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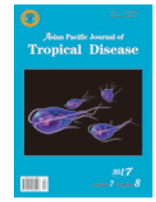

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Anisakis simplex (Nematoda: Anisakidae) from horse mackerel (*Trachurus trachurus*) in Atlantic coast of Morocco

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ABSTRACT

Objective: To focus on the description of the *Anisakis simplex* (*A. simplex*) parasites of *Trachurus trachurus* (Linnaeus, 1758) from the Atlantic coast of Mehdia (Kenitra, Morocco) from December 2014 to November 2015.
Methods: A total of 1 012 *Trachurus trachurus* (Linnaeus, 1758) obtained from commercial fishing were performed autopsy for their parasitic Nematoda. Then 6 695 specimens of *A. simplex* were collected from their abdominal cavity. These parasites were attached on different organs particularly on ovaries and testes. All parasites were counted, measured and photographed under microscopy.
Results: The infection levels of fishes by larval *A. simplex* are expressed by prevalence (35.28%), mean intensity (18.75) and abundance (6.6). The effect of parasitism did not show a significant negative impact on the condition of the examined fishes.
Conclusions: Significant positive correlations were found between host length and *A. simplex* occurrence, and abundance. The variation observed in the infection levels was discussed within the seasons and climatic change.

1. Introduction

Anisakis simplex (*A. simplex*) is a marine nematode of the Anisakidae family that causes the humans anisakidosis disease and also economic damage[1]. In some areas of northern fisheries, the prevalence has increased recently, which makes financial effects[2]. Anisakidae are common parasites of marine fish that are used as definitive, intermediate or paratinic hosts. The definitive hosts are usually the top predators like marine mammals, piscivorous fishes and piscivorous birds. Numerous fish species are paratenic hosts in these life cycles[3]. *A. simplex* is some of these nematodes, which can infect humans after ingestion of raw or undercooked fish[4].

Horse mackerel [*Trachurus trachurus* L. 1758 (*T. trachurus*)] is a fish species in which the larval *A. simplex* has been described by some researchers[5-7]. The definitive hosts for these parasite species include piscivorous fishes and marine mammals[7]. Horse mackerel (*T. trachurus*) is a pelagic fish, eating squids and small fish[8,9] that become infected with *Anisakis* larvae by feeding on planktonic crustaceans, euphausiids[7-9].

This study aims to give information about the morphology of anisakid infesting moroccan *T. trachurus*, using binocular loupe and scanning microscopy, to determine their taxonomy, epidemiological indexes and dynamic of infestation, and to get an insight into the effect of anisakid on biological performances of the host.

2. Materials and methods

A total of 1 012 specimens of *T. trachurus* were sampled of the commercial fisheries at the port of the Mehdia of Kenitra (Atlantic coast of Morocco) from December 2014 to November 2015.

After sampling, death fishes specimens were transferred to the laboratory. For each fish, lengths were measured and its sex was determined. Besides, eviscerated weights, liver and gonad weights were measured. After inspection of the internal, we started dissection of the body and examined the abdominal cavity of organs

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and muscles, which was performed by visual and under microscope examination. The number of parasites and the attachment site were noted for each fish.

After the extraction of parasites to the outside of the fish, we removed the muscles and immersed it in the water for a few minutes. Specimens of Nematoda were collected and examined under a microscope after anatomy and the basis of their morphology was measured using a graduated eyepiece and kept in an alcohol 70% for further use. According to the recent descriptions[10,11], prior to that, visceral cavity and digestive tract were examined.

Parasitological indexes related to prevalence, mean intensity and abundance were calculated in accordance with Hernández-Orts *et al.*[12], Bush *et al.*[10], and Shields *et al.*[11].

Prevalence = [(No. of infected fishes/No. of fishes examined) × 100%]

Mean intensity = (Total No. of parasites/total No. of infected fishes)

Abundance = (Total No. of parasites/total No. of examined fishes)

3. Results

Anisakis larvae were collected from the cavity of *T. trachurus* (Figure 1). The distribution of parasites revealed that most were attached to the abdominal cavity and to other male or female gonads organs (Figure 1B and C).

3.1. Variation in infection levels with host length

Comparion between the lengths of parasitized and non-parasitized fishes showed a variation in parasite prevalence, mean abundance and mean intensity in six categories of host length. Larval *Anisakis* were found in 357 (35.28%) of 1012 horse mackerel (*T. trachurus*). All larvae were found in cavity of body or at the surface of visceral organs and no larvae were observed in the muscle.

When horse mackerel samples were partitioned according to host length, significant positive correlations were found between host length and *A.simplex*. It was not found for any *A. simplex* in a host size class (12–15 cm) and (15–18 cm) of total number of the 352 fishes examined. It was found in a host size class (18–21 cm) that 1 *A. simplex* was found particularly in fish length of 19 cm out of total number of the 119 fishes examined. In a host size class (21–24 cm), 81 out of the 197 fishes examined were infected. The prevalence was 41.12%, abundance 1.59 and mean intensity 3.88. In a host size class (24–27 cm), 65 out of the 84 fishes examined were infected and prevalence was 77.38%, mean abundance 3.68, and mean intensity 4.75. In a host size class (27–30 cm), 45 out of the 61 fishes examined were infected, and its prevalence was 73.77%, mean abundance 9.90, and mean intensity 13.42. In a host size class (30–33 cm), 72 out of the 84 fishes examined were infected and its prevalence was 85.71%, mean abundance 20.65, and mean intensity 24.09. In a host size class (33–36 cm), 55 out of 70 fishes examined were infected, with the prevalence of 78.57%, mean abundance of 23.43, and mean intensity of 29.82. In a host size class (36–39 cm), 25 out of 28 fishes examined were infected, with prevalence of 89.28%, mean abundance of 38.00, and mean intensity of 42.56. In a host size class (39–42 cm), 10 out of 13 fishes were infected and its prevalence was 76.92%, mean abundance 59.46, and mean intensity 77.30. In a host size class (42–45 cm), 3 out of 4 fishes were infected and its prevalence was 75.00%, mean abundance 63.75, and mean intensity 85.00. There was a high significant increase in prevalence and intensity of infection with the increase of the total length of fish. The mean intensity was high in 42–45 cm fishes, but low when the length of the fish was inferior to 18–21 cm. The prevalence was higher in fish exceeding 24 cm of size and lower in fish measuring less than 21 cm (Figure 2). The direct relationship between infestation level and age or length in the horse mackerel is a prevalent phenomenon in many fish species.

3.2. Seasonal evolution of parasite A. simplex

Table 1 shows the prevalence, mean intensity and mean abundance of *A. simplex*, as well as the host length in each year season and the occurrence was significantly different throughout the year.

Table 1

Variation in infection levels with host length class.

Sampling season	Host size class (cm)	No. of fish infected	Prevalence	Mean intensity	Mean abundance
Winter	22–42 cm	90	36.43%	21.45	7.80
Spring	22–40 cm	83	34.00%	17.00	5.70
Summer	19–40 cm	100	41.00%	16.14	6.60
Autumn	22–43 cm	84	31.00%	20.70	6.30
Total		357			

Figure 2 shows the different values between sampling seasons.

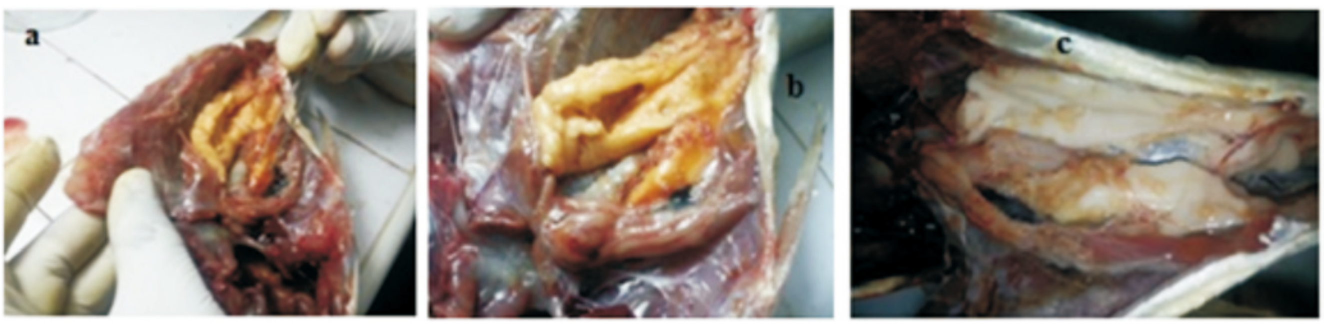

Figure 1. *Anisakis* sp. on viscera, particularly gonads.
a: Abdominal cavity; b: Female gonads organs; c: Male gonads organs.

Highest value of prevalence (41.00%) was recorded in summer. The reason for this was that the fish collected in summer was more than other seasons. So, the lowest value of mean intensity (16.14) was in summer. The highest value of mean intensity was found in winter (21.45), while in autumn and spring, the mean intensity values were 20.70 and 17.00, respectively. In present work only 357 fishes infected were detected in 1012 fishes (The larvae of nematode in moderate size samples appeared poorly and disappeared in small size of fish). The reasons for these variations are not clear. Therefore, the time interval between capture and observation may be responsible for the relative.

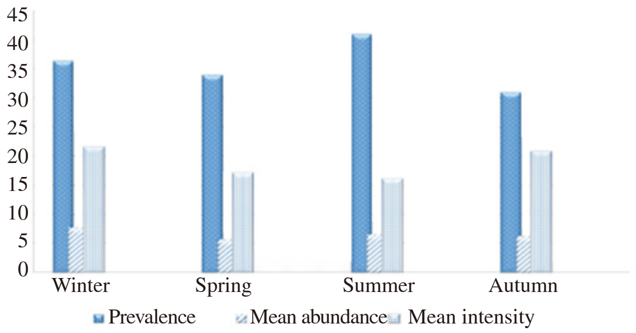

Figure 2. Seasonal variations in the prevalence, mean abundance and mean intensity of horse mackerel (*T. trachurus*).

4. Discussion

Horse mackerel (*T. trachurus*) is a pelagic fish and it is like most pelagic fish that feed on planktonic crustaceans and small fish[8,13] and may therefore become infected with *A. simplex* by consuming small crustaceans (euphausiids), which are intermediate hosts of these nematodes[14].

Distribution of larval anisakid showed a relationship between prevalence and abundance with the host length. The increase of *A. simplex* prevalence with the increase of host length was previously described in *T. trachurus* by Adroher *et al*.[15]. The highest mean intensity was found in the intermediate host size class 40–43 cm. It must be stressed that a relationship between infection level and age or length of the host can be expected for parasites like *A. simplex* which produce a long term infestation[16]. In the most marine fish, there is a wide range of common parasites, such as larvae and adults of *A. simplex*[17-19]. The *A. simplex* appear in fish at the larval stage and its infection of *T. trachurus* was previously reported in the Mediterranean and North Atlantic coasts[15,20,21].

The presence of nematodes in horse mackerel is according to the reports of Kuhn *et al*.[6], Choi *et al.*[7], and Stlva and Elras[22]. *A. simplex* was found in the body cavity[15]. However, *A. simplex* was reported in horse mackerel muscle (it has been reported that post-mortem migration of *A. simplex* into the flesh of some host species[23,24]), but the percentage of larvae found in this tissue varied from very low 1.8% to high 41.8% values[22,25]. It is should be emphasized that very amount of larvae in muscle may depend on the amounts of lipids in this tissue since migration. It is absent in "non-fatty" hosts and exists in "fatty" species[23,26].

Mladineo *et al.*[24] suggested that the hosts have a diet which may affect the selection of the larvae for a microhabitat (visceral organs and body cavity or muscle). The species which feed on euphausiids presented consistently low proportions of larvae in muscle and piscivorous fishes had larvae more widely distributed[6,27]. With higher infestation being obtained for length, the relationship between host length and *A. simplex* infestation level, was earlier described for *T. trachutrus*[6,27].

In the present work, prevalence and abundance are significantly correlated to host length and although intensity does not present a significant correlation, the two lower length classes showed very low mean intensities and the three higher length class's higher mean intensities. As length classes higher than 31 cm showed a marked increase in mean intensity, a positive significant correlation can be expected with the inclusion of longer fish in the sample. On the other hand, the lowest mean intensity was found in the fishes 22–30 cm and the highest mean intensity in the host size class 40–43 cm. Contrary with the study of Adroher *et al.*[15], it is fair to analyse the seasonal variation in the infection levels because of the diverging host length in the sampling seasons. In the present work, the level of infections is close to that in the previous studies in Spain for horse mackerel reports (prevalence of 39.4%)[15] and in Portugal for horse mackerel reports (prevalence of 38.3%)[17]. However, the abundance of *A. simplex* is normal to relate to the host length. During the summer, the observation of the mean intensity and prevalence of *A. simplex* did not present the forecasted values for fishes of those sizes. Summer value was higher than expected (the mean host length is similar to the overall mean, and prevalence, mean abundances, and mean intensity are higher than those observed in the total sample). Autumn value was lower than expected; there are similarity in host length and low on prevalence and mean abundances. The larval burden was related to host length, and was also noted by Kuhn *et al.*[6], Cruz *et al*.[17] and Abou-Rahma *et al.*[27].

A. simplex has a high abundance in winter. The researchers suggested that an increase of euphausiids associated with an increase in the supply of eggs by definitive hosts (northward migrating whales) may be responsible for this types of infection[13,14,28]. Furthermore, Smith[28] suggested that seasonality might not be expected because *A. simplex* eggs shed from final hosts possibly throughout the year. It may develop and hatch at any time. This make it easier for us to know the reason behind the spread of infection in specimens of *T. trachurus*. The pelagic species depends on plankton in the food habits, especially crustaceans, (copepods)[13], which are abundant in the water column and are considered potential intermediate hosts for these parasitic nematodes, strengthening and supporting parasitism and development cycle. The horse mackerel may therefore be infested with *A. simplex* by consumption of euphausiids, which becomes the intermediate hosts of these nematodes[14]. The relationship between the different feeding area and the different diets components make a different results. This discrepancy can be explained by the distinctive recruitment of the larvae. The results obtained during this study remain interesting. On the other hand, the parasites studies of marine fish do not receive enough attention in Morocco[29-31]. However, it is desirable to deepen the work to better understand for Nematoda.

Conflict of interest statement

We declare that we have no conflict of interest.

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