Research Article

Prevalence and Public Health Significance of Anisakis Larvae in some Marketed Marine Fish in Egypt

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Abstract | This study investigated the prevalence rate of Anisakis larvae in two marine water fish commonly consumed in Egypt, namely, herrings and sardine. Samples were collected equally from Suez, Ismailia, Damietta, Port Said and Alexandria. The obtained results revealed overall incidence rates of Anisakis larvae in herrings and sardine at 70% and 50%, respectively. The parasite infested mainly (100%) the visceral organs of the positive samples in the two fish species; while infested the muscles in 30% and 10% of herrings and sardine, respectively. The highest prevalence rates for the two species were recorded in the collected samples from Damietta, followed by Alexandria, Port Said, Ismailia, and Suez, respectively. The public health significance of Anisakis larvae was further discussed.

Keywords | Herrings; Sardine; Anisakis larvae; Egypt

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INTRODUCTION

Fish is considered as a major source for high quality protein, essential amino acids, polyunsaturated fatty acids, minerals, and vitamins (El-Ghareeb et al., 2021; Morshdy et al., 2013, 2019). However, fish is considered as a potential source for foodborne pathogens including bacteria, molds, and parasites. The World Health Organization (WHO, 2012) stated that about 56 million cases of human infection with parasitic diseases are associated with the consumption of fish.

Anisakids are nematodes that associated with a parasitic zoonotic disease named anisakiasis which occurs due to ingestion of the third larval stage of *Anisakis spp*. This parasite infests mainly marine fish and parasitizes crustaceans, cephalopods, and fish as intermediate hosts (Nieuwenhuizen and Lopata, 2013). The disease is caused mainly by *Anisakis simplex*, *Anisakis pegreffii*, and *Anisakis physeteris* (Mattiucci and Nascetti, 2008).

Humans acquire anisakis infestation via ingestion of inadequately cooked fish or raw fish such as sushi and sashimi (famous dishes in Japan) (Pampiglione et al., 2002). In addition, several anisakid antigens are thermostable and therefore, they constitute a major health concern (EFSA, 2010; Caraballo et al., 2011). Human anisakiasis is characterized by acute abdominal pain, nausea, vomiting, and the disease might progress to develop peptic and duodenal ulcers, appendicitis, and peritonitis. Hypersensitivity, urticaria, and anaphylaxis are also among the critical symptoms in some highly susceptible people (Villazanakretzer et al., 2016).

The third Anisakis larval stage is observed in many fish species worldwide. Herrings are among the important fish species in Egypt and Mediterranean countries and play important roles in the economy and fish trading in those countries. Herrings are sold in Egypt either as raw herrings or as smoked ones. The latter is very popular in Egypt because of its specific aroma and flavor. However, herrings are also considered as among the important hosts for *Anisakis spp.* (Bao et al., 2017; Guardone et al., 2017). *Sardine spp.* is another important fish species of high nutritive value that is very common in the fish menu in Egypt. At the same time, this species is among the natural hosts for *Anisakis spp.* (Debenedetti et al., 2019).

Studying the prevalence of anisakis larvae in fish in Egypt, particularly among the marketed herrings and sardine has received less attention. In sight of the previous facts, this study aimed at investigation the prevalence rates of *Anisakis spp.* in the retailed raw herrings and sardines. Discussion of the public health significance of this nematode was followed.

MATERIAL AND METHODS

Collection of Samples

A total of 100 random whole fish, including 50 fish from each of herrings (*Clupea harengus Linnaeus*), and sardine (*Sardinella aurita*). Fish samples were collected equally from fish markets (n = 20 fish/from each locality) in Suez, Ismailia, Port Said, Damietta, and Alexandria, Egypt on a daily basis in the morning and within 2 h from the arrival of the fish into the market. Fish samples were intact, with fresh smell, and reddish gills. Samples were transferred cooled directly without delay to the Laboratory of Food Hygiene, Faculty of Veterinary Medicine, Zagazig University, Egypt for parasitological examination.

Fish examination

Fish samples were dissected and analyzed for the presence of Anisakid larvae by careful inspection of the fish viscera under a stereoscopic microscope. Fish muscles were exposed to an artificial enzymatic digestion according to Llarena-Reino et al. (2013) and the resultant product was examined under a stereoscopic microscope.

Morphological identification

All the detected anisakids were identified according to the morphological characters described before (Gibbons, 2010). The main criteria considered for anisakids classifications were the position of the excretory pore, the shape of the tail, the arrangement and separation of the digestive tract into esophagus, ventricle and the presence/absence of structures such as intestinal caeca and esophageal appendix.

Molecular identification

DNA extraction, quantification, and quality assessment: Total DNA extraction and quantitative estimation of DNA from 1 to 4 larvae per sample was performed according to Guardone et al. (2016). In addition, DNA integrity was further evaluated according to Giusti et al. (2019).

Amplification of the mitochondrial cytochrome c oxidase subunit II gene: Amplification of a 629 bp fragment of the mitochondrial cytochrome c oxidase subunit II (cox2) gene was performed as described in Guardone et al. (2018). The cycling conditions used for the amplification procedures started with an initial denaturation at 95 °C for 3 min, followed by 45 cycles at 95 °C for 20 s, 52 °C for 20 s, 72 °C for 25 s, and a final extension cycle at 72 °C for 10 min was followed.

Statistical Analysis

The prevalence and the distribution frequencies were analyzed according to Bush et al. (1997) for the total anisakid larvae in relation to the place of infestation either the muscle or the viscera and to the origin of the sample collection.

RESULTS

The obtained results in the present study revealed detection of Anisakis larvae in herrings and sardine with overall prevalence rates of 70% and 50%, respectively. Anisakis larvae were detected at 70% and 50% in the viscera of the two species, respectively. The larvae infested the muscles at lower rates 30% (herrings), and 10% (sardine), respectively (Figure 1).

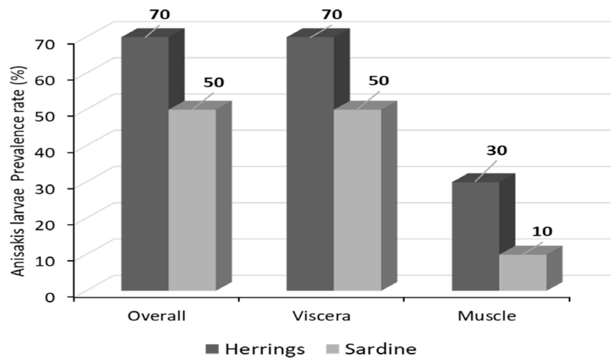

Figure 1: Prevalence rates (%) of Anisakis larvae in the examined herrings and sardine

The results recorded in Figure 2 showed that fish sampled at Damietta had had the highest infestation rate with Anisakis larvae, while that sampled at Suez had the lowest infestation rate.

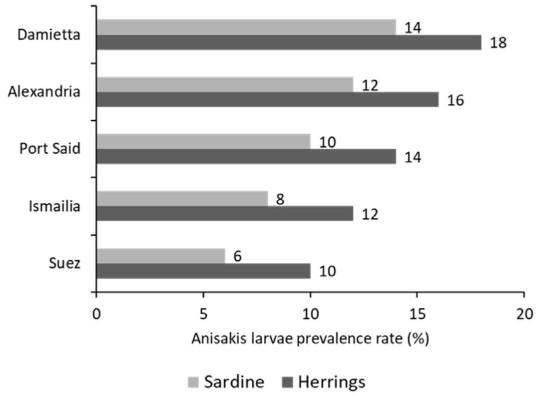

Figure 2: Prevalence rates (%) of Anisakis larvae in the collected herrings and sardine from different localities in Egypt

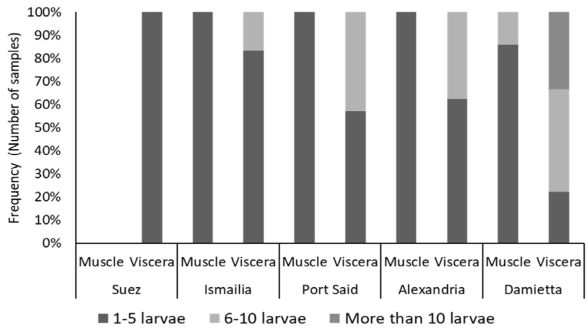

Figure 3: Frequency distribution of Anisakis larvae from viscera and muscle of herrings collected from different localities in Egypt

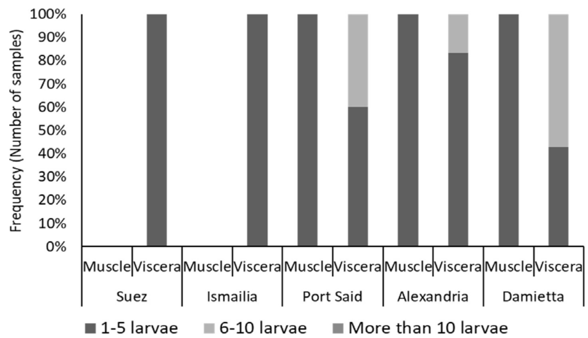

Figure 4: Frequency distribution of Anisakis larvae from viscera and muscle of sardine collected from different localities in Egypt

The presented data in Figure 3 showed detection of more than 10 anisakis larvae in the viscera of 30% of the herrings collected from Damietta representing a high-risk group, while 6 to 10 larvae (intermediate-risk group) were detected at 0%, 10%, 30%, 30%, and 40% in the viscera of the herrings collected from Suez, Ismailia, Port Said, Alexandria, and Damietta, respectively. Sardine located at the intermediate-risk group as Anisakis larvae was detected at only 10%, 20%, and 40% in the viscera of sardine collected from Alexandria, Port Said, and Damietta (Figure 4).

DISCUSSION

Herrings and sardine are among the most important fish species in the Egyptian markets. These fish kinds are used as fried or grilled or pass into further manufacture and processing steps to make new types of fish products such as salted sardine, or smoked herrings. The obtained results in Figure 1 revealed that herrings had higher infestation rate with Anisakis larvae compared with sardine. Anisakis larvae infested the viscera at higher rates compared with the muscles in the two fish species examined. In agreement with the obtained results in the current study, Unger et al. (2014) detected Anisakis larvae in the viscera of the herrings collected from the Baltic-sea at 100%. Bao et al. (2017) detected Anisakis larvae in the visceral organs of the herrings collected from the North Sea at 76%. Similarly, Levsen et al. (2018) detected the parasite in the body cavity of the herrings collected from the North Sea at 81.2% and in the muscles at 17.4%. In addition, Guardone et al. (2019) detected Anisakis larvae at 41.5% from the marketed herrings and herrings; products in Italy. They further reported that visceral organs were the most favorable site for the parasite where, they can detect the larvae at 98% from the positive samples. Anisakis larvae were also detected in sardine collected from Spain at 6.76% (Debenedetti et al., 2019). Generally, sardine is considered as a fish with low risk of anisakiosis because of the low prevalence rate (Cavallero et al., 2015; Gutiérrez-Galindo et al., 2010).

The prevalence of Anisakis larvae in the two examined fish species varied according to the place of the collection. As the highest prevalence rates of the parasite from the two fish species came in the following order: Damietta > Alexandria > Port Said (Mediterranean Sea source) > Ismailia (Suez Canal source) > Suez (Red Sea source) (Figure 2). In agreement with this observation, Debenedetti et al. (2019) observed a clear variation in the prevalence rates of Anisakis larvae in different fish species (hake, mullet, sardine, mackerel, and anchovy) collected from the Atlantic Ocean than same fish species collected from the Mediterranean Sea. Therefore, the origin of the fish should be considered as a critical factor in order to reduce the infection risk by this zoonosis. This assumption agrees with Abollo et al. (2001), Silva and Eiras (2003), and Herrador et al. (2019). Higher infestation rates were observed in the viscera compared with the muscles, particularly the viscera of herrings which represented a high-risk group (Figure 3, 4). Unlikely, there was no high-risk group in sardine, i.e., more than 10 larvae per fish (Debenedetti et al., 2019). The obtained result in the present study agrees with the report of Debenedetti et al. (2019) who demonstrated a clear variation in the frequency of distribution of anisakis larvae in different fish species including anchovy, hake, sardine, mullet, and mackerel. In all data analyzed, the high prevalence of the larvae in the viscera compared with the flesh was observed. People usually consume the fish flesh than the viscera; however cross contamination of the muscle can take place during any step of fish preparation starting from evisceration, cleaning, or even migration of the larvae from the viscera to the flesh (Abollo et al., 2001).

Parasitic infestation of the fish with anisakis larvae has several adverse health effects on the fish starting from inflammation during larval penetration to the different visceral organs with significant reduction in the physiological functions of such organs. In addition, the larvae can excrete some chemical compounds such as pentanols and pentanones which have local anaesthetic effects on the fish muscles and therefore affecting the swimming ability of the fish making them easy targets for their predatory fishes (Buchmann and Mehrdana, 2016; Haarder et al., 2013; Rohlwing et al., 1998).

Consumption of fish contaminated with anisakis larvae might have several adverse health effects including abdominal anisakiasis in the humans due to penetration of the gastric or abdominal mucosa (Audicana et al., 1997; Di Azevedo et al., 2017). In addition, allergic reactions that might reach to anaphylaxes might occur, particularly in the high-risk groups including children, elderly, and debilitating patients (Song et al., 2019; Pozio, 2013).

Among the effective strategies to prevent anisakis infection are to prevent their migration postmortem from viscera to flesh by visual inspection by the fishermen or the consumers and collecting the larvae manually, avoid consumption of undercooked or raw fish, efficient cooking of the fish by allowing an internal temperature of 60°C for 1-3 minutes, adequate freezing of the fish for 24 hours at -20°C, and pickling of the fish in vinegar and salt is also considered as a suitable method for reducing the hazards of the anisakis larvae (EC, 2011).

In conclusion, the obtained results in the present study revealed detection of anisakis larvae in both herrings and sardine collected from different locations in Egypt at variable percentages. Herrings had higher prevalence rates compared with the sardine. The nematodes mainly infested the viscera than the muscle. Therefore, efficient cooking of the fish is recommended as a standard preventive strategy to reduce the risk of human anisakiasis in Egypt.

CONFLICT OF INTEREST

None.

AUTHORS CONTRIBUTION

All authors contributed equally.

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