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Occurrence of *Anisakis* sp. larvae in *Merluccius merluccius* (Teleostei, Gadiformes) of the libyan north coast and evaluation of its zoonotic potential

Présence de larves d'Anisakis sp. dans Merluccius merluccius (Teleostei, Gadiformes) de la côte nord libyenne et évaluation de leur potentiel zoonotique

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Abstract. The occurrence of *Anisakis* sp. larvae (L3) were studied in 94 individuals of *Merluccius merluccius* fish, collected from Libyan North coast. The prevalence value increased with the host length from 22.2% (at length class ≤ 23.5) to a maximum of 86.7% (at length class ≥34cm). Mean intensity increased from 2.25 (at length class ≤ 23.5) to 16.3 (at length class 29-33.5). There wasn't a significant relationship between the sex of the host and the prevalence, however, males showed significantly heavier parasite burden. The migration of the parasites to the flesh after the death of the host was observed in the infected fishes.

Keywords : *Merluccius, Teleostei*, Gadiformes, *Anisakis, Libye.*

Résumé. La présence d'*Anisakis sp*. larves (L3) a été étudiée sur 94 individus du poisson *Merluccius merluccius*, collectés sur la côte Nord de la Libye. La valeur de la prévalence augmente en fonction de la longueur de l'hôte de 22,2% (classe de longueur ≤ 23,5) à un maximum de 86,7% (classe de longueur ≥ 34cm). L'intensité moyenne a augmenté de 2,25 (classe de longueur ≤ 23,5) à 16,3 (classe de longueur 29-33,5). Aucune relation significative entre le sexe de l'hôte et la prévalence n'a été décelée. Par contre, les individus mâles ont présenté une charge parasitaire nettement plus importante. La migration des parasites vers la chair après la mort de l'hôte a été observée chez les poissons infectés.

Mots-clés : *Merluccius, Teleostei*, Gadiformes, *Anisakis, Libya.*

INTRODUCTION

Anisakis sp. are anisakid nematodes which are common parasites of marine fishes. These species have zoonotic importance due to the human consumption of raw or undercooked fish flesh invaded by the nematode larvae after the death of the host (Smith, 1984, Smith & Wootten 1975, Cattan & Carvajal 1984). *Anisakis* sp. has also been identified as an important cause of gastric granuloma (Sey & Petter 1997), allergic reactions "ranging from *urticaria angioedema* syndrome to anaphylactic shock" *In* (Ventura *et al.*, 2008) and even intestinal intussusception in adults (Mura *et al.* 2010). Also, the ingestion of material from dead parasites in food is also potentially dangerous (Audicana *et al*. 2002).

On the other hand, the current increase in prevalence and intensity of larval Anisakids in some northern hemisphere fisheries causes significant economic losses due to condemnation of infected fish (Chen *et al*. 2010)

Although, studies on Anisakid larvae have received a good deal of attention (Wooten & Smith 1976, Bussmann & Ehrich 1979, Smith, 1984, Petter & Maillard 1988, Ruiz-Valero *et al.* 1992, Koie 1993, Valero *et al.* 2006 a, b), nothing is known about the occurrence or the zoonotic potential of the larvae in fishes of the Libyan waters.

The aim of this study is to report the prevalence and intensity of the infection in *Merluccius merluccius* L. (*Teleostei*, Gadiformes) and to evaluate the zoonotic significance of the parasite throughout the study of the migration into the flesh after the death of the host.

MATERIAL AND METHODS

A total of 96 European hake *M. merluccius* specimens were collected from Tripoli port. Most of the fishes were sampled from fishermen catches immediately after the arrival of the fishing boats, and the most of them were still alive when sampled. The specimens were sexed and measured (total length), and the visceral cavity was thoroughly inspected for the presence of parasitic larvae. Twenty five nematode specimens were microscopically observed and measured for identification.

The study of the migration of the parasites into the muscle was done as the infected fishes were gutted 28 hours after their capture (Silva & Eiras 2003) and the nematodes present in the viscera were sampled. For detection of the parasites in the flesh, the whole muscle of the specimens was carefully dissected and the larvae were recovered. X^2 was used for statistical analysis.

RESULTS

The observation of the morphology of the specimens allowed their identification as probably *Anisakis simplex* L3 larvae (Fig. 1). The morphometric values (in mm) of the larvae were: length of the body: 23.9 (18-30); maximal width of the body: 0.4 (0.2-0.5); length of the esophagus: 2 (1.4-2.8); width of the esophagus: 0.1 (0.07-0.16); length of the ventriculus: 0.7 (0.2-1.0); width of the ventriculus: 0.3 (0.2-0.4). The larvae were found mostly in the visceral cavity.

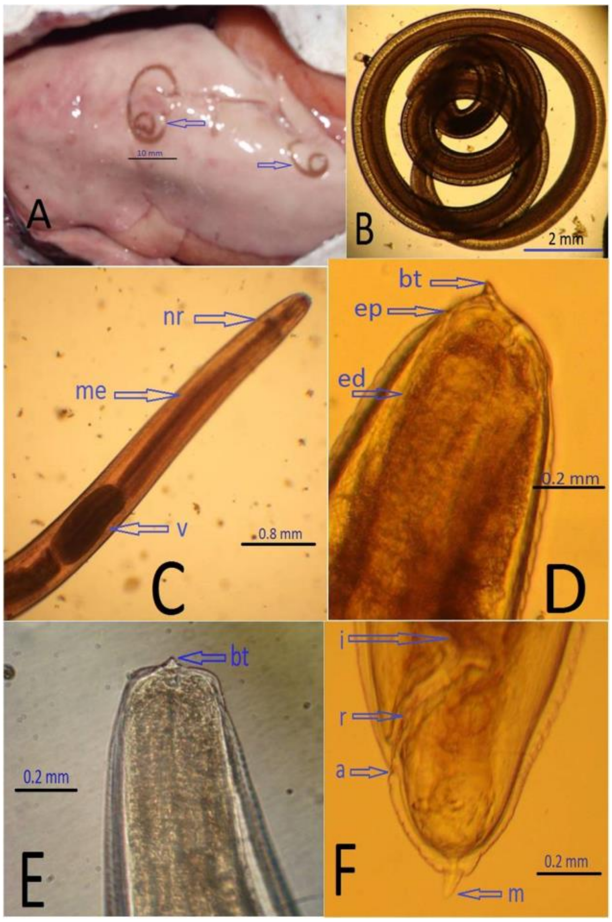

Figure 1. *Anisakis* L3 larva and its morphological aspects. A: two of the detected larvae embedded on the liver surface of *M. merluccius*, B: one of the detected larvae coiled on itself, C: anterior extremity showing muscular esophagus (me), nerve ring (nr) and ventriculus (v), D, E: anterior end showing characteristic boring tooth (bt), excretory duct (ed) and excretory pore (ep), F: posterior end showing anus (a), intestine (i), characteristic mucron (m) and rectum (r).

Larva are detected mostly at the surface of the liver (Fig.1: A) and gonads, and sometimes they were observed penetrating the stomach wall. Sixty of the 94 examined (63.9%) European hake *M. merluccius* revealed infestation with a mean of 10. 8 (1-300 *Anisakis* larvae /fish). The prevalence value increased with the host length from 22.2% (at length class ≤ 23.5 cm) to a maximum of 86.7% (at length class ≥34cm). Mean intensity increased from 2.3 (at length class ≤ 23.5 cm) to 16.3 (at length class 29-33.5 cm) followed by slight decrease 15.5 (at length class ≥34 cm). There was a highly significant increase ($p < 0.001$) in the prevalence and intensity of the infection with the increase in total fish length (Tab. 1).

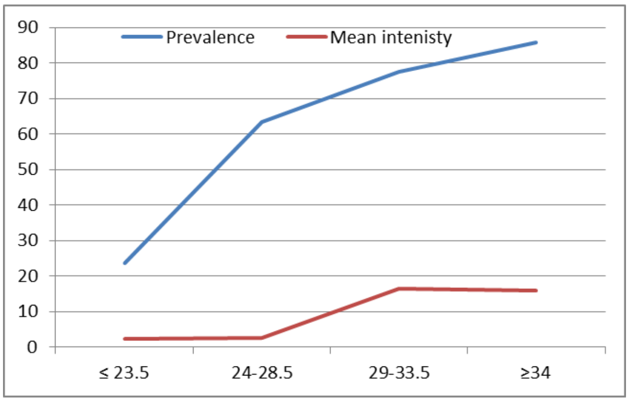

Figure 2. Variation in prevalence and mean intensity of *Anisakis* sp. larvae in *M. merluccius* by host length (cm).

When analyzing the infection rate and intensity of *Anisakis* larvae by host sex shown on Table 1, fourty- one of the examined 64 (64.6%) males were infected with 13.6 (1-300 larvae per fish). On the other hand, of the examined 30 females (fig. 3) were 19 (63.3%) infected with 4.8 (1-26 larvae per fish). There wasn`t a significant relationship between the sex of the host and the prevalence although males showed significantly heavier larval burden ($p<0.05$). Concerning the migration into the muscle, it's occurred in 5 out of the 60 (8.33%) infected fishes.

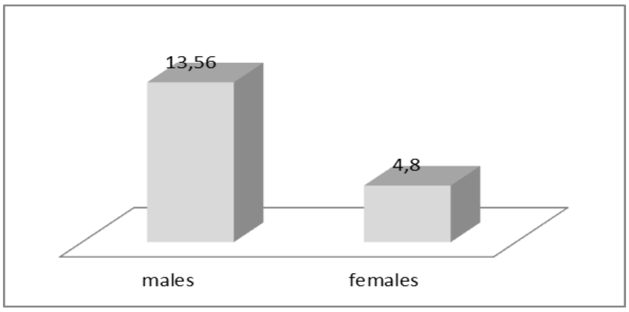

Figure 3. Mean intensity of *Anisakis* sp. larvae by host sex.

Table 1. Parasitological evaluation of *M. merluccius* infected by *Anisakis* sp. larvae.

FL (in cm)	EF/IF (P)	MI (range)	EM/IFM (P) MI (range)	EFM/IFFM (P) MI (range)
≤ 23.5	18/4(22.2)	2.3 (1-4)	12/2(16.7) 1.5 (1-2)	6/2 (33.3) 3 (2-4)
24-28.5	30/19(63.3)	2.5 (1-11)	20/14 (70) 2.14 (1-3)	10/5 (50) 3.4 (1-11)
29-33.5	31/24(77.4)	16.3 (1-300)	21/16 (76.1) 22.8 (1-300)	10/8 (80) 3.3 (1-7)
≥34	15/13(86.7)	15.5 (1-50)	11/9 (81.8) 17.7 (1-50)	4/4 (100) 10.5 (1-26)
Total	94/60(63.9)	10. 8 (1-300)	64/41 (64.1) 13. 6 (1-300)	30/19 (63.3) 4.8 (1-26)

FL. Fish length (in cm), **EF**. Nb of examined fishes, **IF**.Nb of infected fishes, **P**. prevalence, **MI**. mean intensity of infection, **EM**. examined males, **IFM** infected males, **EFM**. examined females, **IFFM**. infected females.

DISCUSSION

Our results showed that *Anisakis* sp. is a common parasite of Libyan marine fish *M. merluccius* and is recorded and descried for the first time in Libya. The morphometric values of our specimens were similar to the ones reported by Sanmartín *et al.* (1994) and Silva & Eiras (2003) for *Anisakis simplex* L3 larvae infecting several host fish species.

The high prevalence of the detected *Anisakis* larvae (63.9%) was higher than that reported from the same fish species in the Mediterranean off southern Spain (41.3%) and lower than that reported from the Atlantic off north-west Africa (88%) as reported by Valero *et al. (*2006 a). Such variation could be attributed to the variation of examined fish length classes.

The present prevalence was slightly higher than those of anisakids occurring in the greater forkbread "*Phycis blennoides"* and forkbread "*Phycis phycis"* (58.9% to 62.1%, respectively) from the Mediterranean costs of southern Spain (Valero *et al.* 2006 b) and much lower (63-100%) than those reported in specimens examined from the Atlantic Ocean (Wooten & Smith 1976, Sanmartín *et al.* 1989, Reuiz-Valero *et al.* 1992). However, a much lower prevalence of anisakid larvae were reported in Kuwaiti food fishes "12.7%" (Sey & Petter 1997); in Marine Fishes sold in Shenzhen, China "31%" (Chen *et al.* 2010) and in Spain fishes "23.5% and 0-11%" (Ruiz-Valero *et al.* 1992 and Gutiérrez-Galindo *et al*. 2010, respectively). Such variation could be attributed to the variation in type of the fish, type of prey and / or the abundance of crustacean intermediate hosts. The mean intensity in the present study (10.8 larvae⁄ fish) was higher than those reported in the same fish species either in the Mediterranean off southern Spain (1.73 larvae/fish) or from the Atlantic off north-west Africa (4.7 larvae /fish) (Valero *et al.* 2006 a).

The prevalence and intensity values of the infection varied significantly with the length of the studied fishes this agreed with Valero *et al.* (2006 a) for the same fish species. Such variation was most probably related to the intensity of the feeding upon the crustacean intermediate hosts or could be due to an accumulation of parasites in the host throughout its life (Bussmann & Ehrich 1979) or to the variations of the fish diet (Valero *et al.* 2006 b). It is important to state that the higher prevalence and intensity values were found in fish upper length classes, which are more intensely exploited. This agreed with a number of studies, which showed that the parasitic burden increases with the age and the length of the fish (McGladery 1986, Platt 1975, Valero *et al.* 2006 b). However, Silva & Eiras (2003) did not find such correlation, despite the tendency of higher values for the fish upper length classes. Also, Costa *et al.* (2004) found a positive but not significant correlation between intensity and length and they attributed the detected high values of prevalence and low values of intensity to the dispersing of larvae within their hosts. On the other hand, Davey (1972) reported that younger fish were often found to be more heavily infested than older fish and it was therefore suggested that the accumulation of larvae in the body cavity may be affected by annual fluctuations either in the population of an infested first intermediate host or in the extent to which the fish were feeding on this host.

The present task also revealed that there wasn`t a significant relationship between the sex of the host although males showed significantly heavier parasite burden ($p<0.05$). In this regard, Silva and Eyras (2003) did not find a significant relation between the sex of the host and either the prevalence or the intensity of *Anisakis* sp. infection, while Al-Zubaidy (2009) reported a slight significant difference between the infection of males and females with the Anisakid *Contracaecum* sp. although the females were more frequently infected than males.

The postmortem migration of the Anisakid larvae into the flesh of some of the studied fishes was also reported by some authors (Smith 1984, Smith & Wootten 1975, Cattan & Carvajal 1984). Apparently, it was believed that the migration occurs only in fatty species in which the lipids are mainly stored in the flesh "like *Clupea harengus* or *Scomber scombrus"* (Silva & Eiras, 2003). While in the non-fatty species, where stored lipids are mainly in the liver or mesenteries (like *Merlangius merlangus*), the migration does not occur (Smith 1984).

In this regard, Silva & Eiras (2003) showed conflicting results between the migration and the lipid content of the fish *Sardina pilchardus*, which has the highest lipid content in the muscle, experienced the smaller degree of migration, while *Trachurus trachurus* and *S. scombrus* (with intermediate amounts of lipids), experienced a very much higher migration levels. On the other hand, *M. poutassou*, clearly a non-fatty species, presented migration levels superior to the ones of *S. pilchardus*. However, it is difficult to explain why the migration occurred only in some specimens of the same host fishes (Silva & Eiras 2003). Therefore, we believe that some unknown factors, other than the lipid content of the flesh, are involved in causing the migration of the parasites.

The observed fish species represent the majority of the annual catches in Libya although there were no reported cases of human infection by *Anisakis*. This may be due to the inexistence of the habits of eating raw fish. However, the existence of human infections cannot be excluded, due to the lack of contact of physicians with *Anisakis* infection symptoms. In Spain, country with similar fish consumption per capita and similar gastronomic habits, the number of reported cases has increased rapidly, as a result of an increased awareness by physicians (Audicana *et al*. 2002, Silva & Eiras 2003).

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