

Incidence of sensitivity to *Anisakis simplex* in a risk population of fishermen/fishmongers

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Background: *Anisakis simplex*, a fish and cephalopodes parasite, can cause either gastrointestinal symptoms or allergic reactions in humans on eating/handling contaminated fish.

Objective: The aim of our study was to determine the capacity of *Anisakis simplex* to induce specific IgE production and allergic reactions following eating and handling fish in a population at risk.

Methods: We determined the levels of total IgE, specific IgE, and eosinophil count in 28 fishermen/fishmongers (group A) and 15 healthy donors (group B). A skin prick test (SPT) with extracts from *Anisakis* and the most common species of fish in our country, were also carried out.

Results: Specific IgE to *Anisakis* were found in 14 subjects of group A (13 of them had a positive SPT to the same extract) and none of group B (only one subject had a positive SPT). The SPT with fish extracts was positive in 4 patients of group A but in none of group B. Subjects in group A with specific IgE to *Anisakis* showed higher total IgE levels and eosinophil counts compared with either other individuals of the same group or to those of group B.

Conclusions: These results indicate that fishermen/fishmongers are a population at risk for *Anisakis simplex* sensitization and suggest that this kind of sensitization should also be investigated not only in subjects like fishermen/fishmongers who live in countries where fish is likely to be contaminated with *Anisakis simplex* parasites, but also in those who handle fish for other reasons.

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INTRODUCTION

Adverse reactions to food can be induced by contaminants that induce sensitization. Among these, there are fish parasites such as *Anisakis nematodes* (mainly *Anisakis simplex* and *Pseudoterranova decipiens*), cestodes of the *Diphyllobothrium* genus and digenetic trematodes of the *Heterophyidae*, *Opisthorchiidae*, and *Nano-*

phyetidae families.¹ Seafood-induced infestations by these helminthes, particularly by *Anisakis simplex*, are usually characterized by gastrointestinal symptoms similar to those observed in gastric ulcer, neoplasms, or acute appendicitis.^{2–7}

Fish infestation by *Anisakis simplex* has been observed in several areas including the Sea of Japan,⁸ the southern and eastern seas of Korea,⁹ the Antarctic Ocean,¹⁰ the East China and Yellow seas,¹¹ the Canadian,¹² and Italian waters.¹³

It has also been reported that *Anisakis simplex* is able to induce an acute urticaria-angioedema reaction and, more rarely, asthma shortly after eating or handling fish.^{14–18} Further, Armentia et al¹⁹ recently reported 2 cases of occupational asthma caused by the inhalation of antigens from *Anisakis simplex*.

In this study, we sought to determine the incidence of *Anisakis simplex* sensitization in a selected population exposed to daily contact with fish (fishermen, fishmongers) and to establish the role of atopy in this setting.

METHODS

Study Population

Two groups of subjects were included in this study. Group A consisted of 28 males in daily contact with fish (16 fishermen and 12 fishmongers), aged between 18 and 48 years (average age 30.6). Eighteen of them reported allergic reactions related to eating or handling fish (13 urticaria-angioedema and 5 bronchospasm). Two subjects out of eighteen reported asthmatic episodes unrelated to fish contact. Group A was subdivided into A1 and A2 according to the presence or not of specific IgE to *Anisakis*. Group B consisted of 15 non atopic healthy males, aged between 20 and 44 years (average age 32.5).

None of the subjects had taken any drug likely to interfere with the study. All subjects signed an informed consent.

Anamnesis

Patients were asked about their habits (contact and ingestion of fish) and history of allergic reactions (rhinoconjunctivitis, asthma, urticaria-angioedema, and anaphylaxis) associated or not with exposure to fish.

Total Serum IgE Determinations

Total IgE levels were determined by the radioallergosorbent test according to the manufacturer's instructions (Pharmacia Diagnostics, Milan, Italy). Data were expressed in International Units per milliliter (IU/mL).

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Table 1. Group A Data

Patient No.	Age (yr)	Anisakis Specif. IgE (IU/mL)	Anisakis SPT	Fishes SPT	Inhalant SPT	A.R. after contact with fish	Total IgE (IU/mL)	Eosinophil count (n × 10 ⁶ /L)
1	31	27.5	+	—	—	+	157	128
2	44	92.1	+	—	Dust mites	+	803	426
3	22	8.3	+	—	—	+	96	174
4	38	40.2	+	—	—	+	169	125
5	33	38.9	+	+	Dust mites	+	258	302
6	34	29.1	+	—	—	+	165	106
7	25	19.4	+	—	—	+	119	186
8	19	31.6	+	—	—	—	202	202
9	26	25.0	+	—	—	+	147	151
10	22	19.5	+	—	—	+	142	133
11	33	14.3	+	—	—	+	88	83
12	20	27.7	—	—	—	+	236	197
13	27	28.2	+	—	—	+	439	146
14	39	34.0	+	—	—	+	184	231
15	26	—	—	—	—	—	78	198
16	25	—	—	—	—	—	93	78
17	18	—	—	+	—	—	132	91
18	21	—	—	—	—	+	264	142
19	41	—	—	—	—	—	67	97
20	32	—	—	+	—	+	124	185
21	40	—	—	—	—	—	47	80
22	48	—	—	—	—	+	58	62
23	35	—	—	—	—	—	179	166
24	24	—	—	—	—	—	55	86
25	36	—	—	+	—	+	95	119
26	31	—	—	—	—	—	49	98
27	43	—	—	—	—	+	72	124
28	26	—	—	—	—	—	151	232

Table 2. Group B Data

Patient Number	Age	Total IgE (IU/mL)	Anisakis Specific IgE (IU/mL)	Anisakis SPT	Fishes SPT	Eosinophil count (n × 10 ⁶ /L)
1	19	82	—	—	—	107
2	25	112	—	—	—	123
3	26	54	—	—	—	95
4	42	105	—	—	—	144
5	44	167	—	—	—	271
6	28	32	—	—	—	88
7	41	56	—	—	—	79
8	25	45	—	—	—	60
9	37	16	—	—	—	85
10	44	98	—	—	—	110
11	31	148	—	+	—	165
12	23	79	—	—	—	109
13	39	134	—	—	—	54
14	29	304	—	—	—	217
15	35	28	—	—	—	63

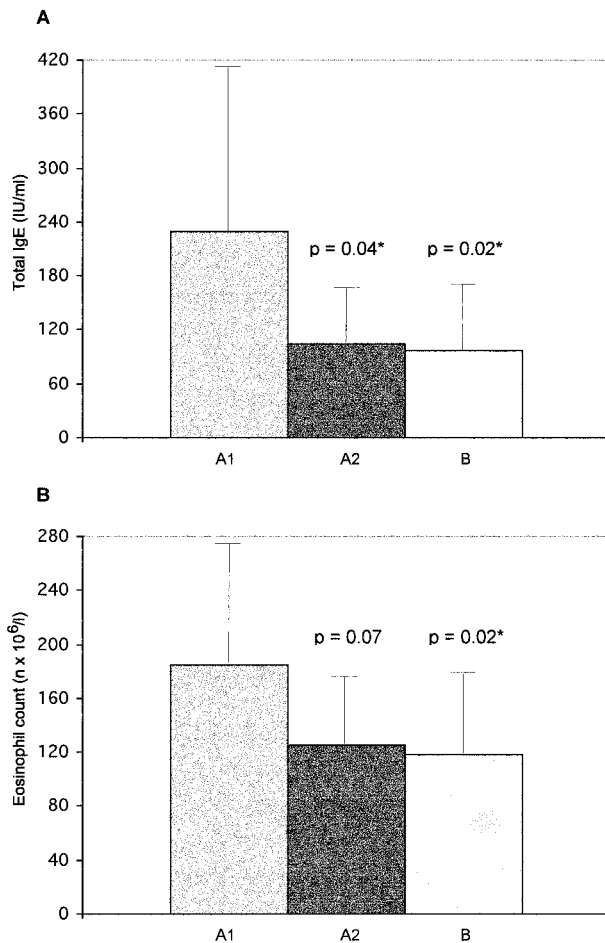


Figure 1. A. Means $\pm$ standard deviations of total IgE in subjects of group A1, A2, and B. (* $p < 0.05$). B. Means $\pm$ standard deviations of eosinophil count in subjects of group A1, A2, and B. (* $p < 0.05$).

Prick Test

Skin prick tests were performed on the volar aspect of the forearm with a standard panel of inhalant allergens and also with *Anisakis simplex* and fish extracts of hake, atlantic mackerel, horse mackerel, red mullet, anchovy (0.05 mg/mL) (Bayer DHS, Milan, Italy) using allergy pricker lancets (Alk lancets, Milan, Italy). Ten milligrams per milliliter histamine and saline solutions were used as positive and negative controls, respectively.

Specific Serum IgE Determinations

Specific IgE to *Anisakis* determinations were performed by radioimmunoassay utilizing a commercial preparation (CAP System, Pharmacia Diagnostics, Milan, Italy). Results

higher than 0.7 kU/L were considered positive.

Eosinophil Count

Blood was anticoagulated with heparin (Sigma, St. Louis, CO, USA), diluted 10 times and stained with an eosin-formalin solution (2.5 mL of formalin and 25 mL of 1% eosin for 250 mL of distilled water). Eosinophils were morphologically identified and enumerated using a Buerker counting chamber.

Statistical Analysis

Data are expressed as means $\pm$ standard deviations (SD) and analyzed by one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test. A value of $P < .05$ was considered significant. Correlation co-

efficients were calculated with the Spearman rank correlation coefficient test. All data were analyzed using the Stat View SE program (Abacus, Berkeley, CA, USA).

RESULTS

As shown in Table 1, only two patients had a positive prick test to dust mites extracts (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). Specific IgE antibodies to *Anisakis simplex* were found in 14 subjects from group A with a median of 31.13 kU/L; 13 of these subjects had also a positive skin prick test to *Anisakis simplex* extract. On the contrary, all subjects from group B were negative for IgE to *Anisakis* and, except one, negative to skin prick test to *Anisakis* (Table 2).

Fish extracts gave a positive reaction in three cases of group A2 (2 for hake, 1 for anchovy) and one of group A1 (for horse mackerel), and none of group B (Tables 1 and 2).

We found a direct correlation between positive prick test to *Anisakis* extract, IgE to *Anisakis* and a positive history of previous allergic reactions to eating or handling fish (13 out of 18 with a positive history, 72.2%, and 1 out of 10 with a negative history, 10%, respectively) (Table 1).

Subjects of group A1 showed higher total IgE levels compared with those of group A2 and B (228.93 ± 186.89 versus 104.57 ± 61.19 IU/mL, $P = .0411$, and versus 97.33 ± 72.87 IU/mL, $P = .0275$, respectively) (Fig 1A).

Similarly, the eosinophil count was higher in group A1 subjects compared with group A2 and B (185.00 ± 89.15 versus $125.57 \pm 51.72 \times 10^6/L$, $P = .0789$, and versus $118.00 \pm 60.30 \times 10^6/L$, $P = .0205$, respectively) (Fig 1B).

As shown in Figures 2A and 2B, specific IgE to *Anisakis* strongly correlated to total IgE levels ($Rho = 0.833$; $P = .0027$). A less significant direct correlation with the eosinophil count was also observed ($Rho = 0.429$; $P = .1223$).

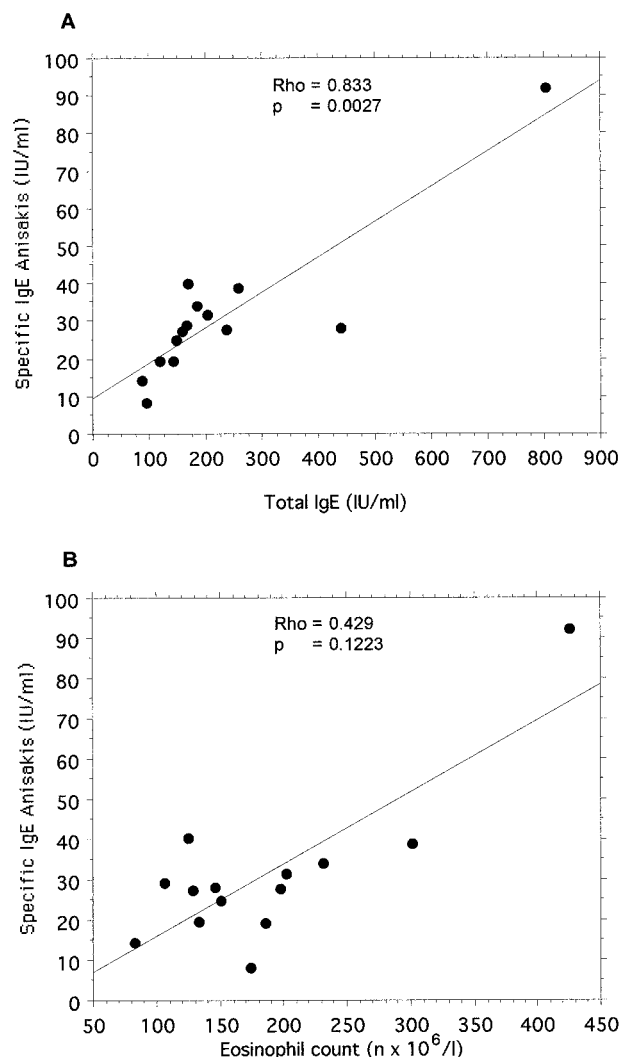


Figure 2. A. Correlation between specific IgE to *Anisakis simplex* and total IgE levels in group A1 (Rho = 0.833; $P = .0027$). B. Correlation between specific IgE to *Anisakis simplex* and eosinophil count in group A1 (Rho = 0.429; $P = .1223$).

DISCUSSION

The first reports regarding problems connected with *Anisakis simplex* infestation date back to 1960 when the first cases were observed in the Netherlands.²⁰ Since then many cases have been reported from several countries in the world, including Japan,²¹ UK,²² USA,²³ Spain,⁶ and France.²⁴

Adult *Anisakis simplex* live in the digestive apparatus of marine mammals (seals, dolphins, etc). Their eggs are expelled by feces into the sea where they become second-stage larvae and are eaten by crustaceans.

Then, crustaceans are eaten by fish and larvae penetrate intestinal tissue, go to the surface of organs, mesenteries or musculature, and change into third-stage larvae. Last, the larvae are ingested by marine mammals and *Anisakis* life cycle start again (Fig 3).

Accidentally, humans can ingest the third-stage larvae by eating the parasitized crustaceans, cephalopods or fish.²⁵ The principal route of sensitization in humans is the digestive tract, but it can also be the respiratory tract, which explains the possible role of this nematode in the pathogenesis of

asthma in subjects who handle contaminated fish.²⁶

This is the first study carried out to define the incidence of *Anisakis simplex* sensitization in a population at risk like fishermen/fishmongers who handle fish daily and also often eat it.

As previously observed,²⁶ the presence of other allergic disease was not an important factor for the development of *Anisakis*-related allergic reactions (Table 1). In fact, only two subjects of group A suffered from dust mites allergy and only 4 patients showed a positive prick test to fish extracts. We observed, however, a high incidence of *Anisakis* positivity in fishermen/fishmongers (Tables 1 and 2). These results indicate that fishermen/fishmongers may represent a population at risk for developing allergic reactions on eating or handling fish. It is interesting to note that in these subjects the incidence of allergy to fish contaminated with *Anisakis simplex* was higher than to other species of fish. These results are consistent with previous reports,²⁷ and suggest that the incidence of this kind of sensitization could have been underestimated.

These results also strongly indicate that fishermen/fishmongers should be considered a population at risk of sensitization to *Anisakis simplex* by both ingestion and inhalation of the allergen.²⁶

In our opinion sensitization to *Anisakis simplex* should be taken into account when evaluating patients with clinical features of urticaria or with asthma attacks especially if they have a history of exposure to fish likely to be contaminated by this kind of parasite. In these cases, if sensitization to *Anisakis simplex* is present, patients should be advised not to handle fish and since *Anisakis simplex* is thermolabile,²⁸ to avoid eating raw or poorly cooked fish, and to maintain salted, pickled or raw fish at -20°C for 24 h.²⁹

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LIFE CYCLE OF *ANISAKIS* spp.

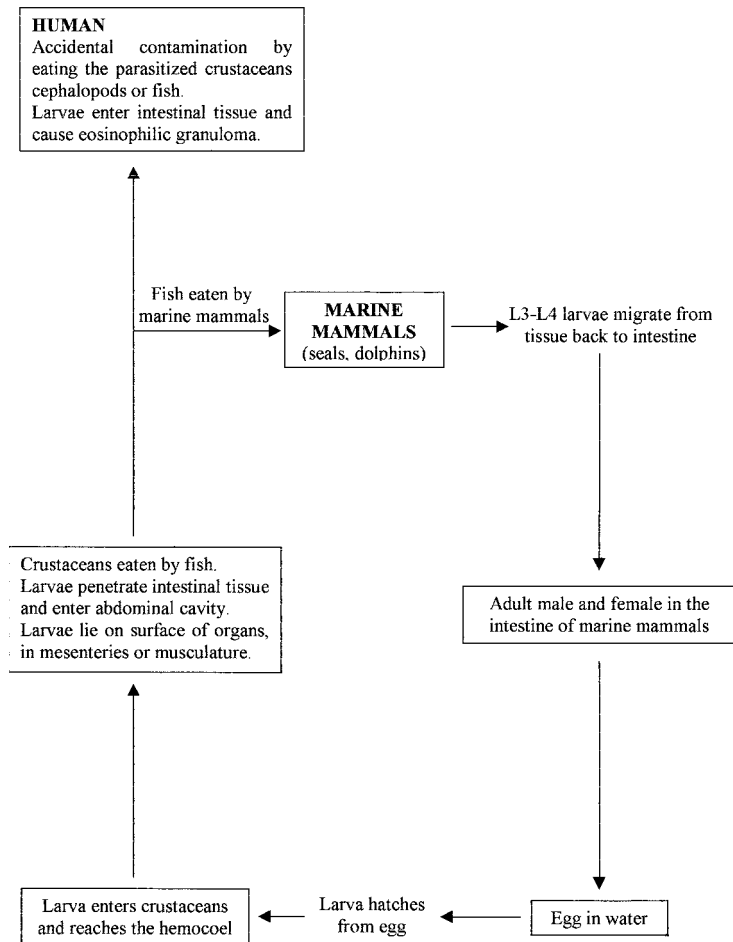


Figure 3. Life cycle of *Anisakis* spp.

Institute of CNR (National Centre of Research), for marine biologic informations.

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