

Parasites of Anisakidae Family—Geographical Distribution and Threat to Human Health

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Abstract: Nematodes of the Anisakidae family are important parasites that can cause anisakiasis—human parasitic infection of gastrointestinal tract. Allergic symptoms can arise as secondary immune response after infection by living parasite. In the life cycle of these parasites, intermediate, definitive and paratenic hosts (fish) might occur. In most cases, human anisakiasis is caused by consumption of fish which are infected with the larvae of the Anisakidae family. The purpose of the article was to assess the definitive host, the geographical spread of the parasites and the risk associated with consumption of raw fish and fishery products. Furthermore, this article describes symptoms and treatment of anisakiasis and kinds of preventive measures that can be taken to prevent anisakiasis.

Key words: Larvae of Anisakidae family, *Anisakis*, *Pseudoterranova decipiens*, anisakiasis.

1. Introduction

Anisakiasis is parasitic zoonosis caused by larval forms of nematodes of the Anisakidae family. Humans become infected by ingesting raw or undercooked meat of marine fish or cephalopods containing third-stage (L3) larvae of these nematodes (usually *Anisakis simplex* s.s., *Anisakis pegreffii* and *Pseudoterranova decipiens*). Live larvae can cause gastrointestinal mucositis, haemorrhages, granulomas and other symptoms by damaging gastrointestinal tract. Gastro-allergic anisakiasis (hypersensitivity to parasite allergens) is more common than gastrointestinal anisakiasis caused by the presence of parasites in digestive tract. The genus *Anisakis* has the greatest importance in the Anisakidae family because of high pathogenicity of the L3 larvae [1-5]. The characteristic of the parasite, geographic distribution (including individual species) and their pathogenicity and risk will be presented in this paper.

2. Life cycle

The nematode *Anisakis* spp. has a complex life cycle. Sexually mature nematodes are found in the stomachs of marine mammals. The fertilized females of nematodes lay eggs into the digestive tract of the definitive host. The eggs are passed in the feces to the water. The eggs become embryonated in water and first stage larvae are formed in the eggs. The larvae moult and become to be second-stage (L2) larvae. In this form, the larvae hatch from the eggs. Released from eggs, free-swimming L2 larvae are ingested by crustaceans. The development of larvae in eggs requires a suitable temperature (5-7 °C). The eggs hatch in 20-27 d. This stage is invasive for the intermediate hosts which usually are crustaceans (mainly from the cluster crustacea). Inside these hosts, the larvae moult into third-stage (L3) larvae. Transmission to other hosts is possible via ingestion of infected crustaceans. Fish and cephalopods appear to be paratenic hosts as the development from second to third stage occurs in crustaceans. In their bodies, L3 larva locates mainly in the peritoneum and the liver

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capsule or between gonads. Once the fish have been caught, as a result of environmental changes (death of the fish, the increase of temperature and change of pH) prior to evisceration, *Anisakis* spp. larvae migrates from the body cavity into the fish muscle, which dramatically increases the risk of infection to human. *Pseudoterranova* spp. larvae are also able to intravitaly migrate into the muscle [1, 2, 6].

3. Identification of Anisakidae Larvae

The most common fish nematodes of the Anisakidae family are parasites belonging to the genera *Anisakis*, *Pseudoterranova*, *Contracaecum*, and *Phocascaris*. The species or genus identification of the Anisakidae larvae using microscopic observation on the basis of several morphological characteristics is performed. During examination, the morphology of cuticula, oesophagus, ventriculus and excretory pore are taken into account. The developmental stage of larvae, which have been detected in fish, as well as geographical distribution and host range are also important [3]. The morphological differentiation is not as accurate as genetic analysis. The developed molecular techniques based on the study of genetic markers, particularly COX-2 gene and the ITS1 and ITS2, are perfect for precise distinction of species of the genus *Anisakis* or *Pseudoterranova*. In Ref. [4], *Anisakis* species are divided into two clades. In one of them, there are *Anisakis pegreffii*, *Anisakis simplex* s.s., *Anisakis simplex* C, *Anisakis typica*, *Anisakis ziphidarum*; while in the other one, there are *Anisakis physeteris*, *Anisakis paggiae* and *Anisakis brevispiculata* [4]. For species identification of Anisakidae larvae, biochemical analysis of allozyme can be applied, allowing species differentiation of *Contracaecum* and *Phocascaris* [7].

4. Distribution

The strict geographic distribution of these species is very difficult to determine. However, studies on

selected fisheries areas allow to identify where the parasite species occur most frequently and where definitive hosts were most commonly reported:

- The most widespread species of the genus *Anisakis* is *Anisakis simplex* s.s.. The parasite occurs mainly in the Northern waters of the Atlantic Ocean and the Pacific Ocean. The most frequent definitive hosts are Minke whales (*Balaenoptera acutorostrata*), common Dolphins (*Delphinus delphis*), Pilot whales (*Globicephala melaena*), White-beaked dolphin (*Lagenorhynchus albirostris*), Killer whales (*Orcinus orca*) and Striped dolphin (*Stenella coeruleoalba*). The species was also found in Gray seals (*Halichoerus grypus*) and Harbor seals (*Phoca vitulina*) from the Baltic Sea;

- *Anisakis pegreffii* was detected more rarely than *Anisakis simplex* s.s.. This species occurs mainly in the Mediterranean Sea and the waters of the Southern Atlantic Ocean. Parasites are quite common in dolphins (*Delphinus delphis*), Cuvier's beaked whale (*Ziphius cavirostris*) and common Bottlenose dolphin (*Tursiops truncatus*);

- *Anisakis simplex* C occurs in the Southern waters of the Atlantic Ocean and Northern Pacific Ocean. This species was usually found in False killer whale (*Pseudorca crassidens*) and Cuvier's beaked whale (*Ziphius cavirostris*);

- *Anisakis ziphidarum* was found mainly in the Mediterranean Sea and the Southern waters of the Atlantic Ocean similar *Anisakis pegreffii*. The most common definitive hosts are true's Beaked whale (*Mesoplodon mirus*), Strap-toothed whale (*Mesoplodon layardii*), the Cuvier's beaked whale (*Ziphius cavirostris*) and all species of the *Ziphiidae* family;

- *Anisakis* sp. A is a species that has only been detected of the coast of Madeira and Spain [8]. It was found only in Blainville's beaked whale (*Mesoplodon densirostris*);

- *Anisakis typica* is a species founded in the warm waters of the Atlantic Ocean. This species was found

in Tucuxi (*Sotalia fluviatilis*), Striped dolphin (*Stenella coeruleoalba*), Pantropical spotted dolphin (*Stenella attenuata*), Spinner dolphins (*Stenella longirostris*), Rough-toothed dolphin (*Steno bredanensis*) and Fraser's dolphin (*Lagenodelphis hosei*);

- *Anisakis physeteris*, like *Anisakis pegreffii* and *Anisakis ziphidarum*, occurs in the Mediterranean Sea and the Atlantic Ocean. The most common definitive hosts are Pygmy sperm whale (*Kogi breviceps*), Dwarf sperm whale (*Kogi sima*), Cachalot (*Physeter macrocephalus*) and rarely Cuvier's beaked whale (*Ziphius cavirostris*);

- *Anisakis brevispiculata* also occurs in the Mediterranean Sea and the waters of the Central Atlantic Ocean. Definitive hosts are the same as in the case of *Anisakis physeteris* (*Kogi breviceps*, *Kogi sima* and *Physeter macrocephalus*);

- *Anisakis paggiae* was detected in Pygmy sperm whale (*Kogi breviceps*) and Dwarf sperm whales (*Kogi sima*) in the Atlantic Ocean, particularly in its central part;

- *Anisakis schupakovi* was detected endemic in the Caspian Sea only in Caspian seals (*Phoca caspica*) [9].

The larvae of *Anisakis* spp. are commonly found in marine fish, which are paratenic hosts for these parasites, all over the world, especially in the waters of the Northern Hemisphere. It is estimated that 40%-80% of marine fish are infected by *Anisakis* spp. larvae [10]. Because these parasites are often observed in Herring and they are called “herring worms”. The invasion may also occur in such species of fish, as Cod (*Gadus morhua*), Mackerel (*Scomber scombrus*) (Fig. 1), Salmon (*Salmonidae*), Garfish (*Belone belone*) and many others. *Anisakis* larvae may be found also in fish living in brackish (salt and fresh water mix) waters, like Rainbow trout (*Oncorhynchus mykiss*) [11]. For example, the presence of *Anisakis* spp. larvae was noted in Zander (*Sander lucioperca*) coming from the Vistula Lagoon [12]. In total, the larvae of nematodes were founded in 12% of these fishes.

Food and Agriculture Organization of the United Nations (FAO) prepare a report, which provides information on the prevalence of *Anisakis simplex* larvae of several species of high economic importance (Table 1). In some cases, 100% of samples from wild fishes were infected with *Anisakis simplex* larvae. However, parasites were not found in any fish from aquafarming [13]. A retrospective analysis of literature data on Anisakidae prevalence suggests that their life cycle depend on water salinity and the presence of small shrimp-like crustaceans (Fig. 2). For example, in a study conducted on fish from the coast waters of Portugal, L3 larvae of *Anisakis* spp. were found in 100% of the European hake (*Merluccius merluccius*), 95.6% of Atlantic mackerel (*Scomber scombrus*), 93.8% Blue whiting (*Micromesistius poutassou*), 75.9% of the examined Atlantic horse mackerel (*Trachurus trachurus*) and 6.8%-28.1% of



Fig. 1 Larvae of *Anisakis* spp. in body cavity of mackerel (*Scomber scombrus*).

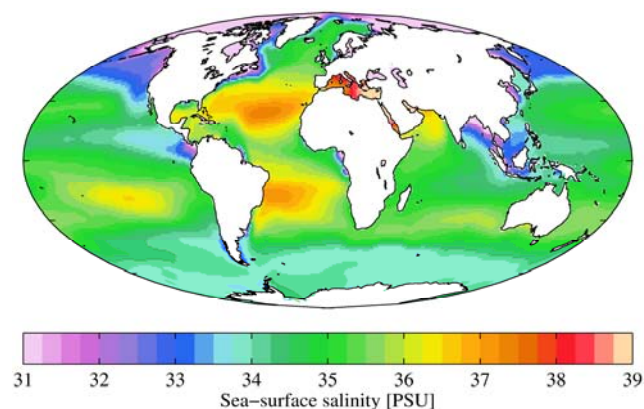


Fig. 2 Sea-surface salinity.

Data was from the World Ocean Atlas 2009.

Table 1 Occurrence of *Anisakis simplex* in wild and farmed marine fish (FAO).

Fish	Origin	Number of samples	% of infected samples
Farmed salmon	Washington	50	0
Farmed salmon	Norway	2,832	0
Farmed salmon	Scotland	867	0
Farmed coho salmon	Japan	249	0
Farmed rainbow trout	Japan	40	0
Wild salmon	Washington	237	100
Wild salmon	North Atlantic	62	65
Wild salmon	West Atlantic	334	80-100
Wild salmon	East Atlantic	34	82
Wild coho salmon	Japan	40	100
Sardines	Mediterranean	7	14
Herring	Mediterranean	4,948	86
Herring	Pacific Ocean	127	88
Cod	Pacific Ocean	509	84

the fish species, such Black seabream (*Spondyliosoma cantharus*), Pout whiting (*Trisopterus luscus*), Sole (*Solea* spp.), gurnard (*Trigla lucerna*) and European pilchard (*Sardina pilchardus*). *Anisakis* spp. larvae weren't detected in Wedge sole (*Dicologlossa cuneata*) and Shore rockling (*Gaidropsarus mediterraneus*) [14]. Research conducted on fish from the coast of Norway found *Anisakis* spp. larvae in the peritoneum of 99.6% coalfish (*Pollachius virens*), 97.8% of Atlantic cod (*Gadus morhua*) and 88% redfish (*Sebastes marinus*) [15]. Similar studies conducted on the coast of Spain showed the presence of *Anisakis simplex* s.s. larvae and *Anisakis pegreffii* in 14 fish species, like Blue shark (*Prionace glauca*), Conger (*Conger conger*), Garfish (*Belone belone*), European hake (*Merluccius merluccius*), Blue whiting (*Micromesistius poutassou*), Blue ling (*Molva dypterygia*), Pout whiting (*Trisopterus luscus*), Frog-fish (*Lophius piscatorius*), Atlantic mackerel (*Scomber scombrus*), Black seabream (*Spondyliosoma cantharus*), Atlantic horse mackerel (*Trachurus trachurus*), Four-spot megrim (*Lepidorhombus boscii*), Grey gurnard (*Eutrigla gurnardus*) and Red scorpion fish (*Scorpaena scrofa*). Extensivity of invasion of *Anisakis* spp. determined by the presence of larvae in a body cavity and organs of these species was from 32% to 100%. Furthermore, in the European hake,

Blue whiting, Frog-fish, Atlantic mackerel, Atlantic horse mackerel, *Anisakis simplex* s.s. larvae and *Anisakis pegreffii* were detected in muscle of 3.2%-41.1% fish tested [11]. The results of the project carried out on fish harbored on Southern Baltic Sea (more than 30,000 Herring, over 3,000 Atlantic cod, over 1,500 Flounders and 3,400 Sprats) showed that the parasites belonging to Anisakidae were most common in Herrings (0%-86%) and Atlantic cods (4%). In Sprats, there were no larvae of these parasites [16].

Detection of *Anisakis* spp. larvae into the peritoneum or other internal organs in freshly caught fish is common. In muscle tissue, larvae are recorded rarely due to the shortage of necessary time to transfer larvae from the peritoneal cavity into the muscle tissue. *Anisakis* spp. larvae are moving into the muscle tissue only a few hours after the death of fish. Thus early evisceration is so important to prevent anisakiasis. Prolongated time of evisceration highly increased the risk of anisakiasis. The internal quality of fish products highly depends on the time of evisceration. For example, cheap products, like smoked Herrings from Lublin hypermarkets, has shown the presence of L3 larvae of *Anisakis simplex* in 83.3% of the samples [6]. Szostakowska et al. [16] also reported the presence of *Anisakis simplex* larvae (live) in the

pickled and frozen Herring.

5. Anisakiasis in Human

Human infection with nematodes of the genus *Anisakis* are caused by consumption of fish containing live larvae. The larvae locate in humans in the gastrointestinal tract, most commonly in the stomach (gastric form) or the small intestine (intestinal form). Clinical symptoms of gastric anisakiasis usually appear in several hours after ingestion. There are primarily acute abdominal pain, vomiting, diarrhea, and sometimes fever. Gastric form is reported quite often in Japan, while the intestinal infections were more common among natives in Europe [17]. The intestinal form of anisakiasis symptoms do not appear until several days after consumption of infected fish and it is not that strong as in the gastric form. In this form, the larvae penetrate the intestinal mucosa of human. But in this case, they do not produce activation agent of white blood cells, which causes infiltrates in the parasite penetration place. This leads to limited inflammation or necrosis of infected tissue. In this case, only surgery intervention can be successful [18]. Complications may occur and may even lead to peritonitis. Also pharyngeal form of anisakiasis is described; when the nematode penetrates the throat mucosa of infected person, it causes strong coughs and vomiting. Under certain conditions, the larvae can pass through the gastrointestinal mucosa to the pancreas, liver, spleen and lungs, and may develop in intestine into four stages (L4). The larvae passed through the wall of the gastrointestinal tract and often settled on the peritoneum or subcutaneous tissue, causing granulomas or ulcerations [19]. So far, no cases of human anisakiasis have been reported in Poland. However, the problem exists but is likely to be underdiagnosed and/or underreported. The number of hypersensitivity for *Anisakis* allergens is growing. *Anisakis simplex* allergens are now considered as the most common among food allergens [20, 21]. Presence of *Anisakis* allergens can cause hazardous

allergic reactions, including anaphylaxis. It has to be pointed that such reactions may also occur after ingestion of processed fish and fishery products containing parasite proteins [22]. It has been proved that *Anisakis* allergens, in Hake (*Merluccius merluccius*) tissues infected with *Anisakis simplex*, were active even after 11 months of freeze storage. Furthermore, this allergen is heat stable and resistant to digestive enzymes. Therefore, in susceptible individuals, hypersensitivity may also occur even after consumption of heat-treated fishery products containing dead larvae or their parts. Even a trace amount of the allergen in food may cause the typical allergic reactions. Allergic reactions to food can be produced by contaminants from poultry fed with fishmeal contaminating with *Anisakis*. Even if fishmeal production process inactivates larvae (high temperature), allergenic factors are still present in final product. *Anisakis* spp. allergens are resistant to digestive enzymes, and may be present in muscle tissues of farmed chickens for 24 h after ingestion of fishmeal [5, 23-28].

6. Treatment

So far, the only effective treatment of anisakiasis is endoscopic or surgical removal of the living parasites. Usually, larvae are dying about three weeks after infection. If they do not induce any clinical symptoms, removing surgery is not necessary. However, when it comes to the formation of granulomas, ulceration or other pathological changes, the surgical intervention is necessary. For pharmacological treatment, drugs like albendazole and mebendazole, are commonly used; however, their effectiveness is discussed. Treatment with albendazole reduces the number of larvae, however the effectiveness of the drug decreases in low pH [29].

There are different factors influencing anisakiasis. For example, lower number of anisakiasis is observed in some Asian societies consuming raw fish with spices, such as ginger or perilla. That phenomenon

caused interest of scientist. The survey about various essential oils showed that α -pinene significantly reduces the damage to the gastrointestinal mucosa of infected rats [30]. In contrast, Hierro et al. [31] have shown that substances, such as perillaldehyde, citral and citronellol, damage *Anisakis* spp. L3 larvae, and thereby they reduce the bleeding in the digestive tract of experimentally infected rats. However, the results of those studies were not transferred to medicine. The best prevention against anisakiasis is consumers' education programme and the recommendation to avoid eating raw or partially cooked marine fish or squid [32].

7. Conclusions

The invasion of nematode of the Anisakidae family poses a high risk to human health, especially among groups where raw fish is consumed traditionally or occasionally as modern exotic food. The risk of infection is multiplied by traveling especially to Asia and consumption of local specialties made with raw fish. In Europe, raw products like sushi become more and more popular and it seems to be the real threat. The current legislation rules protect consumers from the *Anisakis* infection. In accordance to European Union Regulation (EC) No 853/2004, fish and fish products must be subjected to freezing temperatures not higher than -20 °C in all parts of the product and being kept in that temperature not less than 24 h. This also applies to raw eaten fish and partially cooked, cold smoked, preserved, marinated or slightly salted fish, especially species like Herring, Mackerel, Sprat, Atlantic and Pacific Salmon. However, durable and resistant temperature of *A. simplex* allergens is so high that the problem of allergies remains unresolved.

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