Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on allergy to *Anisakis*

Section of Food Safety and Nutrition

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Abstract

Anisakiasis and allergy to *Anisakis* are a particularly significant health concern in countries such as Spain with a high level of fish. Allergy to *Anisakis* may produce immediate signs of allergic reaction (hypersensitivity) ranging from urticaria or angioedema to anaphylactic shock, and mixed symptoms with gastrointestinal and allergic clinical manifestations.

In recent years, progress has been made in the scientific knowledge in this area, in relation to the allergen potential of the dead parasite and the possible clinical appearance with respect to the intake of fish treated in accordance with recommended standards, implying a risk for allergic individuals. Consequently, the Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has been asked to review and assess the currently available information on allergy to *Anisakis*.

The scientific bibliography published up to the date of publication of this report, and consulted in order to write the report, does not show current clinical evidence to confirm that the dead parasite is a health concern for consumers who are allergic to *Anisakis*.

Nevertheless, the immunological recognition of different thermostable antigens, demonstrated even after applying the recommended methods of treatment, does not permit the total rejection of the possibility of risk of allergic reaction in those individuals who are sensitive to these.

Key words

Allergens, food allergy, *Anisakis, a*nisakiasis, parasitosis, fish, prevention.

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1. Introduction

Anisakiasis and allergy to *Anisakis* are a particularly significant health concern in countries such as Spain with a high level of fish consumption. Allergy to *Anisakis* may produce immediate signs of allergic reaction (hypersensitivity) ranging from urticaria or angioedema to anaphylactic shock, and mixed symptoms with gastrointestinal and allergic clinical manifestations. In 2005, the Scientific Committee issued a report on the factors favouring the appearance of allergy to *Anisakis* and the applicable prevention measures for guaranteeing that the parasite is dead prior to the consumption of fish (AECOSAN, 2005).

Since then progress has been made in the scientific knowledge in this area, in relation to dead parasite allergenicity and the possible clinical appearance with respect to the intake of fish treated in accordance with recommended standards, implying a risk for allergic individuals. Consequently, the Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) have been asked to review and assess the currently available information on allergy to *Anisakis*.

1.1 Background

In 2005 the Scientific Committee of the AECOSAN issued a report on the factors favouring the appearance of allergy to *Anisakis* and the applicable prevention measures (AECOSAN, 2005). In 2006, Royal Decree 1420/2006, of 1 December, was published on the prevention of parasitic infection by *Anisakis* in fish products supplied by establishments serving food to end consumers or communities (BOE, 2006). Subsequently, in another report from the Scientific Committee focusing on measures to reduce the risk associated with the presence of *Anisakis* (AECOSAN, 2007), recommendations were issued on the safest heat treatments for fish intended to be eaten cooked or in raw preparation.

The latter document offers recommendations intended for both the consumer and the catering sector regarding the heat treatment of fish intended to be eaten cooked and fish intended to be eaten raw. These recommendations, based on the acceptance of the non-allergenic nature of the dead larvae of the parasite are described below:

1. Regarding fish consumed cooked

Fully cooked products, that is, boiled and fried, are considered safe from the point of view of the inactivation of the parasite, as in these treatments temperatures of more than 90 ºC and 170 ºC respectively are reached.

Guidelines are offered to increase the safety of grilled fish, which include standards such as considering “well-cooked fish” when the “flesh comes away from the backbone easily” and has the “typical matt appearance of coagulated proteins”. With respect to other cooking methods, recommendations are offered for microwave cooking, advising that the fish be turned over and allowed to rest covered for 2 minutes to guarantee adequate heat conditions. If these conditions are not met, the fish should be previously frozen.

2. With respect to fish consumed raw

Freezing: Legislation establishes that fish products which are to be eaten raw or almost raw must be frozen at a temperature equal to or less than -20 ºC obtained throughout the product, not only on the surface, for a minimum of 24 hours or at -35 ºC for a minimum of 15 hours. Marinated and pickled fish products and salted fish should be frozen if the salt concentration does not reach a level of 9 % maintained over 6 weeks. This treatment is also mandatory for cooking processes in which the internal temperature of the fish does not exceed 60 ºC, such as cold-smoked products.

All these measures have been considered safe for the destruction of live larvae, which until now has been considered the only cause of symptoms in individuals that are allergic to *Anisakis*. Nevertheless, since the introduction of these measures, allergic reactions and new advances in the immunological recognition of *Anisakis* antigens, even when dead, have been reported. Therefore, we need to ask whether these recommendations are adequate for guaranteeing consumer safety.

2. Developments in the knowledge of the antigenicity of *Anisakis*

At present, as a part of the standard clinical practice, it is accepted that active infection by living larvae is necessary to trigger an allergic reaction. Therefore, it is assumed that the heat treatments indicated in accordance with the guidelines provided by the Scientific Committee of the AECOSAN (2007) and the European recommendations (EU, 2004) (EFSA, 2010) are safe. This assumption is based on and confirmed by the experimental studies of the authors which have confirmed that sensitized patients with a prior medical history, and on whom a controlled exposure test was conducted, are resistant to the intake of parasites which have been previously frozen (Alonso et al., 1999) (López Serrano et al., 2000) (Alonso-Gómez et al., 2004), lyophilised dead larvae (Sastre et al., 2000) or excretory-secretory antigen (Baeza et al., 2004). It should be noted that a subsequent clinical follow-up of the patients was conducted over long periods of months or years. During these periods, the patients continued with their diet, containing fish treated in accordance with the standard recommendations for avoiding the risk, and no symptoms were recorded.

These findings support the hypothesis that only the living parasite is able to cause the symptoms, or in other words, that the allergens of *Anisakis simplex* only interact with the immune system of the host when the parasite inoculates them into its tissues (Alonso-Gómez et al., 2004), and therefore the intake of viable larvae is necessary to release an allergic reaction. Similarly, in experimental studies on rats inoculated with living larvae and dead larvae of *Anisakis*, an increase in reactivity, measured by the immunological parameters IgM and IgE, was only observed in the animals inoculated with live larvae (Abe and Teramoto, 2014).

Nevertheless, other authors have described that some individuals refer to having experienced symptoms with food which has been frozen, canned or cooked in accordance with the officially established regulations (Alonso et al., 1997) (Audícana, 2001) (Audícana et al., 2002) (Falcão et al., 2002) (Caballero y Moneo, 2004) (Moneo et al., 2005) (Armentia et al., 2006) (Moneo et al., 2007). If this is the case, some thermostable antigens from dead larvae, or allergens resistant to

digestion by gastric proteases, may be able to cause allergic reactions in previously sensitised individuals.

These discrepancies between the two hypotheses may be justified by a number of causes:

- Recognition by some of these patients of antigens resistant to heat treatment. It is possible that there will be different types of allergic patients who are able to recognise different antigens even from dead larvae, which would require the recommendation of different dietary measures, according to the characteristics of the proteins to which they are sensitised.
- The permanence of active larvae even when the recommended measures are followed.
- Low level of adherence to the recommendations.

2.1 High resistance antigens

To date, up to 28 allergens have been characterised for *Anisakis simplex* (Arcos et al., 2014). Some of these allergens have a high resistance to different temperature ranges, and degradation by proteases, indicating that infection with living larvae may not be essential for causing the symptoms (Solas et al., 2008). From the recognition data for these antigens in patients' serum, it can be extrapolated that the recognition may justify the symptoms that occur in some cases.

In addition, exposure to certain allergens of *Anisakis simplex* may also occur as a result of the consumption of certain parts of the fish which contained secretory antigens but in which the intact larvae were no longer present, either because they had been eliminated, or because parts of the fish not usually parasitized, rejecting the most affected, were consumed (Audicana and Kennedy, 2008).

The majority of the studies published recently focus on the description and characterisation of heat-resistant allergens, using serum from sensitised patients (Caballero y Moneo, 2004) (Caballero et al., 2008). Most of these studies were *in vitro* in which serum was included from patients who refer, in up to 20 % of the cases in some series, to having experienced symptoms with fish processed in accordance with the recommendations for avoiding the risk (Moneo et al., 2007). Up to 30 % of the patients recognised *in vitro* allergens following heat treatment of the product. These resistant allergens may explain the appearance of symptoms following the intake of cooked or canned fish.

In addition, the capacity of thermostable antigens to induce a cellular response similar to that of antigens that have not been heat-treated has been described (González-Muñoz et al., 2010). In another study of the same group, accepting "a priori" the allergenic nature of the thermostable proteins, a highly sensitive technique has been developed to quantitatively assess (up to 1 ppm of Ani s4) the presence of antigens recognised by patients' serum, in fish treated by freezing (-20 ºC for 11 months) and in cooked fish (Rodríguez-Mahillo et al., 2010).

Other groups of researchers have identified some of the thermostable allergens of *Anisakis*, which may be of interest for use as recombinant allergens to improve diagnostic specificity, in sensitised patients. The Ani s5 (Caballero et al., 2011), Ani s7, Ani s9 (Kobayashi et al., 2007) (Rodríguez et al., 2008) (Rodríguez-Pérez et al., 2008), Ani s10 (Caballero et al., 2011), Ani s7

(Cuellar et al., 2012) (Fæste et al., 2014), Ani s13 (González-Fernández et al., 2015) and Ani s11 (Carballeda-Sangiao et al., 2016) are thus described.

In addition, the presence of allergens after subjecting the fish to conditions similar to those corresponding to canned conservation, at different temperatures and for different time periods, from 90 ºC for 30 minutes, to 115 ºC for 90 minutes has been determined. A decrease in the recognition of Ani s4 was obtained but residual antigenicity was confirmed to persist (Tejada et al., 2015).

In another publication along the same line, after heating by autoclave at 121 ºC for 20, 40, and 80 minutes (Carballeda-Sangiao et al., 2014), it was observed that the basophile-activating capacity was conserved although the number and intensity of the bands detected by immunoblotting according to the heating time was reduced, especially in the 80 minute period. Therefore, the authors concluded that certain antigens of *Anisakis simplex* maintain their capacity to join IgE and to activate basophiles after being subjected to methods equivalent to those used in industrial conservation and canning, and may represent a risk for sensitised patients.

A different aspect looked at in other publications would be the capacity of dead larvae to produce an immunological response in sensitised subjects, assessed in this case, with prick-test and patch skin testing (Ventura et al., 2008). Using dead, cooked or frozen larvae as antigens for the performance of the test, 70 % of the patients produced a positive result in the prick test, suggesting that dead larvae may evoke an immune response. Along the same line, epicutaneous patch testing revealed that 37.5 % and 12.5 % of the patients recognised frozen and cooked larvae respectively.

All these studies address the allergenic nature, with recognition of thermostable proteins from the treated fish (Moneo et al., 2005) (Fæste et al., 2014) and only measured as a detection of allergens mainly *in vitro*, without this necessarily implying a clinical correlation.

2.2 Vitality of larvae after the application of different heat treatments to the food

Some studies have focussed on the study and confirmation of whether the current recommendations may be considered safe because they guarantee the death of the parasite.

Partial viability of larvae after freezing has been observed (Adams et al., 2005). While all the larvae died when the fish was subjected to 96 hours at -15 ºC, 60 hours at -20 ºC, 12 hours at -30 ºC and 9 hours at -40 ºC, it was observed that after 48 hours at -20 ºC, up to 30 % of the larvae survived, where the thickness of the fish was a more decisive factor than the time for which it was kept at the freezing temperature.

Using electronic microscope and fluorescence techniques, the effect of some heat treatments on the structure of the larvae was examined (Tejada et al., 2006). Fillets of artificially parasitized fish with a dose of 40-60 larvae of *Anisakis simplex* were subjected to freezing treatments (at -20 ºC for 44 h), heating by immersion in water (at 95 ºC for 8 minutes) and cooking (3 minutes in microwave at 900 W). Significant changes were observed in the larvae cuticles in the cooked samples and in some of the frozen samples. However, in these last samples parasites were also identified that

had not undergone any significant structural change (Tejada et al., 2006). Additional studies are required to determine whether the structural changes observed in the larvae cuticles may modify the resistance of the parasites to the action of the gastric enzymes. It was demonstrated in this study that after heating at 60 ºC for 10 minutes, some larvae showed spontaneous movements, which were not observed when the temperatures were maintained at 70 ºC or above for 1 minute or more (Vidaček et al., 2010).

Microwave inactivation studies of the larvae (Vidaček et al., 2011) have confirmed that treatments at 70 ºC in microwaves inactive the larvae more quickly than traditional cooking processes, provided that the microwaves are guaranteed to completely penetrate the fish. Nevertheless, in this study the presence of thermostable allergens was detected in the muscular tissue surrounding the larvae, suggesting that the dead larvae may release allergens into surrounding tissue, which would suppose a risk for patients sensitised to heat-resistant allergens.

2.3 Low level of adherence to the recommendations

Another possibility to be assessed is that, in everyday life, there is inadequate processing either in terms of time or temperature, or that the interpretation of the recommendations based on subjective observation is lax ("change of colour", "comes away from the backbone", etc.), and therefore insufficiently precise, with the result that patient consumes viable larvae and experiences symptoms.

3. Discussion

Without doubt, the safest alternative for the patient who has experienced symptoms of allergy to *Anisakis* is a fish-free diet.

However, the type of dietary restrictions indicated for individuals allergic to *Anisakis*, is an aspect with a significant impact on the daily life of the patient and his/her environment. A fish-free diet, or excessively restrictive recommendations for its preparation, may be difficult to observe in populations with strongly-rooted culinary traditions which include both the frequent consumption of fish and methods of preparation using heat treatment which is inadequate for controlling the appearance of allergic symptoms. Consequently, recommendations have been adopted which are considered adequate for ensuring the death and consequent harmlessness of the parasite (EU, 2004) (AECOSAN, 2005, 2007) but which permit the consumption of fish in conditions which remain organoleptically acceptable for the consumer.

At present, while the possible exposure to the parasite increases due to the increase in the intake of fish for dietary and nutritional reasons, and due to a change in culinary habits, the immunology of the infestation and the allergic sensitisation to *Anisakis* are the subject of further studies.

At present, the *in vitro* information, description and characterisation of high resistance antigens is more developed than the clinical studies, with tests of exposure controlled through consumption that correspond to the same thermostable situation. The clinical evidence that exposure to thermostable antigens causes symptoms has not yet been demonstrated experimentally in

patients. Information about this problem can only be increased with the performance of more clinical studies that include controlled exposure tests with thermostable allergens, on those patients who have referred symptoms with adequately processed fish considered free of live larvae. That is, to date, sensitisations to multiple allergens have been described (immunological recognition of the allergen) but this does not necessarily imply that there is a clinical allergy. This difference, which has been clearly established, and is applicable to both inhalants, and food, or to any potential allergen, including sensitisation without symptoms and allergy, is the basis of the precise clinical diagnosis in any allergic disorder.

Moreover, certain recent approaches (Daschner et al., 2012) look at the debate from a different point of view, highlighting the fact that the allergic pathology caused by *Anisakis* does not follow the same pattern as that equivalent to a food allergy. Therefore, in a classic allergy to foods, the patient always shows symptoms after eating the food, with an intensity that varies depending on the degree of sensitisation and the amount consumed.

In the case of parasitation by *Anisakis*, exposure to the allergen depends on whether or not the fish consumed is parasitised. In the latter case, the intake of an unparasitised fish, even with certain relaxation of the regulations, would not cause symptoms, and therefore gives the patient a false feeling of security which may reduce the degree of adherence to the recommendations. The intake of parasitised fish, cooked in the same conditions as the previously tolerated fish, that is, with living larvae, will however result in the appearance of symptoms.

Along these lines, some previously recommended methods (AECOSAN, 2007) may be subjective, with a margin of interpretation, with recommendations such as "flesh comes away from the backbone easily" or "matt appearance typical of coagulated proteins" as regards "well-cooked fish" in relation to grilling or microwave cooking methods.

Conclusions of the Scientific Committee

The scientific bibliography published up to the date of publication of this report, and consulted in order to write the report, does not show current clinical evidence to confirm that the dead parasite is a health concern for consumers who are allergic to *Anisakis*.

Nevertheless, the immunological recognition of different thermostable antigens, demonstrated even after applying the recommended methods of treatment, does not permit the total rejection of the possibility of risk of allergic reaction in those individuals who are sensitive to these.

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