

First report of IgE sensitization to *Anisakis simplex* in a healthy population in Colombia

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Abstract

The anisakiasis is little known in Colombia and rarely diagnosed. However, reports of fish parasitized by anisakid larvae in South America are increasing.

Objectives: The objective was determine the prevalence of sensitization to *Anisakis simplex* in a healthy population in Colombia

Methods: Cross-sectional survey of 150 volunteers from Universidad del Valle's community, Cali, Colombia. Past medical history, including eating habits associated with fish consumption was assessed. Prevalence of IgE antibodies was determined by the ELISA- Trisakis 170 kit for the recombinant allergens Ani s 1 and Ani s 7.

Findings: Two sera (1.3%) of blood tested were positive to the Ani s 7 antigen, while for Ani s 1 all sera were negative.

Conclusions: This research is the first exploratory study on *Anisakis* prevalence conducted in Colombia, the results do not indicate a major problem. Nevertheless, more epidemiological studies are needed.

Key words: *Anisakis simplex*, IgE, recombinant allergens, allergy, nematodes, fish consumption.

Primer reporte de sensibilización IgE a *Anisakis simplex* en una población sana de Colombia

Resumen

La anisakiasis es poco conocida en Colombia y raramente diagnosticada. Sin embargo, los informes de peces parasitados por larvas de anisákidos en América del Sur están aumentando.

Objetivo: Determinar la prevalencia de sensibilización a *Anisakis simplex* en una población sana de Colombia.

Materiales y métodos: Se realizó una encuesta transversal a 150 voluntarios de la comunidad de la Universidad del Valle, Cali, Colombia. Se evaluó el historial médico, incluidos los hábitos alimentarios asociados con el consumo de pescado. La prevalencia de anticuerpos IgE se determinó mediante el kit ELISA-Trisakis 170 para los alérgenos recombinantes Ani s 1 y Ani s 7.

Resultados: Dos sueros (1,3%) de la sangre analizada fueron positivos para el antígeno Ani s 7, mientras que para Ani s 1 todos los sueros fueron negativos.

Conclusiones: Esta investigación es el primer estudio exploratorio sobre prevalencia de *Anisakis* realizado en Colombia, los resultados no indican un problema mayor. Sin embargo, se necesitan más estudios epidemiológicos.

Palabras clave: *Anisakis simplex*, IgE, alérgenos recombinantes, alergia, nematodos, consumo de pescado.

Introduction

It is widely accepted that diets rich in fish have beneficial effects in the prevention of cardiovascular diseases¹. However, fish consumption varies with culinary traditions along the world and is especially dependent of the geographical position of countries and regional availability of fishes. According to the Food and Agriculture Organization of the United Nations (FAO), the average annual fish consumption in Co-

lombia is particularly low, with only 4.73 kg per capita, which contrast with countries as Spain (38 kg/year), Japan (54 kg/year), and even the mean consumption in Latin America (18 kg/year)². Yet globalization has facilitated the introduction of Mediterranean and Asian foods in diets worldwide, resulting in fish consumption increasing in countries with little or no tradition of it³. Like in other parts of world, several studies in Colombia reported the presence of nematodes of the family Anisakidae in fish for human consumption, both in waters of

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the Atlantic Ocean and the Pacific Ocean⁴⁻⁸, which represents a potential risk for human infections, particularly when consumed raw, semi-raw, in sushi, salted, or marinated.

Although only a clinical case of *Anisakis* infection has been reported in Colombia⁹ until now, the accumulated experience in other countries revealed that *Anisakis* infections are frequently misdiagnosed, mainly when subclinical cases not reporting the typical symptoms as urticaria, angioedema, gastrointestinal allergies and/or anaphylaxis are present^{10,11}. Indeed, this form of anisakiasis can be present a prevalence of 12% in some populations^{12,13}. By this reason, Moneo et al.¹¹ suggested that, from a public health perspective, any epidemiologic study on *Anisakis* infections should include the group of sensitized asymptomatic patients, i.e., individuals with a high titer of anti-*Anisakis* IgE who likely presented an undiagnosed or subclinical gastric anisakiasis without allergic symptoms.

To detect sensitized asymptomatic patients in a population, serological methods with a combination of target allergens can be used providing they are specific and selected to cover together 100% of infected subjects^{13,14}. A combination of recombinant Ani s 1 and Ani s 7 allergens recognized seems to meet these criteria as it was reported that near 100% of patients who have been sensitized during an *Anisakis* infection produced IgE antibodies to at least one of these allergens^{15,16}. Therefore, the objective of this study was to determine the IgE sensitization of the parasite *Anisakis simplex* in a healthy population in Colombia in which fish consumption habits were also investigated in order to enable comparisons with the results of the seropositive subjects.

Materials and methods

A cross-sectional study was carried out with the approval of the Institutional Review Committee of Human Ethics in the Faculty of Health at the Universidad del Valle through Act No. 015-015.

Study population

One hundred and fifty healthy volunteers from members of the teaching and student community of the Universidad del Valle, San Fernando campus, in Cali, Colombia participated in the study. All provided signed informed consent.

Survey

A survey was conducted on all volunteers to determine their fish and shellfish consumption and general medical history, following the protocol proposed by Puente et al. in 2008 and Castellanos-Garzón et al. in 2019^{2,12}. The survey comprised 28 questions: six on general information, 15 on eating habits associated with the consumption of fish/shellfish with multiple choice options, and seven open questions about medical history. The questions included the frequency of consumption of specific food items per month, their place of consumption (restaurants/home), form of consumption and/or preparation (canned, raw, salted), the species of fish consumed, and

participants' personal medical history (Table 1). The survey was previously validated by means of a pilot on 44 volunteers from the same study population.

Inclusion criteria: (a) Being of legal age; (b) consumption of fish or shellfish; (c) a willingness to provide the requested blood serum sample; (d) the ability to provide informed consent, and; (e) the ability and willingness to answer the survey. Exclusion criteria: a) At the time of blood sampling, one sample was insufficient or had hemolysis. b) A request for withdrawal from the study was made by the participant.

Table 1. Questionnaire about fish consumption habits and medical history

Survey	
Dietary habits	
1.	How many times a month do you eat fish?
2.	Do you eat canned fish? Yes/No. If yes, how many times a month?
3.	Do you eat fish in restaurants Yes/No If yes, how many times a month?
4.	When eating in restaurants, how do you like your fish to be prepared? (Various options) 1) Fried, 2) Stewed, 3) Steamed, 4) Oven-baked, 5) Breaded or battered, 6) Raw, 7) Other
5.	Do you eat raw fish? For example, in sushi or ceviche? Yes/No. If yes, how many times a month?
6.	Do you eat smoked or marinated fish? Yes/No. If yes, how many times a month?
7.	Which species of fish do you eat most frequently? (Name three).
8.	Do you eat any type of shellfish? Yes/No If yes, how many times a month?
9.	Which species of shellfish do you eat most frequently? (Name three).
10.	How do you or your family prepare fish at home? (Various options) 1) Fried, 2) Stewed, 3) Steamed, 4) Oven-baked, 5) Breaded or battered, 6) Raw, 7) Other
11.	Which family members enjoy eating fish? 1) Children, 2) Teenagers, 3) Men, 4) Women, 5) Adults, 6) Everyone
12.	Where do you buy fish or shellfish? 1) Supermarket, 2) Markets, 3) Both previous places, 4) I don't buy it.
13.	When you buy fish, where do you store it? (Various options) 1) Freezer, 2) Refrigerator, 3) Food storage containers, 4) I don't store it, 5) Other.
14.	On average, how much time passes between you buying fish and getting it home? (One option only) 1) Less than an hour, 2) Between one and two hours, 3) Half a day to a day, 4) More than a day, 5) I'm not sure/No response.
Medical history	
15.	Have you ever suffered any reaction or ill-health after consuming fish or shellfish? Yes/No. Which? 1) Digestive problems, 2) Skin rash, 3) Other allergy, 4) Other (Please specify).
16.	Are you allergic to any foodstuff? Yes/No. If yes, to which food(s)?
17.	Do you suffer from any respiratory difficulties from an allergy? Yes/No. Which?
18.	Are you allergic to dust mites? Yes/No.
19.	Do you suffer from any other medical conditions? Yes/No. If yes, which condition(s)?
20.	Have you ever been diagnosed with parasites (in particular, nematodes (roundworm). Yes/No. Which type? How long ago were you diagnosed?
21.	Have you suffered from a chronic infectious disease? Yes/No. Which? I don't know/No response
22.	Have you been diagnosed with acute or chronic urticaria (hives)? Yes/No. If yes, which type? Acute/Chronic/Not sure/No response

Sampling

By means of venipuncture, a 10 ml blood sample was taken from each volunteer and centrifuged. The serum obtained was then stored and preserved at -20° C.

Determination of IgE antibodies to rAni s 1 and t-Ani s 7 allergens

Anti-Ani s 1 and anti-Ani s 7 IgE determinations were done using the Trisakis 170 kit developed at the Universidad de Santiago de Compostela (Santiago de Compostela, Spain) using the extended protocol included with the kit. This test uses two recombinant major allergens (Ani s 1 and Ani s 7) as target to assess IgE sensitization to *Anisakis* sp.²⁸. Interestingly, this test proved to be adequate for detection of true *Anisakis* infections and was able to detect 100% of gastroallergic anisakiasis and 95% of cases of *Anisakis* sensitization associated with chronic urticaria¹⁵. Moreover, this method can be considered as a gold standard for specificity as the Ani s 7 recombinant sequence used in the kit has no sequence identity with any known human allergen in line with the FAO/WHO criteria (<https://fermi.utmb.edu/>). Sensitivity differs between recombinant allergens; Ani s 1 is less sensitive (61%; 95% confidence interval, CI 54–68%) compared to Ani s 7 (93%; 90–98%)⁽¹³⁾.

The results of each serum were calculated by subtracting the value of the corresponding negative control from the OD obtained for the wells coated with the rAni s 1 and t-Ani s 7 allergens. The samples were considered positive when the calculated absorbance was greater than the cut-off points of the rAni s 1 and t-Ani s 7, being 0.09 and 0.05 respectively^{15,17,18}.

Statistical analysis

The SPSS program, version 15.0, was employed and a descriptive analysis was carried out to establish frequencies and averages. The Kolmogoroff Smirnov test was applied to determine the distribution of the data. The quantitative variables were: frequency of fish intake, canned fish, raw fish, salted fish, consumption of shellfish and consumption of fish in restaurants. These results were related to the medical history associated with allergies. For each variable, the median and interquartile range were calculated and compared using the Mann-Whitney test. Variables with a P value of ≤ 0.05 were considered significant. The laboratory tests were analyzed with the GraphPad Prism 6 program. The qualitative variables are described in the form of frequency/total and in percentages, and the quantitative variables as mean \pm standard deviation. Comparison between quantitative data between two groups was performed with the Student's t-test or Mann-Whitney's U, according to the distribution of the data. Values of $P < 0.05$ were considered statistically significant with 95% confidence interval (CI)

Results

Of the 150 participants, 84 were women (56%) and 66 men (44%), with ages ranging between 18-67 for women and 18-61 for men. The median consumption of fish was three times

a month [interquartile range (IQR) 2-5], the consumption of canned fish (tuna and sardines) was twice a month [IQR 1-3] and the consumption of raw fish (salted/marinated) and seafood was once a month [IQR 0-1.25]. Eighty-seven percent of the participants reported consuming raw fish. Nineteen percent referred to eat raw fish in restaurants. At home, the preferred form of consumption was fried (70%), while 7% consumed it raw, marinated or salted. The five most commonly consumed fish species were tilapia (*Oreochromis* sp.) with 31%, tuna (*Thunnus* sp.) 26%, salmon (*Salmo* sp.) 17%, trout (*Oncorhynchus mykiss*) 10%, and snapper (*Lutjanus* sp.) with 3%. Regarding seafood, the participants preferred to consume either shrimp (55%) or langoustine (4%). Eighty-seven percent of the participants indicated that their fish products had been frozen before consumption and 13% stated that they preferred to have it refrigerated.

Participants' medical histories show that 11% reported having experienced at some point digestive problems associated with the consumption of fish or shellfish, 12% had allergic-type symptoms, and 2% reported having presented both allergic and digestive symptoms. With regard to food-type allergies, 11% reported being allergic to some type of food. Four percent of respondents reported being allergic to shellfish, 27% reported some type of respiratory allergy and 47% reported dust mite allergies.

The comparative study of the analyzed variables showed that participants who consume salted fish have a greater probability of suffering from hives ($P < 0.03$), of being allergic to mites ($P < 0.02$) and of presenting allergies to some type of food ($P < 0.04$). Regarding seafood consumption, a positive association was observed with the probability of presenting urticaria ($P < 0.04$). In addition, in relation to the frequency of fish consumption per month, the analysis determined that the higher the intake, the greater the probability of suffering from hives ($P < 0.001$) and a trend towards significance with regard to mite allergy ($P = 0.063$).

IgE response to Ani s 1 and Ani s 7 allergens

Using the Trisakis 170 kit, it was found that only two sera (1.3%) showed positive levels of IgE antibodies against the tAni s 7 allergen, while all the sera were negative for the rAni s 1 allergen (Figure 1). Moreover, the data showed that the OD values of the two positive sera (OD= 0.064 and 0.055, respectively) are near the cut-off value of the assay for Ani s 7 (OD= 0.05). Due to the low prevalence observed, as expected, no significant statistical differences were observed between Ani s 7 sensitization and the parameters of fish consumption included in Table (not showed). In addition, both positive subjects reported to consume canned fish (tuna) between three and eight times a month, shellfish, and to have preference of consuming fish either fried, steamed, oven-baked or breaded. Interestingly, one participant (IgE OD value= 0.055) reported to consume raw fish. Nevertheless, none of the two positive cases experienced recent digestive or allergic symptoms associated with the consumption of fish or shellfish.

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation. All participants gave signed informed consent to participate in the study

Right to privacy and informed consent. The authors declare that no data that enables identification of the patients appears in this article.

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Author's contribution. Conception and design of the work: CC and MCP. Collecting data: JACG and LS. ELISA analysis: JACG and FMU. Statistical method: JACG and AD. Interpretation of data for the work: JACG, FMU, MCP, LS, AD and CC. Drafting the work and revising it critically for important intellectual content: JACG, FMU, AD and CC.

Conflict of interest. The authors declare that the revision was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Authors declare absence of conflicts of interest in the planning and development of the study.

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