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Prevalence of Parasites in the Mackerel (*Scomber scombrus*) from the Moroccan Mediterranean Coast

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ABSTRACT

This study represents the first investigation of its kind into the prevalence of animal-derived parasites found in the mackerel (*Scomber scombrus*) harvested from the Moroccan Mediterranean coast. The research shedded light on the potential risks of consuming the mackerel, which may carry various parasites originating from animals. The ecto- and endoparasites found in the catch samples from the M'diq and Jebha ports were addressed over one-year from October 2020 to May 2022, covering four seasons. The analysis involved a combination of metrics, morphological analysis, and identification techniques. A total of 495 fish specimens were examined, with 67.68% found to be parasitized. The results show that the prevalence was similar across the four seasons at each studied port, with some variation in the summer. Based on morphological examination, identified 1184 larvae, including 896 *Anisakis simplex*, 265 *Pseudoterranova decipiens*, and 23 *Contracaecum* were identidied. The study showed a high prevalence of Anisakidae in the collected mackerels, highlighting the need for precautions before consumption or export. To minimize the risk of Anisakidae migration to the flesh and muscles, it is recommended to gut the fish and consume it as fresh as possible.

INTRODUCTION

The global catch of fishing production reached a record as high as 96.4 million tons in 2018, a 5.4% increase from the average of the three previous years, primarily due to marine capture fishing **(FAO, 2020)**. Morocco, which ranks the first in Africa and the 25th globally, produced 1,511,267 tons in 2022, including 18,318 tons from the Mediterranean ports. M'diq and Jebha ports landed 2,335 and 501 tons, respectively **(ONP, 2023)**.

The consumption of raw fish continues to rise globally, including Morocco, where this culinary tradition is growing. However, the removal of cooking steps can pose risks for consumers due to parasitic infestations. Parasites such as Anisakidae, Plathelminths, Nematodes, Acanthocephalans, Crustacea, Isopods, and Copepods can cause serious harm to fish, including injuries, hemorrhages, growth deficiency, reduced reproduction, diseases, and high mortality rates **(Euzet & Pariselle, 1996 ; Ramdane *et al.*, 2009; Van As & Van As, 2019)**.

It is worthnoting that, raw fish may contain parasites from the Anisakidae family, which are commonly found in all species of fish globally caught. The consumption of raw or undercooked fish can lead to anisakiasis, an emerging zoonotic disease. In recent decades, the incidence of *anisakiasis* has risen dramatically, with more than 20,000 recorded human cases, mostly in Japan **(Hochbe Chai *et al*., 2005; Hochberg & Hamer, 2010; Mattiucci *et al*., 2013)**.

Visible worms in fish from various genera consumed by humans can cause economic losses and health problems, but removing all parasites from fish intended for human consumption can be difficult for the processing companies due to limitations in quality control systems **(Seesao, 2015)**.

The *Ascaridoidae* of the Anisakidae family are harmful to humans and are found in more than 200 species of fish and 25 cephalopods **(Seesao, 2015)**. The genus *Contracaecum* is less common but can also pose a public health threat **(Seesao, 2015)**. *Hysterothylacium* spp. are present in the intestines of 12 fish species, but their presence may lead to consumer rejection **(Seesao, 2015)**.

A retrospective survey carried out from 2010- 2014 among university hospitals in France revealed 37 cases of anisakidosis, including 18 cases of allergic anisakidosis and 6 severe allergy cases reported by the Allergo-Vigilance Network **(Dupouy-Camet *et al.*, 2020)**.

Hence, the aim of this study was to evaluate the prevalence of parasites in the common mackerel (*Scomber scombrus*) captured off the Moroccan Mediterranean coast, while additionally comparing the parasitological findings between the two designated ports of study.

MATERIALS AND METHODS

1. Study sites

The research was conducted in two Moroccan Mediterranean ports, M'diq (located 15km from the city of Tetouan at lat. 35° 40' 45" N and long. 5° 18' 50" W) and Jebha (120km from Tetouan at lat. 35°13'10" N and long. 4°40'45" W) (Fig. 1).

Fig. 1. Geographical location of the study ports

2. Sampling

A total of 495 mackerel (*Scomber scombrus*) specimens were collected from fishing vessels at the selected ports during the period of 2020- 2022. The fish samples were promptly transported to the laboratory on the same day in pre-cleaned polyethylene bags, where their total size, weight, and sex were determined. The fish underwent a visual examination for ectoparasites before being dissected with a ventral incision from the anus to the mouth. The dissection targeted various organs and locations: the visceral cavity, digestive tract, liver, and gonads, to search for endoparasites. The worms found were encapsulated and coiled in shape, embedded in the visceral organs, and made visible by pouring water into the cavity.

After collection, the parasites were preserved in 10% alcohol for identification under a light microscope. The identification was based on morphological details and conducted following the guidelines of **Nicolas *et al.* (2000)**.

3. Calculation of parasite indices

To gain a comprehensive understanding of the infestation status within the studied population and to elucidate the level of affinity between the parasite and its host, parasitic calculations were employed. Specifically, three parasitic indices were calculated, as proposed by **Bush *et al.* (1997)**:

The prevalence, abundance, and average intensity were assessed, utilizing the titles and definitions proposed by **Margolis *et al.* (1982)**.

Parasite prevalence (P%)

Parasite prevalence, also known as the parasitism rate, is determined by dividing the number of fish hosts infested by a particular species of parasite (NPI) by the total number of host fish examined (NPE). This ratio is then expressed as a percentage. It's important to note that an individual fish is classified as parasitized if it harbors at least one parasite.

$$P = NPI/NPE \times 100$$

- P: prevalence in%.
- NPI: Number of fish infested with a given species of parasite.
- NPE: Number of fish examined.

Average parasite abundance (Am)

Parasite abundance, often referred to as the infestation rate, is calculated by dividing the total number of individuals of a parasite species found within a sample of hosts by the total number of hosts in that sample (both infested and non-infested). It represents the average number of individuals of a particular parasite species per host examined.

$$A=NP/NPE$$

- NP: Total number of individuals of a parasite species.
- NPE: Number of fish examined.

Average parasite intensity (Im)

It is the ratio of the total number of individuals belonging to a group of parasites found in a sample of hosts to the number of fish that are infested with parasites.

$$Im= NP/NPI$$

- NP: Number of parasites.
- NPI: Number of infested fish.

RESULTS

A total of 495 the mackerel specimens were analyzed in two Moroccan ports (Port M'diq and Port Jebha) facing the Mediterranean Sea, with 293 mackerel specimens analyzed at Port M'diq and 202 at Port Jebha.

1. Parasitic indices

During the fourseasons, a series of sampling events were conducted at the Mdiq and Jebha ports to determine the (P) prevalence, (A) abundance, and (I) parasitic intensity, as shown in Tables (1, 2, 3 & 4).

Table 1. Parasitic indices of M'diq port according to season

	Fish examined	Infested fish	Year	Number of parasites	Prevalence (P)	Abundance (A)	Parasitic intensity (I)
Autumn	74	58	2020	195	78,38%	2.63	3.36
Winter	82	64	2021	163	78,05%	1.99	2.55
Spring	87	69	2021	364	79,31%	4.18	5.27
Summer	50	35	2021	109	70,00%	2.18	3.11
Total	**293**	**226**		**831**	**77,13%**	**2.84**	**3.68**

Table (1) reveals that 74 mackerel specimens were analyzed during the autumn (September - December 2020), 82 in winter (February 2022), 87 in spring (April - May 2021), and 50 in summer (August 2021). The findings suggest a consistent pattern across seasons, with parasite prevalence, abundance, and intensity showing minimal variation, indicating that these indices are not significantly influenced by seasonal factors.

Table 2. Seasonal variations in parasitic indices at Jebha port

	Fish examined	Infested fish	Year	Number of parasites	Prevalence (P)	Abundance (A)	Parasitic intensity (I)
Autumn	53	35	2020	87	66.04%	1.64	2.49
Winter	7	4	2021	8	57. 14%	1.14	2.00
Spring	68	51	2021	56	75. 00%	2.94	3.92
Summer	74	19	2021	200	25. 68%	0.76	2.95
Total	**202**	**109**		**351**	**53,96%**	**1.74**	**3.22**

Table (2) shows that 53 mackerel specimens were analyzed in autumn (August 2020), 7 in winter (February 2021), 68 in spring (May 2022), and 74 in summer (August 2021). The low number of the mackerels analyzed in the winter (7 fish) was due to weather problems and the scarcity of the species at EL Jebha port during this season. The autumn and winter results are similar, with a slight increase in spring (75%). The summer period showed a significant decrease in prevalence (26%) and abundance (0.757). To gain a comprehensive understanding of this non-ordinary case compared to other seasons, we analyzed additional parameters, such as the date of sampling, average weight, and average height. This information is presented in Table (3).

Table 3. Summer season insights: Unveiling additional results from Jebha port

Fish examined	Infested fish	Number of Parasites	P (%)	A	I	Date	Average weight (g)	Average size (mm)	% Immature fish
24	16	53	67	2.21	3.31	**15/08/21**	124.46	234.38	16.7
50	3	3	6	0.06	1	**16/08/21**	24.76	144.93	100

During the summer, two harvests were conducted. The first harvest on August 15, 2021 involved 24 individuals, 83.3% of whom were mature, with an average weight of 124.46g, and an average size of 234.38mm. This resulted in a 67% prevalence and 2.21 abundance, similar to the results recorded in autumn and winter. The second harvest on August 16, 2021 involved 50 individuals, with an average weight of 24.76g, average size of 144.93mm, 100% of which were immature. This confirms that the juvenile mackerels are increasingly less susceptible to Anisakidae parasites.

Generally, upon considering only the harvest of August 15, 2022, when all mackerels were mature, the results suggest that parasitism occurrence between the two ports remained consistent and unaffected by the season. The parasitic indices recorded in Port M'diq were slightly greater than those recorded in Port Jebha (70, 78, 78, and 79% in Port M'diq, and 64, 75, and 67% in Port Jebha), except for the summer prevalence of 57%. However, the limited number of fish analyzed in winter (7 fish) makes it difficult to draw definitive conclusions.

2. The location of parasites in the examined fish

During the examination for parasites in the mackerel specimens, the presence of worms in various organs was recorded. If the parasites were found in non-specific organs (such as the intestine, caecum, gonad, liver, swim bladder, etc.) or were freely floating in the water in the basin (used to enhance visibility of the worms), the location was recorded as the visceral cavity.

Table 4. Localization of parasites in the examined mackerel specimens

Parasite localization	%	Parasitic number
Visceral cavity	68.58%	812
Intestine	19.68%	233
Caecum	5.83%	69
Gonad	4.56%	54
Liver	1.18%	14
Swim bladder	0.17%	2
flesh and muscle	0.00 %	0
Total		**1184**

Table (4) displays the distribution of larvae in different organs of the mackerel specimens under examination. The majority of the larvae were found in the visceral cavity, accounting for 68.58% (n= 812 worms). Ignoring the visceral cavity, the intestine had the highest concentration of larvae (n = 233, 19.68%), followed by the caecum (n = 69 worms, 5.83%), gonads (n = 54 worms, 5.83%), liver (n = 14 worms, 1.18%) and 2 larvae (0.17%) in the swim bladder. Notably, no larvae were detected in the muscle tissue or flesh of the specimens.

3. Differential diagnosis of Anisakidae larvae

Following the guidelines of **Huang (1988)** and **Nicolas *et al.* (2000)** and based on the study by **Dadar *et al.* (2016)** (Fig. 2), we identified the parasites present in the mackerel samples collected from two study ports.

In our study, three genera of *Anisakids* were identified: *A. simplex*, *Pseudoterranova decipiens*, and *Contracoecum.*

Additionnaly, referring to **Huang (1988)** and **Nicolas *et al.* (2000)** and based on the study by **Dadar *et al.* (2016)** (Fig. 2), the morphological characteristics of those genera can be distinguished as follows:

The genus *Anisakis*: all *Anisakis* larvae are yellowish-white in color, 20 to 30mm long, and 0.3 to 0.5mm in diameter (Fig. 2A, B, I).

The genus *Pseudoterranova*: This genus has a large size (from 27 to 4 mm long and 0.8 to 1mm in diameter) and a reddish color, and has been found mostly in the muscles (Fig. 2G, H, M).

The genus *Contracoecum*: The L3 larvae present in the fish are 7 to 30mm long. It has an excretory pore located at its anterior end. These should be considered the most important morphological characteristics when differentiating *Contracaecum* species from the rest of the *Anisakid* nematodes, as they are the most consistent across all developmental stages (Fig. 2F, L).

The genus *Hysterothylacium* is represented by (C, D, E) and (J, K) (Fig. 2).

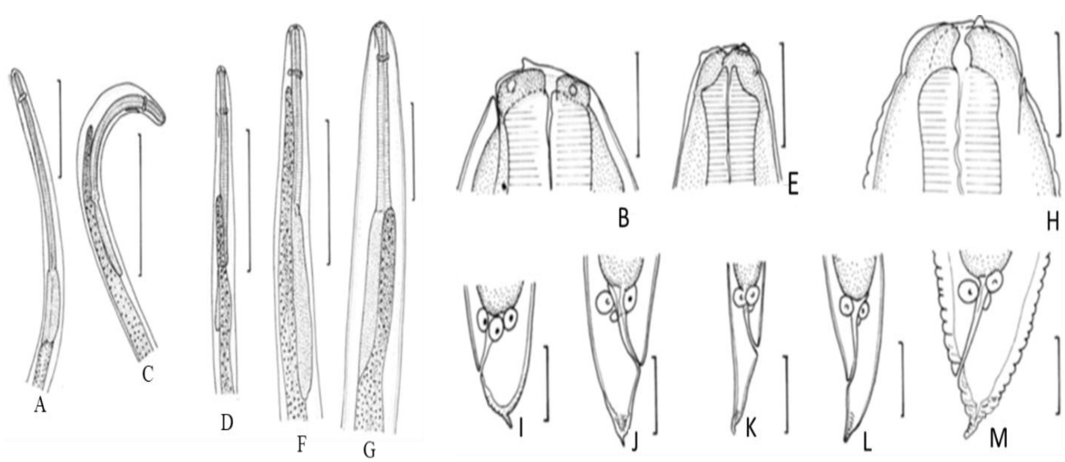

Fig. 2. L3 of *Anisakidae* (A- H: Anterior extremities; A- B: Anisakis simplex; C- D- E:

Hysterothylacium spp. ; F: *Contracaecum* sp. ; G- H: *Pseudoterranova decipiens*; I- M: Posterior ends; I: *Anisakis simplex*; J- K: *Hysterothylacium* spp. ; L: *Contracaecum* sp. ; M: *Pseudoterranova decipiens*, Line length: top designs: 1mm; middle and bottom designs: 100µm).

3.1 M'diq port

Table (5) and Fig. (3) display the percentages of species identified by season.

Table 5. Number of Anisakidae according to season (M'diq Port)

Genera of Anisakidae	Autumn	Winter	Spring	Summer	Total
Anisakis simplex	140	84	325	101	**650**
Pseudoterranova decipiens	50	79	32	8	**169**
Contracaecum	5	0	7	0	**12**
Hystérothylacium	0	0	0	0	**0**
Total	**195**	**163**	**364**	**109**	**831**

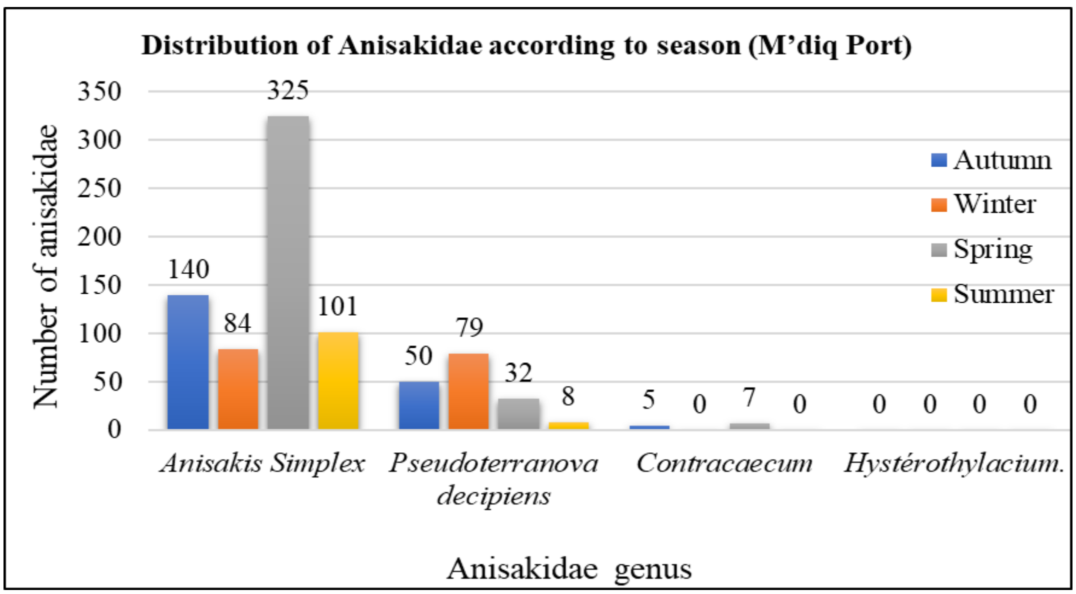

Fig. 3. Distribution of Anisakidae according to season (M'diq Port)

Table (5) and Fig. (3) reveal that *Anisakis simplex* (Fig. 3) is the predominant species in all four seasons, with 71.95% (140/195) in autumn, 89.28% (225/364) in spring, 92.66% (101/109) in summer, and an almost equal distribution with *Pseudoterranova decipiens* (Fig. 4) with 48.46% (79/163) and *A. Simplex* with 51.53% (84/163) during winter. *Pseudoterranova decipiens* accounted for 25.64% (50/195) in autumn, 8.79% (32/364) in spring, and 7.33% (8/109) in summer. The genus *Contracaecum* (Fig. 5) had a minor presence, at 2.56 % (5/195) in autumn, 1.92% (7/364) in spring, and 0% in summer and winter. This study revealed the complete absence of the genus *Hysterothylacium.*

During the year of the study at M'diq port, *simplex anisakis* was the most common species at 78.21% (650/831), followed by *Pseudoterranova decipiens* at 20.33% (169/831) and a small share of the *contracoecum* at 1.44% (12/831).

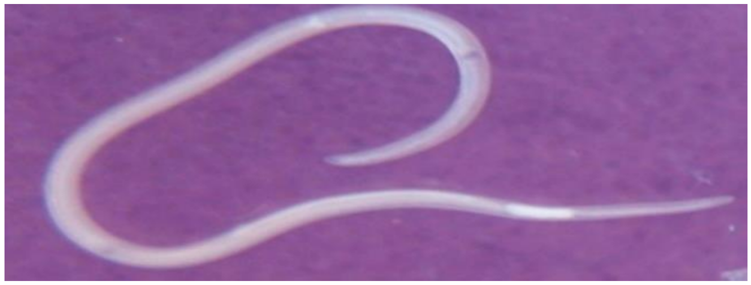

Fig. 4. Picture of *Anisakis simplex*

Fig. 5. Picture of *Pseudoterranova decipiens*

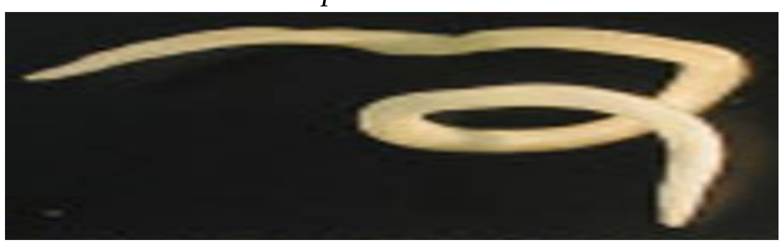

Fig. 6. Picture of *Contracoecum*

3.2 Jebha port

Table (6) and Fig. (4) present the percentages of species identified by season in relation to the port of Jebha.

Table 6. Number of Anisakidae according to season (Jebha port)

Genera of Anisakidae	Autumn	Winter	Spring	Summer	Total
Anisakis simplex	87	8	101	52	**248**
Pseudoterranova decipiens	0	0	89	0	**89**
Contracaecum	0	0	10	4	**14**
Hystérothylacium	0	0	0	0	**0**
Total	**87**	**8**	**200**	**56**	**351**

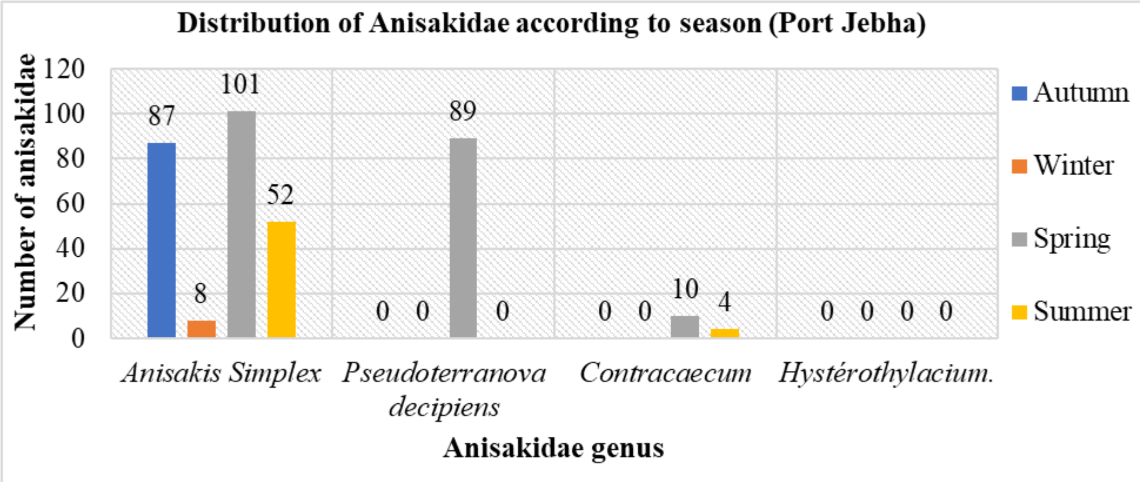

Fig. 7. Distribution of Anisakidae according to season (Port Jebha)

As shown in Table (6) and Fig. (7), *Anisakis simplex* was the most prevalent parasitic genus in the four seasons, at 100% in autumn (87/87) and winter (8/8), and 92.85% (52/56) in summer, followed by *Contracaecum* at 0% in autumn and winter, 7.14% (4/56) in summer and 10/200 (5%). During spring, there was an almost equal distribution of *Anisakis simplex* 101/200 (50.5%) and *Pseudoterranova decipiens* 89/100 (45.5%). Whereas, this study revealed no occurrence of the genus *Hysterothylacium*.

During the year of the study at the port of Jebha, the preponderance of *Anisakis simplex* was recorded to be 248/351 (70.65%), followed by *Pseudoterranova decipiens* at 89/351 (25.35) and *u* at 14/351 (3.98%).

DISCUSSION

Genera within the family Anisakidae, such as *Anisakis*, *Contracaecum*, *Pseudoterranova*, and *Hysterothylacium*, have been documented in various species of edible fish, including the blue whiting, Spanish mackerel, common hake, horse mackerel, red salmon, pink sea bream, *Pagellus acarne*, and sardine, among others **(Abattouy *et al.*, 2011; Ichalal *et al.*, 2015; Molina-Fernández *et al.*, 2015; Seesao, 2015; Cavallero *et al.*, 2019; Keltoum & Ramdane, 2019; Boukhari Benamara *et al.*, 2020)**. Factors influencing infestation with these parasites can vary among fish species, including fishing zone, number, seasonality, the nature of the sample taken (fillets, gutted or whole fish, wild or farmed state), and the method of parasite detection **(Seesao, 2015)**. The author also noted that worldwide prevalence rates are inconsistent due to a lack of standardization, making it challenging to compare the present data with those in the literature, as each study presented results in diverse ways.

Our sample prevalence is similar to that reported in previous studies, such as that of **Levsen *et al.* (2005)**, who reported a 32- 77% prevalence of *Anisakis* larvae in fish fillets in the North East Atlantic. Moreover, our results are consistent with the findings of

Abollo *et al.* (2001), who reported a 74.54% prevalence in 55 mackerels along the Galician coast (Spain) **(Cattan & Carvajal, 1984)**. **Chaligiannis *et al.* (2012)** even reported a 100% prevalence of Anisakidae in the Aegean Sea.

Angelucci *et al.* (2011) reported a prevalence of 77.8% in the Sardinian Sea (Eastern Mediterranean). The mackerel (*Scomber scombrus*) off the Bay of Biscay, Eastern Channel, and Gulf of Lion had an overall prevalence of 54.39%, with 50% in the viscera and 19.17% in the fillets **(Seesao, 2015)**. **Levesen *et al.* (2018)** reported an 87% overall and 52% muscle prevalence after examining 1801 mackerel specimens from the North East Atlantic and the Mediterranean. In this context, **Madrid *et al.* (2016)** reported a 58.4% total prevalence (55.4% in viscera, 26.0% in flesh) in 140 he Atlantic and 91 Mediterranean fish from some Spanish supermarkets. **Mostafa *et al.* (2020)** noted a 42.8% prevalence after examining 140 *Scomber scombrus* from fish markets in EL-Sharkia (Egypt). **Ozuni *et al*. (2021)** reported a 68% prevalence in 100 *Scomber scombrus* out of 856 fish collected over 5 years (2016- 2020).

The overall prevalence of *Anisakis* larvae was 25%, with 172 larvae isolated from viscera and 19 from muscle, which were revealed in 40 imported frozen mackerel (*Scomber scombrus*) from Norway to Turkey purposed for human consumption **(Pekmezci, 2014)**.

Additionally, a 70% prevalence of nematode larvae (27 in total) was found in the abdominal cavity of the Atlantic mackerel (*Scomber scombrus*) collected from the Marmara Sea in Turkey **(Özbakış Beceriklisoy *et al*., 2020)**.

In addition, a total of 83.3% prevalence of parasitism was found in 42 Atlantic mackerels (*S. scombrus*) purchased from the Portuguese Atlantic port auctions between October 2009, January 2010 and June 2010. The most common parasite was *A. simplex* (73%), followed by *A. pegreffii* (27%), and occasional occurrences of *Hysterothylacium aduncum* worms were also identified **(Santos *et al*., 2017)**.

Silva and Eiras (2003) reported a 95.6% prevalence of parasitism in 390 fish collected from the Portuguese west coast vessels, including 45 common mackerels. Another study by **Meloni *et al*. (2011)** examined 285 fish off Sardinia in the eastern Mediterranean over a 15-month period between Jan 2009 and April 2010 and reported a 50% prevalence (5/10) in 10 *Scomber scombrus* examined, with 17 Anisakidae present.

Studies on *Scomber scombrus* have shown a lower parasitic index, with an 11% prevalence after analyzing 447 mackerels in Tarragona, Spain over the period of February 1996- January 2000 **(Gutiérrez-Galindo *et al*., 2010)**. **Biary *et al*. (2021)** reported an 8.4% prevalence in 402 mackerels acquired from the Moroccan wholesale markets from January 2016 to December 2018. Eight mackerels from Turkish coasts (Aegean Sea) were found to be parasitized by 24 *Anisakis*, with an average intensity and abundance of 20.2 **(Pekmezci, 2014)**. Conversely, no Anisakidae larvae were detected in the 6 examined mackerels collected from the Spanish Mediterranean coast **(Pulleiro-Potel *et al*., 2015)**.

As reported, no larvae were found in the flesh of our specimens. This absence could be attributed to the short time elapsed between capture and examination, potentially impeding the migration of parasites into the flesh. It is advisable to consume fresh fish to minimize the risk of Anisakidae infection, as previous studies have shown that the duration of time between capture and consumption impacts the presence of larvae in the flesh. Nonetheless, certain studies have noted larvae exclusively in muscle tissue, a noteworthy observation within scientific literature.

In contrast, **Madrid *et al*. (2016)** reported a 26% prevalence of *Anisakis* larvae in the mackerel flesh. Studies on the distribution of *Anisakis* in fish have revealed variability in its prevalence between the viscera and flesh **(Madrid *et al.*, 2016)**. The migration of larvae to muscle tissue remains incompletely understood and may depend on factors such as host feeding habits or the distance traveled within the host **(Cattan & Carvajal, 1984; Strømnes & Andersen, 1998; Abollo *et al.*, 2001; Silva & Eiras, 2003; Cruz *et al*., 2007)**. However, excessive nutrients and fat content in muscle tissue may stimulate larval migration **(Levsen *et al*., 2018)**.

From this review of the literature and the results of our study, we can conclude that, most of the studies revealed that most of the mackerel (*Scomber scombrus*) examined were parasitized either by low or high prevalence, which requires precautions before consumption.

Given the potential risk underscored by our study regarding the elevated prevalence of *Anisakids* found in the mackerel, we advise the following precautions:

Consume mackerel immediately after catching to prevent the migration of Anisakidae into the flesh.

Promptly eviscerate the abdominal area to remove any larvae present in the abdominal cavity.

Refrain from consuming mackerel raw. If desired, freeze them at -20°C for a minimum of 24 hours, adhering to regulation CE/853/2004, before consumption.

Cooking thoroughly remains the most reliable method for preventing any risk of infection.

CONCLUSION

A total of 495 mackerels (*Scomber scombrus*) were examined from fishing vessels that landed in the M'diq and Jebha ports over the period October 10, 2020 – May 7, 2022, during four seasons per port. A high prevalence of Anisakidae, particularly *Anisakis simplex*, was found at both ports, indicating a greater risk of human contamination through the consumption of the mackerel in the Moroccan Mediterranean region.

Larvae were found only in the viscera, not in the flesh or muscle, likely due to the short time between harvesting and examination of the mackerel. No external parasites (ectoparasites) were identified in *Scomber scombrus*. *Anisakis simplex* was the dominant

Anisakidae larvae found in all seasons in the two monitored ports, with a low presence of *Contracaecum* and total absence of *Hysterothylacium*.

The consumption of third-stage larvae of *Anisakis simplex* and some *Pseudoterranova nematodes* in raw, undercooked, or inadequately vinegared fish can cause gastric illness and abdominal pain **(Mattiucci & Nascetti, 2008; EFSA, 2010)**. Even well-cooked fish infected with these parasites can cause allergic reactions due to their allergenic antigens **(Audicana & Kennedy, 2008)**. The high prevalence of Anisakidae in the examined mackerels calls for caution before consuming or exporting them.

Most of the larvae collected were in the third stage. Anisakidae were found in the viscera but not in the flesh or muscle. No external parasites were found on *Scomber scombrus*. To minimize the risk of *anisakiasis*, it is best to gut the fish and consume it fresh to prevent the migration of Anisakidae to the flesh and muscle before adequate cooking, salting, or vinegar treatment.

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