3(1): 21-28 (2017) doi: 10.3153/JFHS17003

Journal of Food and Health Science

E-ISSN 2149-0473

REVIEW ARTICLE DERLEME MAKALESİ

ANISAKIASIS: PARASITIC HAZARD IN RAW OR UNCOOKED SEAFOOD PRODUCTS AND PREVENTION WAYS

Osman Kadir Topuz, Nalan Gökoğlu

Akdeniz University, Faculty of Fisheries, Department of Seafood Processing Technology, Antalya, Turkey

Received: 25.06.2016

Accepted: 02.11.2016

Published online: 23.11.2016

Corresponding author:

Osman Kadir TOPUZ, Akdeniz University, Faculty of Fisheries, Department of Seafood Processing Technology, 07054, Konyaalti, Antalya,-Turkey

E-mail: oktopuz@akdeniz.edu.tr

Abstract:

Parasitic infections related to the consumption of raw or uncooked seafood products have always been a concern for the consumers and for seafood economy. Anisakiasis is a serious zoonotic disease related with a wide range of syndromes in humans caused by member of Anisakidae. In last decade, an increasing number of anisakiasis disease have been reported, and this has been connected to the increase of globalized eating habits, ready to eat practices, the trend to avoid excessive cooked foods for nutrient preservation, consumption of fresh seafood for health reasons. Raw or slightly cooked ready-to-eat seafood products such as marinated, salted and cold smoked fish products, sushi and sashimi are the tool for transmission of *Anisakis* spp. larvae to human gastrointestinal system. As well as the factors that have yielded to an increase of the Anisakiasis cases, public health issues, anisakiasis symptoms, and methods to kill the *Anisakis* spp. larvae such as freezing, cooking, salting, marinating, irradiation, high hydrostatic pressure and chemicals have been reviewed in this study.

Keywords: Food safety, Parasitic hazard, Anisakiasis incidence, *Anisakis* spp.

Introduction

Parasitic infections regarding the eating of raw and uncooked seafood products have always been a concern for the consumers and for economy. In the last decade, there has been an increased number of reports regarding infections and/or allergic reactions in consumers owing to the increased awareness of doctors and an increased prevalence of these parasites in fish (Pozio, 2013). The main origin zoonoses related with the consumption of raw and uncooked seafood products are mainly due to the trematodes, cestodes and nematodes. Among the last mentioned, the anisakis species are the most common parasites from a sanitary way of thinking, since they are capable of inducing pathologies in consumers (Chai, Murrell, & Lymbery, 2005). Anisakiasis is a disease caused by nematodes having larval stages in aquatic hosts. The one of main nematode known to has caused disease in humans is *Anisakis simplex* (Beldsoe & Oria, 2001). Nematode of the genera *Anisakis* is parasite of sea mammals at the adult stage and of fish and cephalopods at the larval life stage (Anastasio et al., 2016; Pozio, 2013). Parasitic nematode, *Anisakis simplex,* reaches sexual maturity in the intestinal tract of marine mammals. The life cycle of anisakis species is shown at Figure 1. The life cycle of *Anisakis* spp. starts in the feces of an infected marine mammal (1). Marine mammals excrete unembryonated eggs (2). Eggs become embryonated in water and L2 larvae stage form in the eggs (3). After the L2 larvae hatch from eggs, they become free swimming. Free-swimming larvae are ingested by crustaceans and they mature into L3 larvae form (4). Infected crustaceans are eaten by fish such as rockfish, herring, mackerel, salmon and anchovy or squid (5). After the the host's death, larvae move to the muscle tissue, and through predation, the larvae are transferred from fish to fish this ways (6). Marine mammals such as dolphins, seals or humans may become infected from consuming the infected intermediate host (7). In humans, these worms do not mature, but the worms can migrate from the gastrointestinal tract, becoming embedded in the gastrointestinal mucosa and yielding tissue reaction and discomfort that is, gastric pain, diarrhea, vomiting (Beldsoe & Oria, 2001).

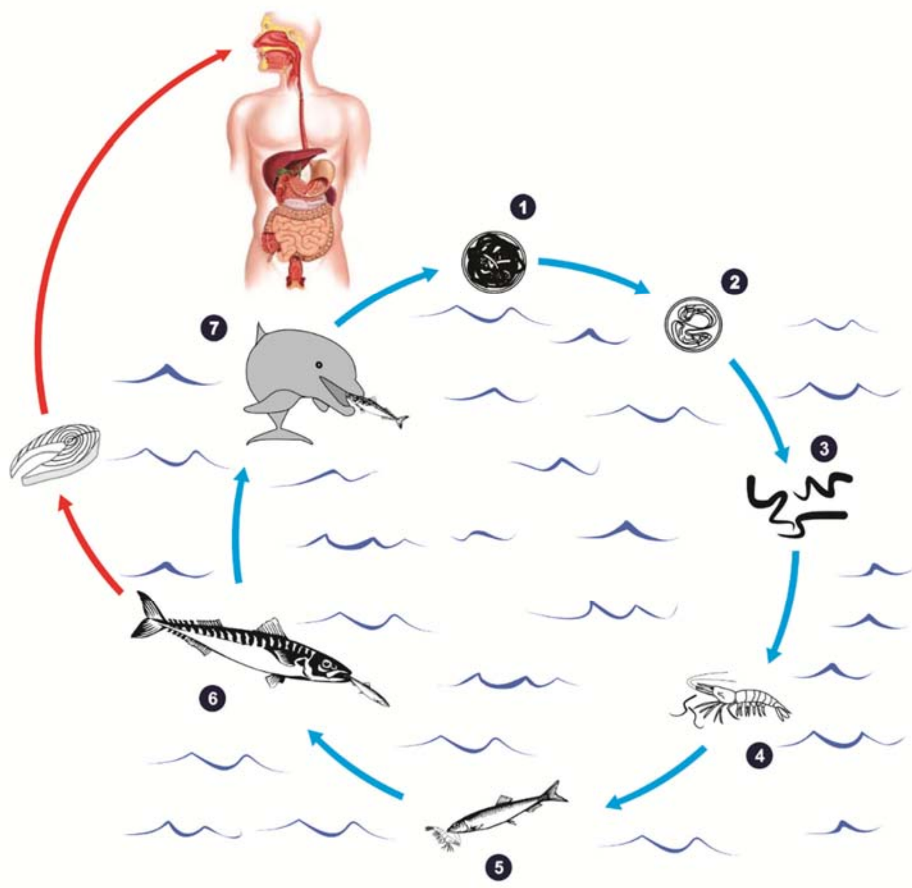

Figure 1. Life cycle of *Anisakis* spp. parasites.

The identification of *Anisakis* species is very difficult owing to the limited species-specific differences in morphological characters. Moreover, these differences are only visible in the matured worm and not in the larvae (Mattiucci et al., 2007). The larvae must have a size that makes them detectable and must be clearly differentiable from the tissues of the fish, even in the absence of optical instruments. In the literature, only two species were determined responsible for zoonotic forms: *Anisakis simplex*, known as “herring worm”, and *Pseudoterranova decipiens*, known as “cod worm”. However, the molecular studies based on genetic markers have reported that many morphospecies of *Anisakis* and *Pseudoterranova* include a certain number of sibling species with identical morphology, but different genetic make-up and geographical location. Currently, nine species of the *Anisakis* genus and six of the *Pseudoterranova* genus have been detected (D'amico et al., 2014).

Public health problems

Humans could become host if they eat raw, marinated or uncooked seafood that is infected at least one L3 viable which may then cause to a severe pathology, named as ‘Anisakiasis’ (Bao, Garci, Antonio, & Pascual, 2013). In general, anisakis larvae may be responsible for four forms of symptoms in consumers: gastric (i), intestinal (ii), ectopic (iii) and allergic (iv) symptoms. Additionally *Anisakis simplex* is now related with occupational seafood allergy (Audicana & Kennedy, 2008).

It known that anisakis nematodes could not be host at the larval life stages. It means that wide range of fish species can play a role as intermediate or host. Larval anisakis can infect through aquatic species by means of predation and may be transferred to larger predator. So, different aquatic species may play an important role in the spread of anisakis in the aquatic environment. Different aquatic species could be main source of infestation in humans, mammals and piscivorous birds (Shamsi, 2014). There is controversy about the effect and pathogenicity of anisakis worms on aquatic mammals and birds. While some researchers believe that infections with anisakis nematodes are not serious in aquatic mammal hosts (Geraci & Aubin, 1987), others have remarked that anisakis can be harmful in the alimentary tract of aquatic mammals (Abollol, Lopezz, Gestall, Benaventez, & Pascual, 1998; Jefferies, Hanson, & Harris, 1990). Anisakis have also been determined in terrestrial mammals, such as dogs and pigs, which are fed fish contains anisakis larvae with pathological changes resembling those found in aquatic mammal final hosts but differing in some aspects, such as in fewer macroscopic granulomata in pigs (Shamsi, 2014).

Over the last 30 years, there has been an increase in the reported prevalence of anisakiasis throughout the world. This increase may be due to a higher infection of captured fishes, improvements in the diagnosis of disease and the incorporation of foreign eating habits (Japanese sushi and sashimi) to food culture, and other typical seafood origin uncooked seafood snacks food dishes (marinated anchovies, etc.) (Bao et al., 2013). Several cases of infection have been reported in countries in which the consumption of uncooked fish is common (e.g. sushi in Japan, cod liver in Scandinavia, marinated fish Mediterranean countries), with a variety of clinical manifestations. Epidemiologically, *A. simplex* infections have been reported globally, with a marked prevalence in Japan. Indeed, Japanese cases alone account for more than 90% of all anisakiasis case reports (Hochberg, Hamer, Hughes, & Wilson, 2010), and some other cases are reported in Europe, in USA, and in Australia (Anastasio et al., 2016; Bucci et al., 2013; Cipriani et al., 2016; D'amico et al., 2014).

A recent survey of patients with generic gastrointestinal disorders in the United States reported that these symptoms were ascribable to parasitic diseases of aquatic origin, with such a frequency requiring preventive controls throughout the national territory (Hochberg et al., 2010). In Europe, the estimated incidence is almost 0.038% and most of the diseases have been reported in Spain, Italy, France, Netherlands and Germany (D'amico et al., 2014). Studies indicated that *A. simplex* was found in 39.4% of the fresh mackerel and 55.6% of blue whiting fish examined from different fish markets in Spain. In Italy, a few cases have been reported, particularly in related with the consuming of marinated anchovies (Bucci et al., 2013). The exact incidence is difficult to establish, but it seems to average 20 cases per country per year. In France, a report in 2003 estimated an incidence of 6 cases every year (D'amico et al., 2014).

The anisakiasis disease in developing countries such as Turkey has also not been considered to be a matter of great importance. Although there are some cases regarding occurrence of *Anisakis* spp. in fish, there is no report of human anisakiasis case in Turkey. Studies showed that anisakis larvae commonly parasitized a great variety of fish from in Aegean and Mediterranean Sea coast of Turkey, excluding Black Sea, similarly to data reported in a number of surveys performed in most of the Mediterranean Sea along the European and African coasts (Keser et al., 2007; Meloni et al., 2011; Pekmezci et al., 2014; Serracca et al., 2013).

The factors that have led to an increase of the Anisakiasis cases over the past 30 years are many and interdependent. The food scares crises, for example the “mad cow disease” and the “avian influenza”, which have shifted the orientation of consumers' attention towards proteins of aquatic origin, have increased the consumption of fishery products. Another factor to be taken into consideration is that the spread of ethnic food on the Western tables has led to the availability of a variety of Oriental dishes, especially Japanese (sushi), characterized by preparations of raw seafood. In fact, many Japanese restaurants are not really authentic, but managed by workers of different ethnicity, especially Chinese. These latter tend to increasingly convert their restaurant activities into Sushi Restaurants, offering cheaper products, often at the low quality. However, the lack of an exact knowledge on the microbiological and parasitic risks regarding dishes based on raw fish could lead to an inappropriate manipulation and treatment of the raw materials. (D'amico et al., 2014).

Symptoms of Anisakiasis

Human anisakiasis can be several forms. Clinically, following its penetration in the human gastrointestinal tract, *A. simplex* can cause gastrointestinal (classified as acute, chronic, or ectopic reactions) or allergy symptoms. The clinical symptoms vary depending on the organ infected and which *Anisakis* spp is ingested (Bucci et al., 2013).

The acute symptom typically involves the stomach and is characterized by abdominal pain, vomiting, and nausea within hours of the ingestion of *Anisakis* spp. contaminated food, mimicking an acute abdominal syndrome. In this type, an upper endoscopy performed within 12 h of the ingestion of larvae is essential to allow the localization and removal of *A. simplex* with a complete resolution (Sugimachi, Inokuchi, Ooiwa, Fujino, & Ishii, 1985). The chronic symptom is due to the localization of *A. simplex* in the intestinal wall. Typically, symptoms continue several months, with mild cramping abdominal pain, losing weight, and diarrhea, and it may be difficult to diagnose. A subtype of this form is determined by the migration of the larvae beyond the gastrointestinal wall, with the localization of the worm in the peritoneal cavity or in solid or hollow organs, causing symptoms related to the involved organ (Bucci et al., 2013).

The allergic symptoms occur within several hours of after the consuming of contaminated fish. In gastro-allergic anisakiasis reactions may take place as secondary immune response after a previous infestation by live larvae. There is an ongoing discussion about whether primary sensitization by antigens from dead larvae can also happen. Four clinical allergic symptoms (gastric, intestinal, ectopic, and systemic) have been associated with *Anisakis* spp., and reactions may rely on the route of sensitization (Fæste et al., 2014). Several cases of anaphylactic shock, hypersensitivity reactions, urticaria, and angioedema have been represented in word association with the consuming of or re-exposure to contaminated fish (Bucci et al., 2013).

Most cases of anisakiasis around the world are because of the *Anisakis* or *Pseudoterranova* larval types (Shamsi, 2014). In Japan, it takes places most commonly as a gastric infection, while intestinal disease is more prevalent in Europe. In the United States, a recent report indicated that these symptoms were ascribable to parasitic diseases of fish origin, with such a frequency requiring preventive controls throughout the national territory (Hochberg et al., 2010). The Australian case of anisakiasis is due to *Contracaecum* larval type. Symptoms such as vomiting, diarrhea, sore throat, abdominal pain, nasal congestion, rhinorrhoea and cough l continue about 3 weeks until a larva is moved in a bowel motion Human infestations take place after consuming a infested seafood such as mackerel (Shamsi, 2014).

The endoscopic removal of the living larvae from the gastrointestinal wall is known as medical treatment of acute type of Anisakis worms. Conversely, the treatment of chronic and ectopic anisakiasis depends on the medical complications produced by the larvae, ranging from the need for surgical removal of the granuloma to the use of steroids to reduce local inflammation. Unfortunately, there is no effective pharmacological treatment to kill the larvae after eaten. The only protection against *Anisakis* spp. is the frozen storage and properly processing of seafood (Bucci et al., 2013). Most cases of anisakiasis have been related to the consumption of raw or uncooked seafood made with anchovy (Anastasio et al., 2016;

D'amico et al., 2014), salmon (Bao et al., 2013), herring (Cipriani et al., 2016), mackerel (Pekmezci, 2014), sardine (Rello, Adroher, & Valero, 2008), bonito/skipjack (Soewarlan, Suprayitno, & Nursyam, 2014), hake (Mattiucci, Abaunza, Ramadori, & Nascetti, 2004), mullet (D'amico et al., 2014), whiting (Llarena-Reino, González, Vello, Outeiriño, & Pascual, 2012), sea bass (Bernardi et al., 2011). *Anisakis* spp. including *Anisakis simplex* and *Pseudoterranova decipiens* are widespread in the raw or uncooked seafood products including marinated (Karl, Roepstorff, Huss, & Bloemsma, 1994), salted (Van Mameren & Houwing, 1970) and smoked (Beldsoe & Oria, 2001) fish products.

Inactivation methods of the parasites

Freezing

The current European Union ruling on food hygiene (the so called ''Hygiene Package") takes into consideration the risk of the presence of parasites in fish products, and permits the consumption of fresh products only when they have been made safe through freezing (-20 ºC at the center of the product) or with other methods of proven efficacy, such as hot smoking at over 60 ºC or acidic marinating treatments sufficient to kill any parasites present (Reg. 853/2004, Section VIII, Chap. III, point D) (Brutti et al., 2010). Unlike bacteria, molds, and viruses, most parasites are easy to destroy by holding the raw material or finished product at freezing temperatures for a specified period of time; of course, this is dependent upon the core temperature of the food stuff (Beldsoe & Oria, 2001). The time required to reach the intended core temperature and fat content of the fish may affect the treatment. With regard to the type of parasite involved, some cestodes are more sensitive to freezing treatment than trematodes. According to the U.S. FDA, in order to inactive the nematode, the product may be subjected to various types of preventive treatments, which provide different time/temperature combinations, including:

i- Freezing at -20 °C followed by a storage minimum 7 days at -20 °C (or lower);

ii- Freezing at -35 °C (or lower) followed by a storage at -35 °C (or lower) for 15 h;

iii- Freezing at -35 °C (or lower) followed by a storage at -20 ° C (or lower) for 24 h.

Freezing, as a preventive treatment, is a procedure expressly required by law and, according to the provisions about the correct information to consumer; the data regarding the process have to accompany the product up to the retail sale. However, this information is usually neglected, especially in catering and food service. Defrosted fish yield a mistrust among consumers, showing a reluctance to purchase and consume it. In this regard, "defrosted" term should not be served on fishery and aquaculture products subjected to a preventive treatment for food safety and health purposes (D'amico et al., 2014).

Salting and Marinating

Although *A. simplex* are sensitive to salt, the high salt concentrations and times needed for its elimination make salting an inadequate method of inactivation (Beldsoe & Oria, 2001). Some European countries, such as Spain and France, have determined the technical conditions of salting and acidic marinating to kill the larvae of the parasite, thus excluding the preventive freezing of the products. In Spain, the Scientific Committee of AESAN, asserted that freezing is not necessary for those fishery products that reach a concentration of NaCl above 9% for at least six weeks, between 10 and 20% for four-five weeks or more than 20% for at least three weeks. With regard to salting, the French Food Safety Agency (AFSSA), reported that in traditional preparations and for small quantities salinity levels of 20% result in the inactivation of the parasite within 21 days, while concentrations of 15% require 28 days. AFSSA also indicated that, according to some scientists, fish marinated with 10% acetic acid and 12% salt, maintained for 5 days at 4 °C, are not hazardous to health as well as marinated seafood products within 12% salt and 6% of acetic acid for 13 days at 4 °C (D'amico et al., 2014).

In the last decade some novel techniques were developed in order to inactivate anisakidae larvae in seafood products, such as irradiation and high hydrostatic pressure even if these methods have showed some negative effect on sensorial properties of these products (Giarratana, Muscolino, Beninati, Giuffrida, & Panebianco, 2014).

Irradiation

The freezing treatment can also be shifted by either irradiation or treatments with high pressures. Irradiation of seafood is an effective method of inactivating nematodes. Earlier studies reported that in order to inactive *Anisakis* spp. in salted herring, doses of as high as 6 to 10 kGy were necessary

(Mameren & Houwing, 1968). Similarly, *A. simplex* larvae was found to be highly resistant to irradiation doses of 2 kGy or 10 kGy in another study (Beldsoe & Oria, 2001). Unfortunately, the irradiation treatment procedure used to kill the nematodes seems to induce negative changes in the organoleptic characteristic (Farkas, 1998). In addition, in the EU the use of ionizing radiation for seafood products is not approved by most of the Member States (D'amico et al., 2014).

High Hydrostatic Pressure

High hydrostatic pressure has been used for treating food to extend its shelf life and has been indicated to inactive *Anisakis* spp. larvae, although the common usage of high hydrostatic pressure technology in food have been to inhibit endogenous enzymes and inactivate microorganisms (Vidacek, de las Heras, Solas, Rodriguez Mahillo, & Tejada, 2009). The hydrostatic pressure required to inactive *Anisakis* spp. larvae is generally much lower than that used for inactivating the microorganisms. Pressures of 200 MPa for 10 min and 207 MPa for 3 min were reported to kill 100% isolated *Anisakis* spp. larvae and larvae in fish muscle (Molina-García & Sanz, 2002). Regardless of its effect on the larvae, hydrostatic pressure may yield some negative sensorial and functional changes in the fish muscle, perceived as changes in texture, color and lipid oxidation, which will differ according to the pressure/time conditions applied (Vidacek et al., 2009).

Chemical or Natural Additives

Concerning chemical additives, only the hydrogen peroxide was recognized for its effect against *Anisakis* spp. larvae, although its use it is not allowed in the European Community. Recently, several studies had reported a significant effect against the L3 larvae of *Anisakis* spp. exerted by various natural products including essential oils of different terrestrial plants such as thyme (*Thymus vulgaris*), chamomile (*Matricaria chamomilla*), tea tree (*Melaleuca alternifolia*), peppermint (*Mentha piperita*), (Barros et al., 2009; del Carmen Romero, Valero, Martín-Sánchez, & Navarro-Moll, 2012; Giarratana et al., 2014; Hierro et al., 2004).

Conclusion

Surveillance of anisakiasis and monitoring of *Anisakis* spp. over decades has demonstrated that seafood related parasite has become a major contributor to human fish-borne disease because of its hospitalization rates related to the organism's invasive qualities. Food attribution studies combined with data from food research, risk assessments and scientific expert opinion may help to determine where the greatest risks are for anisakiasis. The scientific data produced are useful as specific inputs for shelf life at manufacturing and information for consumers around food choice, especially for susceptible populations. However, due to the ready to eat and snack food types and consumption patterns differ around the world, *Anisakis* spp. inspection and control researches may need to be performed on regional basis. Most of the countries have established parasites regulations, industry guidance documents, and consumer educational practicing strategies but impact are still so low. At present, anisakiasis diseases seem to be a lower priority compared to other public health problems but its surveillance in many parts of the world is very limited. The seafood-borne parasitic diseases are of great importance for public health, and the above-mentioned precautions should be taken before serving of raw or uncooked seafood to consumption. It suggests that the critical control points at the Hazard Analysis Critical Control Point (HACCP) programmer should be properly reviewed to reduce the risk of anisakis induced allergies for seafood consumers.

Acknowlegments

The authors wish to thank Yaşar Özvarol for assistance with the drawing of figure.

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