

## Case Report

Human gastric hyperinfection by *Anisakis simplex*: A severe and unusual presentation and a brief review

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## ABSTRACT

Anisakiasis is an emerging marine food-borne zoonosis resulting from the accidental ingestion of *Anisakis* larvae, through the consumption of raw or undercooked infected seafood products. The first case of human gastric hyperinfection by *Anisakis simplex* with an unusual and severe presentation, occurring in a Portuguese woman, is described in this article. Over 140 anisakid larvae were removed by gastroscopy. Massive infection is uncommon in areas where the consumption of raw fish is not part of the traditional diet, as is the case in Portugal. The increased consumption of raw seafood products is considered a health determinant in the rise in cases of anisakiasis. However, clinicians should be aware of the emergence of these infections, not only because of the new dietary habits of the population, but also because of the high prevalence of *Anisakis* larvae in the different fish species usually consumed by the population, collected on the Portuguese coast.

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## Introduction

Anisakiasis is an emerging marine food-borne zoonosis resulting from the accidental ingestion of L3 larvae of parasite nematodes of the *Anisakis* genus. The life-cycle of anisakid parasites follows the general nematode life-cycle, including four larval stages (L1–L4) and the adults in the final hosts. The adult stages of *Anisakis* reside in the stomach of marine mammals (such

as whales and dolphins) and pinnipeds (such as seals and sea lions), and unembryonated eggs are expelled with the faeces. These eggs develop and hatch, releasing free-swimming L3, which are ingested by euphausiid oceanic krill and copepods (intermediate hosts). Sea fish and cephalopods (paratenic hosts) ingest planktonic crustaceans and other fish and cephalopods infected with L3 larvae, contributing to the dissemination of the parasite. The infective L3 (embedded in the viscera and muscle or free in the body cavity) are transferred to the final hosts (marine mammals) by ingestion of the sea fish and cephalopods (in the case of dolphins, seals, sea lions) or via oceanic krill (in the case of whales). In the final host, two moults occur (from L3 to adult) before sexual maturity to produce eggs, and a further cycle is initiated.

All L3-infected seafood types including fish, crustaceans, and cephalopods can cause anisakiasis when ingested by humans. Humans are an accidental host in which the larvae cannot complete the life-cycle. Occasionally, anisakids moult into L4 in

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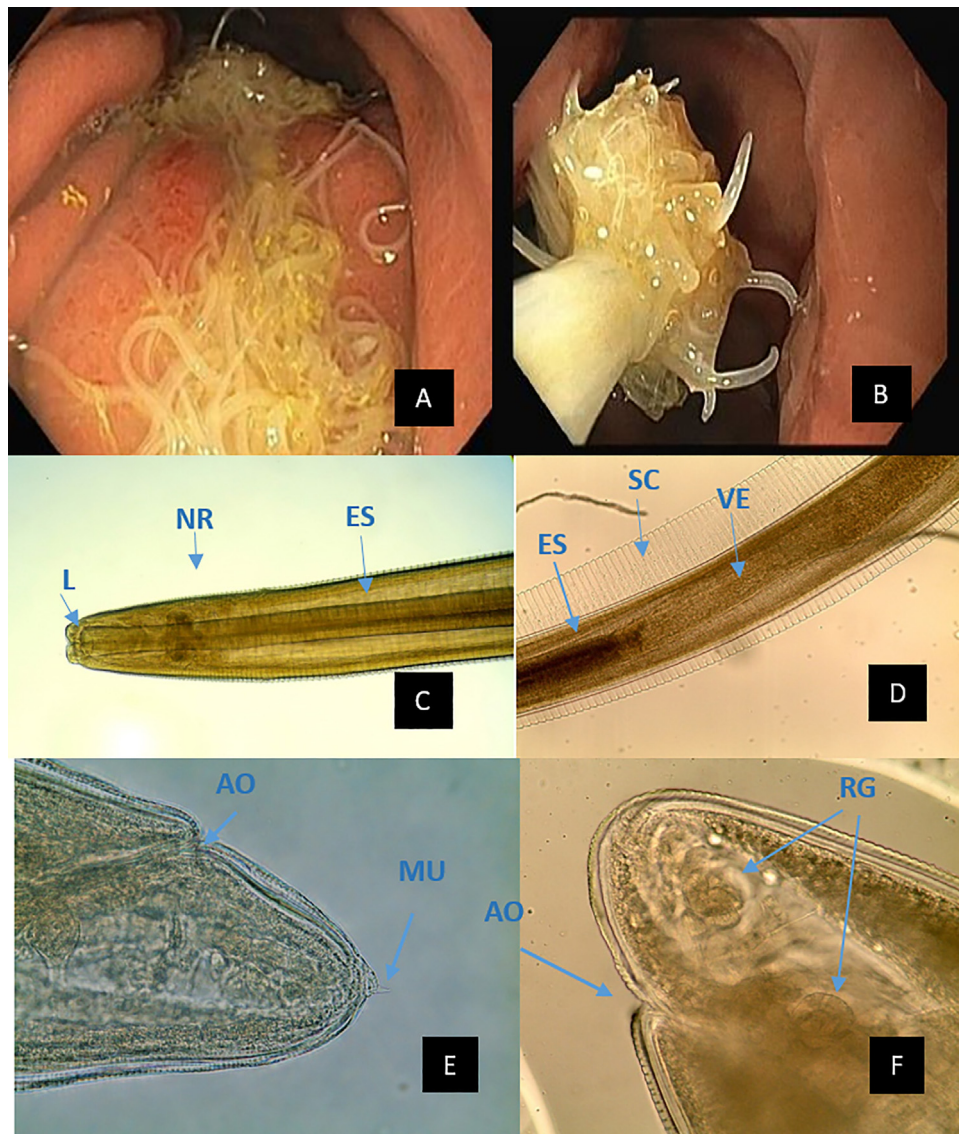
humans, but they do not progress to the adult stage (Audicana and Kennedy, 2008).

Anisakiasis is a cosmopolitan zoonosis, with about 20 000 cases being reported to date, the vast majority (90%) in Japan. The number of human cases of anisakiasis is increasing worldwide, with several cases related to the consumption of international fish dishes, including raw or semi-cooked fishery products harbouring L3 larvae. Japanese sushi and sashimi, Dutch salted or smoked herring, Nordic gravlax (dry, cured salmon), Hawaiian lomi-lomi (raw salmon), German rollmop (rolled fillet of marinated/pickled herring), South American ceviche, and Spanish boquerones (pickled anchovies) are regular pathways of infection. A number of new cases reported from European countries facing the Mediterranean Sea related to the consumption of raw marinated fish have occurred in the last decade. In Spain, the prevalence of infected fish in markets is between 25% and 80%, with very high rates of infection in some particular species (Arcos et al., 2014).

Several authors have reported the presence of different fish species infected by *Anisakidae* along the Portuguese coast, including *Trachurus picturatus*, *Merluccius merluccius*, and *Sardina pilchardus*. On the island of Madeira, Portugal, the prevalence of infected *Aphanopus carbo* reached 97.2%, while in Lisbon the prevalence of infected *T. picturatus* reached 51.4% (Borges, 2008; Costa et al., 2003).

Fish with the viscera preserved after capture, instead of being gutted immediately, are a common source of human contamination, since worms migrate from the viscera to the flesh soon after the fish is dead. Furthermore, the risk of infection may increase with the ingestion of whole fish that have not been gutted, like sardines (*S. pilchardus*), small mackerel (*Trachurus trachurus*), and anchovies (*Engraulis encrasicolus*), as worms are often found in the viscera as well.

After ingestion, anisakid larvae may remain in the gastrointestinal tract without penetrating the tissues, causing an asymptomatic



**Figure 1.** (A) and (B) show high digestive video-endoscopy images: hyperemic and oedematous gastric mucosa with massive infection of *Anisakis* larvae. Images (C)–(F) show the external morphology of *Anisakis simplex* larvae: (C) cephalic end of a larva: L, mouth with developed lips; NR, nerve ring; ES, anterior portion of the oesophagus. (D) Middle section of the larva showing the transition from oesophagus to ventriculus: SC, transversal segmentation of the cuticle; ES, oesophagus; VE, ventriculus. (E) Posterior end of the larva with the anal opening: AO, anal opening; MU, caudal spine or mucron. (F) Posterior end of a larva without mucron: AO, anal opening; RG, rectal glands.

infection. More frequently, they may penetrate the gastric or intestinal mucosa, causing invasive gastrointestinal anisakiasis, producing either acute or chronic symptoms. The clinical manifestations differ depending on their location and according to the histopathological lesions elicited.

The four major clinical syndromes described include gastric, intestinal, ectopic, and allergic diseases. Symptoms of acute gastric anisakiasis may appear as soon as 1–12 h after ingestion, and include sudden stomach pain, nausea, and vomiting caused by penetration of the larvae into the gastrointestinal wall. These often form granulomas, in which the larvae die a few weeks later. If larvae reach the intestine, a severe eosinophilic granulomatous response may also occur 1–2 weeks following infection, causing chronic inflammation and symptoms mimicking Crohn's disease.

Acute infection may be associated with allergic reactions, such as urticaria, angioedema, bronchospasm, and even severe anaphylaxis (Audicana et al., 2002). However, there have also been several reports of allergies resulting from cooked fish intake, or occupational or domestic exposure to allergens from dead worms by food-borne, airborne, or skin contact routes, with symptomatology such as asthma, rhinitis, conjunctivitis, and dermatitis, which suggests that immunological reactions might occur after exposure to *Anisakis* antigens alone (versus live larvae) (Audicana and Kennedy, 2008; Daschner et al., 2012; Nieuwenhuizen and Lopata, 2013). Many issues regarding the immune response remain questionable, requiring further investigation to clarify the exact mechanism of the immune and allergic response triggered by the parasite (Daschner et al., 2012).

The first case of a gastric hyperinfection caused by *A. simplex* in a Portuguese woman, with an unusual and severe presentation, is reported here.

### Clinical case

A 43-year-old Portuguese woman living in Lisbon was admitted to the emergency department complaining of the sudden onset of nausea, epigastric pain, asthenia, intense cold, and low blood pressure (67/45 mmHg), 24 h after consuming a grilled scabbard fish. She presented an intense skin rash on the lower limbs, less pronounced on the upper limbs and abdomen, and mild diarrhoea. The anamnesis was unremarkable, with no history of drug or food allergies.

A total blood count revealed discrete anaemia (red blood cell count  $3.5 \times 10^{12}/l$ , haemoglobin 10.7 g/dl, haematocrit 33.1%) and a high erythrocyte sedimentation rate (69 mm/h). Chemical analyses revealed an elevated C-reactive protein (CRP 20.2 mg/dl), while other parameters remained normal.

Electrocardiogram, echocardiogram, chest X-ray, and abdominal ultrasound results were all normal. Due to previously inconclusive IgE results, specific IgG and IgG4 antibodies (at 1:200 and 1:100 dilution, respectively) were detected by ELISA using *A. simplex* larvae antigen prepared in-house (Benex, 1974), and undiluted serum was tested by counter-immunoelectrophoresis (CIE) to assess specific reactivity of antigen–antibody binding. A strong positive reaction was achieved, with immunoglobulin levels almost 3-fold higher than the established cut-offs (0.5 and 0.4, respectively), while intense immune precipitate bands confirmed specific reactivity of antigen–antibody binding. All immunoassay procedures were performed according to the standardized protocols used in the study laboratory (Romber, 1976).

A gastroendoscopic examination revealed hyperemic and oedematous gastric mucosa with massive infection of *Anisakis* larvae in multiple locations of the gastric lumen. One hundred and forty viable larvae were recovered by aspiration during gastroscopy (Figure 1A, B). The worms were fixed in 10% formalin, cleared in glycerin–alcohol, mounted with glycerin jelly and observed under

a light microscope equipped with a micrometer. The size of the specimens varied between  $13.5 \times 0.3$  mm and  $28.1 \times 0.5$  mm, according to the stage of development. The larvae with typical nematode morphology (Figure 1C–F) had a cephalic end with well-defined lips, a clear nerve ring, and a slender oesophagus followed by the ventriculus. The cuticle was deeply transversely striated, and at the posterior end, the anal pore and the anal glands were visualized. Based on morphology (Sohn et al., 2015) and molecular analysis targeting nuclear rDNA-ITS (internal transcribed spacers 1 and 2, and 5.8S rRNA gene) using primers ITS1 (forward: 5'-TCC GTA GGT GAA CCT GCG G-3') and NC2 (reverse: 5'-TTA GTT TCT TTT CCT CCG CT-3'), the parasite was identified as *A. simplex* s.l. (GenBank accession numbers [KY247095](#), [KY247096](#), [KY247097](#)).

The patient's health status improved shortly after removal of the worms and she was discharged after receiving supportive therapy with albendazole, 400 mg twice a day, for 7 days. During a follow-up period of 6 months, no similar episodes were reported and blood tests returned to normal values.

### Discussion

Although massive infections caused by anisakid larvae are uncommon, even in areas where the consumption of raw fish is part of the traditional diet, there are some cases reported in the literature (Sohn et al., 2015; Jurado-Palomo et al., 2010). Uncooked dishes are not part of the traditional Portuguese diet, thus the present clinical case was quite unexpected. However, due to the uptake of international gastronomy and the high prevalence of *Anisakis* in different fish species usually consumed by the population and collected on the Portuguese coast, this pattern is expected to change.

High levels of IgG and IgG4 (a specific biomarker of active helminth infection) were found in this patient. In contrast, levels of IgE were undetectable, which may be related to immunomodulatory effects induced by the high parasite burden, preventing mast cell degranulation and other effector mechanisms, with subsequent high production of IgG rather than IgE (Nieuwenhuizen and Lopata, 2013; Jurado-Palomo et al., 2010). On the other hand, CIE tests confirmed serum reactivity against the specific antigen and simultaneously excluded cross-reactivity against related antigens.

In conclusion, *Anisakis* infection is recognized as an emerging cosmopolitan zoonosis, and since the increased consumption of raw seafood products is the main health determinant in the rise in human disease, *Anisakis* infection should be considered whenever it is clinically and epidemiologically justified. Further molecular characterization within the *A. simplex* s.l. complex will allow the identification of sibling species frequently involved in human infections and their zoonotic potential, which may clarify the different pathogenicity and clinical presentations associated with anisakiasis.

### Conflict of interest

The authors declare no competing financial interests.

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