

ORIGINAL ARTICLE

Occupational Asthma Due to Grain Pests *Eurygaster* and *Ephestia*

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ABSTRACT

Background: Workers occupationally exposed to grain dust have a high prevalence of asthma. The pathogenesis of their respiratory symptoms remains obscure when sensitization to cereal allergens has not been proved. Given the ubiquity of arthropods in stored vegetable products, we have studied the allergenic potential of two very prevalent grain pests, *Eurygaster* and *Ephestia*, as a cause of occupational asthma. We have also studied the allergenic relationship between *Anisakis simplex* (AS) and these pests. **Methods:** We selected 15 asthmatic workers exposed to cereal dust, in whom sensitization to cereal allergens was not clear. As controls, we selected a patient who suffered from anaphylaxis after the ingestion of cereals, 6 patients sensitized to different arthropods, 1 patient who suffered from asthma after inhaling fish flour contaminated with AS, and a pool of 40 asthmatic patients with different etiologies not due to arthropods or cereals. We performed prick tests with these pests, AS, and pure and parasitized flours, bronchial challenges, specific IgE determination, and RAST inhibition. **Results:** All of the 15 cereal-exposed workers were sensitized to *Eurygaster* and *Ephestia*. Only 2 had detectable levels of serum IgE to these pests, but 13 presented a positive prick test result and IgE to AS without problems after eating fish. Bronchial challenges were positive to *Eurygaster* in 7 patients and to *Ephestia* in 2 patients. Twelve patients had positive prick tests to parasitized flour but not to pure flour. The patient with cereal anaphylaxis presented sensitization to both flours but not to AS. In the control group, the patient who suffered from asthma after the inhalation of AS-parasitized fish flour presented a positive prick test result and high levels of specific IgE to these pests. The RAST inhibition showed cross-reactivity between *Eurygaster* and AS in this patient, but the inhibition was not as evident with the sera of other workers. The same tests in the group of 40 asthmatic patients were negative. **Conclusions:** The clinical importance of pests as a cause of baker's asthma should not be underestimated. Sensitization to *Eurygaster* and *Ephestia* could be important in the

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asthma episodes suffered by our patients, and the parasitized wheat was found to have a higher allergenic potential than pure cereal flour.

Key Words: Cereal pests; *Eurygaster*; *Ephestia*; Baker's asthma; *Anisakis simplex*.

INTRODUCTION

Workers occupationally exposed to grain dust have a high prevalence of respiratory symptoms (1–4). Grain dust contains biological contaminants, including mold spores, pollens, and arthropods. The most prominent allergenic components involved in cereal allergy are salt-soluble proteins, and several allergens associated with flour allergy have recently been purified. All of them exhibit inhibitory activities against arthropod alpha-amylases and trypsin (4). Nevertheless, the pathogenesis of respiratory symptoms in grains workers and bakers remains obscure when sensitization to cereal flour has not been proved.

Allergy to storage mites in the form of bronchial asthma and allergic rhinoconjunctivitis has been reported among farmers, bakers, and grain elevator workers. The most common storage mites involved belong to the family Glycyphagidae (1). Recent publications based on the analysis of farm dust and clinical parameters have pointed out that *Lepidoglyphus destructor* (Ld) is the most important storage mite causing symptoms involving both the upper and lower respiratory tract among farmers in Europe (5). Johansson et al. (5) also found a cross-reactivity between Ld and *Dermatophagoides pteronyssinus*. Our data regarding mite infestation in samples of cereal flour and clinical data are consistent with those of the above-mentioned authors (3).

Numerous reports of occupational allergy have been attributed to members of the Coleoptera order. *Tenebrio molitor* is a member of the coleoptera insect order, which includes beetles and weevils. Mealworms are *Tenebrio molitor* larvae that normally infest stored grain. In addition, they are widely used as fishing bait. Bernstein et al. (6) demonstrated that inhaled particulates from *Tenebrio* exoskeletons are potent sensitizers and elicit IgE-mediated occupational asthma. Schroeckenstein et al. (7) described a subject with occupational allergy to *T. molitor*. By means of immunoblotting techniques, they studied the allergens involved in the patient's immunological response and confirmed the fact that beetles of the Tenebroid family are potentially allergenic for workers exposed to grains or grain products. Immunological cross-reactivity between *T. molitor* and *Alphitobius diaperinus*, as

well as a slight cross-reactivity with blowfly, was shown by RAST inhibition (6). On the other hand, a barley flour inhibitor of *Tenebrio molitor* alpha-amylase is a major allergen associated with baker's asthma disease (8).

We performed in 1997 an epidemiological analysis of the sensitization to these stored grain pests on 4379 patients residing in an area of cereal industries (3). The questionnaire consisted of questions relating to rhinoconjunctivitis, coughing, wheezing and shortness of breath, allergic and occupational precipitants, contact with cereal, animal fodder, observation of insects in flour or cereals by the patient, length of exposure, and other epidemiological data. The presence of specific IgE antibodies to the extracts was demonstrated by prick tests and RAST. The association between respiratory symptoms and occupational exposure was confirmed by bronchial challenge tests. The prevalence of sensitization to storage mites among mite-sensitive patients was 11.88%. Among the cereal workers, the most frequent sensitization was that to *D. pteronyssinus* (58%), followed by *D. farinae* (48%), *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* (38%), *Blomia kulagini* (34%), and *Acarus siro* and *Chortoglyphus arcuatus* (24%). In addition, 22% of these patients presented negative prick tests and RAST for *Dermatophagoides* species and positive test results to storage mites. Fifty percent of the workers were sensitized to *Tenebrio molitor* and 36% to *Blatta orientalis*.

The identification of mites, tenebroids, and cockroaches in dust samples yielded useful data for the diagnosis of our patients. These results, together with the aforementioned studies, confirm the fact that cereal pests are potential allergens for workers exposed to grains or grain products. A surprising finding that we observed in an important number of these workers was the sensitization to the fish parasite *Anisakis simplex* (AS), without showing any symptoms related to fish ingestion. Very recently, Johansson et al. (9) demonstrated cross-reactivity between storage mite allergens and AS. This finding, together with our previously mentioned studies, prompted us to analyze the allergenic potential of two very prevalent cereal pests in our area: *Eurygaster* and *Ephestia*. We also investigated a possible allergenic cross-reactivity between AS and these two parasites.



MATERIALS AND METHODS

Analysis of *Eurygaster* and *Ephestia* Pests in Cereal Dust

We analyzed the stored grain pests in 20 samples of cereal (*Triticum aestivum*) from different storage areas (bakeries, farms, silos, and stables). Samples were collected from dust on or near the floor and placed in clean plastic bags. Identification of pests was performed in accordance with previous publications (3), using the keys of Vodal (1949), Stichel (1957–62), Wagner (1960), and Kis (1984). Live and dead arthropods were counted under a binocular magnifying glass. The most predominant species was *Eurygaster austriaca* (Schränk, 1778), subspecies *E. austriaca seabrai*, family Pentatomidae, and order Heteroptera (China, 1933) (Fig. 1). In *Ephestia* (family Phycitidae, order Lepidoptera), larvae of these moths were found in samples of flours used to bake cookies.

Extract Preparation

For in vivo studies, 15 live *Eurygaster austriaca* were frozen and immediately macerated in 10 mL of 0.9% NaCl. Afterward, they were centrifuged for 2 min at 900 g; and the supernatant was filtered through a 0.22- μ m filter. Serial dilutions were prepared for use in prick test titration. The same method was used with *Ephestia*, but only 30 larvae and no adult specimens were included. In *Anisakis*, the extract was obtained as indicated (11).

For in vitro analysis, *Eurygaster* insect bodies and *Ephestia* and *Anisakis* larvae were extracted in

phosphate-buffered saline (PBS) at 5% w/v with a Potter. After centrifuging, the supernatant was dialyzed and sterilized through a 0.22- μ m-pore-diameter membrane. Protein concentration was determined by the method of Lowry. The extracts were stored at -18°C until use.

Pure and parasitized wheat flours, provided by the ETS Ingeniería Agrícola from Palencia, Spain, were extracted by using the same method except that magnetic stirring instead of a Potter was used.

Selection of Patients

Clinical data and serum samples were obtained from farmers who had previously participated in an epidemiological survey (3). We selected 15 asthmatic workers exposed to cereal dust in whom sensitization to cereal allergens was not clear (previous bronchial challenge tests with pure cereal flours were negative). As controls, we selected 1 patient who suffered from anaphylaxis after the ingestion of cereals, 6 patients sensitized to different arthropods (moth, flea, apis venom, mosquito, horsefly, and spider), 1 patient who suffered from asthma after inhaling fish flour contaminated with AS, and a pool of 40 asthmatic patients with different etiologies.

All participants gave their consent, and the procedure was approved by the local ethics committee.

Skin Prick Tests

Skin prick tests were performed not only with aforementioned extracts but also with a standard panel of allergenic extracts and putative suspected occupational allergens, such as flours and their additives (wheat, barley, rye, oat, gluten, alpha-gliadin, alpha-amylase, and soybean lecithin) provided by LETI laboratories (Barcelona, Spain); pollens (wheat, barley, rye, oat, and other grasses), molds (*Aspergillus fumigatus*, *Alternaria tenuis*, *Cladosporium herbarum*, and *Ustilago* spp.), and animal danders (dog, cat, horse, cow, rat, and pig) from ALK-Abello laboratories (Madrid, Spain); mites (*D. pteronyssinus*, *D. farinae*, *Euroglyphus maynei*, *Tyrophagus putrescentiae*, *Acarus siro*, *Glycyphagus domesticus*, *Lepidoglyphus destructor*, *Blomia kulagini*, *Gohiera fusca*, and *Chortoglyphus arquatus*) provided by BIAL-Aristegui Laboratories (Bilbao, Spain). The extract of *Anisakis simplex* was provided by IPI Laboratories (Madrid, Spain). After 15 minutes, the wheals obtained were measured after tracing their outline on adhesive paper.

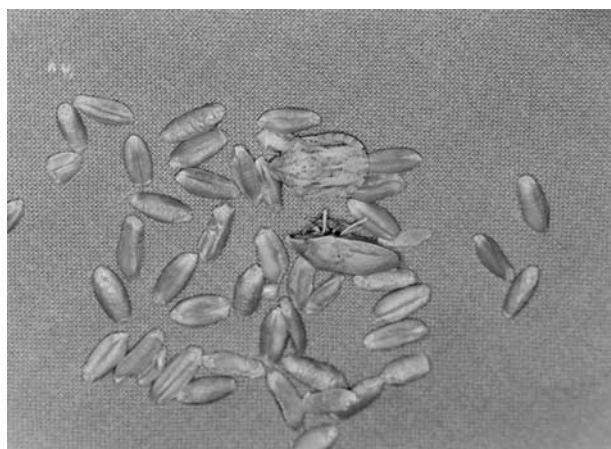


Figure 1. *Eurygaster* and parasitised grains.



Bronchial Challenge Tests

They were only performed in patients 1, 3, 5, 6, 7, 10, 12, and 13 who gave us their consent to perform these challenges. Patients 1, 5, 6, 10, and 12 presented sensitization to parasitized cereal, and these tests would be very useful to elucidate if allergens from the parasite or from the grain were the cause of their asthma. These tests were conducted with cereal flour and with *Eurygaster* and *Ephestia* by using a technique previously described. (2) The initial dose was

the dilution producing a 3-mm-diameter wheal by prick testing.

Specific IgE Determination

For the evaluation of specific IgE to *Eurygaster*, *Ephestia*, and to pure and parasitized wheat flours, paper discs were activated with BrCN and later sensitized with the extracts (protein concentration about 1 mg/mL), as described by Ceska et al. (12) The discs were incubated with 50 μ L of the patients'

Table 1. Clinical data of patients and controls.

No.	Sex	Age	Job	Symptoms	Species with positive prick results (diameters in mm) and specific IgE in kU/L
Patients					
1	M	24	Farmer	RA	Pt (2 $\times$ 2) 7.15; Fa (2 $\times$ 2) 2.95; Wheat (5 $\times$ 5) 0; Barley (5 $\times$ 5) 1.46; Rye (5 $\times$ 5) 0.
2	M	55	Farmer	A	Pt (8 $\times$ 8) 4.6; Fa (12 $\times$ 9) 1.51, Ld (10 $\times$ 4) 0.74; Gly (10 $\times$ 10) 3.4; Wheat (1 $\times$ 1) 0; Barley (5 $\times$ 5) 0;
3	M	44	Baker	A	Wheat (3 $\times$ 3) 0, Barley (2 $\times$ 2) 1.5, Soy (10 $\times$ 10) 2.7
4	M	40	Farmer	RA	Chen (3 $\times$ 3) 3.3; Olea (3 $\times$ 3) 2.3; Wheat (5 $\times$ 5) 0; Barley (5 $\times$ 5) 0, Rye (5 $\times$ 5) 0.
5	M	37	Baker	A	Wheat (10 $\times$ 10) 1.7; Barley (5 $\times$ 5) 0; Rye (5 $\times$ 5) 0.
6	M	35	Carpen	A	Mustard (10 $\times$ 10) 3.8; Wheat (5 $\times$ 5) 0, Barley (10 $\times$ 10) 0; Rye (5 $\times$ 5) 0.
7	M	59	Farmer	RA	Pt (10 $\times$ 10) 5.7; Fa (10 $\times$ 10) 6.2; Wheat (10 $\times$ 10) 0, Rye (6 $\times$ 6) 5.7, Barley (6 $\times$ 6) 0.
8	M	71	Farmer	A	Pt (10 $\times$ 10) 2.8; Wheat (2 $\times$ 2) 0, Barley (10 $\times$ 10) 0, Rye (5 $\times$ 5) 0.
9	M	38	Farmer	RA	Pt (10 $\times$ 10) 6.7, Fa (10 $\times$ 10) 2.3, Wheat (5 $\times$ 5) 0
10	M	58	Farmer	A	A.siro (5 $\times$ 5) 4.9; Tyr (5 $\times$ 5) 4.2; Wheat (5 $\times$ 5) 1.07, Rye (3 $\times$ 3) 0
11	M	37	Farmer	RA	Lolium (10 $\times$ 10), Wheat (3 $\times$ 2) 0, Rye 3 $\times$ 3 (1.03); Barley (3 $\times$ 2) 2.02.
12	F	30	Baker	RA	Lolium (6 $\times$ 10) 25.7; Wheat (6 $\times$ 6) 0, Barley (6 $\times$ 2) 0.6
13	F	55	Farmer	A	Wheat (10 $\times$ 10) 6.05; Barley (10 $\times$ 10) 0.
14	M	43	Baker	A	Wheat (4 $\times$ 4) 0; Barley (3 $\times$ 3) 0
15	M	29	Farmer	RA	Pt (5 $\times$ 5) 0, Fa (5 $\times$ 5) 0, Lolium (5 $\times$ 5) 1.19
Controls					
16	M	25	Student	Ana	Wheat (10 $\times$ 10) class 3; Rye (10 $\times$ 10) class 3
17	M	2	Farmer	A	Moth (10 $\times$ 10)
18	F	12	Student	A	Flea (10 $\times$ 10)
19	M	34	Beekeeper	Ana	Apis venom (10 $\times$ 10)
20	M	57	Poultry-feed	A	Anisakis (10 $\times$ 10)
21	F	18	Student	Ana	Mosquito
22	M	28	Student	Ana	Horsefly
23	M	12	Student	Ana	Spider
24	Pool	20–60	No farmers	A	Different causes except asthma due to arthropods or cereals

Abbreviations: Sex: M, male; F, female. Symptoms: A, asthma; RA, rhinitis asthma; Ana, anaphylaxis. Pricks: PT, Pteronyssinus; Fa, Farine; A. siro, Acarus siro. Chen, Chenopodium.



sera and later with $\approx 100,000$ cpm of ^{125} [anti-IgE monoclonal antibody HE-2. (13). As a reference, we used discs sensitized with *Lolium perenne* and 4 dilutions from a "pool" of sera from grass-allergic patients, which had previously been calibrated against the Pharmacia Phadebas[®] RAST system.

IgE antibodies to the other allergens were measured with the Pharmacia CAP System (CAP FEIA, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions. Results >0.35 kU/L were considered positive.

RAST Inhibition

RAST inhibition experiments were performed with *Eurygaster/Anisakis*-sensitized discs, which were incubated with 50 μ L of patient serum ($1/2$ – $1/4$ dilution) and 50 μ L of inhibitor extract at different protein concentrations (750–3 μ g/mL) and later on with $\approx 100,000$ cpm of ^{125} I-anti-IgE monoclonal antibody HE-2.

RESULTS

The patients' clinical data and the results of prick tests, bronchial challenges, and specific IgE can be seen in Tables 1 and 2. All of the 15 cereal-exposed workers presented cutaneous sensitization to *Eurygaster* and *Ephesia* (Table 2). Two (numbers 10 and 13) had detectable levels of IgE, and seven had positive bronchial challenges to these pests (7 to *Eurygaster* and 2 also to *Ephesia*). Fourteen of the 15 patients presented positive prick results and IgE to AS, and 13 positive IgE without experiencing any problem after eating fish. The patient suffering from asthma due to AS-parasitized fish flour (number 20) had high levels of IgE to *Eurygaster* and *Ephesia*, and bronchial challenge tests with these pests and AS were positive. None of the 40 control patients showed AS or pest sensitization.

Seven of the 15 exposed patients under study presented sensitization to mites, and 14 patients showed a positive prick test with commercial cereal flours. However, only two patients had detectable

Table 2. Diagnostic tests performed in patients and controls.

No.	Prick (mm) and IgE, kU/L, to <i>Ephesia</i>	Prick (mm) and IgE, kU/L, to <i>Eurygaster</i>	Prick (mm) and IgE, kU/L, to pure wheat flour	Prick (mm) and IgE, kU/L, to parasitized wheat flour	Prick (mm) IgE, kU/L, to <i>Anisakis</i>	BCT <i>Ephesia</i>	BCT <i>Eurygaster</i>
1	(3 $\times$ 3)0	(3 $\times$ 3)0	Neg, 0	(5 $\times$ 5)0	(5 $\times$ 5)3.24	Neg	Neg
2	(3 $\times$ 3)0	(5 $\times$ 5)0	Neg, 0	Neg, 0	(3 $\times$ 3)1.14	ND	ND
3	(2 $\times$ 2)0	(10 $\times$ 5)0	Neg, 0	Neg, 0	(2 $\times$ 2)0	Neg	Pos
4	(2 $\times$ 3)0	(3 $\times$ 5)0	Neg, 0	Neg, 0	(5 $\times$ 5)0.41	ND	ND
5	(2 $\times$ 2)0	(12 $\times$ 16)0	Neg, 0	(10 $\times$ 10)1.9	(6 $\times$ 6)13.7	Neg	Pos
6	Neg, 0	(10 $\times$ 10)0	Neg, 0	(10 $\times$ 10)0.4	(3 $\times$ 3)2.4	Neg	Pos
7	(2 $\times$ 2)0	(10 $\times$ 10)0	Neg, 0	(10 $\times$ 10)0	(5 $\times$ 5)3.88	Neg	Pos
8	(2 $\times$ 3)0	(10 $\times$ 10)0	Neg, 0	(8 $\times$ 8)0	(5 $\times$ 5)2.8	ND	ND
9	(3 $\times$ 3)0	(10 $\times$ 10)0	Neg, 0	(5 $\times$ 5)0	(3 $\times$ 3)0	ND	ND
10	(10 $\times$ 10)4.4	(5 $\times$ 5)2.1	Neg, 0.9	(10 $\times$ 10)1.1	(10 $\times$ 10)42	Pos	Pos
11	(3 $\times$ 8)0	(8 $\times$ 8)0	Neg, 0	(8 $\times$ 10)0	(3 $\times$ 3)51	ND	ND
12	(3 $\times$ 5)0	(6 $\times$ 6)0	Neg, 0	(6 $\times$ 6)0.5	(6 $\times$ 6)28	Neg	Pos
13	(10 $\times$ 10)0.7	(2 $\times$ 2)4.8	(2 $\times$ 2)6.2	(10 $\times$ 10)6.8	(3 $\times$ 3)3.9	Pos	Pos
14	(2 $\times$ 2)0	(6 $\times$ 6)0	Neg, 0	(4 $\times$ 4)0	(2 $\times$ 2)1.9	ND	ND
15	(5 $\times$ 5)0	(5 $\times$ 5)0	Neg, 0	(5 $\times$ 5)0	(18 $\times$ 8)59.7	ND	ND
16	(2 $\times$ 3)0	(10 $\times$ 10)9.2	(3 $\times$ 3)15.6	(10 $\times$ 10)14.8	Neg, 0	ND	ND
17	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg	Neg
18	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND
19	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND
20	(10 $\times$ 10) >17.5	(10 $\times$ 10) >17.5	Neg, 0	(8 $\times$ 8)19.6	(10 $\times$ 10)100	Pos	Pos
21	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND
22	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND
23	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND
24	Neg, 0	Neg, 0	Neg, 0	Neg, 0	Neg, 0	ND	ND

Abbreviations: BCT, bronchial challenge test; Neg, negative result; Pos, positive result; ND, not done.

levels of specific IgE to pure wheat flour, and none presented a positive bronchial challenge tests with pure cereal flours. Besides, five presented specific IgE to parasitized wheat flour and positive bronchial challenge with these cereal posts.

The patient who suffered from anaphylaxis after cereal ingestion (number 16) demonstrated high levels of IgE (RAST class 4) to parasitized wheat flour and also pure wheat flour. The same high level of specific IgE to parasitized wheat flour was seen in the patient with asthma due to inhalation of parasitized fish flour (number 20). In the other control patients, sensitization to parasitized wheat flour was not demonstrated.

The RAST inhibition experiments showed cross-reactivity between *Eurygaster* and AS in the patient sensitized to fish flour (Fig. 2), but the inhibition was not as evident with the sera of other workers (Fig. 3).

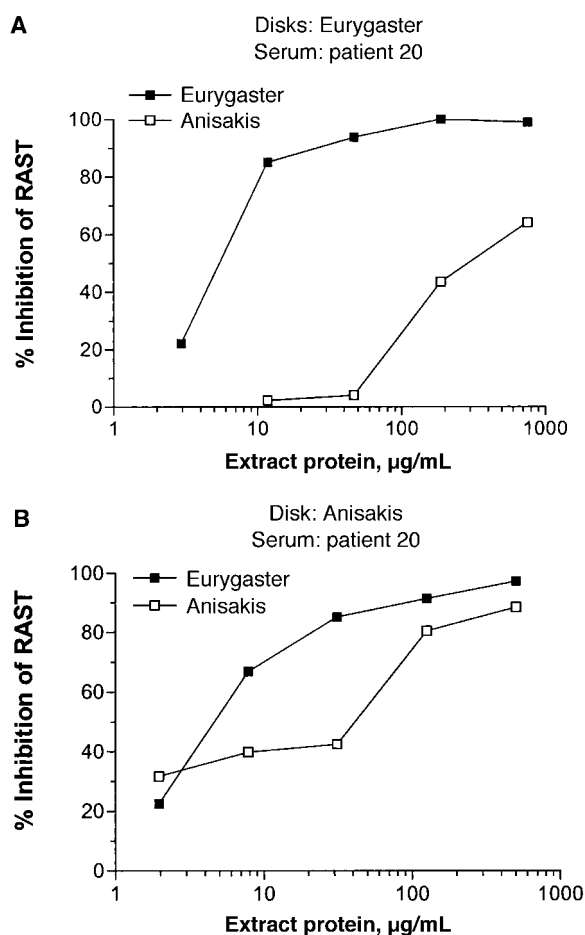


Figure 2. RAST inhibition with *Eurygaster* on the solid phase (A) or *Anisakis* (B) and with serum of patient number 20.

DISCUSSION

Given the ubiquity of arthropods in stored vegetable products, it comes as a bit of a surprise that there are so few reports in the medical literature on allergy to pests as inhalants, compared with the many studies dedicated to other allergens. It seems important, therefore, to evaluate its prevalence in a more numerous series of patients and to study not only the distribution of arthropods and their relationship with clinical symptoms but also the nature of their potential as occupational allergens (14). The clinical relevance of two very prevalent pests in our area, *Eurygaster* and *Ephestia*, as a cause of hypersensitivity reactions, was the primary aim of our study. We also investigated a possible allergenic cross-reactivity between AS and these two parasites.

Eurygaster, subspecies *Austriaca austriaca*, family Pentatomidae, order Heteroptera, which has more than 5000 species, is a stinkbug characterized by its foul-smelling secretions (Fig. 1). Preharvest infestation by *Eurygaster* causes serious damage to stored wheat. Bug-infested wheat contains protease enzymes, which degrade gluten proteins (10). *Ephestia* or *Anagasta*, family Phycitidae, order Lepidoptera, is a cosmopolitan pest of cereal products and other stored foods (Fig. 2). The larvae cannot live on pure starch because they require vitamins A and B. They spin a web in flour, grain, or seeds. These pests appear between May and August in our country.

Anisakis simplex can act as a food allergen and induce IgE-mediated reactions (urticaria, angioedema, and anaphylactic reactions). We described two cases of occupational asthma after inhalation of contaminated fish flour (11). One of these patients (a chicken breeder) was one of the controls in our study (number 20). IgE antibodies to AS have been detected with a high frequency among individuals without symptoms, including blood donors and atopic children (15). We have found a surprisingly high incidence of sensitization (38%) in a series of 134 farmers and bakers (data not published). Nevertheless, these patients did not experience symptoms related to fish ingestion. Cross-reactivity with other arthropods may be a possible explanation. Antigenic cross-reactivity between *A. simplex* and nematodes is well known (16), as well as between AS and arthropods (17). The studies conducted by Johansson et al. (9) showed allergenic cross-reactivity among *Lepidoglyphus*, *Tyrophagus*, *Acarus siro*, and *Anisakis* and revealed that 20% of dust mite-sensitized farmers had IgE to AS. We found an important association between sensitization to AS and *Eurygaster* and *Ephestia*. Considering this and the



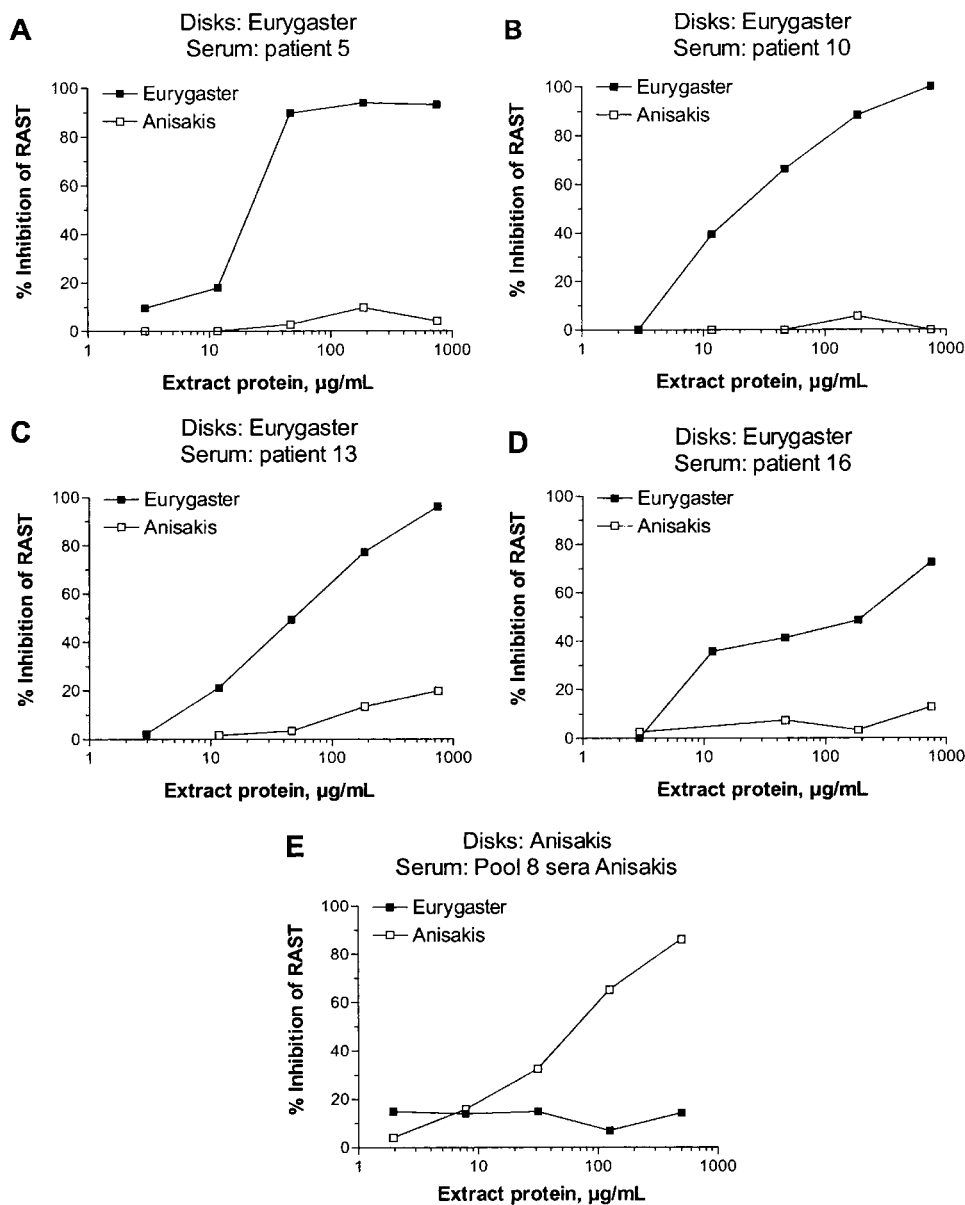


Figure 3. A–D: RAST inhibition with *Eurygaster* on the solid phase and with serum of patients number 5, 10, 13, and 16. E: RAST inhibition with *Anisakis* on the solid phase and a pool of 8 sera of patients highly sensitised to *Anisakis*.

fact that of the 40 asthmatic control subjects none of them were positive to AS, we can state that there is an association between exposure to parasitized cereal grains and sensitization to AS. In this regard, it is worth pointing out that the control patient with asthma after inhalation of fish flour contaminated with AS had high levels of specific IgE to *Eurygaster* and *Ephestia*. The results of RAST inhibition showed allergenic cross-reactivity between AS and *Eurygaster* in this patient. The poor inhibition found in the other cases

deserves further analysis. The cross-reactivity between AS and cereal pests might be the cause of sensitization to AS, not related to fish ingestion, in cereal workers.

Our results also suggest that sensitization to the cereal pests *Eurygaster* and *Ephestia* was important in the asthmatic symptoms of our patients and that the parasitized wheat had a higher allergenic potential than pure (non-parasitized) cereal flour. In this sense, the major cereal allergens responsible for baker's asthma are alpha-amylase and trypsin inhibitors, which carry a



single glycan, containing both α 1 $\rightarrow$ 3fucose and β 1 $\rightarrow$ 2-xylose, and these residues are key epitopes responsible for cross-allergenicity among unrelated glycoproteins from vegetables and arthropods (18). In addition, an interaction between proteins from cereal grains and pest proteins might be a source of neoantigens or a way of enhancing their allergenicity. Major wheat and rye allergens associated with baker's asthma have been identified as specific inhibitors of *D. pteronyssinus* alpha-amylase (Der p 4). The most active inhibitors were the wheat tetrameric allergen and rye the dimeric RDAI-1. These data suggest that interaction between mite and cereal allergens (alpha-amylase/inhibitor complexes) might occur in house dust mite-infested flours (19).

There are few studies in which the manipulations of food sources contaminated with mites are described as a possible cause of occupational asthma. Nevertheless, cases of anaphylaxis developed after eating food contaminated by storage mites has been reported (20). In one of the control patients with anaphylaxis, we found high levels of IgE to *Eurygaster*. Thus, the possibility of anaphylaxis due to the ingestion of flours contaminated by this parasite should be taken into account.

We have only found two studies on the allergenicity of *Ephestia* (21,22) and none on occupational asthma due to inhalation of *Eurygaster*, despite the prevalence of these pests in cereals. Perhaps the reasons for the relative lack of interest in arthropod allergy include the difficulty of collecting live arthropods for preparing extracts useful for diagnosis. The use of biologically standardized extracts of stored grain pests should improve the accuracy of prick testing in the diagnosis of allergic respiratory disease. In fact, we found skin prick testing to be the most sensitive diagnostic criteria.

In summary, we consider that the clinical importance of pest as a cause of baker's asthma should not be underestimated.

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