**ANISAKIDOSIS (*Anisakis* spp. and *Pseudoterranova* spp.): AN ESSENTIAL PRATICAL REVIEW**

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***Resumo:*** *A anisaquidose é uma doença veiculada por alimentos causada por larvas em terceiro estadio (L3) de nemátodes marinhos parasitas da Família Anisakidae, comummente conhecidos por anisaquídeos. Os anisaquídeos mais frequentemente envolvidos integram os géneros Anisakis e Pseudoterranova e são parasitas cosmopolitas que infetam muitas das espécies de teleósteos (peixes) e cefalópodes (lulas) comercialmente importantes nos principais pesqueiros do mundo. Apesar da distribuição epidemiológica original desta zoonose estar associada a países cuja gastronomia inclui tradicionalmente especialidades à base de produtos da pesca crus ou insuficientemente confecionados, atualmente a sua ocorrência é generalizada devido à globalização e à popularização de tendências culinárias exóticas. Clinicamente, esta doença envolve frequentemente sintomas gastrointestinais devido à ação física invasiva das larvas após ingestão, mas também poderá incluir reações alérgicas potencialmente fatais em pacientes sensibilizados. Dado o número crescente de casos reportados recentemente, esta revisão tem como objetivo compilar e sumarizar os aspetos mais relevantes desta doença zoonótica de uma maneira prática e fácil de consultar.*

***Palavras-chave****: Anisaquidose, segurança alimentar, Anisakis spp., Pseudoterranova spp., transmissão, prevenção*

***Abstract:*** *Anisakidosis is a food-borne disease mainly caused by third-stage (L3) larvae of marine parasitic nematodes (or roundworms) of the Family Anisakidae, commonly known as anisakids. The anisakids most frequently involved belong to the genera Anisakis (also known as herring or whale worms) and Pseudoterranova (also referred to as cod or seal worms). These are widespread parasites that infect most commercially important exploited teleost (fish) and cephalopod (squid) species in the main fishing grounds of the world. Despite the original epidemiological distribution of this zoonosis in countries where raw or undercooked seafood prevails, today its occurrence is widespread due to globalization and the popularization of exotic culinary trends. Clinically, this disease usually involves mainly gastrointestinal symptoms due to the physical action of the invasive larvae after ingestion, however it can also include potentially fatal allergic reactions in sensitized patients. Given the increasing number of cases reported recently, this review aims to compile and summarize the main aspects of this zoonotic disease in a practical and user-friendly way.*

***Keywords****: Anisakidosis, food safety, Anisakis spp., Pseudoterranova spp., transmission, prevention*

1. **HISTORICAL NOTES**

In 1876, Leuckart reported for the first time a case of a marine nematode that was vomited by a Greenlandic child, but it was not until 1960 that Van Thiel associated a larva of *Anisakis* with gastrointestinal disease in humans due to the ingestion of marinated herring (the traditional Dutch *nieuwe*) in the Netherlands (cited by EFSA, 2010).

In Japan, cases of *Ascaris*-like larvae associated with gastrointestinal lesions in humans had been documented since the 1940’s, however only in 1965 was it possible to determine that the responsible agents were L3 larvae of *Anisakis*, similar to those reported by Van Thiel a few years earlier. These events, and the increasing number of reported cases, led to the creation of a research group, financially aided by the Japanese government, consisting of parasitologists, pathologists, surgeons and marine biologists that investigated the disease in that archipelago. Interestingly, they found that several previous cases of the disease had been misdiagnosed and surgically removed as cancer and that the ingestion of raw fish was indeed on the genesis of that zoonotic morbid entity (Oshima, 1972).

Similarly, extensive research work in the 1950’s and 1960’s in the Netherlands suggested that a change in the eating habits (lightly salted “green herring” had been recently introduced and popularized) as well as a transformation in the processing of fish due to the introduction of onboard refrigeration (evisceration was delayed and larvae would easily migrate to the fish muscle) were the cause of the sudden increase in anisakidosis numbers. As such, in 1968 the Netherlands became the first country in Europe to legislate and implement sanitary measures (freezing treatment of fishery products) to prevent this food-borne disease (EFSA, 2010).

1. **CAUSATIVE AGENTS**

Most reported causative agents of anisakidosis belong to the genus *Anisakis*, namely *A. simplex* sensu stricto (s.s.) and *A. pegreffii* (Umehara *et al*., 2007; Guardone *et al*., 2018), in which case the disease is more specifically known as anisakiasis. These two sibling species (along with *A. berlandi*) form the *A. simplex* complex, formerly known as *A. simplex* sensu lato (s.l.).

Human infection may also be caused by members of the genus *Pseudoterranova*, namely *P.* *decipiens* s.s., *P. cattani* and *P. azarasi* (Arizono *et al*., 2011; Weitzel *et al*., 2015; Brunet *et al*., 2017), a condition usually known as pseudoterranovosis. Previously, these 3 sibling species (as well as *P. bulbosa*, *P. krabbei* and *P. decipiens* E) were known as *P. decipiens* s.l., or *P. decipiens* species complex.

On the other hand, *Hysterothylacium aduncum* and *Contracaecum osculatum* have also been reported as causative agents of human infection, even though to a lesser extend (Nagasawa, 2012; González-Amores *et al.*, 2015). Therefore, and given their prominent sanitary relevance, the present review will focus on *A. simplex* s.s., *A. pegreffii*, *P. decipiens* s.s., *P. cattani* and *P. azarasi* (**Table 1**).

**Table 1** Taxonomic classification of the parasites in the genera *Anisakis* and *Pseudoterranova*.

|  |  |
| --- | --- |
| Kingdom  | AnimaliaNemathelminthesNematodaAscaridaAscaridinaAscaridoideaAnisakidaeAnisakinae |
| Phylum |
| Class |
| Order |
| Suborder |
| Superfamily |
| Family |
| Subfamily |
| Genus | *Anisakis* | *Pseudoterranova* |
| Species | *simplex* sensu stricto\* | *decipiens* sensu stricto\* |
|  | *pegreffii\** | *krabbei* |
|  | *berlandi* | *bulbosa* |
|  | *typica* | *azarasi\** |
|  | *ziphidarum* | *cattani\** |
|  | *paggiae* | *decipiens E* |
|  | *physeteris* | *kogiae* |
|  | *nascettii* | *ceticola* |
|  | *brevispiculata* |  |

\*Species reported in human cases of anisakidosis

* 1. **IDENTIFICATION OF THE LARVAE**

Third-stage larvae are commonly found coiled and often encapsulated in the visceral organs and muscles, or freely in the body cavity, of various commercially important fishery products (**Figure 1**). *Anisakis* L3 larvae are whitish worms with approximately 11 to 30 mm of length (Quiazon *et al*., 2008), whereas *Pseudoterranova* larvae are characterized by a brownish/yellowish to reddish color and up to 43 mm of length (Hernández-Ortz *et al*., 2013).



Figure 1 Fish hosts infected with anisakid third-stage larvae. Detail of *Anisakis simplex* s.s. larvae (arrow) penetrating the peritoneum (A) and encapsulated in the muscle (B) of chub mackerel (*Scomber japonicus*). Numerous larval anisakids (arrowheads) coiled on the liver of an Alaska pollock (*Gadus chalcogrammus*) (C). Scale bar: 10 mm.

When observed under a light microscope *Anisakis* and *Pseudoterranova* larvae can be distinguished based on the morphology of their alimentary tract, in which the latter has an intestinal caecum anteriorly projected whereas the former does not (**Figure 2**). Conversely, *Contracaecum* and *Hysterothylacium* L3 larvae have both an intestinal caecum and a ventricular appendix (in opposite directions) (Navone, Sardella and Timi, 1998; Shamsi, 2019).

It has been suggested that the length of the ventriculus (glandular digestive compartment, see **Figure 2**) can be used to morphologically distinguish between *A. simplex* s.s. (0.9–1.5 mm) and *A. pegreffii* (0.5–0.78 mm) (Quiazon *et al.*, 2008). Nonetheless, the exact species within each genus may only be identified with certainty through molecular techniques such as PCR-RFLP, DNA sequencing or allozyme markers (Mattiucci *et al*., 2018).



**Figure 2** Main morphological features of the anterior portions of *Anisakis* (I) and *Pseudoterranova* (II) L3 larvae.

Abbreviations: bt – boring tooth; ep – excretory pore; e – esophagus; ed – excretory duct; v – ventriculus; ic – intestinal caecum; i – intestine.

Scale bar: approximately 1 mm.

1. **LIFE CYCLE AND HOSTS**

The life cycles of these parasites include five stages, separated by four molts. Gonochoristic adults (distinct male and female individuals) are found inside the gastric compartments of marine mammals, which are their final or definitive hosts. *Anisakis* *simplex* s.s. and *A. pegreffii* are preferentially found in Delphinidae, Balaenopteridae and Phocoenidae cetaceans (e.g. dolphins, baleen whales and porpoises, respectively), whereas *P. decipiens* s.s., *P. azarasi* and *P. cattani* have mainly Phocidae and Otariidae pinnipeds (e.g. seals and sea lions, respectively) as final hosts. After mating, the eggs are then eliminated through the host’s feces and start to develop once they reach the water column (Mattiucci *et al*., 2018). Inside the eggs, the larvae develop and molt before hatching (Køie, Berland and Burt, 1995). Free-swimming larvae are then consumed by crustaceans which serve as intermediate hosts, particularly euphausiids of the Family Euphausiidae (krill) for *Anisakis* spp. and copepods of the Subclass Copepoda for *Pseudoterranova* spp. (McClelland, 2002). Subsequently, L3 larvae are transferred to the transport or paratenic hosts, which are fish and cephalopods that predate on infected crustaceans. These paratenic hosts may be directly eaten by the final host (where the parasite molts to the L4 and then the adult stage) or by another fish or cephalopod and the parasite is transferred along the trophic web (Levsen and Berland, 2012; Mattiucci *et al*., 2018). Only saltwater fish, including anadromous ones (those that live in the ocean and migrate to rivers to breed such as salmons) will have the chance of getting infected as these are marine parasites.

These anisakids are known to have low paratenic host specificity. Larval *Anisakis* spp. have been recorded in at least 65 Families of teleosts and cephalopods, particularly Gadidae (cods and pollocks), Merlucciidae (hakes), Scombridae (mackerels, tunas and bonitos), Carangidae (jacks and pompanos) and Salmonidae (salmons) (Mattiucci *et al*., 2018). On the other hand, larval *Pseudoterranova* spp. have been molecularly identified from at least 11 Families (most notably Gadidae) (Mattiucci and Nascetti, 2008).

Humans may become accidental hosts when they ingest raw or undercooked infected seafood.

1. **GEOGRAPHIC DISTRIBUTION**

*Anisakis simplex* s.s. has been detected in different fish species in the North Pacific and Atlantic as well as in the Arctic Ocean, whereas *A. pegreffii* is mostly identified in the Sea of Japan, East China Sea and Mediterranean Sea (Quiazon, Yoshinaga and Ogawa, 2011; Mattiucci *et al*., 2018, Gomes *et al.*, 2020). In fact, a recent report has suggested that temperature is an important factor affecting the distribution of these two sibling species, as the early biological forms (eggs, free-swimming recently hatched larvae and L3 larvae from fish) of *A. simplex* s.s. exhibit a better adaptation to lower temperatures while those of *A. pegreffii* tend to be comparatively more tolerant to higher temperatures (Gomes *et al.*, 2023).

*Pseudoterranova decipiens* s.s., on the other hand, is mostly distributed in North Atlantic fish, *P. azarasi* is recorded mainly in the northern Pacific Ocean and *P. cattani* is usually reported in fish from the southern Pacific coasts of Patagonia (Mattiucci and Nascetti, 2008).

Logically, the geographic distributions of these two genera are also directly correlated to the distributions of their final hosts: cetaceans (*Anisakis* spp.) in both coastal and offshore areas, and pinnipeds (*Pseudoterranova* spp.) mostly in coastal regions (Measures, 2014).

1. **DESCRIPTION OF DISEASE IN HOSTS**

Anisakiasis is mostly characterized by the penetrative action and migration of the larvae in the hosts by means of its boring tooth and secretory enzymes (Levsen and Berland, 2012) (see **Figure 2**).

* 1. **PARATENIC HOSTS - FISH**

In fish, the larvae usually pierce through the gastrointestinal wall and enter the body cavity. Depending on the host, larvae are mainly found on the liver, mesentery and gonads, where they are usually encapsulated (Gomes *et al*., 2020). Some larvae may even migrate to the body muscles, particularly the abdominal masses or “belly flaps”, either intra vitam or postmortem (Mattiucci *et al*., 2018). Generally, fish hosts appear healthy regardless of their parasitic load (Levsen and Berland, 2012) but mortality has been reported in exceptional cases of heavy infection with *Anisakis* larvae (Klimpel and Palm, 2011).

A distinct form of the disease has been observed inwild Atlantic salmons (*Salmo salar*), wherethe migration of *Anisakis* larvae in large numbers to the skin, muscle and body cavity of the vent area, is associated with a condition commonly known as red-vent syndrome. Although the definitive cause remains unclear, the presence of these anisakids has been associated with swelling, hemorrhage and skin erosion. There is no evidence that this condition causes mortality or prevents salmon from spawning, but further research is needed (Noguera *et al*., 2009).

* 1. **ACIDENTAL HOSTS – HUMANS**

In humans, infective anisakidosis may vary depending on the specific affected site (**Table 2**). The least invasive of these forms is transient luminal anisakidosis, in which larvae (typically *Pseudoterranova* spp.) do not penetrate tissues but may remain in the open spaces (lumen) of the gastrointestinal tract, usually causing milder symptoms such as “tingling throat syndrome”. In contrast, *Anisakis* spp. are generally more disruptive, frequently invading the gastric or intestinal mucosas and causing eosinophilic granulomas (Audicana *et al*., 2002). Nonetheless, a case of hepatic anisakidosis mimicking metastatic liver cancer and caused by *Pseudoterranova decipiens* has also been reported (Murata *et al.*, 2018).

On the other hand, *Anisakis simplex* s.s. and *A. pegreffii* have been credited as the only known parasites of fishery products capable of inducing allergy in human consumers (EFSA, 2010) and even just their proteins can originate an allergic reaction in sensitized patients (Audicana *et al*., 2002; EFSA, 2010). There are currently 14 known allergens of *Anisakis* *simplex* (Ani s 1 to Ani s 14) (Kobayashi et al., 2015), which are either integrating proteins of the muscle and skin (paramyosin and tropomyosin) or secreted/excreted by the parasite. This form of disease may lead to anaphylactic shock, which is potentially fatal due to the risk of glottis edema (Audicana *et al*., 2002; Ivanović *et al*., 2017). Ocular, cutaneous and respiratory contact with *Anisakis* allergens may also occur and have been described to cause occupational conjunctivitis (Añíbarro and Seoane, 1998), dermatitis (Nieuwenhuizen *et al*., 2006) and asthma (Armentia *et al*., 1998), respectively.

1. **HUMAN INFECTION RISK FACTORS**

Naturally, countries where fish consumption is high face an increased risk of infection particularly in places where traditional raw, lightly cooked, smoked, fermented or marinated gastronomic specialties are common. The Asia-Pacific region is especially rich in these foods including Japanese *sushi* (raw fish on vinegared rice) and *sashimi* (sliced raw fish), Filipino *kinilaw* (cubed, marinated fish) and *bagoong* (salted, fermented fish), Malaysian *hinava* (sliced marinated fish), Indonesian *gohu ikan* (spiced marinated tuna), Korean *hoe* (sliced raw fish), Thai and Lao *koi pla*/*paa* (minced, marinated fish), Tongan (also Samoan and Tahitian) *‘ota ‘ika* and Fijian *kokota* (marinated fish with coconut milk). Apart from that, *Yusheng* is a popular new year delicacy among Chinese communities in several countries, which consists in a tossed raw fish salad.

In Europe anisakidosis may be associated with Dutch *nieuwe* and *maatje* (soused herring), Scandinavian *gravlax* (dry lightly cured salmon), Spanish *boquerones en vinagre* (pickled anchovies) and Italian *alici marinate* (marinated anchovies) and *carpaccio* (sliced tuna or salmon). South American *ceviche* (marinated fish) and Hawaiian *lomi-lomi* (raw salmon salad) may also be relevant causes of this zoonosis. However, globalization and the spread of exotic eating habits have increased the risk throughout the world. ­­

In addition, top predatorial fish species will tend to accumulate larger quantities of larvae in their tissues, which increases the risk to human consumers (Mattiucci *et al.*, 2018).

1. **ECONOMIC IMPORTANCE**

These nematodes are a major cause of economic loss in the fishing industry not only due to the reduced marketability and commercial value of visibly infected fishery products but also the damage it inflicts to consumer confidence (Llarena-Reino *et al*., 2015). For instance, a study has revealed that 77% of Spanish consumers were willing to pay 10% above the usual fish price at market for parasite-free fishery products (Bao *et al.*, 2018). This necessity for inspection controls to remove visible parasites has increased the costs associated with the commercial processing of fishery products (Abollo, Gestal and Pascual, 2001; Llarena-Reino *et al*., 2015). Such procedures are currently required by the European Union, which decrees that fishery products that are obviously contaminated with parasites must not be placed in the market for human consumption (Regulation 853/2004/EC). In fact, anisakids were ranked 4th out of 24 food-borne zoonotic parasites in terms of relevance to international trade because of regulations imposed by countries on imports regarding food safety (FAO/WHO, 2014).

In Canada alone, the economic losses to the fish industry have been estimated in 27–50 million CAD$ per year associated with the removal of *Pseudoterranova* larvae (McClelland, 2002).

1. **EPIDEMIOLOGICAL DATA**

Approximately 90 percent of the 20 000 global human anisakiasis cases reported so far are from Japan, where 2 000 are reported annually (Audicana *et al*., 2002; EFSA, 2010). In Europe, approximately 500 cases have been reported, mostly in Italy and Spain (Audicana *et al*., 2002), but also in Croatia, France, the Netherlands, Belgium, Norway, Denmark, Germany, Austria, Russia, the United States, Canada, South Korea, Thailand and Taiwan (Mattiucci *et al*., 2018). Countries with no traditional raw fish consumption habits have recently reported cases for the first time due to the importation of exotic food trends, such as Portugal (Carmo *et al.*, 2017; Baptista-Fernandes *et al.*, 2017; Bernardo and Castro-Poças, 2018) and Brazil (Cruz *et al.*, 2010).

While in South Korea and Italy most zoonotic cases are caused by *A. pegreffii* (Lim *et al*., 2015; Mattiucci *et al*., 2018), in Japan the main causative agent is *A. simplex* s.s. (Umehara *et al.*, 2007).

Generally, pseudoterranovosis is less commonly reported than anisakiasis (almost 350 cases by 1993). Japan is the territory with the most cases, but this condition has also been diagnosed in the United States, South Korea, the United Kingdom, Canada, Greenland (Ishikura *et al*., 1993), Chile (Weitzel *et al*., 2015), Argentina (Meghi *et al*., 2020), Denmark (Nordholm *et al.*, 2020) and France (Brunet *et al*., 2017).

1. **MODES OF TRANSMISSION**

Transmission of these parasites among aquatic animals occurs along the food chain, by predation, via the digestive tract (Mattiucci *et al.*, 2008).

Infective anisakidosis may occur in human patients upon consumption of raw or undercooked seafood infected with viable L3 larvae, which is the infective stage for mammals. Because neither *Anisakis* spp. nor *Pseudoterranova* spp. can mature and reproduce in humans and other nondefinitive hosts, these are considered dead-end, or cul-de-sac, hosts and larvae generally have limited survival after infection (Measures, 2014).

On the other hand, allergic anisakiasis can be triggered by the consumption of fish containing larvae or contaminated with larval proteins even after freezing or cooking because many of the allergens are pepsin-, heat- and freeze-resistant (Ivanović *et al*., 2017). Contact with allergens may also occur occupationally through the skin, eyes or respiratory tract.

Human to human transmission of these parasites has never been reported.

1. **PREVETION AND CONTROL MEASURES**

Neither the infection with *Anisakis* nor with *Pseudoterranova* spp. are OIE-notifiable diseases.

Currently there are no sea fishing grounds that can be considered free of anisakids (EFSA, 2010). However, in some aquaculture conditions the risk of anisakid infection from consuming some farmed species (e.g. salmon) may be considered negligible if fish are raised in sea pens, cages or onshore tanks and fed commercial feed throughout their life cycle (Inoue *et al*., 2000; Lunestad, 2003; EFSA, 2010).

Anisakid larvae are generally very resistant to environmental conditions. *Anisakis* larvae can survive in fish flesh up to 25 days in traditional salt and vinegar marinades (EFSA, 2010), in some domestic freezing temperatures (Podolska *et al*., 2019) and in commercial cold smoking conditions (Gardiner, 1990). Rapid evisceration of freshly caught fish and refrigeration may prevent postmortem larval migration to the muscle but viscera discards should not be dropped at sea to prevent further larval recruitment (Mattiucci *et al*., 2018). Visual inspection and removal of visible larvae may be practical for superficial parasites. Also, candling (use of a strong light) can be used commercially to visualize larvae deeper in fish fillets (particularly *Pseudoterranova*), although its efficiency is questionable (Audicana *et al*., 2003). As a control measure, temperature is widely accepted as the most efficient and safe treatment to kill the larvae and reduce the risk of infection. For instance, cooking the fish product for at least one minute at > 60 °C on its core is advised (EFSA, 2010). Also, in many parts of the world, fishery products meant to be eaten raw must undergo a freezing treatment. In the European Union, for instance, all such products must be subjected to at least –20 °C for not less than 24 hours, or −35 °C or below for 15 hours, by law (Regulation 853/2004/EC; Regulation 1276/2011/EC). Similarly in the United States, fish shall be frozen and stored at an ambient temperature of –20 °C or below for not less than seven days, for example (FDA, 2017). Despite these control measures the risk of allergic anisakiasis remains but recent advances in food technology have opened new possibilities for the removal of *Anisakis* allergens from some processed fishery products (such as *surimi*) to make them safer for sensitized consumers (Olivares *et al*., 2014).

1. **FINAL CONSIDERATIONS**

While it may be virtually impossible to irradicate these resilient and widespread parasites from wild fish stocks, several approaches can be put in place to minimize its risk to human consumers. Subjecting fishery products intended to be eaten raw to a freezing treatment is undoubtedly one of the primal steps towards prevention, but it should not be regarded independently. For instance, even though Japanese sanitary authorities recommend fish freezing, Japanese law does not decree it (as freezing changes the texture and quality of highly appreciated specialties such as *sushi* and *sashimi*), instead a thorough training program is implemented to capacitate all professional chefs and raw fish handlers regarding food safety. In fact, most cases of human anisakiasis are a consequence of incautious home fish preparation using inadequate techniques or fish species (Oshima, 1987). Other relevant efforts to minimize the risk of anisakidosis would include further investment in the aquacultural industry and the production of parasite-free fishery products as well as the development of new food technologies to neutralize these nematodes and its allergens. It has been previously demonstrated (for instance in the 1950’s Netherlands) that the sudden change in eating habits could introduce new hazards to consumers (EFSA, 2010). The recent rise in popularity of restaurants serving raw fish (for instance South American *cevicherías* and Japanese *sushi* restaurants) outside of their native cultural environment has presented these nematodes to previously naïve consumers and even unprepared/unaware food operators. These realities underline the need for further research and information divulging, increasing awareness, not only among consumers and food business operators but also physicians, veterinarians, researchers, sanitary authorities and policy makers.



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