

Seropositivity to a major allergen of *Anisakis simplex*, Ani s 1, in dyspeptic patients with *Helicobacter pylori* infection: histological and laboratory findings and clinical significance

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ABSTRACT

Previous studies have demonstrated a high prevalence of seropositivity to the Ani s 1 protein in dyspeptic patients with *Helicobacter pylori* infection, but it is not known whether this represents episodes of anisakiasis misdiagnosis or previous exposure to the parasite without clinical relevance. To investigate the clinical significance of seropositivity to the Ani s 1 protein, a cohort study was performed with 87 consecutive dyspeptic patients who were treated for *H. pylori* infection. Fourteen (16.5%) patients were seropositive for the Ani s 1 protein, which was associated with the consumption of uncooked fish ($p < 0.0002$). There were no differences in histological findings between subjects seropositive or seronegative for Ani s 1, but seropositive patients had increased eosinophil and basophil leukocyte counts ($p < 0.05$). Anti-Ani s 1 IgE was associated with a lack of improvement in the group of patients with non-ulcer dyspepsia after successful eradication of *H. pylori* ($p < 0.016$). Thus, in at least a subset of patients with *H. pylori* infection, seropositivity to Ani s 1 could have clinical relevance. In addition, these data highlight that only anisakiasis associated with severe allergic or gastric symptoms is currently being diagnosed.

Keywords Ani s 1 protein, *Anisakis simplex*, diagnosis, dyspepsia, *Helicobacter pylori*, IgE

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INTRODUCTION

Anisakis simplex is a nematode that has a world-wide distribution and is found frequently in a large variety of fish consumed by humans. Infection is caused by eating uncooked fish or seafood containing the third-stage larvae of *A. simplex*. After ingestion, larvae can penetrate the host stomach or intestinal wall. The resulting disease, known as anisakiasis or anisakidosis, can mimic other gastrointestinal disturbances and allergic reactions, such as urticaria, angioedema or anaphylaxis [1,2]. Fibreoptic gastroscopy within 24 h of fish ingestion, or an exploratory laparotomy, is required for diagnosis when anisakiasis is suspected [1,3]. Therefore, it is suspected that only the most severe cases requiring invasive techniques

are diagnosed, and that many mild cases remain unrecognised [4]. Although serological techniques for the detection of *A. simplex* infection have been developed, cross-reactivity with other nematodes is a serious limitation [5–8]. In an attempt to improve diagnosis, a major IgE-binding protein has been purified from the parasite and characterised. This major parasite allergen, termed Ani s 1, is a protein from the excretory gland that shows no homology with other proteins described previously [9]. Detection of specific IgE for Ani s 1 has been shown to be a useful tool for the diagnosis of anisakiasis [10].

A high prevalence of seropositivity to Ani s 1 has been reported in dyspeptic patients with *Helicobacter pylori* infection [11], but the clinical significance of this finding is unknown. It is possible that episodes of anisakiasis in combination with *H. pylori* infection may have been misdiagnosed, or that the findings may reflect previous exposure to the parasite without clinical relevance.

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The present study investigated whether seropositivity to Anis 1 might have a clinical role in this setting by performing a cohort study in a group of dyspeptic patients with *H. pylori* infection.

PATIENTS AND METHODS

Patients

Consecutive dyspeptic patients ($n = 87$) who underwent a programmed upper endoscopy and were treated for *H. pylori* infection were enrolled in the study. Endoscopy procedures were performed in the Gastroenterology Unit at Hospital Carlos III (Madrid, Spain) between February 1998 and January 1999. Excluded were patients who had been treated previously to eradicate *H. pylori* infection, or who had received antimicrobial or antacid agents during the previous 3 months; patients who were taking non-steroidal anti-inflammatory drugs (including low doses of aspirin) within a month of endoscopy; and patients with underlying diseases, gastrointestinal bleeding, documented gastro-oesophageal reflux or endoscopic evidence of oesophagitis, previous gastric surgery, cholelithiasis, or any abnormal physical and laboratory findings. The present study was approved by the Ethics Committee of the Hospital Carlos III, with informed consent to participate being obtained from each patient.

Dyspepsia was defined as an intermittent or persistent pain, or discomfort in the upper abdominal region or lower part of the chest, heartburn, nausea, a feeling of postprandial fullness, or any other symptoms thought to be related to the upper gastrointestinal tract that lasted for ≥ 3 weeks [12]. Ulceration was diagnosed when there was an area of complete loss of mucosa >0.5 cm in diameter. Erosive duodenitis is considered to be a variant form of duodenal ulcer, with symptomatic and histological improvement after eradication of *H. pylori* infection. For these reasons, this condition was included in the group of ulcer dyspepsia (UD) conditions [13,14]. Patients with normal endoscopy findings (i.e., five or fewer gastric erosions and no gastric or duodenal ulcer) were diagnosed with non-ulcer dyspepsia (NUD) [15].

Data collection

All patients were interviewed with the aid of a standard questionnaire before endoscopic examination. The first part of the questionnaire was an abbreviated version of the Gastrointestinal Symptom Rating Scale, which includes the main ulcer-like symptoms (abdominal pain, heartburn, acid regurgitation and sucking sensations in the epigastrium) and uses a validated four-point Likert scale [16]. The severity of the associated symptoms was scored as 0 (absent), 1 (low intensity), 2 (moderate intensity) or 3 (severe). In addition, the patients answered questions designed to reveal the presence of any IgE-mediated diseases (urticaria, angioedema, anaphylactic reactions or other symptoms). Finally, tobacco and alcohol consumption were recorded as described previously [11], and the patients were asked about their fish-eating habits during the previous year, including the type and form of consumption (uncooked, i.e., raw or smoked, and cooked). The patients were classified, with respect to the

frequency of consumption, as non-consumers or sporadic consumers (monthly or less) and habitual consumers (more than once-monthly).

Endoscopy

All patients were examined by upper endoscopy with a conventional video-endoscope (FG-100FP; Fujinon, Tokyo, Japan). All endoscopy devices were disinfected with glutaraldehyde 2% v/v. During the endoscopy procedure, the presence of macroscopic lesions in the stomach was evaluated carefully, and at least five biopsy samples, three from the antrum and two from the corpus, were obtained.

H. pylori infection and histology

During endoscopy, biopsy samples were taken for histological examination (two antrum and two corpus biopsies) and culture (one antrum biopsy). *H. pylori* infection was diagnosed by positive culture and/or histological results. Neutrophil infiltration, mononuclear cell infiltration, glandular atrophy, intestinal metaplasia and *H. pylori* density in the gastric biopsy samples were assessed on a scale of 0–3, in accordance with the updated Sydney System [17], using formalin-fixed biopsy tissues stained with haematoxylin–eosin. Eosinophil infiltration was analysed using the same approach. All histological evaluations were performed by a single pathologist with no knowledge of the clinical and serological results.

Differential blood counts

At the time of the endoscopy, venous blood samples were collected in EDTA-treated tubes. Total leukocyte counts were determined using a Coulter AcT Diff 2 haematology analyser (Beckman Coulter, Fullerton, CA, USA). Differential blood counts were made from blood smears stained by Diff-Quik. Counts were performed by one investigator in a blinded fashion, and the mean of two slides was calculated (300 cells counted/slide).

Determination of Anis 1-specific IgE

Sera. At the time of endoscopy, a serum sample was obtained from each patient and stored at -80°C . All patients were tested for the presence of IgE against *A. simplex* extract. IgE directed specifically against Anis 1 was studied in subjects who were reactive to the *A. simplex* extract.

Antigens. The *A. simplex* extract was prepared with larvae obtained from muscle tissue of *Merluccius merluccius* and *Micromesistius poutassou*. Larvae (c. 200) were ground in a mortar with 5 mL of phosphate-buffered saline. After centrifugation for 10 min at 4500 g, the pellet was discarded. The protein content of the supernatant was 2 mg/mL, as measured by the Bradford method [18], with bovine serum albumin as the protein standard. Anis 1 was isolated from larvae and purified from the crude parasite extract by ethanol precipitation and reverse-phase HPLC as described previously [9].

Immunoblotting. Anis 1 (0.1 mL of a 100 mg/L dilution) or crude *A. simplex* extract (0.1 mL of a 1000 mg/L dilution) was mixed with 0.1 mL of phosphate-buffered saline. The mixture was electrophoresed (150 V) in an acrylamide 16% w/v mini-gel with standard SDS-PAGE conditions, and then transferred by diffusion to two nitrocellulose sheets for 18 h. After being

blocked in Nonidet P-40 3% v/v for 30 min, the membranes were placed on a Mini-Protein II multiscreen (Bio-Rad, Hercules, CA, USA). For specific IgE determination, the different sera were diluted 1:6 in incubation buffer (125 mM Tris, pH 6.8, SDS 4% w/v, glycerol 2% v/v, bromophenol blue 0.042% w/v) and incubated overnight in independent chambers. After incubation and washing, the membranes were incubated for 3 h with 10 mL of a 1:1000 dilution of a monoclonal human anti-IgE antiserum (Ingenasa, Madrid, Spain). After further washes, the membranes were incubated with 10 mL of a 1:4000 dilution of an alkaline phosphatase-labelled goat anti-mouse antiserum (Biosource, Camarillo, CA, USA). Finally, the membranes were washed, and the signal was visualised by staining with 5-bromo-4-chloro-3-indolyl phosphate-4-nitroblue tetrazolium (Amresco, Solon, OH, USA) for 30 min.

Drug administration and follow-up

Patients received either omeprazole 20 mg, amoxycillin 1000 mg and clarithromycin 500 mg, or ranitidine bismuth citrate 400 mg, amoxycillin 1000 mg and clarithromycin 500 mg, each given twice daily for 1 week. All patients were reviewed at 2 months after treatment was completed. At this time, the urea breath test was performed to determine whether *H. pylori* had been eradicated. The overall effect of treatment was assessed by comparing the severity of dyspeptic symptoms before and after treatment with the Likert scale. A score of 0–1 was considered as resolution, while a score of ≥ 2 was considered to indicate persistence of dyspeptic symptoms. This cut-off point was assessed by constructing a receiver operated characteristic curve in which the specificity was plotted against 1 minus sensitivity for the full range of post-treatment dyspepsia scores. All follow-up was performed with neither the patients nor the interviewer being aware of the results of the *A. simplex* serology and recent breath tests, and whether or not the infection had been eradicated.

Statistical analysis

Categorical variables were analysed with the chi-square test, Fisher exact test or analysis of variance (ANOVA), as appropriate. Linear regression models were used to compare the

number of blood leukocytes (log-transformed data) between anti-Ani s 1 IgE-seropositive and -seronegative subjects, adjusting for age, gender, tobacco and alcohol consumption. A *p* value <0.05 was considered to be significant. SPSS v.11 (SPSS Inc., Chicago, IL, USA) was used for all analyses.

RESULTS

Of the 87 patients included in the study, two were lost to follow-up. The mean age of the 85 patients studied was 47.4 years (range 21–78 years) and 45 were male. No cases of active parasitism were diagnosed during endoscopy procedures. However, anti-Ani s 1 IgE antibodies were detected in 14 (16.5%) of the 85 patients. Twenty-three (27.1%) patients were habitual consumers of uncooked fish, with pickled anchovies being implicated in 18 (78.3%) patients. There was a significant relationship between seroreactivity to Ani s 1 and the habitual intake of uncooked fish (10/23; *p* 0.0002). The clinical and epidemiological features of the patients seropositive for Ani s 1 are shown in Table 1.

Thirty-seven patients showed ulceration by endoscopy (29 patients with duodenal ulcers, seven with gastric ulcers, and one with both), and 48 patients were diagnosed with NUD (37 patients with active chronic gastritis and 15 with intestinal metaplasia/atrophy). The distribution of endoscopy and histological findings was similar for anti-Ani s 1 IgE-seropositive and -seronegative patients. In addition, there were no differences between anti-Ani s 1 IgE-seropositive and -seronegative subjects with respect to the grade of eosinophil, neutrophil and mononuclear cell infiltration in biopsy samples (data not

Table 1. Clinical and epidemiological data for the 14 patients positive for IgE against Ani s 1

Patient	Gender/age	Dyspepsia	Uncooked fish consumption ^a		Dyspepsia after <i>Helicobacter pylori</i> eradication	Allergy symptoms
			Frequency	Form		
1	M/50	UD	Habitual	Smoked	No	No
2	M/48	UD	Habitual	Pickled	No	No
3	F/61	UD	None or sporadic	–	No	No
4	F/55	UD	Habitual	Smoked	No	Urticaria/angioedema
5	F/50	UD	None or sporadic	–	No	No
6	M/45	UD	Habitual	Pickled	No	No
7	F/57	NUD	Habitual	Pickled/smoked	Yes	No
8	F/47	NUD	Habitual	Pickled	Yes	Urticaria/angioedema
9	F/50	NUD	None or sporadic	–	Yes	No
10	M/48	NUD	Habitual	Pickled	Yes	No
11	F/35	NUD	Habitual	Raw	Yes	Urticaria/angioedema
12	M/23	NUD	Habitual	Pickled	No	No
13	M/50	NUD	None or sporadic	–	^b	No
14	M/56	NUD	Habitual	Pickled/raw	^b	No

UD, ulcer dyspepsia; NUD, non-ulcer dyspepsia; M, male; F, female.

^aNon-consumers or sporadic consumers, once or less/month; habitual consumers, more than once/month.

^bNo eradication of *H. pylori* infection.

shown). However, the total numbers of eosinophils and basophils were increased significantly in patients with anti-Ani s 1 IgE antibody, compared with seronegative subjects ($p < 0.05$). The histological findings and blood leukocyte counts for anti-Ani s 1 IgE-seropositive and -seronegative patients are shown in Table 2.

The validity of the abbreviated version of Gastrointestinal Symptom Rating Scale was assessed by comparing the dyspepsia scores in 110 healthy volunteers, 110 patients with NUD, and 47 patients with a peptic ulcer. The mean scores in these three groups were 0.32 (range 0–3), 5.8 (1–11), and 5.7 (2–12), respectively. The scores in the patients with NUD and UD were similar, and both were significantly higher than those in the healthy control group ($p < 0.001$).

In the group of 36 patients with UD, all but one (97.3%) showed successful eradication of *H. pylori* infection. The median dyspepsia score at initial presentation for these 36 subjects was 5.6 (range 2–11). At reassessment, only three (9.1%) had persistent symptoms (score ≥ 2). Eradication of *H. pylori* infection was achieved in 42 (87.5%) of 48 NUD subjects. At endoscopy, the median score of these 42 subjects was 5.9 (range 2–12). A high proportion (15/42; 35.7%) of these patients had persistent symptoms following successful treatment for *H. pylori* infection, which was a higher proportion than that observed with the UD group ($p 0.004$).

Of the 18 subjects with persistent dyspeptic symptoms after *H. pylori* eradication, five (27.8%) were positive for anti-Ani s 1. This proportion was higher than that observed among subjects whose symptoms had been relieved (7/60;

11.7%), although the difference was not statistically significant. When this analysis was performed only in the subset of subjects with NUD, anti-Ani s 1 antibodies were significantly more frequent among patients with persistent symptoms than among those who had alleviated symptoms (33.3% vs. 3.7%; $p 0.016$).

Ten patients reported episodes of urticaria/angioedema. These allergy symptoms were more frequent in anti-Ani s 1 IgE-seropositive patients (3/14; 21.4%) than in seronegative patients (7/71; 9.9%), although the difference was not statistically significant ($p 0.357$).

DISCUSSION

The present study was performed with a cohort of dyspeptic patients treated for *H. pylori* infection. The enrolment criteria excluded patients who required an urgent endoscopy in whom anisakiasis is usually diagnosed; in fact, no cases of active parasitism were detected at the time of endoscopy. Nevertheless, the observed rate of seropositivity to Ani s 1 was high, and was similar to that reported in a previous more extensive epidemiological study [11]. The high seroprevalence of Ani s 1 IgE in the present study may be caused by several factors: first, Spain has a high level of fish consumption (85 g/person per day) [19]; second, a high level of fish parasitism of $\geq 45\%$ has been detected, depending on the species examined [20]; and third, the habitual consumption of uncooked fish, especially pickled anchovies, was very high.

Endoscopy and histological findings were associated mainly with *H. pylori* infection, and there were no differences between anti-Ani s 1 IgE-seropositive and -seronegative patients. This is to be expected, as anisakiasis is an infection that is difficult to diagnose and does not produce ulcers or major histological changes [21]. Although intestinal infestation with parasites is a frequent cause of eosinophilic gastroenteritis, no differences in eosinophil cell infiltration between seropositive and seronegative patients were found. However, the number of biopsy samples obtained was limited, and eosinophil cell infiltration is restricted mainly to the site at which the larvae penetrate. Anisakiasis is rarely considered to be a cause of eosinophil gastritis, despite *A. simplex* being a relatively common parasite in fish [1]. In contrast, the total numbers of eosinophils and

Table 2. Comparison of histological findings and blood leukocyte counts (log-transformed values) between anti-Ani s 1 IgE-seropositive and -seronegative patients

	Anti-Ani s 1 IgE-positive $n = 14$	Anti-Ani s 1 IgE-negative $n = 71$	p
Histological findings ^a			
Ulcer	6 (42.9)	31 (43.7)	0.955
Active chronic gastritis	5 (53.7)	32 (45.1)	0.173
Atrophy/metaplasia	3 (21.4)	8 (11.2)	0.379
Blood leukocyte count ^b			
Total leukocytes	3.87 (3.79–3.97)	3.85 (3.76–3.92)	0.092
Neutrophils	3.67 (3.50–3.77)	3.61 (3.51–3.70)	0.14
Lymphocytes	3.41 (3.35–3.47)	3.41 (3.32–3.48)	0.684
Monocytes	2.70 (2.60–2.78)	2.60 (2.48–2.70)	0.084
Eosinophils	2.21 (0.00–2.62)	0.00 (0.00–2.03)	0.027
Basophils	0.05 (0.00–2.02)	0.00 (0.00–0.00)	0.02

^aCategorical data are absolute numbers (%).

^bContinuous variables are median (interquartile range) and comparisons were performed after adjusting for age, gender, tobacco consumption and alcohol intake by linear regression analysis.

basophils in blood were increased significantly in patients seropositive for Ani s 1 after adjusting for variables that can affect the blood leukocyte count. Both eosinophilia and basophilia are common in subjects who suffer from anisakiasis [1,22]. All of these data illustrate the limited value of endoscopy and histological examination in diagnosing many cases of this parasitic infestation, and underline the need to implement serological approaches.

The persistence of dyspeptic symptoms was analysed after successful eradication of *H. pylori* infection. Most patients experienced symptomatic relief; however, as reported previously, the clinical benefit was higher among UD patients than among NUD patients [23–26]. Anti-Ani s 1 IgE was found significantly more frequently among NUD patients showing a lack of improvement after successful eradication of *H. pylori*. It is noteworthy that anisakiasis is a condition that is difficult to diagnose and that ulcer formation is rare [21]. Therefore, the data seem to suggest that many cases of anisakiasis with mild symptoms could be misdiagnosed as NUD, but the results must be interpreted cautiously because of the limited sample size. More studies are needed to link therapeutic intervention with clinical improvement to confirm these results.

Although Ani s 1 is a major allergen of *A. simplex*, no association was found between detection of anti-Ani s 1 IgE and allergy symptoms. In contrast, previous studies have reported the frequent finding of IgE-mediated diseases in patients with anisakiasis [2]. However, this association has been described in individuals with a severe allergic reaction that required urgent endoscopy. These data could suggest that most cases of anisakiasis with mild allergic symptoms remain undiagnosed.

The underlying mechanism of anisakiasis is based on IgE-mediated reactions [27,28]. Thus, the development of disease requires previous sensitisation to the parasite. Individuals seropositive for *A. simplex* in the present study were regular consumers of uncooked fish. Therefore, it can be hypothesised that these seropositive individuals may have had previous episodes of anisakiasis. These episodes would have been indolent, or with mild allergic or gastric symptoms. It is noteworthy that sensitised subjects with repeated exposure to the parasite will be at risk of developing acute anisakiasis with severe symptoms. This

form of anisakiasis represents the tip of the iceberg of the disease and constitutes most cases diagnosed currently.

In conclusion, the results from the present study seem to suggest that, in at least a subset of individuals with *H. pylori* infection, seropositivity to *A. simplex* could have clinical significance. Laboratory findings and an accurate history of fish-eating habits would be helpful in identifying possible episodes of anisakiasis with clinical relevance. These data also highlight the fact that only cases of anisakiasis causing severe allergic or gastric symptoms requiring invasive techniques are currently being diagnosed.

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REFERENCES

1. Ishikura H, Kikuchi K, Nagasawa K *et al.* Anisakidae and anisakidosis. *Prog Clin Parasitol* 1994; **3**: 43–101.
2. López-Serrano MC, Gómez AA, Daschner A *et al.* Gastroallergic anisakiasis: findings in 22 patients. *J Gastroenterol Hepatol* 2000; **15**: 503–506.
3. Sakanari JA, McKerrow JH. Anisakidosis. *Clin Microbiol Rev* 1989; **2**: 278–284.
4. Verhamme MA, Ramboer CH. Anisakidosis caused by herring in vinegar: a little known medical problem. *Gut* 1998; **29**: 843–847.
5. Fernández-Caldas E, Quirce S, Marañón F, Díez-Gómez ML, Gijón-Botella H, López-Román R. Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*. *J Allergy Clin Immunol* 1998; **101**: 554–555.
6. García M, Moneo I, Audicana MT *et al.* The use of IgE immunoblotting as a diagnostic tool in *Anisakis simplex* allergy. *J Allergy Clin Immunol* 1997; **99**: 497–501.
7. Iglesias R, Leiro J, Ubeira FM, Santamarina MT, Navarrete I, Sanmartín ML. Antigenic cross-reactivity in mice between third-stage larvae of *Anisakis simplex* and other nematodes. *Parasitol Res* 1996; **82**: 378–381.
8. Pascual CY, Crespo JF, San Martín S *et al.* Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. *Allergy* 1997; **52**: 514–520.
9. Moneo I, Caballero ML, Gómez F, Ortega E, Alonso MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol* 2000; **106**: 177–182.
10. Caballero ML, Moneo I. Specific IgE determination to Ani s 1, a major allergen from *Anisakis simplex*, is an useful tool for diagnosis. *Ann Allergy Asthma Immunol* 2002; **89**: 74–77.

11. Toro C, Caballero ML, Baquero M *et al*. High prevalence of seropositivity to a major allergen of *Anisakis simplex*, Ani s 1, in dyspeptic patients. *Clin Diagn Lab Immunol* 2004; **11**: 115–118.
12. Barbara L, Camilleri M, Corinaldesi R *et al*. Definition and investigation of dyspepsia. Consensus of an international *ad hoc* Working Party. *Dig Dis Sci* 1989; **34**: 1272–1276.
13. Cheli R, Giacosa A, Bovero E. Clinical significance of duodenal erosions. *Endoscopy* 1984; **16**: 105–108.
14. Gisbert JP, Boixeda D, de Argila CM, Bermejo F, Redondo C, de Rafael L. Erosive duodenitis: prevalence of *Helicobacter pylori* infection and response to eradication therapy with omeprazole plus two antibiotics. *Eur J Gastroenterol Hepatol* 1997; **9**: 957–962.
15. Blum AL, Talley NJ, O'Morain C *et al*. Lack of effect of treating *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. *N Engl J Med* 1998; **39**: 1875–1881.
16. Svedlund J, Sjödin I, Dotevall G. GSRS-A clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988; **33**: 129–134.
17. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161–1181.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–254.
19. Audicana MT, Ansotegui IJ, Fernández de Corres L, Kennedy MW. *Anisakis simplex*: dangerous—dead and alive? *Trends Parasitol* 2002; **18**: 20–25.
20. Adroher FJ, Valero A, Ruiz-Valero J, Iglesias L. Larval anisakids (Nematoda: *Ascaridoidea*) in horse mackerel (*Trachurus trachurus*) from the fish market in Granada, Spain. *Parasitol Res* 1996; **82**: 319–322.
21. Sugimachi K, Inokuchi K, Ooiwa T, Fujino T, Ishii Y. Acute gastric anisakidosis. Analysis of 178 cases. *JAMA* 1985; **253**: 1012–1013.
22. Cocheton JJ, Cabou I, Lecomte I. Anisakiasis and *Anisakis* infections. *Ann Med Interne (Paris)* 1991; **142**: 121–130.
23. Graham DY, Lew GM, Klein PD *et al*. Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. *Ann Intern Med* 1992; **116**: 705–708.
24. Jaakkimainen RL, Boyle E, Tudiver F. Is *Helicobacter pylori* associated with non-ulcer dyspepsia and will eradication improve symptoms? A meta-analysis. *BMJ* 1999; **319**: 1040–1044.
25. Laine L, Schoenfeld P, Fennerty MB. Therapy for *Helicobacter pylori* in patients with nonulcer dyspepsia. A meta-analysis of randomized, controlled trials. *Ann Intern Med* 2001; **134**: 361–369.
26. McColl KE, El-Nujumi A, Murray LS *et al*. Assessment of symptomatic response as predictor of *Helicobacter pylori* status following eradication therapy in patients with ulcer. *Gut* 1998; **42**: 618–622.
27. Asaishi K, Nishino C, Ebata T, Totsuka M, Hayasaka H, Suzuki T. Studies on the etiologic mechanism of anisakiasis. 1. Immunological reactions of digestive tract induced by *Anisakis* larva. *Gastroenterol Jpn* 1980; **15**: 120–127.
28. del Pozo V, Arrieta I, Tunon T *et al*. Immunopathogenesis of human gastrointestinal infection by *Anisakis simplex*. *J Allergy Clin Immunol* 1999; **104**: 637–643.