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Anisakis – immunology of a foodborne parasitosis

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Summary

Anisakis species are marine nematodes which can cause zoonotic infection in humans if consumed in raw, pickled or undercooked fish and seafood. Infection with *Anisakis* is associated with abdominal pain, nausea and diarrhoea and can lead to massive infiltration of eosinophils and formation of granulomas in the gastrointestinal tract if the larvae are not removed. Re-infection leads to systemic allergic reactions such as urticarial or anaphylaxis in some individuals, making *Anisakis* an important source of hidden allergens in seafood. This review summarizes the immunopathology associated with *Anisakis* infection. Anisakiasis and gastroallergic reactions can be prevented by consuming only fish that has been frozen to −20°C to the core for at least 24 hours before preparation. Sensitization to *Anisakis* proteins can also occur, primarily due to occupational exposure to infested fish, and can lead to dermatitis, rhinoconjunctivitis or asthma. In this case, exposure to fish should be avoided.

KEYWORDS

allergy, anisakiasis, *Anisakis*, fish parasites, nematode

1 | INTRODUCTION

Through consumption of animal products, including fish, humans are susceptible to a variety of parasitic foodborne zoonoses, many of which are caused by helminths. Helminth infections can be transmitted from freshwater, brackish and marine fish and include liver fluke diseases such as clonorchiasis, opisthorchiasis and metorchiasis, intestinal trematodiasis caused by heterophyids and echinostomes, anisakiasis and pseudoterranoviasis caused by *Anisakis* and *Pseudoterranova* nematode larvae, and diphyllobothriasis (tapeworm infection).[1] Traditional and modern habits of eating raw or incompletely cooked fish are the major reason for acquired zoonotic infections, in most cases. Due to the recent worldwide popularity of sushi and dishes in which fish or seafood is lightly cooked, the consumption of raw or improperly cooked fish is even increasing in some regions.[2,3] This review will focus on *Aniskais*, a marine nematode capable of causing severe infection and allergy, and the immunology of anisakiasis.

1.1 | Anisakiasis – a zoonotic helminth infection acquired from marine fish

Marine fish are wild animals and can also be parasitized with a variety of helminths, including cestodes (tapeworms) and nematodes (roundworms).[1] A family of nematodes, the anisakids (family Anisakidae), is common in marine fish, which are paratenic hosts, and is able to cause symptomatic infections in humans, which are accidental, nonpermissive hosts. Symptoms of anisakiasis include mild-to-severe abdominal pain, nausea and diarrhoea and can also include strong allergic reactions.[4,5] The genus most commonly causing human infections is *Anisakis*, particularly the two sibling species *A. simplex* sensu stricto (s.s). and *A. pegreffii*, but also *A. physeteris*.[6–9] These nematodes have cetaceans (whales and dolphins) as a definitive host. A closely related anisakid, *Pseudoterranova decipiens*, is the second most commonly reported cause of human infection by a marine nematode and has seals and sea lions as a final host.[10,11] *Contracaecum* (definitive host: birds) and *Hysterothylacium* (definitive host: fish, birds reptiles or marine mammals) [12] are other anisakid parasites which have been reported to rarely cause disease.[13–15] The term "anisakidosis" is used to describe disease caused by any member of the family Anisakidae, while "anisakiosis" or "anisakiasis" is usually used to describe disease caused by *Anisakis* species alone.[4,16,17] Since the first reported case of anisakiasis in the 1960s,[18] thousands of cases have been reported, primarily from Japan but also hundreds from Europe and other parts of the world.[4]

1.2 | Infection of fish by anisakids

The life cycle of *Anisakis* (Fig. 1) includes larval stages, which use crustaceans as intermediate hosts and fish as paratenic hosts, and adult worms, which reside and reproduce in marine mammals.[4] Adult worms living embedded within the stomach of sea mammals release eggs which are expelled into the ocean via the faeces. On the ocean floor, the eggs embryonate, and larvae develop and hatch. Free-living stage 2 larvae (L2) are ingested by planktonic crustaceans, in which they develop into L3. The crustaceans are in turn consumed by fish or directly by whales. Fish are consumed by sea mammals such as dolphins. Within the definitive hosts, whales and dolphins, *Anisakis* moult into stage 4 larvae and then adult worms, and the life cycle begins anew. If, however, the larvae are ingested by humans, who are accidental hosts, the larvae cannot complete their life cycle. A large number of fish species act as paratenic hosts for *Anisakis* and other anisakids, with different host ranges found between species, most likely due to different geographic distribution and feeding habits.[1,19–22] Molecular analysis indicates at least nine *Anisakis* species: the *A. simplex* complex consisting of *A. simplex* sensus stricto (s.s), *A. pegreffii* and *A. simplex* C; the *A. physeteris* complex consisting of *A. physeteris*, *A. paggiae* and *A. brevispiculata*, *A. typica*, *A. ziphidarum* and *A. nascettii*.[23–27] Distribution patterns of different *Anisakis* species within different climate zones and oceans, congruent with their final hosts, may be affected by changes in climate patterns and host numbers.[1,22] In fact, parasites such as *Anisakis* can be used as biological indicators for host distribution and abundance.[22]

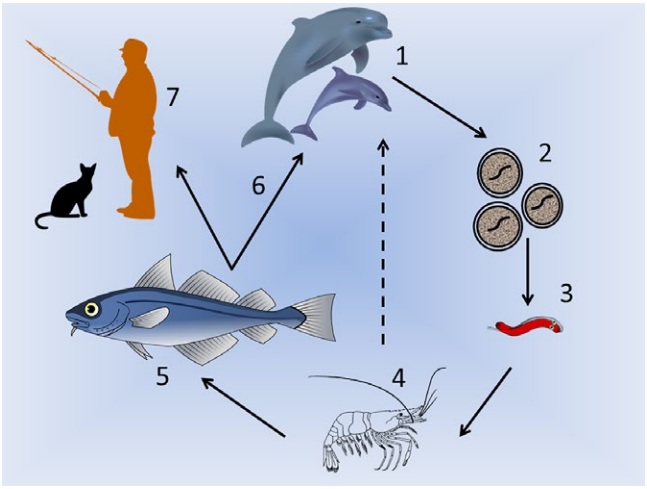

FIGURE 1 Life cycle of *Anisakis* species. (1) Marine mammals such as dolphins and whales are the definitive hosts of *Anisakis* species. *Anisakis* adult worms produce eggs in the intestine of the cetaceans, which pass into the water in the faeces. (2) *Anisakis* larvae embryonate and develop within the eggs, which hatch to release (3) free-living stage 2 larvae (L2). Planktonic crustaceans (4) ingest L2, which moult into L3. (5) Fish or squid ingest crustaceans and become infected with L3, which reside in the gut. Larger fish that are piscivorous can become infected many times. (6) Ingestion of infected fish or crustaceans (4) by marine mammals allows *Anisakis* larvae to enter its definitive host. The larvae moult to become adult worms and the life cycle continues. (7) Ingestion of infected fish by humans or other land mammals interrupts the *Anisakis* life cycle, as the larvae cannot moult into adult worms or reproduce. Humans may experience gastrointestinal pain or allergic reactions as a consequence of the immune response to the parasite

1.3 | Anisakiasis

Infection with *Anisakis* larvae can lead to a range of diseases, which can be grouped into gastric anisakiasis, intestinal anisakiasis, ectopic (extra-gastrointestinal) anisakiasis and gastroallergic anisakiasis.[4,5] Symptoms of acute gastrointestinal anisakiasis include sudden abdominal pain from a few hours to a few days following ingestion of parasitized raw fish, depending on whether it attaches to or penetrates the stomach (gastric anisakiasis) or the intestine (intestinal anisakiasis).[4,28,29] In the case of gastric anisakiasis, the larvae can be seen by gastroscopy and physically removed with forceps. In intestinal anisakiasis, it is not easy to remove the worm and often requires surgery due to severe abdominal pain and/or intestinal obstruction caused by inflammation.

Kikuchi et al.[29] describe intestinal anisakiasis as occurring in mild or fulminant forms. The mild form is thought to be primarily due to a primary infection, with pain that is often tolerated by the patient, which means the worm is not removed and may cause the formation of a granuloma that is later misdiagnosed as a tumour, ulcer, chronic appendicitis or another intestinal disease. The fulminant form, with more severe symptoms, is suggested to be due to a reinfection and allergic inflammatory reaction against larvae and their proteins. The thickness of the intestinal wall can increase by threefold to fivefold due to oedema and cell infiltration. The most intense histological changes occur around the larva, with inflammation and oedema accompanied by exudates of fibrin and small haemorrhagic lesions.

Purely gastrointestinal cases can occur without any allergic reactions, often accompanied by pain and inflammation, and in this case, it is preferable to physically remove the larvae so that it does not cause tissue damage that may lead to chronic symptoms. On the other side of the spectrum is gastroallergic anisakiasis, where there are often acute allergic symptoms (ranging from urticarial or angioedema to anaphylaxis) with minor or no gastrointestinal symptoms, and the larvae are normally expelled by the violent allergic reactions (vomiting, diarrhoea) so that gastroscopic removal is not necessary.[5,30,31]

Cases of ectopic anisakiasis are less common include penetration of the larvae into tissues such as the pharynx, tongue, lung, peritoneal cavity, lymphatic ganglia or pancreas are possible.[9,32]

1.4 | Immune pathology in anisakiasis

In their definitive hosts, cetaceans, adult *Anisakis* worms live in the stomach [33,34] (Fig. 2). The first compartment of the stomach of cetaceans (the forestomach) is nonglandular and does not secrete gastric juice.[35] In one study, parasites were found primarily located in the forestomach in necropsied cetaceans, associated with gastric ulcers,[34]

FIGURE 2 *Anisakis simplex* parasitizing the glandular stomach of a common minke whale, exhibited in the National museum of Nature and Science, Tokyo, Japan. Photograph from Wikipedia commons, licensed under the Creative Commons Attribution-Share Alike 3.0 Unported licence. Photograph credit: Momotarou 2012

while in another study, parasites occurred in all compartments and were mostly free living.[33] In contrast to the multichambered cetacean stomach, the one-chambered human stomach is highly acidic and has been suggested to present a challenge to the larvae, which may penetrate the gastric mucosa to avoid it.[35] Studies indicate that *A. simplex* is more resistant to acidic conditions than *A. pegreffii*.[35,36] *Anisakis* releases proteases that are able to degrade major components of gastrointestinal tissues, allowing it to invade the mucosa and submucosa by creating tunnels and burrows.[37]

Parasites were found associated with gastric nodules in whales, at the caseous necrotic centres of granulomas, which were filled with degenerating eosinophils.[38] Around the eosinophilic centre was a broad zone of epithelioid granulation tissue, surrounded by fibrous tissue. Similarly, in other cetaceans, ulcers occurred within the gastric mucosa, sometimes associated with oedema and haemorrhage.[33,39] Chronic lymphoplasmacytic gastritis, eosinophilic and granulomatous inflammation with giant cells, hemosiderosis, fibrosis and necrosis were associated with the location of the parasites.[33,39] Similar eosinophilic granulomas and abscesses or ulcers have been identified in gastric and intestinal lesions occurring in humans after attachment to the gastrointestinal mucosa or penetration of the gastrointestinal wall and submucosa by the ingested larvae and migration through the tissues.[14,15,38,40]

The larvae usually die within a few days in humans [41] and are broken down in about eight weeks, during which time larval remains are surrounded by oedema, necrosis and cellular inflammation composed mostly of eosinophils but also neutrophils, lymphocytes and monocytes, deposition of fibrotic tissues and formation of foreign body giant cells and lymphocytes, and ultimately, a granuloma.[8,15] The main role of granulomas is to protect the host by walling off pathogens or persistent irritants.[33] Dead *Anisakis* larvae are not easily degradable; therefore, they are typical of stimuli that incite a granulomatous response.[33] *Anisakis*-induced granulomas have been mistaken for tumours in the past, but gradually disappear in most patients, leading to the term "vanishing tumours".[8,42] In some patients, the inflammation takes longer to resolve, resulting in symptoms of chronic anisakiasis. It has been suggested that invasion of bacteria into the lesions formed around larval remains could exacerbate and prolong the ulceration.[33,43] Over time, the larvae become broken down and degenerate, and the remains are surrounded by eosinophils and neutrophils and sometimes surrounded by foreign body giant cells. With formation of a granuloma and clearance of larval debris, lymphocytes start to predominate instead of eosinophils, and it can be hard to recognize that the granuloma was caused by a parasite. The process of granuloma formation is summarized in Fig. 3.

Anisakis infection is sometimes associated with systemic neutrophilia or eosinophilia.[14,44] The extent of tissue damage and inflammation resulting from infection with the small (1–3 cm long) larvae points to interaction between the host immune system and substances secreted by or contained within the larvae as the cause of the pathology.[35,41] *Anisakis* extract was shown to be chemotactic for eosinophils but not neutrophils[45,46]; however, tissue damage itself may induce the neutrophils.[4,47] Eosinophil major basic protein and inducible nitric oxide synthase, molecules that aid in helminth killing, were detected in the inflammatory infiltrate of biopsies from patients with anisakiasis,[48] and serum levels of eosinophil cationic protein were found to be raised in the first 72 hours after gastrointestinal infection.[44] The release of these substances and other products such as peroxidases and eosinophil-derived neurotoxin probably aid in killing the larvae, but also cause local tissue damage.[47] PCRs on intestinal biopsies from anisakiasis patients detected expression of T-cell receptor and the Th2 cytokines IL-4 and IL-5, but not IFN-gamma or IL-2, indicating a Th2 type immune response.[48] IL-4 stimulates IgE production, while IL-5 is responsible for inducing eosinophil proliferation, differentiation and activation and can increase the killing ability of eosinophils.[49]

1.5 | *Anisakis* and cancer

Anisakiasis granulomas are occasionally misdiagnosed as gastric cancer; however, the two diseases can also be concurrent.[40,50] An interesting characteristic of *Anisakis* larvae is that they tend to attach to vulnerable mucosa, such as ulcers,[40] and possibly to cancerous mucosa because of the local defect in acid secretion, change in mucin and other structural alterations.[50] Sonoda et al.[40] list 29 case reports in which *Anisakis* parasites were found attached to cancerous tumours, usually early gastric cancer, and subsequently

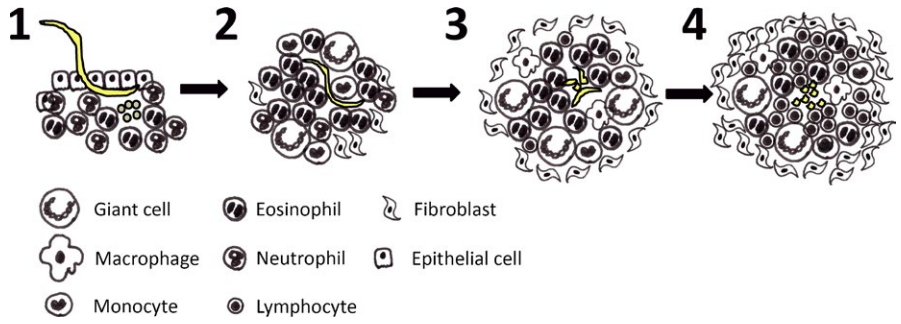

FIGURE 3 Formation of a granuloma after primary infection with *Anisakis*. (1) The larva penetrates the tissue, secreting proteins that are chemotactic for eosinophils. Tissue damage induces neutrophil recruitment. (2) The larva is surrounded by large numbers of eosinophils which release toxic killing molecules, as well as neutrophils, lymphocytes and monocytes. Fibroblasts are recruited and deposit connective tissue to form a granuloma. Formation of foreign body giant cells occurs. (3) Larval remains are surrounded by eosinophils and necrosis in the centre of the granuloma, surrounded by foreign body giant cells and connective tissue. Lymphocytes begin to appear. (4) Larval remains are broken down and unrecognizable. The cellular infiltration becomes composed primarily of lymphocytes, making it hard to recognize that a parasite triggered the formation of the granuloma. The generation of memory T and B cells during primary infection sensitizes the host, who may experience allergic reactions upon subsequent exposure to *Anisakis*

performed studies with another nematode, *Caenorhabditis elegans*, that indicated that nematodes may be able to identify tumours by scent detection.[51] On the other hand, it has also been suggested that *Anisakis* infection could be a cofactor for development of gastric cancer.[52] Recently, higher levels of specific IgA directed at recombinant *Anisakis* proteins rAni s1 and rAni s5 were detected in patients with gastric cancer compared to controls; however, there was no significant difference in levels of *Anisakis*-specific IgG1 or IgE, which would normally be detected after *Anisakis* infection.[53] Therefore, to date, there is no evidence linking *Anisakis* infection to the development of gastric cancer. Many pathogens associated with intense or persistent inflammation and tissue damage, including DNA damage, are associated with carcinoma.[54] Infection with fish trematodes is associated with carcinoma of the bile duct or pancreas in some cases.[1,55] Other helminths associated with cancer are *Schistosoma haematobium* (bladder cancer), *Clonorchis sinensis* (liver cancer, cholangiocarcinoma) and *Opisthorchis viverrini* (liver cancer, cholangiocarcinoma).[54]

1.6 | Immune responses to nematode infections

Upon a primary exposure to a helminth, various degrees of inflammatory responses are triggered, characterized by the recruitment of inflammatory effector cells such as neutrophils and eosinophils and changes in blood vessel permeability and blood flow.[56,57] These changes can be initiated by the activation of the alternative complement pathway and the nonspecific degranulation of mast cells. Basal levels of eosinophils are always present, and the presence of helminths can cause them to rapidly infiltrate to the site of infection.[58,59] In mice, helminth infections are associated with eosinophilia, mastocytosis, goblet hyperplasia, high total (polyclonal) IgE production, helminth-specific IgE and IgG1 production and the accumulation of alternatively activated macrophages.[57,60–63] Similar features are evident in humans and livestock.[64,65] Nonspecific inflammation is minimal in most natural host–parasite systems, but is often enhanced in zoonotic infections where the host is unnatural or nonpermissive.[57] *Anisakis* species and *Ancylostoma caninum*, a canine hookworm, are examples of unnatural parasites of humans that can elicit significant eosinophilic enteritis during a primary infection.[66] Natural parasites have evolved evasive mechanisms and immunosuppressive molecules, and enhanced effector responses mediated by the adaptive immune system are required for their expulsion.[67–69]

Experimental models indicate that the effector mechanisms required for expulsion or destruction of nematodes vary between species and between larval and worm forms of the parasite.[57,60,67,70] For example, eosinophils appear to play a role in damaging or killing infective larvae and encysted stages of the parasite life cycle, but do not appear to play a role in the expulsion of mature worms, except in unnatural or nonpermissive hosts.[57,71,72] Eosinophils release potent mediators such as eosinophil peroxidase and major basic protein that can damage and kill helminth larvae.[73] Specific antibody is thought to enhance eosinophil-mediated killing of larvae through antibody-dependent cytotoxicity.[57,74,75] In particular, IgE production has been associated with acquired immunity to helminth infection in humans [76] and sheep,[77] and vaccine-mediated protection against larval *Onchocera volvulus* in mice required both eosinophils and IgE.[74]

In contrast, expulsion of intestinal dwelling adult worms requires mast cells, goblet cells and altered activity of nonhaematopoietic cells such as smooth muscle cells, goblet cells and epithelial cells, which render the gut lumen environment intolerable for the establishment or survival of nematodes, all effector mechanisms orchestrated by Th2 cytokines.[57,67,78] As helminth larval stages migrate through the lungs or skin, similar effector responses can be initiated at these sites.[79] The Th2 cytokines driving these responses are the same as those that drive allergic diseases and are responsible for the symptoms of gastroallergic anisakiasis, such as diarrhoea, vomiting, itching, angioedema, urticaria and anaphylaxis, as well as *Anisakis* allergy, which can have symptoms such as asthma, rhinoconjunctivitis, urticaria and atopic dermatitis, primarily driven by mediators released from mast cells.[80,81]

Sneezing, coughing, tearing, diarrhoea and vomiting are other examples of "expulsion" responses that can be observed in allergy.[82] The strong immunopathological responses seen in allergy usually do not occur during helminth infection, although both situations involve Th2 responses, including IgE production and eosinophilia. It is thought that this is because chronic and/or high burden parasite infections also induce strong regulatory responses, such as TGF-beta and IL-10-producing cells, which suppress excessive immune responses, facilitating the survival of the parasite and also reducing host tissue damage.[83] While allergic symptoms are not frequent in helminth infections, they occur more commonly in zoonotic infections such as anisakiasis.[31] Infection with trematodes from fish can also cause bronchial asthma and allergic lesions in the early stages of infection.[1,55] Allergic reactions due to infection with *Anisakis* and other zoonoses could be due to the relatively low numbers of larvae to which most patients are exposed and the fact that no mutual adaptation has evolved.[84]

The outcome of any parasite–allergy interaction appears to depend upon the balance between parasite induction of suppressive regulatory (IL-10/TGF-beta driven) responses and allergic Th2 (IL-4/IL-13 driven) responses.[85] It has been suggested that there is a correlation between the amount of tissue damage caused by a helminth (which would often correlate to the parasite burden) and its ability to induce regulatory immune responses. Helminths can cause extensive tissue damage while migrating through the host, which may explain why wound-healing pathways are strongly linked to antihelminth responses.[86,87] In an experimental infection model, rats infected with high numbers of *Anisakis* larvae developed weaker IgE responses than those infected with few larvae, suggesting the ability of high burden worm infections to stimulate a suppressive response.[88]

1.7 | Induction of Th2 responses

Infection with *Anisakis* larvae induces strong Th2 responses in mice, and evidence of Th2 responses is also seen in humans.[30,48,80] As helminth infections are consistently associated with Th2 type responses, it is possible that there are molecular features common to helminths that are recognized by the host.[60,82,86,89,90] Pattern recognition receptors for helminths have not yet been identified, and in general, the trigger for Th2 cell differentiation is not understood, although it appears to require IL-4.[91] It is possible that the tissue injury caused by these large multicellular parasites, or molecular proxies of this tissue damage, may be the trigger for type 2 immune responses.[82,92,93] For instance, it has been shown that the Th2-promoting cytokines IL-33 and thymic stromal lymphopoietin (TSLP) are released by epithelial cells and epidermal cells, respectively, after damage [94,95] and that ATP released from dying cells promotes Th2 responses in the lungs.[96]

The The2-inducing ability of some allergens also seems to rely on their enzymatic activity, for example the house dust mite protease Der p 1 [97] and bee venom phospholipase.[98,99] In addition, experimental data demonstrates that concomitant administration of the house dust mite allergen Der p 1 or mould proteases with normally tolerogenic antigen renders it allergenic. Protease allergens such as Der p 1 have the ability to cleave proteins that make up the tight junctions between epithelial cells lining the lungs, nasal passages and gut. In this way, they bypass the physical barriers of the body and gain abnormal access to subepithelial immune cells. Access to subepithelial immune tissue rich in antigen-presenting cells is considered a primary risk factor for the development of allergic sensitization. Parasitic helminths such as *Anisakis* release large amounts of proteases which facilitate the penetration of host tissues and the digestion of tissues for nutrients.[98,100] Proteases in excretory–secretory products are therefore among the first *Anisakis* antigens to which the host is exposed. It has been shown that pathogen and allergen proteases can directly influence immune responses by cleaving various receptors and costimulatory molecules on dendritic cells, macrophages, T cells and mast cells, including CD40, CD23, CD25, TLR3, DC-SIGN, CD14 and protease-activated receptors.[98,101–107] However, the strong Th2 responses induced by *Anisakis* and its extracts are not yet completely understood.

1.8 | *Anisakis* allergy

Anisakis allergy and gastroallergic anisakiasis are relatively common in Spain [108,109] and Italy [7,110] and have been reported from Japan [111,112] and Korea,[113] but are rarely reported from other parts of Europe or the rest of the world.[114] This is likely due to differences in dietary consumption of raw or pickled fish, with awareness and diagnosis of the disease possibly a contributory factor. In both Spain and Italy, most cases of gastroallergic anisakiasis occur after consumption of raw marinated anchovies, a popular local dish, as well as after consumption of undercooked hake or cod.[7,115,116] Gastroallergic anisakiasis was also reported from Korea in connection with consumption of flatfish, congers, squid, whelk and tuna.[113] In Spain, *Anisakis* sensitization accounts for a high number of initially unexplained acute allergic episodes, as well as chronic urticaria.[4,108,116–118] In Italy, *Anisakis* is also a frequent cause of chronic urticaria,[119] and *Anisakis* parasites in mackerel were found to be the main cause of seafood-associated urticaria in Japan, which was described as occurring commonly.[111] Elimination of raw fish from the diet improved chronic urticaria in one study,[119] while in another, total elimination of fish from the diet did not improve urticaria, and it was suggested that oily fish may in fact improve urticaria because of its high content of antiinflammatory omega-3 fatty acids.[120]

Infection with parasites generates specific IgE against parasites; therefore, the presence of specific IgE or a positive skin prick test alone does not indicate allergy, which can only be assessed by a clinical history, but only sensitization.[114,121,122] Eosinophilic inflammation is typically induced against the parasite as part of the host defence responses, but allergic reactions such as urticaria, angioedema and anaphylaxis appear to involve a separate mechanism, as they do not occur in all patients. Patients with allergic reactions do not usually recall a previous episode of anisakiasis, but must have had one to generate the IgE antibodies causing their allergic reactions, indicating that the primary infection was mild or asymptomatic.[84] The violent allergic reaction (including vomiting and diarrhoea) usually expels the parasite, suggesting that the allergic response functions as an immune defence mechanism.[31]

It is often debated whether live *Anisakis* larvae are required for allergic reactions.[4,115,122] Overall the evidence suggests that while living larvae are in most cases required for both the initial sensitization and subsequent gastroallergic reactions, in a small number of cases sensitized individuals may react to proteins of dead larvae.[123–127] The allergenicity of *Anisakis* proteins is evident from the fact that fish processing workers or others who frequently work with fish can develop *Anisakis*-induced asthma, rhinoconjunctivitis and dermatitis.[80,128–130] However, patients with previous gastroallergic anisakiasis reactions do not react to dead larvae or larval proteins administered as a challenge, and a diet excluding raw fish seems to prevent most reactions, with the removal of unfrozen fresh fish preventing additional reactions due to the possibility of the fish not being cooked thoroughly.[123,126,131,132] A wide range of *Anisakis* allergens have been identified, which include molecules such as protease inhibitors, tropomyosin and haemoglobin.[133–135]

1.9 | Animal studies of *Anisakis* immunology

Animal models have been used to try to dissect immunological mechanisms of disease, clarify aspects of responses to live and dead larvae and determine the pathogenicity of various *Anisakis* species.[136–142] There has been speculation that some species of *Anisakis* may be more infective than others; however, *A. simplex*, *A. pegreffii*, *A. paggiae* and *A. physeteris* are all able to infect rats.[143,144]

In both rats and mice, *Anisakis* infection leads to similar pathology to humans, with infiltration of eosinophils and neutrophils and formation of granulomas with multinucleate giant cells. In primary infection in rats, oedema was mild, with fibrous and granulomatous changes appearing around day 7, foreign giant cells by week 5 and a granuloma by week 11. After re-infection, oedema was more severe.[29,139] In mice, neutrophils accumulated around week 1, and at two weeks, most larvae were still viable and surrounded by granulocytes, occasional multinucleate giant cells, and mature granulomata consisting of eosinophils, fibroblasts and collagen.[139] After three weeks, the larvae had been invaded by inflammatory cells and were dead, surrounded by granulomata consisting of connective tissue, eosinophils and multinucleate giant cells. Antibodies were at first generated to excretory–secretory products of the larvae and later to somatic antigens after the larvae had been broken up.[138] IgG1 was the predominant IgG isotype produced,[80,138] and Th2 cytokines were induced in wild-type mice.[80,145] Re-infection caused an increase in specific IgE antibodies in both rats and mice, indicating that multiple infections with *Anisakis* larvae promote IgE production.[80,88,136] Only live larvae were found to induce antibodies in rats,[146] supporting the idea that allergic sensitization requires live infection in humans.[122] However, challenge of sensitized mice with *Anisakis* protein extract was able to induce allergic symptoms such as itching, diarrhoea and mucus hypersecretion in the lungs,[80] suggesting that it is possible for sensitized patients to react to larval proteins, if they are exposed to high amounts.

Symptoms of *Anisakis* infection in humans are varied, ranging from asymptomatic or mild infection to gastrointestinal anisakiasis to gastroallergic anisakiasis. The important of the genetic background of the host in the immune responses to *Anisakis* is demonstrated by the varying responses in mice of different strains and knockout mouse strains.[80,145] Wild-type BALB/c mice mount a Th2 response to infection, associated with allergic symptoms after subsequent exposure to *Anisakis* proteins, whereas IL-4Rα-deficient mice mount a Th1 response to infection have increased levels of infiltrating immune cells in the intraperitoneal cavity and show no signs of allergic disease upon exposure to *Anisakis* proteins.[80] This is similar to a human study in which raised IFN-gamma and lower IgE was associated with predominantly gastrointestinal symptoms and weak or no allergic symptoms, whereas raised IgE and production of Th2 cytokines was associated with milder or absent gastrointestinal symptoms but urticarial, angioedema and anaphylaxis.[30] This suggests that symptoms of anisakiasis may be largely dependent on the host immune response rather than the larvae themselves, with strong Th2 responders exhibiting allergic reactions that help expel the larvae and Th1 responders developing a more inflammatory response associated with gastrointestinal pain. In accordance with this, a patient parasitized with over 200 larvae was found to have low IgE levels and increased neutrophils,[147] and another patient parasitized with 56 larvae presented with epigastric pain and nausea but no allergic symptoms.[148] However, as previously mentioned, it is also possible that a high parasite burden and the ensuing tissue damage suppress allergic responses.

1.10 | Prevention of anisakiasis and exposure to *Anisakis* proteins

Anisakiasis can occur after consumption of infected seafood that is served raw, undercooked, pickled, citrus juice-marinated or smoked. To prevent anisakiasis, it is recommended to cook seafood well (above 60°C for at least 1 min at the core or to freeze it for at least 24 hours at −20°C or 15 hours at −35°C to kill the parasites [4,114,149,150]). In some studies, anisakid larvae in fish from Pacific regions were able to survive −20°C for longer periods (52 hours to 4 days); therefore, the FDA recommends freezing for 1 week at −20°C or blast freezing at −35°C for 15 hours.[4] The freezing time can depend on the thickness of the fish. Overall, thorough cooking or freezing renders the fish safe for consumption. Anisakiasis cases in the Netherlands due to consumption of raw herrings virtually disappeared after strict rules for freezing of fish to be consumed raw were enforced. However, cultural preferences for fresh raw or pickled fish mean that cases of anisakiasis are likely to continue to occur.

Additional measures to reduce infections aim to decrease the number of parasites present within seafood products by harvesting and storage methods.[151] The type and size of fish harvested should be considered.[151] Many parasites accumulate in the host over the course of lifetime of the fish, with larger, piscivorous fish more heavily parasitized.[1,14,151] Certain geographic locations of fish stocks also develop reputations for being parasite-rich due to the relative abundance of intermediate or definitive hosts and can be avoided by fishing vessels.[1,151] After being caught, fish may be gutted immediately to prevent larvae migrating from the viscera into the flesh after the death of the fish and/or the increase in temperature.[1,14,18,21,151,152] Some

authors were unable to detect migration of *Anisakis* larvae to the muscles of fish post-mortem,[1,153] while others did find increased larvae in the muscles over time.[14,152,154] After gutting of fish, disposal of infected viscera at sea may lead to infections of fish which feed on the discarded materials[1,21]; therefore, safe disposal should be encouraged to prevent re-entry of *Anisakis* into the food chain. At cold temperatures, most *Anisakis* larvae remain encysted and attached to the viscera of the fish, but smaller numbers are found within the flesh of the fish, suggesting *intra-vitam* migration.[152] The distribution of larvae within tissues of fish appears to be affected by the species of parasite, the species of fish, water temperatures and the storage conditions of the fish after capture.[1] In a Japanese study, *A. simplex* was more commonly found in the flesh of the fish than *A. pegreffi*, which was suggested to explain why most cases of anisakiasis in Japan are caused by this species.[155]

Aquaculture, for example farming of salmon, can produce fish in which parasites are reduced or absent.[1,114,151,156] Salmon fed on pelleted feed and housed in cages raised off the sea bed are primarily nematode-free due to the break in the life cycle of the nematodes [114] and may also be trematode free if they originate from hatcheries in snail-free environments.[151] Farmed cod are often fed with unprocessed marine fish or offal, so the risk is high that parasites can be transferred to the cultured fish.[114]

2 | CONCLUSIONS

Zoonoses such as anisakiasis link animal and human populations and may be affected by activities such as intensification of fishing, cultural changes in eating habits, environmental alterations including climate change, fishing practices and movement of human and animal populations.[1] Zoonotic infection with the marine nematode *Anisakis* is primarily associated with infiltration of eosinophils and the formation of granulomas in the gastrointestinal tract. However, infection gives rise to an unusual situation in which re-infection leads to severe allergic reactions in some infected individuals that appears to aid in expelling the parasites, putting anisakiasis on the border between parasitism and allergic disease.

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