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Anisakis spp. as etiological agent of zoonotic disease and allergy in European region – an overview

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ABSTRACT. Nematodes of the genus *Anisakis* are worldwide distributed marine species parasitized many fish and cephalopod species as larvae and sea mammals as adult form. Anisakiosis as food-borne disease is an important public health problem worldwide. Human become infected by eating raw or undercooked fish or squids. Well documented are gastrointestinal response to infection but increasingly allergic symptoms were observed also after eating well cooked fish. This is because some of allergens of *Anisakis* are thermostable and resistant to pepsin treatment. Due to a significant increase in human mobility and global transport of fresh products like fish on ice, food-borne diseases require educational campaigns that pay attention to threats in various parts of the world.

Keywords: anisakiosis, food-borne diseases, allergy, emergent zoonosis, health education

Introduction

Parasites are natural and significant components of all ecosystems. Many parasites have a complex life cycle and entering into interactions with invertebrate and vertebrate hosts [1], also humans. The increasing importance of zoonoses, especially food-borne diseases, transmitted between vertebrate animals and humans, in the public health have been observed. The popularity of raw fish in the recent years is growing considerably in many countries, becoming a new culinary habit. Raw fish poses some potential hazards for consumers, also parasitic diseases and allergy. In good American and European sushi bar dishes has been prepared properly according to regulations by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) which minimizes the risk of infection. Sometimes people prepare raw or lightly cooked fish dishes at home and they don't known that they must use frozen fish, not fresh. Globalization causes that people more travelling, and also food from all over the world is available in markets but people don't know the regional threats.

Anisakiosis is one of the food-borne parasitic diseases transmitted by fish and some cephalopods. The agents are nematodes from the family Anisakidae, most commonly from genera *Anisakis, Pseudoterranova* and *Contracaecum* [2,3]. *Anisakis* can cause not only parasitic disease but also allergy. The ingestion infected fish products with a live larva of *Anisakis* can cause a various symptoms such as gastric, intestinal or allergic reaction and with death larva various allergic symptoms. Currently the number of both, infection and allergy, have increased worldwide [4], but the exact number of gastrointestinal anisakiosis cases and allergic reaction is unknown due to lack of epidemiological data. EFSA reported about 20 000 cases of anisakiosis worldwide before 2010, where of 90% concerns Japan. In Europe, most cases are reported from Spain, Italy and less from the Netherlands and Germany [5–7].

The prevalence of allergic disease, in this food allergy, increased worldwide and become an important public health problem [8]. Some parasitic infection or contact with parasite proteins associated with allergic reaction *e.g.* food allergy, asthma like syndrome, urticaria, anaphylaxis. Some people think that have allergies to fish flesh and thus they are forced to eliminate all fish from their diet. But sometimes the allergic reaction may not be to the fish protein but to the fish parasites. *Anisakis* produces 14 substances that may provoke allergies in humans and several novel probable allergens [9,10]. Some of antigens are thermostable therefore allergic reaction occurred also after consumption of heat treated fish. If allergy testing confirm only reaction to *Anisakis* antigens and rule out to fish, patient must avoid in his diet only fish species known as *Anisakis* hosts and *i.e.* just to be sure, eat only freshwater fish. Because in markets are available fresh fish from the various fishing grounds around the world it is important to share information about hosts, appropriate cooking methods and potential risk of allergy caused by *Anisakis*.

The aim of this paper is to draw attention of the public health specialists to need resource for about the problem of anisakiosis worldwide as well as food allergy, and the most effective ways to risk avoidance.

Biology – distribution and life cycle of *Anisakis*

Nematodes of the genus *Anisakis* are marine species with worldwide distribution. Nowadays, based on the molecular methods, genus *Anisakis* includes nine species: *A. simplex sensu stricto*, *A. pegreffii*, *A. berlandi* (=*A. simplex* sp. C), *A. typica*, *A. nascettii*, *A. paggiae*, *A. physeteris*, *A. brevispiculata* and *A. ziphidarum* [11–16]. These species are morphologically similar but genetically different. They have also different distribution, life cycle and host preferences [11–13].

The most important for public health are *A. simplex s.s.* distributed in northern hemisphere, especially North Atlantic, *A. pegreffii* in central Atlantic and Mediterranean Sea, *A. physeteris* in Atlantic and Mediterranean and *A. berlandi* in Pacific and Antarctic regions [12,13,17].

According to Klimpel et al. [14,18,19] the life cycle of *Anisakis* is sophisticated and still poorly understood because of low host specificity and modifications dependent on the geographical region and feeding ecology of local animal community.

Anisakis is a nematode parasitizes alimentary tract, particularly stomach, of cetaceans like dolphins, porpoises and whales [12,13,20–23]. A total of 53 sea mammals species worldwide were known as final hosts [18]. In the European waters adult nematodes have been noted mainly from harbour porpoise (*Phocoena phocoena*) [21,22, 24–27], common bottlenose dolphin (*Tursiops truncatus*) [18,23,24], white-beaked dolphin (*Lagenorhynchus albirostris*) [24], common minke whale (*Balaenoptera acutorostrata*) and long-finned pilot whale (*Globicephala melas*) [18,23]. Harbour porpoise act as local final host in the North Sea and the Baltic Sea [18,25]. After Smith and Wootten [24], sometimes, but less frequently *Anisakis* were noted in pinnipeds. Skrzypczak et al. [28] found small number of L3 and L4 of *A. simplex*, but no adults, in grey seal (*Halichoerus grypus*) and common seal (*Phoca vitulina)* from the southern Baltic Sea and they suggests that seals are rather accidental hosts.

Eggs produced by adult females in the alimentary tract of the final host are shed into the water where embryonated and develop. Nagasawa [29] described free-living L2, but now, it is known that one or two molts occur within the egg and L2 or L3 hatch to the environment and free-living [30]. Marine crustaceans feeding on free-living stages, L2 or L3, became infected. After ingestion larvae migrate to the haemocoel. Fish are infected with eating infected crustaceans or other smaller fish. In fish organism larvae penetrate intestinal wall and located on the pyloric caeca, mesentery of intestine, liver or gonads. Some of larvae migrate to the muscles [15]. *Anisakis* has been noted from more than 200 fish and 25 cephalopods throughout the world [18]. When the infected fish is eaten by a sea mammal, the life cycle is closed.

Human [31], some fish eating mammals like brown bear (*Ursus arctos*) [20], river otter (*Lutra canadensis*) [32], Eurasian otter (*Lutra lutra*) [33], as well as birds like great cormorants (*Phalacrocorax carbo*) [34,35], northern fulmar (*Fulmarus glacialis*) [2,26,37], Atlantic puffin (*Fratercula arctica*), Brünnich's guillemot (*Uria lomvia*) [37] or common gull (*Larus canus*), [cf. 34] are known as accidental hosts.

European fish-hosts and public health

The third stage larvae of *Anisakis* infected the viscera and/or the musculature of many fish species and some cephalopods [13,14]. *Intra vitam* larval migration can occur [38,39] and sometimes also *post mortem* migration is possible [38,40,41]. Because of worldwide distribution of these nematodes and global transport of fresh fish on the ice to the markets in distant regions makes anisakiosis an emerging public health problem.

In both, Atlantic and Pacific regions, wild salmons species are commonly infected with nematodes [15,42,43]. According to some papers farmed salmons are free from anisakid larvae because of fact that they are feed on processed food [*e.g.* 42,44,45]. Nonetheless a very low risk of infection after consuming of raw farmed salmons also exists. Farmed marine fish such as salmons are cultured in open cages and can be infected by parasites *via* intermediate host entering the cages or free stages in water. Mo et al. [46] found *A. simplex* larvae in farmed Atlantic salmons in Norway. Certainly nematodes in farmed fish occurred less frequently than in wild salmonids.

In European waters fish like the European hake (*Merluccius merluccius*) [47,48], European anchovy (*Engraulis encrasicholus*) [3,41,49,50], European pilchard (*Sardina pilchardus*) [50], Atlantic mackerel (*Scomber scombrus*) [48,51], horse mackerel (*Trachurus trachurus*) [48,52], blue whiting (*Micromesistius poutassou*) [48,53] are a common hosts. Silva and Eiras [48] also found larvae of *Anisakis* in European pilchard (*Sardina pilchardus*), pouting (*Trisopterus luscus*) and less in black seabream (*Spondyiliosoma cantharus*), sole (*Solea* sp.) and tub gurnard (*Chelidonichthys lucerna*). In the Atlantic and the North Sea some commercial fish species, like herring (*Clupea harengus*), saithe (*Pollachius virens*), cod (*Gadus morhua*), redfish (*Sebastes marinus*) [18,54], and also European sprat (*Sprattus sprattus*) [55] are infected with larvae of *A. simplex*. Also larvae were noted in small fish pearlside (*Maurolicus muelleri*) without commercial importance but ecologically valid [18].

In the Baltic Sea the most infected fish species is herring (*Clupea harengus membras*) [*e.g.* 56–59]. Less frequently *Anisakis* infected cod [59–64]. Sporadically nematodes occurred in other fish species like flounder (*Platichthys flesus*) [59,62], zander (*Sander lucioperca*) [65] or garfish (*Belone belone*) [66].

Occasionally *Anisakis* infected gobies [67,68] and three-spined stickleback (*Gasterosteus aculeatus*) [69]. Experiments conducted by Køie [70] as well Rolbiecki et al. [71] showed that larvae from herring viscera are infective if were ingested by other fish, like three-spined stickleback or flounder. European sprat noted as host in the Atlantic and the North Sea, in the Baltic Sea is not infected [55,59].

Prevalence of *A. simplex* in the Baltic herring, and also in cod decreased from south-western to the south-eastern Baltic fishing grounds [59,64]. In the Baltic Sea many populations of herrings are living [72] and seasonal fluctuation in the occurrence of *A. simplex* was observed. Infected specimens of herring appeared from autumn to spring [59,73]. The Baltic herrings from spring coastal stock were infected with intensity from a few to hundreds larvae *Anisakis* per fish located mostly on mesentery, pyloric caeca, gonads but also in the muscles [59]. Herring infection increased with fish age and size. Herring 1-2 years old are free of nematodes and oldest fish accumulated parasites [73–75]. Podolska [75] found parasites only in herring more than 21 cm in length and herrings up to 20 cm in length are free from parasites. Really similar correlation occurred also in case of Baltic cod [59,64] as well as sardines in Spain [76].

Anisakiosis – human infection

Human is not a natural host, but can be an accidental host for *Anisakis*. The larvae are able to survive in humans a few days up to weeks [49]. Humans acquire infection by eating raw fish dishes, e.g. sushi, sashimi, ceviche, anchovies marinated in vinegar anchovies, gravlax, lomi-lomi, herring roe and also inadequately cooked fish or squids [31]. This fish-borne disease was first described in the 1960s in the Netherlands [49,77,78]. It was a result of consumption of „green" herring, very popular in this country, lightly salted herrings. Nowadays cases of infection are reported worldwide [49,79], in Asia countries as Japan, Korea, Taiwan, China, Malaysia [10,49,80–83], in Europe, as Spain, Italy, the Netherlands, Denmark, Germany, France, United Kingdom, Norway and Croatia, in North America noted in Canada and the United States of America with Alaska and Hawaii, in South America countries like Brazil or Chile [9,10,49,80–83], in Africa in Egypt [49], South Africa [84], Morocco [85,86] also in Australia [87] and New Zealand [49].

In the European Union totally 236 cases of anisakiosis were noted from 2000 to 2016. Most of them were detected in Spain 66.9% and Italy 28.4%. In 62 cases, infection was observed after ingesting European anchovy (*Engraulis encrasicholus*). The main symptoms that have been noted include itching, abdominal pain and cutaneous symptoms, less frequently vomiting, diarrhea, fever and pain in the chest. In most cases, the diagnosis was based on serological tests (86.4%) and gastroscopy (10.2%) [88]. Nowadays Spain is second, after Japan country with highest number of cases of anisakiosis [89].

Well documented are gastrointestinal response to infection but nowadays more often allergic reaction to *Anisakis* proteins were observed also after eating properly cooked fish infected with *A. simplex* [9,49]. Nematodes can parasitize humans but never mature. Larvae penetrate gastrointestinal mucosa or rarely other organs with clinical manifestation, also can cause allergic reaction, i.a. urticaria and angioedema [31,90]. Despite this anisakiosis is a relevant zoonotic disease especially since the increased popularity of sushi bar in the world. A human health risk is after eating traditional dishes from raw or low salted or marinated fish like sushi, sashimi, green herring, ceviche, gravlax, salmon tartare, lomi-lomi, anchovies marinated in vinegar or other regional cuisine [5,31,77,91,92]. In the United States, anisakiosis reported mostly after eating of pacific salmons (e.g. *Oncorhynchus nerka*, *O. keta*) [15,49,92]. In the Western Europe it is herring. Eating of flounder or sprat practically does not health hazard [59]. At the beginning of the 21st century prevalence of *Anisakis* in cod was low and risk for health was very low but nowadays infection clearly increasing [64,93]. In Spain, Portugal, France, Italy and Croatia it is pickled anchovies (*Engraulis encrasicholus*) [49] and raw sardines European pilchards (*Sardina pilchardus*) [10]. Also European hake (*Merluccius merluccius*) [47], Atlantic mackerel (*Scomber scombrus*) [51] or horse mackerel (*Trachurus trachurus*) [52], salmon (*Salmo salar*) [10] represents a risk of *Anisakis* infection.

Eating raw, lightly salted or cold smoked huge specimens of Baltic herring or their roe carries a health risk from autumn to spring. Every year, during this time larvae of *Anisakis* in herrings, mostly 25 cm in length and above, both fresh and smoked, in the fish markets were observed. These nematodes were found in smoked herring by Guz et al. [94]. Szostakowska et al. [59] found larvae, also alive, in preserves from herring – smoked and marinated. Bilska-Zając et al. [95] documented *Anisakis* larvae in frozen salmon filets from markets. Spirally convoluted third stage larvae of *A. simplex,* lived in the viscera, gonads or muscles of fish and cephalopod are visible to the naked eye.

Clinical manifestation

Gastrointestinal infection

Parasites located in the gastric or intestinal mucosa. Consumption raw or undercooked fish with living larvae provoke local irritation, severe epigastric pain, nausea, vomiting, diarrhoea, low-grade fever. Symptoms usually appear up to 12 hour after fish consumption in the case of gastric infection and 5–7 days in the case of intestinal infection. Mucosal oedema around the area of penetration is observed and also eosinophilic intestinal lesion or perforation sometimes occurs. Symptoms may be similar to i. a. gastric or duodenal ulcer, appendicitis or peritonitis. Because symptoms are not specific patients with anisakiosis sometimes were misdiagnosed [10,49,96–98]. According to Guardone et al. [90] pathologists should consider the possibility of anisakiosis when eosinophilic granuloma of the gastrointestinal tract, the mesentery or the peritoneum occurred. The gastric or intestinal infections may be accompanied by allergic reaction like urticaria, angioedema or also anaphylactic shock [10,49,96.97].

The diagnosis is based on endoscopy, laparotomy or radiological examination and various immunological tests [113]. Endoscopic extraction of *Anisakis* is the most common method of treatment. Small bowel infection may be treated also with albendazole therapy [49,99,100].

Allergy

Kasuya et al. [101] described for the first time *A. simplex* allergy in Japan. Since then, many cases of allergy were noted worldwide, especially in Spain [*i.e.* 10,49,102,103]. Allergy may be accompanied by invasion but also was observed after eating properly cooked fish and canned fish – exposure to allergenic proteins in food [5,10,104]. Clinical symptoms were also observed in patients after eating frozen fish [5,105,106]. According to Moneo et al. [107] in endemic countries more than 7% of population could be sensitized.

Anisakis can cause to induce IgE-mediated reactions with clinical symptoms as urticaria, angioedema, asthma and anaphylaxis after consumption of parasitized fish [4,108]. Some cases of allergy were diagnosed also after eating meat of chicken feeding on fishmeal, which contained nematodes [109]. Cases of occupational allergy were also noted after contact or inhalation of *Anisakis* allergens. Hypersensitivity reactions as contact dermatitis, conjunctivitis and asthma in fishermen, fishmongers and a chicken breeder were demonstrated after contact with infected fish or fishmeal [84,109–112].

The allergic reaction described for anisakiosis includes urticaria, angioedema, in this facial angioedema, gastrointestinal reaction as nausea, vomiting and/or diarrhea and also respiratory reaction. Anaphylactic shock and respiratory arrest were also noted [*i.e.* 10,49,102,103].

Anisakis produces 14 proteins that may provoke allergies in humans accepted in the International Union of Immunological Societes and published in website allergen.org [10]. Several novel probable allergens also were noted [9,10]. In allergome database were noted 46 items [www.allergome.org]. Allergic proteins of *Anisakis* are classified to tree groups:

- Excretory/secretory proteins – expressed by larvae during infestation as Ani s 1, Ani s 4–9,
- Somatic proteins – constituents of the larvae body as Ani s 2, Ani s 3,
- Cuticular proteins – occurred on the body surface of larvae and protect them from digestion [9,98,113].

Some of allergens of *Anisakis* are thermostable, show resistance to pepsin digestion and low pH therefore allergic reaction is possible also after eating dead larvae with fish flesh for example after conventional or microwave heat treatment [114–116]. Allergic reaction occurred also after freezing fish products [115]. Humans are primarily exposed to somatic and cuticular antigens also from death larvae contained in food and excretory/secretory antigens when alive larvae penetrate tissue. Allergy recognized by more than 50% patients was most often caused by allergens Ani s 1, Ani s 2 and Ani s 7 [98,113,117,118].

In diagnosis are used the skin prick test and the detection of specific IgE using antigen from nematode, but they are interfered with cross-reactivities of other ascarides, mites, shrimps and some microorganism [107,114,119]. Ivanovic et al. [98] also lists method like complement fixation test (CFT), immunofluorescent-antibody test (IFAT), immunodiffusion test (IDT), immunoelectrophoretic assays, enzyme-linked immunosorbent assay (ELISA). According to Lorenzo et al. [120] and Carballeda-Sangiao et al. [121] the fluorescence enzyme immunoassay (CAP FEIA) is the most used method for laboratory diagnosis of *Anisakis* allergy because of high sensitivity and low specificity. The major problem in diagnosis of allergic reactions is the presence of cross-reactivity of *Anisakis* allergens e.g. tropomyosin, paramyosin, with antigens of other nematodes, insects, mites, crustacean and mollusks [10].

Prevention

A. simplex infection may pose a health risk if people eat raw, undercooked, pickled and cold-smoked fish dishes. Parasite infection is not a health hazard when the fish or other seafood is thoroughly cooked but allergy is possible because of thermostable allergens. How Moneo et al. [107] concluded, information about potential health risk of consumption habits would avoid contacts with parasite allergens and consequently decrease the appearance of acute or chronic episodes induced by the parasites.

The best preventive measure on anisakiosis is educating people about the risk of eating raw, inadequately cooked, lightly pickled or salted marine fish or squids, also information about the fish species carrying the greatest risk of disease.

The risk of human infection can be reduced:

- the fresh fish as soon as possible should be carefully eviscerated and wash;
- visual examination of fish: extraction of visible parasites and elimination of heavily parasitized fish – this method is also applicable at home;
- visual inspection of the whole fish abdominal cavity used as standard method in the European Union does not guarantee larvae detection, especially when they can migrate to the fish muscles [117,122]. Presence of Anisakidae larvae in fish products can be assessed by various, more effective laboratory methods, i.a. direct observation of body cavity, liver and gonads, compressor method with illuminated table and UV transillumination detected parasites in muscles, or chlorine-peptic digestation of samples of muscle tissue, similar to methods of detecting *Trichinella* in muscles of pigs, wild boars and other animals [122–124].
- thermal processing – heating or freezing. The European Food Safety Authority (EFSA) and the USA Food and Drug Administration (FDA) recommends:
  - cooking fish at > 60°C (> 145°F) for 15 seconds is sufficient to kill parasites. This means that the common fish fillet must be heat treated for about 10 minutes;
  - fish for raw or lightly cooked consumption must be freezing at –20°C (–4°F) for not less than 24 h or at least four days in a domestic freezer at –15°C (EFSA). FDA recommended freezing at –20°C (– 4°F) for at least 7 days or blast freezing to – 35°C (– 31°F) for ≥15 h [6,125]. This recommendation prevent

gastrointestinal infection, but the study of Rodriguez-Mahillo et al. [115] indicates that the antigenicity of *Anisakis* larvae is preserved after freezing and may sensitized humans after ingestion of infected frozen fish.

More restrictive FDA recommendation is a result of the presence of more resistant *Pseudoterranova* in the North America. European recommendation should also be tightened because the prevalence of fish, like cod, infected with *Pseudoterranova* increased in the last twenty years, also in the Baltic Sea [64,93,126]. *Pseudoterranova* like *Anisakis* can cause both, gastrointestinal infection and allergic reaction in humans [127,128]. However, the allergic potencial of other Anisakidae nematodes, such as *Pseudoterranova* and *Contracacecum*, has not yet been well studied and requires further research [129]. In addition, the latest research carried out by Podolska et al. [130] on freezing cod fillets and whole herrings and the survival rate of nematode larvae have shown that both, *Pseudoterranova* and *Anisakis*, died in cod fillets at a temperature −15°C or lower but in whole herring some *Anisakis* larvae survived at −20°C. These results demonstrate that in the freezing procedure the capability of the freezing device, as well the thickness and type of the fish products being frozen are important [130].

In conclusion, prevention of fish-borne disease requires monitoring of parasites, early fish evisceration and control fish products [122–124]. Very important are education campaigns about home preparation methods, especially fish freezing capacity and properly cooking techniques, also widely available information about hosts of parasites. It is important to known that visual inspection of the whole fish abdominal cavity, possible to use at home, does not guarantee larvae detection. In addition properly cooking or freezing fish products can protect from gastrointestinal infection but not allergy because some proteins have been heat, freezing and pepsin resistant e.g. Ani s 1, Ani s 4, Ani s 5, Ani s 8, Ani s 9, Ani s 10, Ani s 11.0201 [104,114–116,129]. Allergy cases after consuming marine fish (raw, stewed, fried, frozen, smoked, marinated, canned) should be verified by an allergist, whether it is an allergy to fish protein or parasites.

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