Original Article

Anisakis simplex Allergy After Eating Chicken Meat

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Abstract. *Background:* Allergic reactions to food can be produced by contaminants that induce sensitization. Among these, *Anisakis simplex* can cause seafood infestation, and allergic symptoms (urticaria–angioedema, anaphylaxis, and asthma) can follow the eating or handling of affected fish. Although seafood is the principal source of human infections by this parasite, we have found allergic symptoms in 8 patients previously diagnosed as having *A simplex* sensitization after they ate chicken meat. Chicken feed usually has a high proportion of fishmeal, which might possibly be contaminated by this nematode.
Objective: The aim of our study was to determine whether parasite proteins present in chicken meat could be responsible for the symptoms reported by these subjects.
Methods: We carried out in vivo tests (prick, bronchial challenge, and double-blind placebo-controlled challenge with meat chicken) in these 8 patients. We performed immunoblotting using the sera from the 8 patients and controls in order to detect *A simplex* sensitization. We also investigated the presence of *A simplex* proteins in sera from chickens fed with fishmeal and in other sera from chickens fed only with cereals. We excluded sensitization to other chicken nematodes by serologic methods.
Results: All 8 patients presented positive prick and challenges to *A simplex*. When we used serum from chickens fed with fishmeal as the antigen in blotting, patients 3, 4, 5, 6, 7, and 8 recognized a band of 16 kd, also obtained when using pools of fish–shellfish and *A simplex* larva. No detection was observed with sera from chickens fed with only cereals.
Conclusion: We provide evidence, based on in vivo and in vitro tests, that subjects highly sensitized to *A simplex* can detect the presence of *Anisakis* species allergens in chicken meat.

Key words: *Anisakis simplex.* Allergy to fish, fishmeal, chicken, nematodes.

Resumen. *Antecedentes:* Las reacciones alérgicas a alimentos pueden producirse por contaminantes de los mismos que inducen sensibilización tras su ingesta. Entre ellos, el *Anisakis simplex* puede infestar alimentos marinos y puede causar síntomas alérgicos (urticaria, angioedema, anafilaxia y asma) tras comer o manejar pescados. Aunque los animales marinos son la mayor fuente de infestación en el humano, hemos encontrado 8 pacientes previamente diagnosticados de sensibilización a *A simplex* que tuvieron síntomas tras comer carne de pollo. La dieta de los pollos puede contener gran proporción de harinas de pescados que podrían haber sido contaminados por este parásito.
Objetivo: El objetivo de nuestro estudio fue verificar la posibilidad de que proteínas procedentes de este nematodo fueran responsables de los síntomas sufridos por nuestros pacientes.
Métodos: Realizamos pruebas in vivo (prick, provocación bronquial, provocación oral doble ciego controlada con placebo con carne de pollo) en estos 8 pacientes. También immunoblotting utilizando los sueros de nuestros 8 pacientes y controles para detectar alérgenos de *A simplex*. También investigamos la presencia de proteínas de AS en el suero de pollos alimentados con harinas de pescado y pollos alimentados sólo con cereales. La sensibilización a otros nematodos se excluyó por métodos serológicos.
Resultados: Los 8 pacientes presentaron pruebas cutáneas y provocaciones positivas a *A simplex*. Cuando utilizamos sueros de pollos cebados con harina de pescado como alérgenos en el blotting, los pacientes 3, 4, 5, 6, 7, y 8 reconocieron una banda de 16 kda, que también se obtuvo utilizando pools de pacientes alérgicos a marisco y larva de *A simplex*. No se observó detección con los sueros de pollo alimentados sólo con cereales.
Conclusión: Los pacientes con alto grado de sensibilización a *A simplex* pueden detectar alérgenos de *Anisakis* en la carne de pollo.

Palabras clave: *Anisakis simple.* Alergia al pescado, pollo, nematodos.

Introduction

Anisakis simplex, or herring worm; *Pseudoterranova decipiens*, or cod/seal worm, formerly known as *Phocanema decipiens* or *Terranova decipiens*; *Contracaecum* species; and *Hysterothylacium* species, formerly *Thynnascaris* species, all belong to the Anisakidae family. They are nematodes (roundworms) that have been implicated in human infections after the intake of raw or undercooked seafood [1]. Anisakiasis is the term generally used when referring to the acute disease in humans. Seafood is the principal source of human infection by the larvae and its incidence is expected to increase [2].

The prevalence of allergy to *A simplex* in Valladolid, Spain, is notable. Over the last year 231 patients have been diagnosed of sensitivity to this worm based on reported symptoms, prick tests with *A simplex* and specific IgE radioimmunoassay. Of these patients, 46 (20%) presented radioallergosorbent test class 5-6 to *A simplex*. We detected IgE against low molecular weight *A simplex* antigens in 4 fishmongers with asthma after they handled fish (2 also presented contact urticaria if the fish was contaminated with *A simplex*) [3], and in 8 patients previously diagnosed as having *A simplex* sensitization after they ate chicken meat. In the first series the larvae might have been alive and might have sensitized the patients by excretory allergens but that would not have occurred in the other 8 cases, as 2 of them were chicken breeders who usually handled fishmeal in poultry diets. Prick tests, IgE antibodies against chicken meat and double-blind, placebo-controlled food challenges ruled out sensitization to chicken meat. Nevertheless, their symptoms disappeared after the elimination of chicken from their diet. This raised the possibility of sensitization due to the manipulation or intake of chicken meat contaminated with *Anisakis* species proteins from the fishmeal used for feeding chicken. Fishmeal is recognized by animal nutritionists as an excellent source of proteins and is included in commercial diets for poultry, dairy cattle, mink, and fish. Fishmeal is made principally from the offal of fish processed in factory ships by cooking, pressing, drying and grinding the fish [4].

Cutaneous primary sensitization to protein food allergens is likely to occur when the food is handled for cooking. Furthermore protein contact dermatitis caused by *Anisakis* species has been demonstrated [5] and recent studies indicate that persons who handle fish (fishermen/fishmongers and similar) are a population at risk for *A simplex* sensitization [6]. In the case of chicken meat ingestion, a highly sensitized subject might react to the presence of parasite allergens in the event that these allergens pass through the digestive barrier of the chicken and resist the cooking process. Caballero et al [7] recently reported that several allergens from *A simplex* are highly resistant to heat and pepsin treatments [7], and Moneo et al [8] have isolated a heat-resistant allergen from this parasite.

Another possibility is that these patients detected other allergens with cross-reactivity with *A simplex*. Cross-reactivity between cockroach, Chironomidae and *A simplex* has been found [9] and the molecular cloning and characterization of an IgE-reactive protein from *A simplex* (Ani s 1) found that the recombinant protein showed a strong similarity to nematode troponins [10]. Chickens can be parasited by other nematodes (*Ascaridia galli, Heterakis gallinae*) [11] and our patients might have detected them after handling or eating the chicken meat due to cross-reactivity with *A simplex*. In order to explore the possibility of parasite proteins able to produce reactions being present in chicken meat we carried out the present study.

Methods

Patients

We reviewed the data of the 231 patients diagnosed with *A simplex* allergy in the last year. All patients were asked about their habits (contact with and ingestion of fish) and history of allergic reactions (rhinoconjunctivitis, asthma, urticaria–angioedema and anaphylaxis) associated or not with exposure or ingestion of fish. To patients with minor symptoms, we recommended that all fish and shellfish intended for raw consumption (or semi-raw intake, such as when marinated or partly cooked) be blast frozen to −35ºC or below for 15 hours, or be regularly frozen to −20ºC or below for 7 days, following the recommendations of the Food & Drug Administration of the United States of America (www.fda.gov). To patients with marked sensitization (radioallergosorbent test results more than class 4) or severe symptoms, we recommended avoidance of fish and seafood in the diet. Eight patients who had totally avoided fish suffered symptoms again; thus their response was not related to eating fish but rather to the intake of chicken meat (rare chicken steak in all cases). The diagnosis of *A simplex* allergy in these 8 patients was based on medical history, prick tests, and high serum levels of specific *A simplex* IgE antibodies. The data obtained from these patients can be seen in the table. Patient 1 was a fishmonger and patient 2 worked in a poultry feed factory. Patient 3 worked as a butcher and suffered from contact urticaria after handling pig and chicken meat. Two patients (4 and 7, a man and a woman) were chicken breeders; both suffered from asthma after the inhalation of poultry feed based on fishmeal and urticaria–angioedema related to eating or handling fish. Two patients, 5 and 6, were cooks and only 1 patient, number 8, was not clearly a handler of chicken meat or fishmeal.

Test Series

Prick Tests

Skin prick tests were performed with a standard panel of inhalant and food allergens and also with *A simplex*

(IPI Laboratories, Madrid, Spain), fish extracts (anchovy, hake, mackerel, red mullet, cod, haddock), cockroach, chicken, and pig meat (Bial-Aristegui Laboratories, Bilbao, Spain). Histamine and saline solutions were used as positive and negative controls, respectively.

Specific Serum IgE Determinations

Tests for specific IgE to *A simplex* were performed by radioimmunoassay using a commercial preparation (CAP System, Pharmacia Diagnostics, Uppsala, Sweden). Results higher than 0.7 kU/L were considered positive.

Specific IgE to *A simplex* in chicken serum was determined in the same way (CAP system, Pharmacia Diagnostics) after obtaining information about the similarity between human and chicken IgE from a veterinarian specialized in poultry. We selected 2 sera pools, one from chickens fed with fishmeal and the other from chickens fed naturally with cereals. We also determined IgE against *Ascaris lumbricoides* and *Taenia equinococcus* (CAP System, Pharmacia Diagnostics) both in patients and in chicken sera.

Double-Blind Placebo-Controlled Food Challenge

All 8 patients gave signed informed consent to undergoing a double-blind placebo-controlled food challenge with chicken meat. We followed recommended methods [13]. One patient consented to a challenge with meat from a chicken that presented *A simplex* IgE in its serum. We used aqueous raw meat extract in doses of 20 mg/mL in lemon juice. These doses were doubled every 2 hours. Symptoms were monitored until 48 hours after the challenge.

Bronchial Challenge

Bronchial challenge with *A simplex* extract was performed in patients suffering from asthma as recommended [3]. An aqueous extract of *A simplex* was prepared from frozen larvae. The initial dose was the dilution that had produced a 3-mm wheal by prick testing.

Allergen Extracts

Whole body *A simplex* extract was prepared from the third larval stage of parasites obtained from hake bought at a local fish market. After zoological identification the larvae were homogenated in liquid nitrogen for 5 minutes with a pestle. The powder was resuspended with phosphate-buffered saline (10% wt/vol). After 4 hours of extraction in a cool room, the mixture was centrifuged at 15 000*g* for 30 minutes at 4ºC. The supernatant was dialyzed (Spectrapor, Spectrum Medical Industries, Los Angeles, California, USA; molecular weight cutoff, >3.5 kd) at 4ºC for 24 hours against 10 mmol/L of glycine, centrifuged at 30 000*g* for 30 minutes and passed through a 0.45-mm Millipore filter (Millipore, Bedford, Massachusetts, USA). The material was lyophilized and the protein content was determined by the Lowry method [12].

Secretor *Anisakis* species allergen was kindly donated by Prof. Carmen Cuellar (Pharmacy School Universidad Complutense, Madrid). Pooled sera from chickens raised with natural grain diets and sera from chickens fed with standard fishmeal were also used as the antigen and samples for immunoblotting.

In brief, following the procedure as previously described [9], after electrophoresis proteins were transferred to a PDVF (Millipore) membrane and incubated with sera of each of the 8 patients (lanes 1-8), sera of chickens fed with cereals (lane 9) sera of chicken fed with fishmeal (lane 10), pooled sera of patients allergic to seafood (lane 11), pooled sera of patients allergic just to fish (lane 12) and, pooled sera of patients parasited with *Anisakis* species (lane 13). The blotted allergens were also incubated with a pool of sera from all 8 patients (lane 14) and a negative pool of sera of patients sensitized to pollen (lane 15).

Results

All patients presented positive results on prick tests and had elevated serum levels of IgE to *A simplex*. The bronchial challenge tests were positive in patients who suffered from asthma and the food challenge was positive in a patient who also presented angioedema. The results are summarized in the table.

The immunoblotting results (Figure 1) revealed very light bands in the low molecular weight zone and 2 bands over 60 kd in lanes 13 and 14 when sera from chickens fed only with cereals were used as antigen (1 mg/mL), (A1). In contrast, when we used sera from chickens fed with fishmeal as the antigen for blotting (A2), for patients 3, 4, 5, 6, 7, and 8, a band was recognized over 16 kd, as for the sera from chickens feeding with fish flour and pools of fish-shellfish and *A simplex* larva allergic patients.

In the second immunoblotting (Figure 2) with *A simplex* body extract at a concentration of 1 mg/mL (B1) all patients (our 8 patients and pools from patients allergic to fish, shellfish and *A simplex* larva) recognized bands over 60 kd. Patients 2 and 3 had similar patterns with higher intensity in the band of 92 kd. The 2 chicken sera had different patterns with very light bands at 62 kd levels in the case of cereal feeding sera similar to individual patients, whereas the bands recognized by fishmeal feeding chicken sera were similar to those recognized by sera from shellfish allergic patients. Different bands were also recognized by the 3 other sera pools, from fish allergic patients (lane 12), *A simplex* larva sensitized patients (lane 13), and our 8 patients (lane 14).

When the antigen in blotting was the secretor *A simplex* antigen (Figure 2, B2) the sera from patients sensitized to *A simplex* larva (lane 13) recognized more bands (30, 45 and 60 kd). Patients 1 and 3 had a light recognition of one band around 60 kd (patient 1) and 45

Characteristics, Clinical Data, and Diagnostic Test Results*

Patient	Sex	Age, y	Site	Job	Symptoms	CAP AS kU/L	Wheal, AS Prick Test, mm	Wheal, Chicken Prick Test, mm	Other Allergen	Challenge Tests	Symptoms After Avoiding Chicken and Fish in Diet
1	F	52	Urban	Fm	U, A	47.7	12 × 18	–	–	Not done	Improved
2	M	26	Rural	Fw	RC, asthma	32.4	5 × 5	–	–	Bronchial +	Improved
3	M	49	Urban	B	U, A	24.7	10 × 10	–	Cereals	Not done	Improved
4	F	61	Urban	CB	Asthma	12.1	10 × 10	–	Mites	Bronchial +	Improved
5	F	69	Urban	C	U	3.74	6 × 6	–	–	Not done	Improved
6	F	53	Urban	C	U, A	18.4	10 × 10	–	–	Not done	Improved
7	M	54	Rural	CB	U, asthma	>100	12 × 16	–	Mites	Bronchial +	Improved
8	M	20	Urban	S	A	57.3	5 × 5	–	Egg	Food +	Improved

*AS indicates *Anisakis simplex*; F, female; M, male; Fm, fishmonger; Fw, animal feed factory worker; B, butcher; CB, chicken-breeder; S, student; C, cook; U, urticaria; A, angioedema; RC, rhinoconjunctivitis.

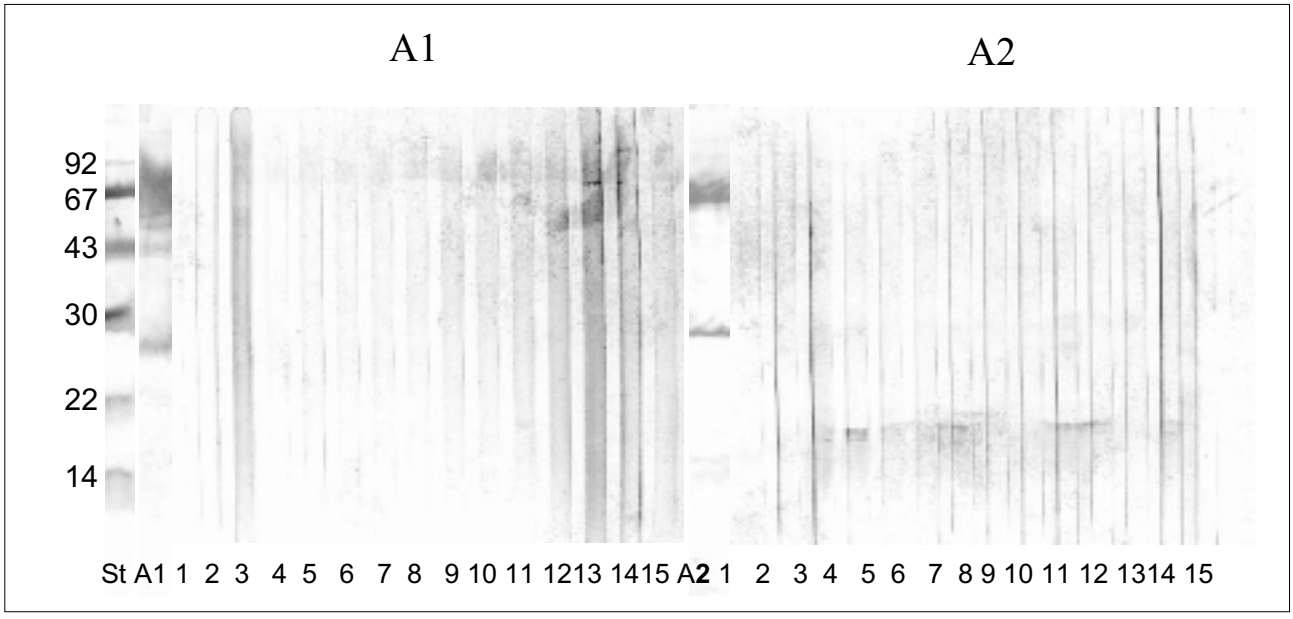

Figure 1. A1—Cereal-fed chicken sera used as antigen:1 to 8, patients' sera; 9, cereal-fed chicken sera; 10, fishmeal-fed chicken sera; 11, pooled sera from seafood-allergic patients; 12, pooled sera from fish-allergic patients; 13, pooled sera from patients parasited with *Anisakis simplex*; 15, pool of sera from 8 patients (1-8);15, negative pool.
A2—Fishmeal-fed chicken as antigen for blotting: 1 to 8, patients' sera; 9, cereal-fed chicken sera; 10, fishmeal-fed chicken sera; 11, pooled sera from seafood-allergic patients; 12, pooled sera from fish-allergic patients; 13, pooled sera from patients parasited with *A simplex*; 15, pool of sera from 8 patients (1-8);15, negative pool.

kd (patient 3). Sera from the other patients (2, 4, 5, 6, 7, and 8) did not recognize bands, indicating that these patients were not parasited. Sera pools from patients allergic to fish and shellfish did not recognize *A simplex* antigen either, since these patients were not parasited.

Discussion

The first reports regarding problems with *A simplex* infestation date back to 1960. Since then, many cases have been reported from several countries [8] and new manifestations of this disease have recently been described: rheumatic symptoms [14], contact dermatitis [5], Crohn's disease and eosinophilic gastroenteritis [15,16], conjunctivitis [17], and asthma [3,18].

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 [6, 16]. In 1998 we described 2 patients who suffered from occupational asthma with exposure to

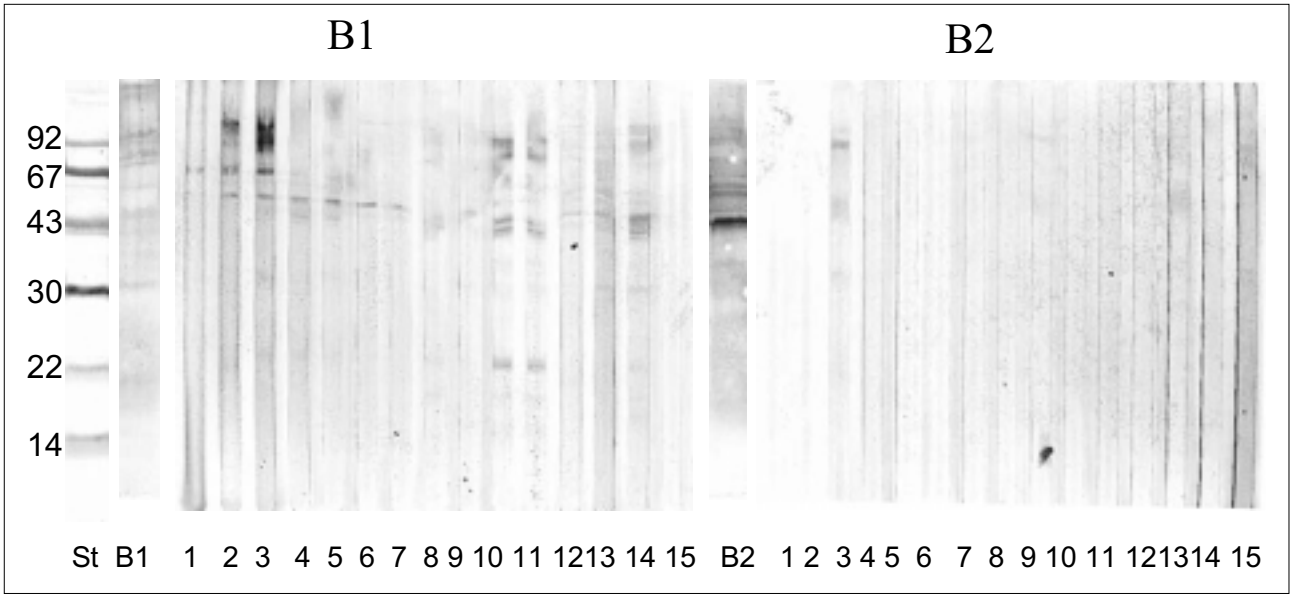

Figure 2. B1—*Anisakis simplex* full body as antigen for blotting. 1 to 8, patients' sera; 9, cereal fed chicken sera; 10, fishmeal-fed chicken sera; 11, pooled sera from seafood-allergic patients; 12, pooled sera from fish-allergic patients; 13, pooled sera from patients parasited with *A simplex*; 15, pool of sera from 8 patients (1-8);15, negative pool.
B2—*A simplex* secretor as antigen for blotting. 1 to 8, patients' sera; 9, cereal-fed chicken sera; 10, fishmeal-fed chicken sera; 11, pooled sera from seafood-allergic patients; 12, pooled sera from fish-allergic patients; 13, pooled sera from patients parasited with *A simplex*; 15, pool of sera from 8 patients (1-8);15, negative pool.

A simplex after handling fish (a fishmonger) and fishmeal (a chicken breeder) [3]. We found that the sera from both patients had IgE against multiple bands of *A simplex* extract. Interestingly, their IgE recognized a band of low molecular weight when incubated with fishmeal extract. By that time, sera from patients with urticaria and anaphylaxis had been found reactive to *A simplex* proteins around 42 kd, but this response was also present with samples from asymptomatic blood donors. Moneo et al [8] described the isolation of a major allergen from the fish parasite *A simplex*. They concluded that *A simplex* contains a potent allergen of 24 kd (now named Ani s 1] in the excretory gland, that could have important clinical relevance in patients who suffer from urticaria–angioedema after fish ingestion. Previous reports have indicated that sera from truly sensitized patients recognize multiple allergens of the crude extract similarly to the recognition observed in atopic patients when tested with common allergens [7]. Añibarro and Seoane [17] reported that symptomatic patients tolerate the ingestion of dead larvae and stated that "the patients are probably sensitized to other *A simplex* antigens, such as the secretor antigens". The same authors, after a double-blind, placebo-controlled food challenge with *A simplex* lyophilized larva and antigen intake, did not obtain a clinical response, but 10 of the 11 patients challenged showed positive conjunctival challenge test results. These findings suggest that different clinical responses depend on the route of sensitization or a loss of allergenic power of lyophilized larvae. Recently, it has also been reported that symptomatic patients suffering from gastroallergic anisakiasis tolerated the ingestion of dead larvae, an observation that led the authors to state that these patients are probably sensitized to secretor *A simplex* antigens [16]. Other authors have also claimed that *A simplex* sensitized patients tolerate ingestion of dead larvae [21], whereas others believe that cooking and freezing may not protect against allergenic reactions to ingested *A simplex* antigens in humans [22]. It has been proposed that heat-and/or pepsin-resistant allergens from *A simplex* could explain reactions and symptoms after ingestion of well-cooked or canned fish in which the larvae are dead [7, 8]. Our results are consistent with the last studies because our patients' sensitization seems to have occurred through the handling or ingestion of chicken meat contaminated with *A simplex* proteins. Fishmeal is a powder that will average between 60% and 70% protein that can be stable to heating and drying processes [4]. Animals can also be parasited by *A simplex*, as observed in cats that were fed with fishmeal [20] and this may also be possible in chickens, as we have shown.

In summary, here we provide evidence, based on in vivo and in vitro tests, that highly *A simplex* sensitized subjects can detect the presence of parasite allergens in rare chicken meat.

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