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***Anisakis simplex* only provokes allergic symptoms when the worm parasitises the gastrointestinal tract**

Received: 7 November 2003 / Accepted: 15 January 2004 / Published online: 18 June 2004
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Abstract We analysed patients with allergic or digestive symptoms after seafood ingestion in order to assess a correct diet in *Anisakis simplex* sensitised individuals. A total of 120 patients who suffered allergic and/or digestive symptoms after marine food ingestion were studied. We performed skin prick tests for *A. simplex* and seafood, total serum and specific serum immunoglobulin E to *A. simplex* in the acute stage and 1 month later. A gastroscopy was carried out to find larvae in those patients with persistent abdominal pain. A challenge with non-infective larvae was performed to assess a correct diet. Some 96 patients were sensitised to *A. simplex*. Gastroscopy was performed in 47 and we detected larvae in 24. We compared symptoms, skin tests, total and specific IgE and the latency of appearance of symptoms in patients positive for *Anisakis* larvae, patients without larvae at gastroscopy and patients without digestive symptoms. There was no difference among the groups. We challenged 22 patients with frozen *A. simplex* larvae. After allowing deep-frozen seafood in the diet for more than 2 years, no patient suffered a reaction. At this time, we allowed all our patients well-frozen seafood without any allergic reaction occurring. Allergic symptoms are the most frequent manifestation of *A. simplex*

parasitism. We could not find any patient allergic to the thermostable proteins of parasite.

Introduction

Anisakis simplex is a nematode that parasitises, in its adult stage, sea mammals. It is included in the Nematoda, Secernentea, Ascaridida, Ascaridina, Ascaridoidea and Anisakidae. In 1960, Van Thiel reported 11 cases of larval anisakiasis in humans with third-stage larvae as the aggressor parasite (Van Thiel 1960; Van Thiel et al. 1962; Cheng 1978; Eugène et al. 1983; Cocheton et al. 1991). The more important intermediary host species are codfish, hake, sardine, anchovy, salmon, red mullet, tuna, mackerel, horse mackerel and squid (Huang 1988; Orecchia et al. 1989; Pereira Buena 1992; Sanmartin et al. 1994).

Human infestation, known as anisakiasis or anisakidosis, occurs accidentally when eating raw or undercooked seafood such as sushi, sashimi, ceviche (Ishikura et al. 1994; Bouree et al. 1995). The luminal form is generally asymptomatic and larvae die and are expelled in vomit or faeces (Kliks 1983).

The acute form is known as gastric or intestinal anisakiasis and is caused by third-stage larvae; after ingestion; the larvae penetrate the host stomach or intestinal wall and can be found in the mucosa or submucosa. This is characterised by epigastric pain, due to a wall reaction to peptidases, nausea and vomiting (Asaishi et al. 1980a; Sugimachi et al. 1985; Kakizoe et al. 1995; Muraoka et al. 1996).

Chronic anisakiasis results from an invasion that causes abscessing or eosinophilic granulomas, with fever eosinophilia diarrhoea and abdominal pain. It can mimic appendicitis, gastroduodenal ulcer, inflammatory bowel disease and intestinal obstruction (Pinkus and Coodlidge 1975; Appleby et al. 1982; Matsui et al. 1985; Kim et al. 1991). Rarely, anisakiasis can affect the lungs, spleen, liver and pancreas (Sakanari and McKerrow

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1989). Arthritis has seldom been described (Fabresse et al. 1984; Areal Vera et al. 1991). Immunoglobulin E and eosinophils have been implicated in the aetiological mechanism of local lesions due to parasite attachment (Kuipers 1964; Asaishi et al. 1980a; Miyazato 1980; Petithory et al. 1986; Akao et al. 1990; Huang et al. 1990; Iglesias et al. 1993). Acute forms may be caused by hypersensitivity reactions in the gastrointestinal wall (Asaishi et al. 1980a, 1980b; Petithory et al. 1986). Occasionally, cutaneous lesions have been reported (Asaishi 1980b; Sugimachi et al. 1985; Sakanari and McKerrow 1989).

Dietary habits make anisakiasis frequent in Japan (Asaishi et al. 1980a; Kliks 1983; Sugimachi et al. 1985; Ishikura et al. 1994; Kakizoe et al. 1995; Bouree et al. 1995; Muraoka et al. 1996). From 1955 to 1968, many cases were reported in Holland (Van Thiel 1960; Van Thiel et al. 1962; Bouree et al. 1995). Other cases have been reported in France, the United Kingdom and the United States from 1975 (Pinkus and Coodlidge 1975; Lucas et al. 1985; Mudry et al. 1986; Bouree et al. 1995). In Spain, until 1996, only a few cases had been reported (Arenal Vera et al. 1991; Mira Gutiérrez et al. 1995; Acebos Rey et al. 1996; Canut Blasco et al. 1996).

In the 1990s, patients with allergic symptoms after the consumption of supposedly "well-cooked" fish were described. These patients had a positive skin prick test and specific IgE to *A. simplex*, and were negative to fish (Kasuya et al. 1990; Audicana et al. 1995a, 1995b, 1995c; Del Pozo et al. 1996; Moreno-Ancillo et al. 1997). These reports showed that the thermostable proteins provoke the hypersensitivity symptoms (Audicana et al. 1995b, 1995c; Del Pozo et al. 1996).

We reported three cases of anaphylaxis with *A. simplex* in the gastric mucosa visualised by a fiberoptic gastroscopy (Alonso et al. 1997), and another dealing with a massive infection in a patient who consulted because of allergic symptoms (Daschner et al. 1997b). Our group has also shown the importance of parasitism on the development of an *A. simplex* allergy (Alonso et al. 1997, 1999; Alonso-Gomez et al. 1997; Daschner et al. 1997a, 1997b, 1999, 2000; Lopez Serrano et al. 2000).

The aim of this article is to confirm whether live parasites are the cause of most allergic reactions due to *A. simplex* ingested with seafood.

Materials and methods

Subjects

Adult patients who attended our emergency room from January to December 1997 with symptoms of allergy (urticaria, angioedema, bronchospasm, and/or anaphylaxis), and/or digestive symptoms (nausea, vomiting, diarrhoea or abdominal pain), and the ingestion of any kind of marine food over the last 36 h, were selected. Their sensitisation to *A. simplex* was determined. The

patients sensitised to *A. simplex* [positive skin prick test (SPT) and/or positive specific IgE] were included in the second phase of the study.

Procedure

Anamnesis

A detailed questionnaire included data about: (1) marine food and way of cooking, and (2) symptoms and latency of symptoms (period of time from ingestion of implicated food to onset of symptoms).

Skin prick tests

SPTs were performed on the underside side of the forearms using 10 mg/ml *A. simplex* (International Pharmaceutical Immunology Laboratory, Madrid, Spain), and the implicated food extract as well as with a selection of fish and shellfish that included hake, anchovy, tuna, sole, prawn, cod and octopus. SPT responses were considered positive with a mean diameter of a least 3×3 mm. Histamine at a 1% concentration and saline solution (0.9% NaCl) were the positive and negative controls, respectively.

Gastroscopy

A fiberoptic gastroscopy was performed on patients with digestive symptoms which persisted for more than 8 h (necessary fasting for an adequate gastric emptiness that allows a good display of the worms).

In vitro tests

Total serum IgE was assessed (the MEIA-IMX-system, Abbot Diagnostics, Chicago, USA), and specific IgE against *A. simplex* and implicated seafood in the reaction was also measured (CAP-system, Pharmacia, Sweden). Both parameters were determined in the emergency room within 24 h of the onset of symptoms, and 1 month later. Specific IgE was considered positive at levels greater than 0.35 kU/l.

Statistics

Statistical analysis was performed using SPSS SV (program SPSS for Windows Student Version 6.0.1). Mean latencies were calculated for each group and compared. Mean values for total serum IgE and specific IgE levels to *A. simplex* in each group were calculated and compared. The *t*-test was used for the comparison of latencies, age and eosinophil counts. Mann-Whitney U-tests and Wilcoxon rank-sum tests were used for a comparison of the total and specific IgE values of each group.

The challenge test

We obtained larvae from blue whiting, selecting worms with a length of more than 2 cm that had been identified by our Microbiology Service as *A. simplex*. These were frozen into caps for more than 48 h at -20°C to obtain non-infective parasites.

The patients included in the study gave their written consent. All patients who developed anaphylaxis were excluded from the challenge. Selected patients took caps containing a placebo, one, four and six larvae with a 1-h interval the same day. The second time, patients with anaphylactic symptoms were also included and they took 20 larvae in this way: placebo, four, six and ten worms.

Results

From 120 patients with allergic/digestive symptoms after seafood ingestion, 96 *A. simplex* sensitised patients were found (Table 1).

The mean age of these patients was 46.6 years (17–85 years). There were 45 males and 51 females. Raw anchovy in vinegar sauce was the implicated seafood in 76 patients, nine had ingested undercooked hake, two raw anchovies, two raw cod and in seven patients the symptoms were related to the ingestion of other cooked fish.

Sixty-seven patients developed epigastric pain, 60 vomiting, 19 diarrhoea and 14 did not develop any abdominal symptoms. Twenty-seven patients developed anaphylaxis, 63 presented with urticaria and angioedema, and six did not have any allergic symptoms.

The mean latency of gastric symptoms was 257 min and that of allergic symptoms was 286 min.

In vivo and in vitro tests

A total of 96 patients had a positive SPT and/or specific IgE to *A. simplex*, and were negative to seafood except for one patient with a positive SPT and IgE to *A. simplex* and octopus.

Gastroscopy

Fibre-optic gastroscopy was performed on 47 patients. One or two *A. simplex* larvae were found in 24 of these 47 patients; in the remaining 23, we could not find any larvae but in several cases indirect signs such as erosion or ulcers, suggesting previous parasite presence, occurred. Forty-nine patients did not fulfil the criteria for gastroscopy.

Primary analysis of the results

We divided sensitised patients into three groups (Table 2): (1) those with positive gastroscopy (G+; finding and removal one or several *A. simplex* larvae), (2) those with negative gastroscopy (G–; no larvae found), and (3) those without gastroscopy (No-G) did not fulfil the criteria for gastroscopy.

Patients with positive gastroscopy

The mean age of the G+ group ($n=24$) was 48 years (17–73 years). There were 14 females and 10 males. Raw anchovy ingestion was implicated in 20 patients while another four had eaten undercooked hake which had, however, supposedly been well cooked.

Epigastric pain was observed in 22 of 24 patients, vomiting in 16 and diarrhoea in eight. The mean latency of digestive symptoms was 186 min, (range: 15 min–8 h). Eleven patients developed urticaria and/or angioedema, nine anaphylaxis and four patients did not develop any allergic symptoms. The mean latency of allergic symptoms was 299 min (range: 15 min–26 h).

Positive SPT to *A. simplex* was found in all these patients. All of them had negative SPTs to the implicated fish and to the marine food on our protocol. The initial geometric mean of the total IgE was 121.5 kU/l (9–1,277 kU/l); 1 month later this was 482.2 kU/l (26–15,258 kU/l). The initial geometric mean of the specific IgE was 21.5 kU/l (2.16–100 kU/l). At 1 month, this was 72.2 kU/l (4.5–100 kU/l). The increase in both values was significant ($P < 0.05$).

Table 1 Patients ($n=96$) sensitised to *Anisakis simplex*

<i>A. simplex</i> sensitised patients	96
Sex (female:male)	51:45
Mean age (years)	46.6 (range 17–85)
Seafood	78 Raw anchovy, 9 hake, 2 raw cod, 6 other
Allergic symptoms	63 Urticaria/angioedema, 27 anaphylaxis, 6 none
Digestive symptoms	67 Epigastric pain, 19 diarrhoea, 60 vomiting, 14 none
Mean allergic latency (min)	286
Mean digestive latency (min)	257
As SPT	Positive in all
Implicated sea-food SPT and IgE	Negative in 95, 1 positive to octopus
Initial geometric mean IgE total (kU/l)	143.6 (9–1,536)
Initial geometric mean specific IgE <i>A. simplex</i> (kU/l)	20.6 (2.14–100)
Day 30 geometric mean total IgE (kU/l)	423.9 (26–15,258)
Day 30 geometric mean specific <i>A. simplex</i> IgE (kU/l)	51.4 (1.4–100)

Table 2 Results for patients in different groups according to gastroscopy

	<i>A. simplex</i> larvae in gastroscopy	No <i>A. simplex</i> larvae in gastroscopy	No gastroscopy
Total	24	23	49
Sex (female:male)	14:10	12:11	25:24
Mean age (years)	48	41.6	50.4
Implicated seafood	20 Raw anchovy, 4 hake	21 Raw anchovy, 1 hake, 1 grilled tuna	37 Raw anchovy, 4 hake, 2 raw cod, 6 other
Allergic symptoms	11 Urticaria/angioedema, 9 anaphylaxis, 4 none	17 Urticaria/angioedema, 5 anaphylaxis, 1 none	35 Urticaria/angioedema, 13 anaphylaxis, 1 none
Digestive symptoms	22 Epigastric pain, 8 diarrhoea, 16 vomiting	15 Epigastric pain, 8 diarrhoea, 18 vomiting, 2 none	30 Epigastric pain, 3 diarrhoea, 26 vomiting, 12 none
Mean allergic latency (min)	299	287	272
Mean digestive latency (min)	186	300	286
<i>A. simplex</i> SPT	All +	All +	All +
Implicated sea-food SPT and IgE	All –	All –	1 (+) octopus
Initial geometric mean IgE total (kU/l)	121.5 (9–1277)	131.2 (24–663)	178.2 (19–1,536)
Initial geometric mean specific IgE <i>A. simplex</i> (kU/l)	21.5 (2.16–100)	16.3 (2.16–100)	24 (2.14–100)
Day 30 geometric mean total IgE (kU/l)	482.2 (26–15,258)	374.6 (48–1,426)	415 (53–3,853)
Day 30 geometric mean specific <i>A. simplex</i> IgE (kU/l)	72.2 (4.5–100)	43.8 (4.44–100)	38.4 (1.4–100)

Patients with negative gastroscopy

The G– group ($n=23$) included patients without any larvae detected by gastroscopy. The mean age of this group was 41.6 years (19–64 years). There were 12 females and 11 males. Raw anchovy ingestion was implicated in 21 patients while another patient had eaten undercooked hake and another grilled tuna, both of which were supposedly well cooked.

Digestive symptoms included epigastric pain in 15 patients, vomiting in 18 and diarrhoea in eight; two patients did not develop any digestive symptoms. The mean latency of digestive symptoms was 300 min (30–270 minutes). Seventeen patients developed urticaria and/or angioedema, five presented with anaphylaxis and one patient did not develop any allergic symptoms. The mean latency of allergic symptoms was 287 min (30–720 min). Positive SPT to *A. simplex* was seen in all patients. All had negative SPTs to the implicated fish and to the marine food on the protocol. The initial geometric mean of the total IgE was 131.2 kU/l (24–663 kU/l); 1 month later it was 374.6 kU/l (48–1,426 kU/l). The initial geometric mean of the specific IgE to *A. simplex* was 6.3 kU/l (2.16–100 kU/l) and 1 month later it was 43.8 kU/l (4.44–100 kU/l). The increase in both was significant ($P<0.05$).

Sensitised patients without gastroscopy

The mean age of the No-G group ($n=49$) was 50.4 years (25–85 years). There were 25 females and 24 males. Raw anchovy ingestion was implicated in 37 patients, four

had eaten undercooked hake, two raw cod, and six had eaten different fish (five supposedly well-cooked and one frozen hake). Epigastric pain was present in 30 patients, vomiting in 26, diarrhoea in three, and 12 patients did not develop any digestive symptoms. The mean latency of such symptoms was 287 min (3–1,440 min). A total of 35 patients developed urticaria and/or angioedema, 13 anaphylaxis, and one patient did not develop any allergic symptoms. The mean latency of allergic symptoms was 272 min (3–1,440 min). Positive SPT to *A. simplex* was seen in all patients. Forty-eight had negative SPTs to the implicated fish and to the marine food on the protocol. One patient was sensitised to octopus; he had an allergic reaction after the ingestion of octopus. In this case we think that his *A. simplex* sensitisation depended on another previous contact. The initial geometric mean of the total IgE was 178.2 kU/l (19–1,536 kU/l); 1 month later it was 415 kU/l (53–3,853 kU/l). The initial geometric mean of the specific IgE was 24 kU/l (2.14–100 kU/l) and 1 month later it was 38.4 kU/l (1.4–100 kU/l). The increase in both was significant ($P<0.05$).

Why did we have to carry out a challenge?

There were no differences between sex, age, latency of symptoms, and the geometric mean of total IgE and specific IgE to *A. simplex* when we compared the G+ and G– groups. There was a significant increase in the in vitro parameters in both groups 1 month later. The two groups were therefore statistically very similar and were pooled. The No-G group did not differ from this

pooled group. In some patients the parasite was in contact with the digestive mucosa long enough to induce symptoms, but not long enough to be detected.

Perhaps *A. simplex* had no clinical implications for some patients in the No-G group on initial contact, which would explain the lower increase of specific IgE. This sensitisation may only show a previous contact with *A. simplex*.

After analysing all data, we realised that: (1) involvement with raw or undercooked fish was common (89 from 96 patients), (2) parasitism was frequently confirmed, (3) fish was an important component in our patients' diet, and (4) there were few allergic reactions in spite of the large number of parasitised fish in our markets.

Therefore, we challenged our patients with non-infective larvae of *A. simplex* as a way to determine whether an allergy to thermostable proteins was present (Alonso et al. 1999).

Non-infective *A. simplex* challenge results

In a first stage, five patients from the G+ group gave their written informed consent for challenge. The non-infective frozen larva challenge was negative. All patients had been eating well-frozen fish for 2 years without any symptoms (Alonso et al. 1999).

In a second stage, 17 patients, two from the G+ group, five from the G- group and ten from the No-G group, were challenged. Four patients had suffered anaphylaxis and 13 urticaria and/or angioedema. All of these challenges were also negative, and all of these patients had been eating well-frozen fish for 2 years without any trouble.

Of 96 sensitised patients, all challenged patients ($n=22$) tolerated frozen *A. simplex* well.

Dietary assessment

After analysis of the challenge results and the clinical picture in most patients, we allowed the rest of our patients to eat well-frozen fish. We contacted all patients 1 year later to determine fish ingestion and the existence of new reactions. A total of 76 indicated that they ate no raw fish, seven were afraid to eat any kind of fish, and 13 were not available for comment.

Discussion

Gastro-allergic anisakiasis (GAA) has been reported as an entity in which acute parasitism by *A. simplex* is accompanied by an IgE-mediated systemic allergic reaction. Diagnosis includes a detailed medical history, fibre-optic gastroscopy, SPT, IgE to *A. simplex* and fish implicated in the reaction, and sometimes a challenge

test (Alonso et al. 1997, 1999; Daschner et al. 1997a, 1997b, 1998, 1999, 2000; Alonso-Gomez 1997; López Serrano 2000).

The clinical picture in many patients can be confused, so that the initial approach to the allergic reaction has to be particularly exhaustive. Allergic symptoms are the main reason for consulting at the emergency room. Symptoms can vary from a slight upper body erythema to anaphylactic shock with cardiac-respiratory arrest; but the most frequent symptom is erythema with urticaria/angioedema. Abdominal symptoms were considered unimportant and data could only be collected after directed inquiry. Nausea, vomiting and epigastric pain are the most frequent digestive symptoms.

The time interval between the ingestion of infected fish and the onset of symptoms distinguishes this disease from another food allergies. Usually, in IgE-mediated food allergies, 1 h or less is time enough to develop symptoms. In GAA, the mean latency of allergic symptoms was 286 min with a range of 3–1,560 min. This is more than 24 h, and is perhaps related to the time during which excretory-secretory proteins are released from the live larvae. This long interval complicates the diagnosis if anamnesis is delayed.

Raw anchovy in vinegar sauce (a typical Spanish raw fish speciality) and hake (insufficiently cooked in northern Spain) are the most frequently involved foods in this disease in Spain. However, other raw seafood specialities can be involved in other countries, such as sushi in Japan.

If a patient suffered an allergic reaction after the ingestion of a well-cooked hake and we detected a worm in his stomach, there were two main possibilities: (1) this hake was not as well cooked as the patient thought, and (2) he had previously eaten an aperitif with raw anchovy in vinegar sauce or some other raw marine food that he had forgotten to tell us about.

In the emergency room, there is another important diagnostic and therapeutic tool: gastroscopy. If epigastric pain persists for long enough, this technique must be used to find and remove the worm that would lead to the diagnosis of parasitism and, at the same time, to provide the treatment, as the symptoms disappear when the nematode is removed. Sometimes no worm is seen using gastroscopy, but indirect findings were usual in group G-, such as erosions that suggested previous parasitism.

SPT to *A. simplex* was usually positive. In our experience, it is a safe and useful technique, and no patient of ours has developed systemic symptoms with cutaneous tests.

We found a significant increase in the total and specific IgE to *A. simplex* between days 0 and 30. We previously postulated that this is due to acute parasitism (Daschner et al. 1999). The specific IgE response does not necessarily mean an allergic response, and seems to be typical to parasitism, as demonstrated by animal experimentation. The initial amount of total or specific IgE is not a predictor of allergic symptoms and does not correlate with clinical features.

Thinking only of thermostable proteins, the existence of sensitised patients without allergic symptoms has made some authors look at the possibility of cross-reactivity or a false positive result, sometimes employing more expensive in vitro methods (Kasuya et al. 1990; Audicana et al. 1995a, 1995b, 1995c; Del Pozo et al. 1996; Lorenzo et al. 2000); however, and without completely ruling out a possible sporadic false positive, our experience points to an IgE-mediated response in sensitised patients, mainly corresponding to previous contact (transitory parasitism in the luminal form or asymptomatic) with live parasites.

Despite the possibility of employing in vitro tests, as in other types of food allergy, we consider the challenge test as a diagnostic method (Williams and Bock 1999). If any doubt exists, challenge is the gold standard diagnostically because it allows us to advise the eating of well-frozen or well-cooked fish to patients to whom we had previously forbidden all seafood. This diet is less restrictive and easier to maintain than unnecessary total prohibition (Alonso et al. 1999).

Statistical comparisons among patients allowed us to postulate that they were all similar independently of group. In some, the parasite attaches to the stomach mucosa long enough to cause the allergic reaction (G—and some patients in the No-G group), but its tendency to come off does not allow it to be detected using gastroscopy.

The immunological response to a parasite could explain most of the signs and symptoms in anisakiasis. This nematode uses proteases to fix itself within the gastrointestinal mucosa, where local factors, such as previous mucosal lesions, gastrointestinal motility and pH, influence it in its fixation capacity, duration, and even the possibility of penetrating the digestive wall. Once a worm is fixed in the mucosa, it activates a protein secretion that starts the defensive response of rich lymphoid digestive tissue. The anti-parasite response is predominantly of the Th2 type. The pattern of released cytokines, such as IL4 and IL5, provokes eosinophil recruitment and hyperplasia of the mastoid intestinal tissue, stimulates the production of IgE, and therefore increases the total and specific IgE for the parasite (Kuipers 1964; Asaishi et al. 1980a; Miyazato 1980; Petithory et al. 1986; Akao et al. 1990; Huang et al. 1990; Iglesias et al. 1993; Daschner et al. 1999). The major allergen of *A. simplex*, Ani s1, which is only present in the excretory gland, could be important in the development of hypersensitivity symptoms (Moneo et al. 2000).

If the response occurs in a local framework, it would appear as an acute gastric anisakiasis. Moreover, the immune response can promote systemic IgE-mediated hypersensitivity symptoms, which appear very frequently, i.e. GAA.

The best treatment for anisakiasis is prophylaxis, i.e. avoiding raw or undercooked fish. The rates of parasitism in fish are very important (Huang 1988; Orecchia et al. 1989; Pereira Buena 1992; Sanmartin et al. 1994). The prophylactic measures suggested by the European

Union, which include the obligatory freezing of every fish that is to be ingested raw at a temperature lower than -20°C for 24–48 h, have drastically decreased the incidence of anisakiasis in many Europe countries. However, in Mediterranean Europe, restaurants and cooks have not been well informed about this regulation.

Challenge tests and tolerance to well-frozen seafood in all patients confirm our initial suspicion. Our aim is to progress from clinical investigation to clinical practice. A diet without any kind of raw seafood is safe for *A. simplex* sensitised patients. If an allergy specialist has any doubts, or if symptoms are severe or there is a clear relationship with well-cooked or deep-frozen seafood without seafood hypersensitivity, a challenge with non-infective *A. simplex* will provide us with the recommended diet.

Our experience supports the hypothesis that *A. simplex* only provokes allergic symptoms when the worm parasitises the gastrointestinal tract.

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