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A REVIEW OF *ANISAKIS* SPP. AND ITS VARIOUS ALLERGENS' PROFILING AND CHARACTERISATION

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Abstract: This review article focuses on the identification and characterisation of the allergens present in Anisakis spp., parasitic worms found in coastal fish that can cause anisakiasis in humans who consume raw or contaminated seafood. The study aims to fill a gap in the research by profiling and analysing the allergens present in *Anisakis simplex* s.s., *Anisakis pegreffii* and the hybrid haplotype. To conduct the analysis of *Anisakis* spp. and its various allergens, a comprehensive bibliographical search was conducted using online sources such as Google Scholar, PubMed Central, ScienceDirect, Springer Link and Wiley Online Library. These sources provided diverse articles, research papers and other relevant literature on the subject. The findings of this analysis were based on an extensive review of the sources and provided insights into the allergenicity of *Anisakis* spp. and the various allergens that it contains. According to the study, the most important allergens are Anis 1, Anis 7, and Anis 12. In contrast, Anis 4, Anis 5, Anis 6, Anis 8, Anis 9, Anis 10, and Anis 11 are minor allergens present in the somatic and excretory/secretory (ES) components of third-stage *Anisakis l*arvae. Anis 1 and Anis 4 are the main causes of anaphylaxis associated with anisakiasis because they both need heat to cause allergic reactions. Anis 1 (24 kDa), Anis 5 (15 kDa), Anis 7 (139 kDa), and Anis 9 (14 kDa) are frequently targeted by antibodies in people with anisakiasis. Some of these allergens, including Anis 1, Anis 5, and Anis 9, are heat-stable, which is a concern because they can linger even after cooking contaminated seafood. These allergies include Anis 4, Anis 8, Anis 11, and Anis 10, as well as Anis 1, Anis 5, and Anis 9. Immunoassays frequently employ the pan allergens Anis 2 (paramyosin) and Anis 3 (tropomyosin), although these assays have a low sensitivity, which limits their diagnostic relevance. The most prominent excretory/secretory (ES) allergen nowadays is Anis 7, the only one that all patients with *A. simplex* infection recognise. The characterisation and profiling of *Anisakis* spp. allergens have yet to be fully explored in the literature, making this review a unique contribution to the field of allergy research.

Keywords: *Anisakis* spp., allergens, Anisakiasis, parasites, fish.

Introduction

Anisakis spp. is a member of the Ascaridoidea group, the Ascaridina suborder, the Ascarida order, the Anisakinae subfamily and the Anisakidae family (WoRMS, 2021). According to phylogenetic studies, *Anisakis* spp. is most closely related to the human parasite *Ascaris* sp. *Anisakis* species are widely dispersed across the planet; however, each species is associated with a specific host species and exhibits a unique distribution pattern based on that host species. Numerous commercial fish species are parasitised by nematodes of the genus *Anisakis*, which also cause allergic reactions and anisakiasis, a zoonosis spread by fish. Additionally, *Anisakis* can harm the fish business financially and foster customer mistrust of fishing products (Bao *et al.,* 2019).

Three related species, including the morphologically different *Anisakis ziphidarum*, *Anisakis typica*, *Anisakis physeteris*, *Anisakis schupakovi,* and *Anisakis brevispiculata*, are part of the *Anisakis simplex* complex

(Nieuwenhuizen & Lopata, 2013). This parasitic nematode can spread illness and infect people. Whilst *A. simplex* s.s. and *A. pegreffii*, two of the nine species that make up the genus *Anisakis* spp., have been identified as the primary agents of the spread of zoonotic illnesses (Laffon–Leal *et al.,* 2000; Ferrantelli *et al.,* 2015; Bao *et al.,* 2017; Ángeles-Hernández *et al.,* 2020). *Anisakis* spp. have invaded and multiplied in people, resulting in the disease known as anisakiasis. Foodborne parasite sickness can result from eating raw or undercooked fish, seafood, or other marine products that contain live third-stage larvae (L3) (Aibinu *et al.,* 2019). At this stage, marine fish function as paratenic hosts or carriers of the L3 larva in which the larvae encyst or remain attached to the internal tissue (Ferrantelli *et al.,* 2015; Ángeles-Hernández *et al.,* 2020). While the parasite has the ability to reside in the coelomic cavity or undergo larval migration to the epiaxial muscle of the infected fish (Mattiucci *et al.,* 2011; Castellanos *et al.,* 2017; Ángeles-Hernández *et al.,* 2020).

The seafood particularly fish including cod, mackerel, rockfish and salmon can be frozen at -20°C for 48 hours in advance to avoid this disease. The risk of contamination decreases when the fish is frozen before eating because the live third-stage larvae (L3) in the edible tissue are encapsulated (Faeste *et al.,* 2014; 2015). L3 larvae were found either free-living in the gastrointestinal tracts (GI) of the hosts or encapsulated in the exteriors of the gastrointestinal tract and other visceral tissue (Shih *et al.,* 2010). Following this, the larvae will break through the digestive epithelium, causing the release of toxic chemicals that cause inflammation. The gastrointestinal system can be harmed by live *Anisakis* spp. larvae, which can result in gastrointestinal mucositis, granulomas, haemorrhages, and other symptoms. Angioedema, hives, and even anaphylactic shock (Polimeno *et al.,* 2021) are allergy symptoms that can result from gastrointestinal anisakiasis, which is brought on by hypersensitivity to allergens from parasites and their presence in the digestive tract (Bilska-Zajc *et al.,* 2015).

The debate over whether or not live L3 larvae are necessary for the emergence of allergic symptoms, as well as whether or not exposure to parasite allergens alone could result in unpleasant reactions like anaphylactic shock or urticaria-angioedema syndrome, even after the L3 larvae have been removed by freezing or boiling the fish, which is still ongoing (Ventura *et al.,* 2008). It is because some allergens, such as Anis 1, cystatin Anis 4, and members of the SXP/Ral family (Anis 8, Anis 9, and Anis 5), have been found to maintain a high level of resistance to enzyme digestion or heat therapy. These proteins are to blame for the hypersensitive reactions to *A. simplex* proteins tainted with marine products. In Japan, where over 2000 Anisakiasis cases are recorded each year, more than 90% of human cases of zoonosis have been documented (Arizono *et al.,* 2012; Lim *et al.,* 2015; Amir *et al*., 2016; Shimamura *et al*., 2016; Rezapour *et al*., 2017; Ángeles-Hernández *et al*., 2020) and in South Korea with 200 cases (Arizono *et al.,* 2012; Lim *et al.,* 2015). In Europe, 500 cases/year are reported including France, Germany, Spain, and the Netherlands, while 70 cases were registered in the US (Audicana *et al.,* 1995; Menéndez *et al.,* 2005; Hochberg & Hamer, 2010; Arizono *et al.,* 2012; Lim *et al.,* 2015; Ángeles-Hernández *et al.,* 2020). Patients who have anisakiasis will display allergic symptoms brought on by allergens. The term "allergen" refers to a substance that causes allergic reactions. Allergens are instantly recognised by the body after they have entered as invaders or foreign substances that cause the immune system to respond by producing antibodies. As a result of the thermostability of several *Anisakis* allergens, immunoglobulin E (IgE-mediated hypersensitivity) reactions can also manifest after the consumption of highly processed seafood products containing such allergens (Bao *et al.,* 2019).

Additionally, *Anisakis* spp. has been linked to allergic IgE-mediated reactions, which happen after secondary parasite infection and include angioedema, urticaria, asthma, and in rare instances, anaphylaxis in highly sensitive people. It should come as no surprise that in the

past, allergic reactions to *Anisakis* spp. have been mistaken for other conditions like acute urticaria or a seafood allergy. Additionally, healthy individuals without any clinical symptoms who are allergic to *Anisakis* spp. were found to have high amounts of specific IgE (Mazzucco *et al.,* 2018).

Anisakis spp. Protein

Proteins are essential for the parasite's survival in hostile settings and play important roles in the parasite-host interface in a number of ways. Enzymatic substances known as excretion–secretion (ES) helps larvae appear and shed, permit parasites to enter tissues, stop blood from clotting, shield the parasite from the host's immune response, and support its diet and nourishment (D'Amelio *et al.,* 2020). Because of this, the immunobiology of *Anisakis* spp. infections depend primarily on ES parasite product components, allowing for the discovery and creation of novel therapeutic approaches for other gastrointestinal worms. An in-depth examination of ES goods could also aid in creating specialised and effective diagnostic techniques and identifying parasite signals in marine products. It has been discovered that *Anisakis* spp. proteins are connected to allergies and antigenicity.

Anisakiasis can cause allergic reactions mediated by IgE in highly sensitive individuals. Patients with anisakiasis may be exposed to antigens from three different species of *Anisakis*. The first possible sources of antigens are somatic, cuticular, and all ES antibodies. This is because tissue perforation and larval degeneration lead to exposure to the entire antigen signature of the parasite. Next, ES antigens, which the parasite has completely discharged, are likely present after penetrating the gut tissue. Finally, cuticular and somatic antigens from dead larvae have been found in food, while ES antigens may be present in small amounts (Audicana & Kennedy, 2008).

Parasites have a unique ability to manipulate the immune system to their advantage, as their ES products are a crucial element in their interaction with their host (Tritten *et al.,* 2017). A study on *A. pegreffii* examined three types of proteins that play an antigenic role: A.peg-7 (armadillo-like helix), A.peg-1 (Kunitz serine protease inhibitor), and A.peg-13 (globin) (D'Amelio *et al.,* 2020). The study analysed the expression patterns of these proteins at regulated temperatures that mimic both ectothermic and homeothermic host states. ES products from *Anisakis* spp. are now used for diagnostic purposes and ongoing research suggests they may have medicinal potential for immune-related disorders (Mehrdana & Buchmann, 2017).

Characterisation of Allergens

According to Caraballo and Coronado (2018), *A. simplex* is the worm that causes the most allergies. The negative consequences are based on the fact that they elicit an allergic response in the host, not on the fact that they are parasites. A catalogue of proteins has been classified as allergens in the Allergome database (Mari *et al.,* 2009). Among them are proteinase inhibitors (Anis 1, Anis 4), somatic paramyosin (Anis 2), and tropomyosin (Anis 3), but there are also some unidentified proteins whose functions are unknown (Faeste *et al.,* 2014). Table 1 shows the characteristics of 14 antigens obtained from *A. simplex*.

Table 1: The 14 antigens in the Anisakis simplex that have been identified

Allergen	Molecular Weight (kDa)	Protein Function	Products Location	IgE Reactivity (%)
Anis 1	24	Animal Kunitz serine protease inhibitor	E/S major allergen	85
Anis 2	97	Paramyosin	S major allergen	88
Anis 3	41	Tropomyosin	S major allergen	13
Anis 4	9	Cystatin	E/S	27
Anis 5	15	SXP/RAL-2 family	E/S	25
Anis 6	7	Cysteine-rich trypsin inhibitor-like domain	E/S major allergen	18
Anis 7	139	Armadillo ARM-like	E/S major allergen	94
Anis 8	15	SXP/RAL-2 family	E/S	25
Anis 9	14	SXP/RAL-2 family	E/S	14
Anis 10	22	Unknown	S	39
Anis 11	55	Unknown	S	50
Anis 11-like	Unknown	Pepsin and heat resistant	S major allergen	Unknown
Anis 12	33	Unknown	Major allergen	57
Anis 13	36.7	Haemoglobin	E/S	64
Anis 14	23.5	ARM-like	Unknown	54

Note: E/S: Excretory-secretory, S: Somatic

Source: Nieuwenhuizen and Lopata (2014); Tang (2021)

Identification of Allergen Groups

Anis 1

Anis 1 is a well-known allergy associated with *Anisakis simplex* (Quiazon *et al.,* 2013). It is a significant allergen found in the sera of 80% of people allergic to *Anisakis* (Kobayashi *et al.,* 2008) and can be useful in diagnosing *A. simplex* allergies (Kobayashi *et al.,* 2008). Anis 1 exhibits two variants (D'Amelio *et al.,* 2020), one with a molecular weight of 24 kDa and the other with 21 kDa. It is a highly specific and sensitive IgE targeted for anisakiasis identification, with no cross-reactivity with other allergenic proteins due to its unique amino acid structure. Although no details about its biochemical function were found, Anis 1 shows nearly 91% similarity to troponin C in *Onchocerca volvulus* nematode but no similarity to any *A. simplex* serine protease inhibitors. Anis 1 was isolated and examined in the excretory glands of parasites, where Moneo *et al*. (2000) showed that trypsin and elastase are the only serine proteases that can inhibit chymotrypsin. Anis 1 is highly specific and sensitive for detecting anisakiasis, with a specificity of 90% and a sensitivity of 86%. The amino acid structure of Anis 1 may explain its lack of cross-reactivity with other allergenic proteins. Although no further details about its biochemical function were found due to its dissimilar amino acid sequence, Anis 1 does exhibit nearly 91% similarity to troponin C in *Onchocerca volvulus* nematode, but not to any *A. simplex* serine protease inhibitors (Moneo *et al.,* 2000; Aibinu, 2018).

Anis 2 (Paramyosin)

Anis 2, the main allergen of *A. simplex*, is a well-preserved protein (100 kDa) found in the muscle of crustaceans (D'Amelio *et al.,* 2020). Perez-Pérez *et al*. (2000) reported that Anis 2 is a paramyosin and a major allergen, sharing 89% amino acid sequence similarity with paramyosin from *A. simplex* and *O. volvulus*. Immunological cross-reactivity with paramyosin has been observed in a variety of arthropods, trematodes, and worms (Rodriguez-Mahillo *et al.,* 2007). Anis 2 contains many IgE binding sites (88%) and is highly immunogenic in parasite infections caused by Schistosoma, Taenia, Onchocerca, and Dirofilaria. The *A. simplex* genome includes two genes that encode for paramyosin, and it is possible that the released Anis 2 structure modulates the host immune response, as seen in Taenia solium (D'Amelio *et al.,* 2020).

Anis 3 (Tropomyosin)

Anis 3 is considered a minor allergen in anisakiasis as it is only present in individuals who have developed specialised IgE antibodies against *A. simplex* allergens due to cross-reactivity with proteins from other sources such as insects, pollens, or house dust mites (Rodriguez-Mahillo *et al.,* 2007).

Anis 4 (Cysteine Protease Inhibitor)

Anis 4, a minor allergen found in *A. simplex*, has been found to be resistant to high temperatures and pepsin digestion. Individuals who exhibit anaphylactic responses to *A. simplex* can often identify Anis 4. Research shows that anaphylactic symptoms are more commonly observed in those who evaluate positive for this allergen, with approximately 27% of sensitised individuals recognising it. Anis 4 is the most frequently identified parasite protease inhibitor that causes allergies and recent studies have demonstrated its resistance to autoclaving and pepsin digestion. Consequently, Anis 4 may have therapeutic potential for treating individuals who are sensitised to this allergen and re-exposed to *A. simplex* allergens after consuming seafood contaminated with treated parasites (Ventura *et al.,* 2008).

Anis 5, Anis 8 and Anis 9 (SXP/RAL-2 Family)

The SXP/RAL-2 protein family, exclusive to worms, has been identified as playing a crucial role in nematode parasitic diseases. This family consists of the *Anisakis* allergic members Anis 5, Anis 9, and Anis 8. A human serum that had been sonosensitizer to Anis 5 did not recognise Anis 9, which overlaps 34% of its sequence with Anis 5. According to this preliminary finding, Anis 5 and Anis 9 do not communicate. The sequence similarity with Anis 8 is 35%, despite the absence of any evidence of cross-reactivity between Anis 8 and Anis 9. It seems that nematode parasitic illnesses depend on the SXP/RAL-2 proteins (Rodriguez-Perez *et al.,* 2008). The thermally stable protein found in excretory/secretory products, the Anis 9 allergens (SXP/RAL-2 family protein and a troponin C-like protein; 14 kDa) and the As-14 allergen from *Ascaris suum* share 60% of the amino acid sequence.

Fifty percent (50%) of those infected can identify the Anis 9 allergen, a weak allergen (Rodriguez-Perez *et al.,* 2008). Immunohistochemical localisation of the parasite's ventriculus, excretory gland, and mucous surface of the intestinal epithelium all used Anis 5, which was also found among the ES product produced by larvae (Caballero *et al.,* 2008). This group contains the heat-stable 15-kDa allergen Anis 8, which shares two amino acid patterns with Anis 5, as well as other SXP/RAL-2 protein family members (Kobayashi *et al.,* 2007). The SXP/RAL2-protein was inferred as the source of a 14 kDa protein band with a strong affinity for Spanish plasma (Anis 8). Anis 8 and other excretory/secretory (ES) proteins are regulated during host infection. S10-S13 had the greatest levels of binding to this allergen in their serum when they had anisakiasis (Faeste *et al.,* 2014).

Anis 6 (Serine Protease Inhibitor)

One of the unimportant (minor) allergens in *A. simplex,* Anis 6 (cysteine-rich serine protease inhibitor), with a molecular weight of 7 kDa, was found in 50% of hypersensitive people (Aibinu,

2018). Anis 6 is the first protease inhibitor that research has identified as an allergen in worms. There are many similarities between Anis 6 and serine protease inhibitors from various animals (Kobayashi *et al.,* 2007). A serine protease inhibitor with 84 amino acid residues was discovered by D'Amelio *et al*. (2020) to block alfa-chymotrypsin rather than trypsin.

Anisakis simplex Allergen Proteins with Repeated Sequences

Anis 7

Anis 7 (139 kDa), one of the allergens in *A. simplex* with repetitive sequences, is known as a significant excretory/secretory (ES) and has a high diagnostic value because the majority of infected people recognises it. This molecule's allergenicity is attributed to the presence of the distinct CX17-25CX9-22CX8CX6 base pairs sequence which was not found in any previously characterised proteins (Anadón *et al.,* 2009). According to D'Amelio *et al*. (2020), the parasitic larval status of Anis 7 determines its allergenic potential and rats' immune systems will only respond to it if the larvae are still alive during the acute phase of infection. Additionally, unlike *Anisakis* 1, people who had acute Anisakis infections (gastroallergic anisakiasis) or *Anisakis* sensitisation brought on by chronic urticaria consistently had high IgE antibody levels to *Anisakis* 7 (Ubeira, 2014).

Anis 12

Anis 12, the third main allergen, is structurally distinct from the other two and is an *A. simplex* allergen with five tandem copies of the CX17-25CX9-22CX8CX6 sequence, compared to Anis 7's 19 repeats. The most varied nucleotide and amino acid sequences were found in Anis 12. (Quiazon *et al.,* 2013). Compared to Anis 11 and Anis 11-like proteins, Anis 12 has a longer tandem repeat structure (cysteine C and other residues X: CX13-25CX9CX7-8CX6, comprising 40–52 amino acid residues).

Anis 14

The sixth most frequent allergen in *A. simplex* is Anis 14. However, compared to Anis 1, Anis 2, Anis 7, and Anis 13, which are recognised by 80% or more of patients, Anis 14 and Anis 12 exhibit a lower incidence of reactivity with patients' sera. Our team identified Anis 14 allergens as a new significant allergen in *A. simplex* using the chemiluminescent immunoscreening method. Recombinant Anis 14 (rAnis 14) allergy testing may be used to identify people who are allergic to *A. simplex* because it has been shown to be IgE reactive. Even though the Anis 14 amino acid sequence is partially homologous with Anis 7 and Anis 12, it is structurally different and does not link to any identified protein families (Kobayashi *et al.,* 2015).

Anis 10

The expression, a collection of replicated DNA sequences that were complementary to an organism's extracted mRNA (cDNA library) obtained from L3 *A. simplex* larvae, was immunoscreened in order to find Anis 10. The protein with 212 amino acids was found as a 22-kDa protein band in an ethanol-fractionated parasite preparation. Anis 10 are distinct from all other known proteins and consists of seven virtually identical 29-amino-acid repeats in their sequence (Caballero *et al.,* 2011). The fact that this allergen is a heat-stable protein means that the usual methods of cooking fish might not be adequate to stop an allergic response. According to Caballero *et al*. (2011), allergy reactions to well-cooked or canned fish have been related to heat-stable allergens.

Anis 11 and Anis 11-like Allergens

According to Kobayashi *et al*. (2011), the Anis 11-like allergen was reported as an *Anisakis* spp. allergy because it shares the same main formation as Anis 11 allergens. These proteins share 78% of their sequences despite having noticeably different chain lengths. Anis 11 has 307 amino acids and a protein that is similar to it has 160 amino acids. Based on the similarity of their shorter sequences of 6–15 amino acid residues, they were divided into six categories (Kobayashi *et al.,* 2011). With a 43% sequence similarity to the Anis 11-like protein, Anis 10

shares this structural feature as well. Anis 11 and similar allergies to Anis 11 are proteins with an unknown biological purpose (Carballeda-Sangiao *et al.,* 2016).

Anis 13 (Haemoglobin)

Anisakis pegreffii stage 3 larvae were the first to find the haemoglobin molecule (Nieuwenhuizen & Lopata, 2013). There is no IgG cross-reactivity with Ascaris haemoglobin, making *Anisakis* spp. haemoglobin (Anis 13) is the most significant allergen in Anisakis patients. Additionally, Anis 13 was found by an antigen-capture ELISA with high detection rates in 64.3% to 80.9% of people with gastro-allergic anisakiasis. Gonzalez-Fernandez *et al*. (2017). Its robust expression and connection to the excretory-secretory ducts were found (Aibinu, 2018). Five epitopes in this protein were found, with epitopes 2 and 5 being the most crucial for IgE binding and affinity (Gonzalez-Fernandez *et al.,* 2015).

Materials and Methods

For this study on the characterisation of peer-reviewed scientific journals and the profiling of *Anisakis* spp. and its differential allergens, electronic databases including SpringerLink, ScienceDirect.com, and PubMed were used. For this review, only English-language pieces and full-version publications will be considered. The magazine articles used ranged in date from 2004 to 2021. Keywords like 'allergen', '*Anisakis* spp.', 'anisakiasis disease', 'characterisation', 'proteome profiling', and 'protein' were used to explore the database. The reference manager programme used in this study was Zotero.

Results and Discussion

Anisakidosis is a parasitic zoonotic disease brought on by members of the Anisakidae family; *Anisakis*, Pseudoterranova, Hysterothylacium, and Contracaecum (Albinu *et al.,* 2019). Humans can contract a variety of zoonotic diseases by eating foods contaminated with parasites. Nematodes, which are present in a variety of marine animals, are among the parasites that cause zoonoses that are spread indirectly. Nematodes are particularly important in this context for populations used to consuming marine goods (Mattiucci *et al.,* 2006; Audicana & Kennedy, 2008; Ferrantelli *et al.,* 2015; Cavallero *et al.,* 2015; Carballeda-Sangiao *et al.,* 2016; Rahmati *et al.,* 2020; Polimeno *et al.,* 2021). Consumption of seafood, particularly fish, contaminated with the infectious stage (third-stage larvae [L3]) of this parasite results in human as an allergy termed 'gastroallergic anisakiasis' (Audicana & Kennedy, 2008). The diseases brought on by members of the genus *Anisakis, A. simplex and A. pegreffii*, and these two members are increasingly being blamed for gastrointestinal illnesses and allergic responses (Albinu *et al.,* 2019). On the other hand, Anisakiasis triggered by the third-stage larvae of nematodes, *Anisakis* (Nieuwenhuizen & Lopata, 2013; Albinu *et al.,* 2019) can resemble other diseases or present as a sickness with few symptoms like abdominal pain, nausea, and vomiting within hours of ingesting the larvae (Lee *et al.,* 2009; Takabayashi *et al.,* 2014; Shimamura *et al.,* 2016) or even by exposure to the parasite, dead, or alive (Audicana & Kennedy, 2008). *Anisakis* larvae may cause an inflammatory mass to form in the small intestine and this manifestation may result in signs and symptoms similar to those of Crohn's disease (McConnaughey, 2007; McConnaughey, 2014). The human immune system starts a complex chain of events in response to the infection. The creation of particular antibodies known as Immunoglobulin E (IgE) is a vital component of this response.

The WHO/IUIS nomenclature committee recognised *A. simplex* as the parasite with the greatest number of known allergies (Fitzsimmons *et al.,* 2014a; Fitzsimmons *et al.,* 2014b; Albinu *et al.,* 2019) and it has been suggested that more allergens for these parasite nematodes are yet to be discovered. *A. simplex* main allergens are among the food allergens that have been found to be both heat-tolerant and trypsin/pepsin tolerant. Notably, several number of research (Caballero & Moneo, 2004; Moneo *et al.,* 2005; Vidaek *et al.,* 2009; 2011; Rodrguez- Mahillo *et al.,* 2010; Faete *et al.,* 2015) have found that at least one main allergen of *A. simplex* exhibits significant resistance to freezing, heating and digestion.

Anis 1 is a well-known allergy associated with *A. simplex* (Quiazon *et al.,* 2013). It is a significant allergen found in the sera of 80% of people allergic to *Anisakis* (Kobayashi *et al.,* 2008) and can be useful in diagnosing *A. simplex* allergies (Kobayashi *et al.,* 2008). Anis 1 exhibits two variants (D'Amelio *et al.,* 2020), one with a molecular weight of 24 kDa and the other with 21 kDa.

Fourteen allergens have been identified in both the excretory/secretory (ES) and somatic components of the parasite. Among individuals with anisakiasis, the most common antibodies are directed against Anis 1 (24 kDa), Anis 5 (15 kDa), Anis 7 (139 kDa), and Anis 9 (14 kDa). Some of these allergens, including Anis 4, Anis 1, Anis 5, Anis 9, Anis 8, Anis 11, and Anis 10, have been shown to be heat-stable, which can pose a problem even if contaminated seafood is cooked. Two pan allergens, Anis 2 (paramyosin) and Anis 3 (tropomyosin), are commonly used as targets for immunoassays, but their poor specificity can limit their usefulness. On the other hand, Anis 7 is the only one all patients with *A. simplex* infection recognize; it is now the most significant excretory/secretory (ES) allergen (Anadón *et al.,* 2009).

These statements clarify the number of allergens identified and their presence in specific parasite components. Additionally, it gives more information about the specific allergens that are frequently targeted in people with anisakiasis, emphasising the gastrointestinal mucosa, where *Anisakis* larvae frequently enter and trigger an inflammatory reaction. This demonstrates how some of these allergens are heat stable and can endure the stomach and small intestine digestive processes. Finally, it adds further details regarding the limitations of two pan-allergen immunoassays and their use in them, providing insights into the diagnostic difficulties brought on by the dynamic interactions between these allergens and the immune system of the host.

Conclusions

Anis 1, Anis 7, and Anis 12 are the three most significant allergens, while Anis 4, Anis 5, Anis 6, Anis 8, Anis 9, Anis 10, and Anis 11 are the seven minor allergens that make it into other categories (Ubeira, 2014). In conclusion, high IgE reactions to somatic and excretory antigens induce *Anisakis* spp., hypersensitivity, which results in a range of clinical symptoms. *Anisakis'* zoonotic infestation appeared to promote the activation of mechanisms meant to release the parasite, triggering allergic reactions in some people.

When symptoms appear after consuming seafood, it is essential to take an *Anisakis* allergy into consideration because seafood proteins can cause allergic reactions even when there is no infection. Before consumption, seafood should be frozen or appropriately prepared to reduce the risk of unpleasant side effects (Nieuwenhuizen & Lopata, 2014). Further study, patient diagnosis, the development of treatments, and identification of the protein allergens in *Anisakis* spp. can all benefit significantly from this information. Our research seeks to encourage informed food choices and eventually improve public health by increasing awareness of this disease in the general population. Perhaps in the future, our government will collaborate with medical professionals to create a diagnosis tool that anyone can use to identify an allergic reaction like anisakiasis quickly. In addition, tools to detect *Anisakis* spp. in fish or marine products can be created, which would help to avoid the disease altogether.

With a focus on the parasite's rising frequency in hosts like fish and crustaceans, this review offers an updated understanding of *Anisakis* as a foodborne pathogen. It explores allergen detection and complex immunological cross-reactivity with invertebrate proteins, which adds to the worrisome public health implications of this parasite.

In conclusion, the interaction between allergen characteristics produced by *Anisakis* larvae and the IgE-mediated response of the

human immune system delicately shapes the development of inflammation inside the small intestine and subsequent allergic reactions. Understanding the intricacies of infections associated with Anisakis and their significant effects on human health require a thorough understanding of these complicated pathways.

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