**RESEARCH ARTICLE**

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# First Record of *Anisakis* sp. Third-Stage Larvae (Nematoda) Occurrence, as a Human Risk, in Pacific Jack Mackerel from a Fish Processing and Packaging Plant in the North of Iran

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**ABSTRACT**

**Purpose:** This research was performed with the aim of hygienic tracking for fish-borne parasitic contamination imported by Jack mackerel fish from certain parts of the world in a fish processing unit during a health inspection in the north region of Iran in the summer of the year 2019.

**Methods:** A sample of 150 jack mackerel fish specimens was selected at random. All the fish had the same length and weight sizes at average measures of 53.6 cm and 2.04 kg. The cysts consisting of robust parasites seen on mesentery and muscles were then removed larval from their locality and placed in small glass jars containing 10% formalin and transferred to the lab.

**The results:** A total of 100% of Jack Mackerel (*Trachurus symmetricus*) fish cargo, as a portion of human food, imported from New Zealand were severely contaminated with *Anisakis* sp. worm parasite. The parasite intensity ranges were between 25 and 70 individuals per fish, and the total of parasites was 7125 individuals. The mean intensity reached 47.5. The encysted parasite specimens were observed in the muscles, mesentery, and peritoneum of viscera around the abdominal cavity of mackerel whereas seeing as twisted third-stage larvae.

**Conclusion:** As an aspect of human sanitary, the *Anisakis* species due to being zoonoses has significant importance. For this reason, people should avoid consuming raw or semi-cooked marine fish. The present study is the first available report of *Anisakis* sp. contamination in Jack mackerel imported to Iran.

**Key words:** Cargo, Contaminate, Encapsulate, Jack mackerel, New Zealand

## Introduction

Pacific jack mackerel *Trachurus symmetricus* (Ayres, 1855) is fished both for commercial and sport. They are frequently caught on baited hooks from docks and boats. Pacific jack mackerel is processed and canned in the same procedure as salmon. The Pacific jack mackerel, also known as the Californian jack mackerel or simply jack mackerel, is an abundant species of pelagic marine fish in the jack family, Carangidae. The species are spread along the western coast of North America, extending from Alaska in the north to the Gulf of California in the south, inhabiting both offshore and onshore environments. Pacific jack mackerel travel in large groups, ranging up to 600 miles offshore and to depths of 400m, usually moving through the upper part of the water column. They are famous to inhabit bays and very shallow waters, in more coastal environments.

The studied fish are obviously not caught in Iran and are occasionally imported into the country as seafood from other parts of the world. There are other types of fish imported into the country consisting of live trout, salmon (fresh and frozen fish, fillet), Tilapia (fillets and other parts of the fish), tuna (products and canned food), live ornamental fish and other kinds of fish as live, frozen, processed food products with fish origin and fillets. The monitoring and control over the quality and hygiene of imported products is the responsibility of the country's veterinary organization, and regular inspection is carried out by health inspections.

Nematode larvae of the genus *Anisakis* Dujardin (Nematoda: Anisakidae) are common parasites in a variety of marine fish as intermediate hosts with a global distribution

(Ubeira et al., 2000). It is estimated that 40%-80% of marine fish are infected by *Anisakis* spp. larvae (Yubero et al., 2004). Typically, hot spots for *Anisakis* spp. are considered to be the waters of the north/northeast Atlantic, the Mediterranean, the west and east Pacific Ocean, as well as the coasts of Japan, China, and North America. Landing distance, temperature, depth, salinity, currents, nutrients upwelling, and water productivity are factors to be considered influential in *Anisakis* spp. distribution (Lopata & Lehrer, 2009). The lifecycle of anisakid parasites follows the general nematode lifecycle, including four larval stages ($L_1$–$L_4$) and the adults in the final hosts. The adult stages of *Anisakis* reside in the stomach of marine mammals (such as whales and dolphins) and pinnipeds (such as seals, Porpoises, and sea lions), and unembryonated eggs are expelled with the feces. These eggs develop and hatch, releasing free-swimming $L_3$, which are ingested by euphausiid oceanic krill and copepods (first intermediate hosts). Fish is the second intermediate host of the parasites, whereas marine mammals typically cetaceans and pinnipeds are the definitive ones (Akbar & Ghosh, 2005). Sea fish and cephalopods (paratenic hosts) ingest planktonic crustaceans and other fish and cephalopods infected with $L_3$ larvae, contributing to the dissemination of the parasite. The infective $L_3$ (embedded in the viscera and muscle or free in the body cavity) is transferred to the final hosts (marine mammals) by ingestion of the sea fish and cephalopods (in the case of dolphins, seals, and sea lions) or via oceanic krill (in the case of whales). In the final host, two molts occur (from $L_3$ to adult) before sexual maturity to produce eggs, and a further cycle is initiated. As stated by the authors, the genus *Anisakis* has the greatest importance in the Anisakidae family because of the high pathogenicity of the $L_3$ larvae (Grabda, 1981; Lorenzo et al., 2000). They present potential risks to human health since these larvae can infect humans following ingestion of raw or undercooked fish (Silva & Eiras, 2003; Mattiucci et al., 2011). While the *Anisakis* nematodes and their impact on public hygiene have been investigated in many fish species worldwide, there have been a few studies of them in commercial fish in Iranian waters. This research was conducted in order to detect fish-borne parasitic zoonoses in seafood imported to Iran, which is associated with the public health of the human community.

## Materials and Methods

Frozen fish were taken out of the cold storage of the fish processing and packaging unit, all 150 fish specimens were selected at random, then both the length and weight of the individual fish body were carefully measured individually before the autopsy. The fish had an average length and weight size of 21.1 inches (53.6 cm) and 4.5 pounds (2.04 kg), respectively. Next, longitudinal incisions were made along the abdomen using a sharp knife. The abdominal cavity was precisely examined according to standard protocol, using a magnifying glass. The encapsulated parasite specimens were observed in the muscles, mesentery, and peritoneum of viscera, around the body cavity of mackerel whereas seen as twisted third-stage *Anisakis* larvae. The cysts consisting of the robust nematode larvae were then removed from their locality. Next, the larvae came out of the cyst using a dissecting needle and started to count with the aid of a dissecting microscope. Later, some parasites were placed in small glass jars containing 10% formalin and transferred to the laboratory of the aquaculture inland waters research center in Port of Anzali for further purposes. Upon arrival at the laboratory, all parasites were then fixed in A.F.A (ethanol, formalin, acetic acid) (Humason, 1979), and a few specimens were stained with aceto-carmine, clarified in Lacto phenol, and mounted in Canada balsam on a slide under a coverslip for permanent preparation in accordance with common methods in Parasitology experiments, given by Fernando et al. (1972) and Roberts (2001). Classical epidemiological variables such as prevalence value and mean intensity rate were calculated in accordance with Bush et al. (1997). Finally, the parasites were identified morphologically according to the valid diagnostic key (Moravec & Manoharan, 2013), and photographed with the camera attached to a stereo microscope.

## Results and Discussion

There is a paucity of knowledge about the epidemiology and ecology of anisakid infections. Researchers discovered that the genus *Anisakis* consists of nine species and mainly two species of the *A. simplex* complex: *A. simplex* sensu-stricto (s.s.) and *A. pegreffii* have been associated with infections in humans (Audicana & Kennedy, 2008; Mattiucci & Nascetti, 2008).

As Davey (1971) claims, *Anisakis simplex* (Rudolphi, 1809; Krabbe, 1878) in its larval stage is a common nematode parasite of marine fish, with the adult parasite widely distributed in marine mammals. Scientists declared that indeed in the case of ingestion by humans via eating raw or uncooked infected marine fish, $L_3$ will molt twice and develop into adult worms that penetrate the gastric and intestinal mucosa, causing the symptoms of *Anisakiasis* (Kagei, 1968; Klimpel et al., 2004; Nagasawa, 1990). According to recent studies, *Anisakiasis* is a parasitic zoonosis caused by accidental ingestion of $L_3$ nematodes of the Anisakidae family upon consuming raw or lightly preserved infected fish (Baptista-Fernandes et al., 2017).

In a recent review, 100% of Jack Mackerel, *Trachurus symmetricus* (Ayres, 1855) fish cargo, as a portion of human food, imported from New Zealand were severely contaminated with a fish-borne *Anisakis* sp. worm parasite (Figure 1). The parasite intensity ranges were between 25 and 70 individuals per fish, and a total number of 7125 individual parasites were

estimated. The mean intensity reached 47.5. So far this parasite has been detected in various fish species in Iran. The larval stages of *A. simplex* were isolated from sprats (Shamsi et al., 1998), *Acipenser stellatus* (Mokhayer, 1974), and *Rutilus kutum* (Eslami & Kohneshahri, 1978). Mirghaed (2017) found *A. simplex* in the intestine, mesenteric, and liver of *Alosa caspia* from the southeast part of the Caspian Sea with a prevalence value of 33.3%. Nevertheless, there was no reported sign of infection with this parasite in examining fish previously, and the present research is the only available survey in the country.

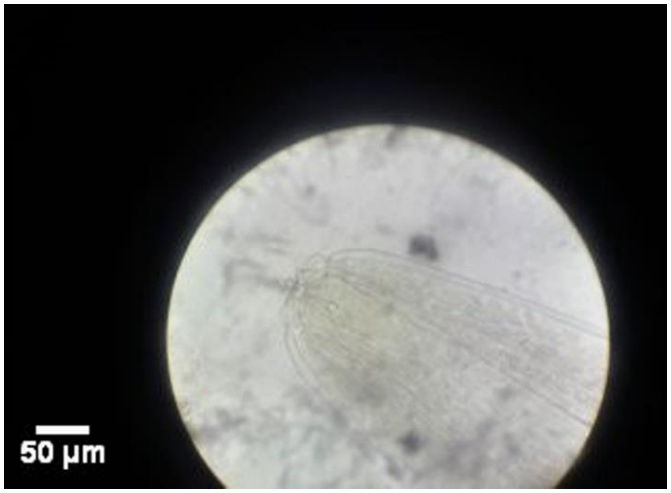


**Figure 1.** *Anterior part of Anisakis sp. third-stage larva from Abdominal cavity of Jack mackerel mag. 400x.*

*Anisakis* larvae was first observed in marine crustaceans by Uspenskaya (1963), who introduced amphipod *Caprella septentrionalis*, decapod *Hyas araneus* and euphausiid *Thysanoessa raschii* as the first intermediate hosts of *Anisakis* larval in the Barents Sea. Also, Smith (1971) has identified *Anisakis* sp. larvae in three *euphausiid* species (*T. inermis*, *T. longicaudata*, and *Meganyctiphanes norvegica*) from the northern North Sea and North-East Atlantic. Euphausiid *Nyctiphanes australis* and the galatheid crab *Munida gregaria* are suggested to be the first intermediate hosts of *Anisakis* larvae in New Zealand waters (Hurst, 1984b). The presence of *Anisakis* larvae has been recorded in a variety of fish in New Zealand (Hewitt & Hine, 1972; Sharples & Evans, 1995). Some specialists have also shown larval Anisakids can pass across various fish species via predation and can be accumulated in larger fish (Burt et al., 1990; Jensen, 1997). In agreement with our survey, Hurst (1984a) has recorded Jack mackerel to be infected commonly with *Anisakis* sp. Larvae.

Researchers revealed *Anisakis* spp. larvae can penetrate the digestive tract and migrate along the intestinal wall, tongue, lung, lymphatic ganglia, or pancreas in humans (Rosales et al., 1999), and result in remarkable clinical signs such as; allergic reaction, stomach pain, vomiting, nausea, and gingivostomatitis (Ancillo et al., 1997; Audicana et al., 2002). The severity of destruction, necrosis of the liver parenchymal, and ruptures of the wall of the blood vessels in abdominal cavity organs are caused by migration of *Anisakis* spp. larvae across infected tissues (Hauck & May, 1977: Smith, 1984). Migration of *Anisakis* larvae shortly after capture while fish are not frozen or filleted into the body musculature, has been observed in Europe and North America (Smith & Wootten, 1975; Smith, 1984). Postmortem migration has also been demonstrated in herring and mackerel through some investigations (Carvajal et al., 1979; Smith, 1984).

Several types of research exhibited that the severity of *Anisakiasis* varies from mild to severe and can have gastric, intestinal, ectopic, and allergic forms (Anibarro et al., 2007; Audicana & Kennedy, 2008; Baird et al., 2014). As displayed by Woo (1995) the symptoms and severity of the disease can vary considerably depending on factors, such as the species of fish, the intensity of the infection parasite on the fish, and the particular organs invaded. The disease when the anisakid larvae infect the liver and cause fibrosis of the liver is the most severe which can lead to atrophy of this organ and significant loss in body weight. Immunologists noticed an acute infection may be associated with allergic reactions, such as urticaria, angioedema, bronchospasm, and even severe anaphylaxis (Audicana et al., 2002). These facts make *Anisakis* spp. dangerous both dead and alive (Audicana et al., 2002; Audicana & Kennedy, 2008).

Since the first reports acknowledge the pathogenic effects of *Anisakis* species in humans (Van Thiel et al., 1960, 1962), the awareness of fish-borne parasitic diseases have been increased (Zhu et al., 1998; Smith & Wooten, 1978; Olson et al., 1983). As the gut of a marine mammal is functionally very similar to that of a human, *Anisakis* species are able to infect humans who eat raw or poorly cooked fish fillets containing infective larvae (Sugawara et al., 2004). Audicana *et al.* (1997) and Morenoancillo *et al.* (1997) described exposure to *Anisakis* antigens, which can cause a severe allergic reaction, even in well-cooked or frozen fish. Also, Oshima (1972) and Ishikura *et al.* (1989) have shown the parasite causes eosinophilic granuloma in the alimentary tract of man when raw or inadequately cooked marine fish with live larvae are ingested.

As a view aspect of public sanitary, *Anisakis* species due to being zoonoses have significant importance. For this reason, people should avoid consuming raw, semi-cooked/smoked, or superficially salted marine fish meat caught from the sea.

## Conclusion

Employing more educational and promotional programs is essential at all levels, considering the high number of *Anisakiasis* cases worldwide, focusing on the consumption

only of fish that was previously frozen or properly cooked. Only a few hours after the death of fish *Anisakis* spp. larvae are moving into the muscle tissue, thus early evisceration is much more important to prevent *Anisakiasis*. The risk of *Anisakiasis* is increased highly by a prolonged time of evisceration. Therefore, it is recommended that fish should be immediately gutted onboard the boat (Knoff et al., 2007, 2013; D'amico et al., 2014; Fonseca et al., 2016).

According to Lopata and Lehrer's (2009) statement, the allergenic proteins are exceptionally resistant to denaturation, leading to IgE antibody-mediated allergic reactions. Hazardous allergic reactions, including anaphylaxis, are due to the presence of *Anisakis* allergens. In 2014, the first case of *Anisakiasis* presented as a bowel obstruction in a child in Croatia (Juric et al., 2013). In the end, this is the only existing record of contamination with *Anisakis* larvae on New Zealand Jack mackerel, which has been imported to Iran across seafood.

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