular appendix and the intestinal

caecum are absent. There are also two nearly spherical rectal glands. The tail is conical, and the mucro is present. The morphological differentiation of *Anisakis* larvae was performed according to Smith and Wootten (1978) and Rello-Yubero *et al.* (2004) based on the two main morphological features: length of ventricular zone of the esophagus (shorter for *A. physeteris*) and the presence of a mucro at the extreme end of the tail in *A. simplex* s. str. Most of the larvae we observed belong to these two species, with predominance of *A. simplex* s. str. (ventricle esophageal longer than wide, presence of mucro, oblique union ventricle-intestine). In fact, a molecular study conducted by Farjallah *et al.* (2008) confirmed that in samples from the waters of Tangier, the highest frequencies for *A. simplex* s. str. was recorded. According to Mattiucci *et al.* (1997) and Mattiucci & Nascetti G (2006), *A. simplex* s. str. was the most prevalent species reported from the North-East Atlantic coasts, mainly from the North of Straits of Gibraltar. In accordance with Farjallah *et al.* (2008), 83% of the fish infected by *A. simplex* s. str. are pelagic and we think that the most species of *Anisakis* spp. we observed correspond probably to *A. simplex* s. str.

We observed that variations in the length of fish sampled each month correlated with variations in *Anisakis* spp. prevalence throughout the year. Furthermore, *Anisakis* spp. mean prevalence was higher in the second half of the year. According to Smith and Wootten (1978), seasonal variations in infection levels are probably due to changes in the population of infected euphausiids in the zooplankton.

The highest levels of infection intensity were found in March and June, with a mean intensity of 34 and 48.6 in the same order, similarly to Adroher *et al.* (1996).

We also observed that parasite mean intensity varied directly with the length (age) of horse mackerel.

Adroher *et al.* (1996) studied variations in the prevalence of *Anisakis* spp. according to the host size in horse mackerel. Similarly to our results, they found that fishes measuring more than 30 cm in length were infected more frequently than those measuring less than 23 cm. Most larvae were observed in body cavity, and only some of them were found in muscles. These results are similar to those reported by Huang (1988) and Adroher *et al.* (1996) who reported that most larvae occurred in the body cavity (61.2%).

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