

Quantification of *Anisakis simplex* Allergens in Fresh, Long-Term Frozen, and Cooked Fish Muscle

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Abstract

Fish-borne parasitic zoonoses such as Anisakiasis were once limited to people living in countries where raw or undercooked fish is traditionally consumed. Nowadays, several factors, such as the growing international markets, the improved transportation systems, the population movements, and the expansion of ethnic ways of cooking in developed countries, have increased the population exposed to these parasites. Improved diagnosis technology and a better knowledge of the symptoms by clinicians have increased the Anisakiasis cases worldwide. Dietary recommendations to *Anisakis*-sensitized patients include the consumption of frozen or well-cooked fish, but these probably do not defend sensitized patients from allergen exposure. The aim of our work was to develop a sensitive and specific method to detect and quantify *Anisakis simplex* allergens in fish muscle and its derivatives. Protein extraction was made in saline buffer followed by preparation under acid conditions. *A. simplex* antigens were detected by IgG immunoblot and quantified by dot blot. The allergenic properties of the extracts were assessed by IgE immunoblotting and basophil activation test. We were able to detect less than 1 ppm of *A. simplex* antigens, among them the allergen Ani s 4, in fish muscle with no cross-reactions and with a recovery rate of 82.5%. *A. simplex* antigens were detected in hakes and anchovies but not in sardines, red mullets, or shellfish. We detected *A. simplex* allergens in cooked hakes and also in hake stock. We proved that *A. simplex* allergens are preserved in long-term frozen storage ($-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 11 months) of parasitized hakes. Basophil activation tests have proven the capability of the *A. simplex*-positive fish extracts to induce allergic symptoms.

Introduction

THE NEMATODE *ANISAKIS SIMPLEX*, a parasite of fish and marine mammals, can cause gastrointestinal and allergic reactions in humans when larvae in the third developmental stage are eaten with infested fish and seafood. The main symptoms associated with the *A. simplex* allergy include urticaria, angioedema, vomiting, dyspnea, asthma, conjunctivitis, and even anaphylactic shock.

Food is the cause of 33%–50% of the anaphylactic episodes in the United States, Europe, Asia, and Australia (Brown *et al.*, 2001; Sampson, 2003; Smith *et al.*, 2005). Many of the patients suffering from food anaphylaxis declare that they were aware of their allergies before the life-threatening episodes, although they did not know that they were eating the cause of the allergy, as it was not declared on the food label (Bock *et al.*, 2001; Clark *et al.*, 2004; Uguz *et al.*, 2005). These substances, present in foods in an insufficient proportion to be declared on the label but enough to elicit allergic reactions, are considered hidden allergens. In this way, *A. simplex* can be considered an important hidden allergen of fish and seafood, as it is the

cause of 45.3% of the allergic food reactions and 58% of the anaphylactic reactions caused by hidden allergens in an adult population of Madrid (Spain) (Añibarro *et al.*, 2007). Each year, 50 new cases of anisakiasis are reported in the United States, 500 in Europe, and 2000 in Japan (Audicana and Kennedy, 2008).

Authors have debated largely whether parasitization of the patients' gastrointestinal tract by live larvae is necessary to develop allergic symptoms (Daschner *et al.*, 2000; Audicana *et al.*, 2002). Although an agreement has not been reached, there are a few reports about patients suffering allergic symptoms related to the consumption of canned or properly cooked fish, where the larvae are dead and, therefore, unable to parasitize the gastric tract (Audicana *et al.*, 2002; Santolalla, 2006; Moneo *et al.*, 2007; Ventura *et al.*, 2008). This is not surprising, due to the heat and pepsin resistance of some *A. simplex* allergens (Caballero and Moneo, 2004). To date, it has been impossible to reproduce any allergic symptom in provocation tests, using either frozen or lyophilized larvae, or larvae protein extracts (Alonso-Gómez *et al.*, 2004; Baeza *et al.*, 2004; Sastre *et al.*, 2000), but an Italian group obtained positive

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results in prick-by-prick and patch tests using live, cooked, and frozen *A. simplex* larvae (Ventura *et al.*, 2008).

To assess if fish is parasitized by anisakid larvae, current methods include visual inspection under white or ultraviolet light. This method is time consuming and not sensitive if the worms are located deep within the fish fillets (Stormo *et al.*, 2007). An enzymatic degradation of the fillets is a more effective method (Levsen *et al.*, 2005), but it destroys the fish. To our knowledge, some molecular methods, such as the polymerase chain reaction, have only been used to identify the anisakid species (Farjallah *et al.*, 2008); as far as we know, there has been one attempt to measure *A. simplex* allergens by immunological methods, but only in *A. simplex* extracts and commercial fish extracts, to standardize these reagents used in the allergy diagnosis (Arilla *et al.*, 2008).

The objective of our work was to develop a sensitive method to extract and measure *A. simplex* proteins directly from fresh or processed fish muscle and fish derivatives, and to characterize their allergenicity.

Materials and Methods

Fish sample preparation

Fresh hakes (*Merluccius merluccius*), anchovies (*Engraulis encrasicolus*), sardines (*Sardina pilchardus*), red mullets (*Mullus barbatus*), blue whittings (*Micromesistius potassou*), clams (*Tapes decussatus*), shrimps (*Parapanaeus longirostris*), and cockles (*Cerastoderma edule*) were obtained in local markets.

To study the effects of cooking on the *A. simplex* allergenicity, four infested hake belly flaps were divided into two parts. One of them was immediately processed to extract the possible *A. simplex* allergens, while the other was boiled for 30 minutes in distilled water (1:1, w:v) before being processed. The water in which the fish was boiled (fish stock) was also studied.

To study the effects of long-term frozen storage in *A. simplex* antigenicity and allergenicity, 34 hakes were caught by trawling from the fishing grounds of the Little Sole Bank in the Bay of Biscay during the 310 research cruise of the German research vessel Walther Herwig III in March 2008. Skin off fillets (dorsal and belly flaps) were vacuum packed, frozen onboard in a blast freezer at -20°C , and stored until analyzed at $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Guts from each hake were digested for ≥ 4 hours at 37°C in Solution A (0.5% [w/v] pepsin, 0.063 M HCl, and 200g of gut/L of solution A), the residue was strained and washed in 0.9% NaCl and *A. simplex* larvae were counted. Hakes were classified according to the viscerae parasitization rate: the count less than 300 *A. simplex* larvae was considered low parasitization, between 300 and 900 larvae was considered medium parasitization, and more than 900 larvae was considered high parasitization. The belly flaps from 12 hakes (3 low parasitized, 4 medium parasitized, and 5 highly parasitized) were thawed and studied 2 weeks after capture; 11 (3 low, 4 medium, and 4 highly parasitized) were thawed and studied 4 months after capture; the other 11 (3 low, 4 medium, and 4 highly parasitized) were thawed and studied 11 months after capture.

A. simplex extracts

Live *A. simplex* larvae were obtained manually from blue whiting viscera, washed twice, and stored at -20°C . Five grams of larvae were ground in a mortar, diluted in 5 mL of

phosphate-buffered saline, and sonicated for 5 seconds. Extracts were incubated at room temperature for 30 minutes before centrifuging them for 10 minutes at 16000 g to obtain a crude extract, whose protein content was measured using Protein Quantification Kit-Rapid (Fluka; Sigma-Aldrich Inc, St. Louis, MO).

Patients' sera

Patients were considered *A. simplex* sensitized if allergic symptoms elicited within 12 hours after the ingestion of fish and skin prick test and serological test (Immuno-CAP; Phadia, Uppsala, Sweden) were positive for *A. simplex* and specific IgE against fish was not detectable. A pool of six sera from patients with anti-*A. simplex* specific IgE > 17.5 kUA/L (class 4 or higher) were pooled for revealing the IgE immunoblots.

A pool of six nonatopic sera was used as experiment control.

Proteins extraction method

Skin, viscera, and bones were taken out from fish before the protein extraction. Shell was also taken out from shellfish before processing it.

Fish fillets (10 g) were minced 1:3 (w:v) in a TBS solution using an Ultra-Turrax T25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). The resulting mixture was sonicated in a Microson ultrasonic cell disruptor XL (Misonix, Farmingdale, NY) and centrifuged (6000 g, 20°C , 30 minutes). The pellet was discarded and the supernatant was acidified to pH < 1 by adding 0.075 mM (final concentration) HCl, incubated for 15 minutes at room temperature, neutralized to pH 7 with NaOH, and centrifuged (16000 g, 30 minutes, 20°C). The pellet was discarded and the supernatant was analyzed.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and immunoblotting

Protein electrophoresis was performed on 16% Tris-tricine minigels according to standard procedures. After electrophoresis, proteins were transferred to nitrocellulose by overnight diffusion, and the membranes were revealed using polyclonal anti-*A. simplex* crude extract (AC) or anti-recombinant Ani s 4 (A4) rabbit antisera (to detect *A. simplex* antigens), or a pool of *A. simplex*-sensitized human sera (to detect *A. simplex* allergens), and the corresponding secondary antisera, according to previously published methods (Moneo *et al.*, 2000; Rodríguez-Mahillo *et al.*, 2007). Color developing with 5-bromo-4-chloro-3-indolyl phosphate-nitro blue tetrazolium (BCIP-NBT) was performed for 30 minutes when using the polyclonal rabbit antisera and for 5 hours when using the pool of human sera.

Negative controls with a preimmune antiserum and a pool of nonatopic human sera were performed.

A result was considered positive if the band pattern of a fish sample was consistent with that obtained for an *Anisakis simplex* crude extract.

When needed, membranes were scanned and measured by densitometry using Quantity One software (Bio-Rad, Hercules, CA).

Quantification of *A. simplex* antigens in fish

Five microliters of the positive samples was dotted in duplicate to a nitrocellulose membrane and dried for 30 minutes

at 37°C. After blocking, membranes were subsequently incubated with polyclonal A4 rabbit antiserum (1/10000 dilution, 1 hour, room temperature) and alkaline-phosphatase-labeled goat anti-rabbit IgG (1/5000 dilution, 30 minutes, room temperature), and revealed for 30 minutes with BCIP-NBT.

A reference curve was made in duplicate, dotting 5, 2.5, 1.25, 0.62, 0.31, and 0 µg of an *A. simplex* crude extract.

Membranes were scanned and we used Quantity One software (Bio-Rad) to define the boundaries of protein dots, to assess pixel number and density, and to obtain final numerical data for quantifying.

Recovery rate and detection limit

Anisakis-free fish fillets (10 g) were injected with 20 µL of decreasing concentrations of *A. simplex* crude extract (200, 100, 50, 25, 0 µg/mL) and recombinant Ani s 4 (rAni s 4) (10, 5, 2.5, 1.25, 0 µg/mL) obtained as described (Rodriguez-Mahillo *et al.*, 2007). After an incubation of 10 minutes at room temperature, proteins were extracted and studied as described above. Assay controls were performed using a rabbit pre-immune serum and a mixture of six nonatopic patients' sera. These extractions were performed in triplicate.

Basophil activation test

Patients blood (100 µL) was incubated as described (Gonzalez-Munoz *et al.*, 2005), with 1 µg/mL of *A. simplex* crude and mature hake (fresh, boiled, or frozen), red mullet and juvenile hake (weight ≤ 150 g) extracts, and with hake stock. To analyze basophil activation, cells were stained with 20 µL CD63(IgG1)-FITC/CD123(IgG1)-PE/HLA-DR(IgG2a)-PerCP reagent mixture (BD Biosciences, Erembodegem, Belgium) for 20 minutes. Activation was determined by flow cytometry measuring the increase of the basophils CD63 expression when incubated with the fish extracts, using a FACSort Cytometer (BD Biosciences) equipped with an argon laser (488 nm). PMT voltages (V) were as follows: FL1 = 570, FL2 = 539, and FL3 = 680.

Nonsensitized patient blood was tested as assay control.

Statistics

Statistical analysis was performed using SPSS 13.0 software (SPSS, Inc., Chicago, IL). Variables are described as median and interquartile range. Pearson's correlation coefficient is given to measure the association between quantitative variables.

Results

Evaluation of the extraction–detection method

Blue whittings and anchovies with no *A. simplex* larvae were injected with decreasing concentrations of a mixture of *A. simplex* crude extract and rAni s 4. After the protein extraction, the intensity of the blots revealed with the AC and A4 antisera correlated for each sample ($r = 0.889$, $p < 0.001$). We recovered 82.5% (interquartile range 59.25%–100%) of the *A. simplex* crude extract injected in fish.

When testing blue whittings, we could detect 200 ng *A. simplex* crude extract/g of fish and 2.5 ng of Ani s 4/g of fish. When testing anchovies, we could detect 625 ng *A. simplex* crude extract/g of fish and 5 ng of Ani s 4/g of fish.

A mixture of *A. simplex*-sensitized patients' sera did not detect any band even when the highest concentration of *A. simplex* extract (0.4 µg/g of fish) was injected.

Negative controls using preimmune antisera and a pool of nonatopic human sera gave completely negative immunoblots (data not shown).

Detection of *A. simplex* antigens in different fish species

Several fish and shellfish species were tested for *A. simplex* antigens and allergens detection (Table 1). Neither the rabbit antisera nor the pool of *A. simplex*-sensitized patients' sera were able to detect any band in immunoblot when clams, shrimps, or cockles were tested. No *A. simplex* antigens or allergens were detected in sardines or red mullets. We could detect *A. simplex* antigens and allergens in mature hakes (>1500 g) but not in juvenile (<150 g) hakes. We could also find *A. simplex* antigens in anchovies.

When *A. simplex* antigens were detected by immunoblot, using rabbit anti-AC antiserum (Fig. 1), the extracts from parasitized fish had several antigenic bands with estimated molecular weights ranging from 9 to more than 75 kDa. When using the A4 antiserum to reveal the immunoblot (Fig. 1), a band around 10 kDa identified as A4 was also detected.

When *A. simplex* allergens were detected by immunoblot, using a pool of sensitized patients' sera (Fig. 1), several allergenic proteins with molecular weights from 10 to more than 75 kDa were detected.

The intensity of the bands was proportional in all the immunoblots: the most intense extracts in the rabbit immunoblot revealed with the AC antiserum were also the most intense extracts in the rabbit immunoblot revealed with the A4 antiserum and the human immunoblot.

Quantification of *A. simplex* proteins in parasitized fish

When positive by immunoblot, the *A. simplex* antigens in fish samples were quantified by dotblot, spotting 5 µL of the fish extracts in a nitrocellulose membrane and comparing the intensity of the resulting dots with a reference curve made spotting decreasing concentrations of an *A. simplex* crude extract. The coefficient of variation between the intensity of the pair of dots proceeding from the same sample was <10%.

TABLE 1. ANISAKIS SIMPLEX PROTEINS DETECTION AND QUANTIFICATION IN DIFFERENT FISH AND SHELLFISH SPECIES

Fish/shellfish	Tested (n)	Positive (n)	µg <i>A. simplex</i> antigens/g of fish [M (IQR)]
Clam	12	0	NM
Shrimp	12	0	NM
Cockle	12	0	NM
Sardine	22	0	NM
Red mullet	10	0	NM
Mature hake	46	44	319.67 (183.54–622.00)
Juvenile hake	20	0	NM
Anchovie	52	10	462.92 (99.23–1752.25)

Fish and shellfish were obtained at local markets from Madrid (Spain). M, median; IQR, interquartile range; NM, not measured.

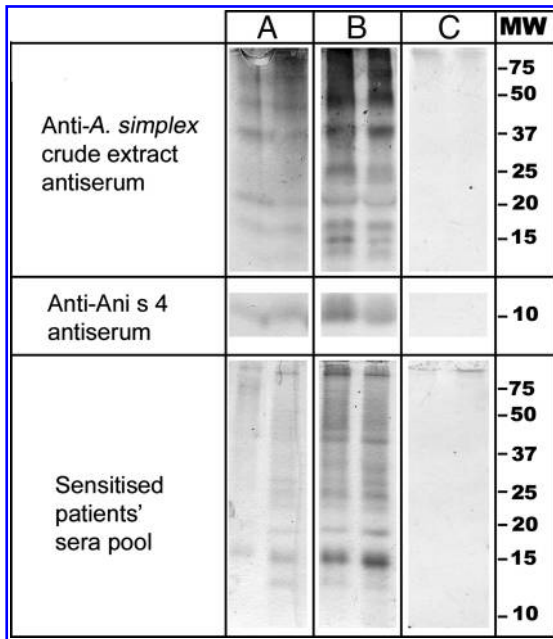


FIG. 1. Immunoblots of two anchovies (A), two adult (B), and two juvenile (C) hakes extracts revealed with polyclonal anti-*Anisakis simplex* crude extract, polyclonal anti-rAni s 4 antisera or a pool of sensitized patients' sera. Molecular weights (kDa) are indicated.

The *A. simplex* antigens content in positive hakes and anchovies are summarized in Table 1.

Effect of cooking on the *A. simplex* allergens

Boiling parasitized hakes did not destroy the antigenic recognition, but it altered the recognition pattern by the polyclonal antisera (Fig. 2). Some of the *A. simplex* antigens, Ani s 4 among them, were detected in the water used for boiling the hakes (hake stock). When measuring the intensity of the band corresponding to Ani s 4, boiling the fish led to a reduction of 54.03% in the Ani s 4 band's intensity in the immunoblot.

Study of the long-term frozen storage of fish in *A. simplex* proteins

Antigens and allergens were detected in the muscle from all the samples, even in the less parasitized group stored 11 months (Fig. 3).

The *A. simplex* larvae in guts for each group of hakes, expressed as median and interquartil range, was 22 and 22–154.5 for low parasitized hakes, 468.5 and 320–639.75 for medium parasitized hakes, and 1191 and 1092–1677 for high parasitized hakes. No relation was obtained between the parasitization rate in guts and the intensity of the band corresponding to Ani s 4 in fish muscle.

Basophil activation

The basophils from four *A. simplex*-sensitized patients (Table 2) were activated by *A. simplex* crude extract, by fresh, boiled, and frozen adult hake extracts, and by the hake stock, whereas the red mullet extracts were not able to activate them (<5% of activated basophils). The basophils of two

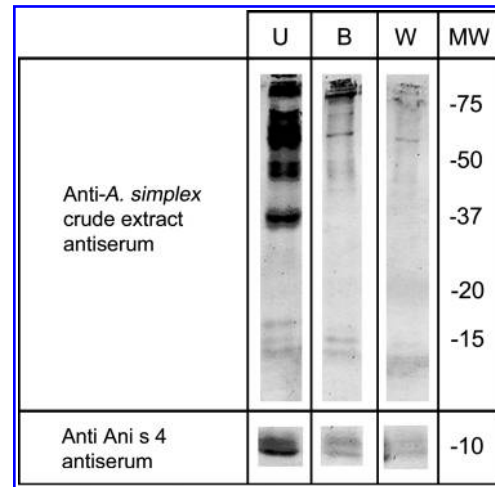


FIG. 2. Immunoblots revealed with an anti-*A. simplex* crude extract antiserum and an anti-rAni s 4 antiserum of untreated (lane U), and boiled (lane B) hake belly flaps. The water used for boiling was also tested (lane W). Molecular weights (kDa) are indicated.

nonsensitized patients were not activated by any of the extracts (<5% of activated basophils) (data not shown).

Discussion

In this article, we describe a new and simple method to extract and detect *A. simplex* antigens in fish muscle and its application in fresh, frozen, and cooked fish, and even in testing the water used to boil fish.

If we assume that the presence of *Anisakis simplex* antigens implies presence of the parasite in fish, the use of a polyclonal anti-*A. simplex* crude extract antiserum led us to discriminate

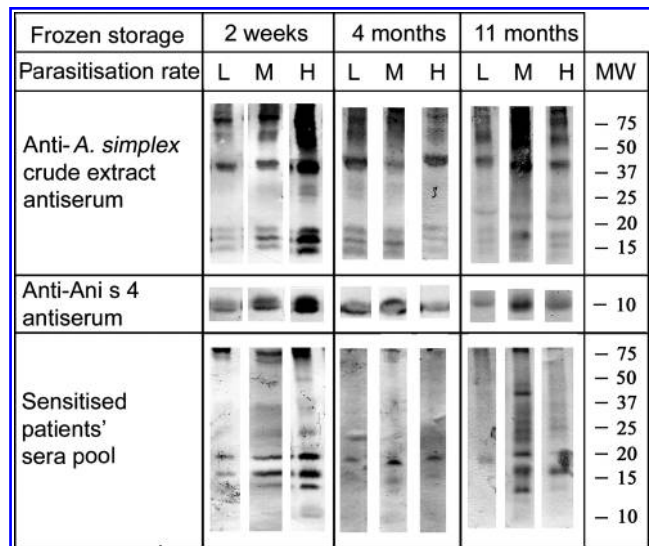


FIG. 3. Immunoblots revealed with an anti-*A. simplex* crude extract antiserum, an anti-rAni s 4 antiserum, and a pull of *A. simplex*-sensitized patients sera of low (L), medium (M), and highly (H) parasitized hakes frozen stored for 2 weeks, 4 months, and 11 months. Molecular weights (kDa) are indicated.

TABLE 2. BASOPHIL ACTIVATION PERCENTAGES WHEN INCUBATING PATIENTS' BLOOD WITH AN *ANISAKIS SIMPLEX*, FRESH, FROZEN ($-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ FOR 2 WEEKS), AND COOKED (100°C FOR 30 MINUTES) MATURE HAKE (*MERLUCCIUS MERLUCCIUS*) MUSCLE EXTRACTS AND HAKE STOCK (100°C FOR 30 MINUTES)

Extract	A. simplex	Fresh hake	Cooked hake	Frozen hake	Hake stock	Red mullet
Patient 1	23.9	24.91	10.84	11.29	10.42	0.56
Patient 2	92.47	72.5	49.36	60.61	19.74	1.2
Patient 3	84.13	91.18	93.75	97.48	85.53	0.22
Patient 4	87.32	95.18	64.04	90.43	44.50	0.59

Nonparasitized red mullet (*Mullus barbatus*) extract was used as negative control.

between parasitized and not parasitized fish, with larvae in the intestinal tract and other viscera, excreting proteins detectable in muscle, or in the muscle itself. *A. simplex* proteins such as paramyosin and tropomyosin cross-react with other allergen sources such as dust mites, cockroaches, or crustaceans (Wild and Lehrer, 2005; Guarneri et al., 2007). Our extraction and detection method has proved to be free of cross-reactivities with shellfish such as shrimps, cockles, or clams, and therefore it can be used to test anisakid parasitization in shellfish.

On the other hand, using a polyclonal antiserum against one allergen, Ani s 4, in the detection of *A. simplex* antigens in fish, enabled us to quantify the total amount of *A. simplex* proteins. Quantifying one allergen to measure the content of an allergenic substance in food has been used previously with peanuts (Pomés et al., 2003).

For other common allergies, the detection tests should ensure a sensitivity of 10 ppm for egg, 24 ppm for peanut, 30 ppm for milk proteins, and 5 ppm for oils (Morisset et al., 2003). Our method is able to detect less than 1 ppm of the allergen Ani s 4, whose sensitization has been associated to life-threatening symptoms in allergic patients (Moneo et al., 2000; Rodriguez-Mahillo et al., 2007). This limit of detection would allow to determine, in a more in-depth study, if certain species of fish are not parasite allergen sources. Therefore, sensitized patients who report allergic episodes after properly cooked or canned fish intake could reintroduce fish in their diets.

Boiling and freezing parasitized fish did not destroy the allergenic epitopes of some *A. simplex* proteins, and after the thermal treatments the *A. simplex* allergens were present in parasitized fish in enough concentration to activate basophils of sensitized patients, as demonstrated in the basophil activation tests. Part of these allergens are also preserved in the fish stock and, again, in enough amounts to activate basophils. Health Authorities recommend freezing fish to be consumed raw to kill the larvae and, therefore, avoid the risks of parasitization and new sensitization. However, our data suggested that consuming properly cooked or frozen fish contaminated with *Anisakis* allergens, or fish stock from parasitized fish, can represent a health risk for some patients sensitized to allergens resistant to heat and freezing, such as these allergens that are not eliminated during the thermal treatments.

We previously described the heat resistance of some of the *A. simplex* allergens (Caballero and Moneo, 2004; Moneo et al., 2007), but, as far as we know, this is the first time that *A. simplex* allergens have been extracted and detected in boiled fish and in the hake stock. Although some experts in this field consider that the relationship between the ingestion of frozen, properly cooked, or canned fish and the

development of allergic symptoms is not demonstrated, several articles in literature describe how patients describe symptoms after eating these products (Audicana et al., 2002; Santaolalla, 2006; Moneo et al., 2007; Ventura et al., 2008). The data obtained in this work support this fact.

The *A. simplex* antigens and allergens in hake muscle are preserved when fish are stored long-term, being detectable by polyclonal rabbit antisera and sensitized human sera at least 11 months after fish capture and freezing. This finding suggests that highly sensitized patients may jeopardize their health by consuming fish frozen for months in a standard industrial or domestic freezer. *A. simplex* migration from viscera to muscle after or before the death of the fish is still a matter of debate (Chai et al., 2005). Although it was not the aim of our study, we could ascertain that *A. simplex* larvae also migrate from the intestine to the muscle of hakes before the death of the fish, as *Anisakis* larvae were found in muscle, mainly in the belly flaps, in hakes eviscerated immediately after capture. After measuring the density of the immunoblot bands corresponding to Ani s 4, we could not find a relation between the quantity of *A. simplex* larvae in viscera and the amount of antigens and allergens in the musculature of hakes, supporting the lack of relation between parasitization rates in viscera and muscle found by other authors (Herrerias et al., 2000; Valero et al., 2006).

Although it was not the purpose of this study, and therefore the number of fish of different species studied was limited, and again assuming that the presence of *Anisakis simplex* antigens means presence of the parasite, the parasitization prevalence that we have found is consistent with literature surveys. Researches have reported allergic symptoms related to sardine consumption (Ventura et al., 2008), but we could not find *A. simplex* parasitizing sardines. Other authors could not find *A. simplex* in sardines either (de la Torre Molina et al., 2000; Rello et al., 2008). We could find *Anisakis* antigens in 19.2% of the anchovies tested. Parasitization prevalence in anchovies described in literature range from 1.4% (anchovies from the Alboran Sea) to 21.9% (anchovies from the Ligurian Sea) (Rello et al., 2009). Although a prevalence of up to 24.5% of *Anisakis simplex* in red mullets from the Ligurian Sea has been described (Manfredi et al., 2000), we could not detect *Anisakis* in any of the red mullets tested. Other authors could not find *A. simplex* in red mullets either (de la Torre Molina et al., 2000). We found *A. simplex* allergens in 95.6% of the hakes tested, which is a higher percentage than described in the literature (de la Torre Molina et al., 2000; Valero et al., 2006).

Benefits of fish consumption are widely demonstrated. Fish intake provides good-quality proteins, vitamins, minerals, and omega-3 fatty acids, which have been associated with

cardiovascular health benefits. Nowadays, clinicians recommend *A. simplex*-sensitized patients to avoid the consumption of fish and shellfish for a certain time, in which they are deprived of these benefits. Using the method described in this article to assess the contamination with *A. simplex* allergens of the different fish species, and even the different parts of a fish, would allow establishing a detailed record of fish and shellfish that do not constitute an *A. simplex* allergen source that could be eaten by sensitized patients who do not tolerate even properly cooked or canned fish without jeopardizing their health.

Conclusion

We have developed an extraction–detection method of *A. simplex* proteins in fish muscle that allowed us to detect and quantify parasite antigens/allergens in fresh, frozen, and cooked fish and in fish stock, with high sensitivity and specificity. This method can be useful in the discrimination of fish products that are not an *A. simplex* allergen source and therefore can be safely consumed with low or no risk for sensitized patients.

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Disclosure Statement

No competing financial interests exist.

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