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# Occupational rhinitis and asthma due to crickets

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**Background:** Insects may cause airborne hypersensitivity reactions. However, few reports exist on specific allergy to crickets.

**Objective:** To report a case of occupational rhinitis and bronchial asthma in a cricket farm worker.

**Methods:** A 28-year-old woman developed rhinitis and bronchial asthma related to her job in a farm where she was exposed to crickets: *Gryllus campestris*, *Gryllus bimaculatus*, and *Acheta domestica*. Extracts were prepared from whole and crushed bodies and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Skin prick tests, specific IgE assays (enzyme allergosorbent test [EAST], immunoblotting, EAST inhibition assays), serial peak expiratory flow monitoring at work, and specific (*A domestica*) and nonspecific bronchial challenge tests were performed.

**Results:** Skin prick test results were positive for *G campestris*, *G bimaculatus*, and *A domestica*. Levels of specific IgE were 2.9, 2.4, and 5.4 kU/L, respectively. The total IgE level was 131 kU/L. Serial peak expiratory flow monitoring at work was consistent with occupational asthma. The result of a bronchial challenge test with *A domestica* was positive with a dual response and elicited an increase in nonspecific bronchial hyperresponsiveness. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting revealed a similar pattern of IgE-binding bands with the 3 cricket extracts (bands of 78 and 64 kDa appeared in nonreducing conditions, whereas bands of 107 to 80, 58, and 52 kDa appeared in reducing conditions). None of these bands was detected by control sera. EAST inhibition studies showed a high degree of cross-reactivity among the 3 species.

**Conclusion:** Crickets are responsible for occupational rhinitis and asthma by an IgE mechanism. Cross-reactivity among the crickets tested in our study was found.

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## INTRODUCTION

Inhalation of insect particles may cause hypersensitivity symptoms, such as rhinitis, conjunctivitis, and asthma. Most cases are due to occupational exposure in laboratories (eg, entomology, biology).<sup>1</sup> Several reports have been made of respiratory allergy to Orthoptera: locusts (*Locusta migratoria* and *Schistocerca gregaria*)<sup>2,3</sup> and grasshoppers (*Melanoplus sanguinipes*).<sup>4</sup> However, few reports have been made on specific allergy to crickets. We have found only 4 reports on cricket hypersensitivity.<sup>5–8</sup> Patients in these reports were laboratory personnel who bred crickets as food for frogs or people who used crickets as fish bait. In all cases, patients developed symptoms of rhinitis and bronchial asthma.

Crickets (family Gryllidae) are arthropods that belong to the Orthoptera order and insect class. Allergic (IgE antibody-mediated) reactions to arthropods may be induced by primary sensitization to major allergens or by cross-reactivity among certain insect species. In this study, we report a case of occupational rhinitis and bronchial asthma in a cricket farm worker.

## METHODS

### Case Report

A 28-year-old, white woman was referred to our clinic with the following symptoms: perennial itching; stuffy nose and

sneezing, which she had for 2 years; and dyspnea, cough, and wheezing, which she had for 1 year. She related the symptoms to exposure to several types of crickets bred on a farm where she had been working for the last 3 years: *Gryllus campestris*, *Gryllus bimaculatus*, and *Acheta domestica*. She was asymptomatic during long holidays but not on weekends (because her work included weekends). She denied exposure to pets. She had been treated with antihistamines, inhaled long-acting bronchodilators, and steroids, but her symptoms did not change.

### Extracts

Extracts were prepared with the 3 species of crickets bred on the farm and with their food. The insects were killed by freezing, and 2 types of protein extraction were performed: one with whole bodies and another with crushed ones (bodies were crushed in a mortar after freezing with liquid nitrogen). The extraction was performed in phosphate-buffered saline at 1/10 (wt/vol) for 2 hours. The samples were centrifuged during 30 minutes at 12,000g, dialyzed against distilled water, and finally sterilized through a 0.22- $\mu$ m-pore-diameter membrane.

### Cutaneous Tests

Skin prick tests (SPTs) were performed in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology Subcommittee on Skin Tests.<sup>9</sup> Tests were performed on extracts of *G campestris*, *G bimaculatus*, and *A domestica* (whole and crushed), the food for the insects, as well as a standard series of aeroallergens, including mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Acaro siro*, *Lepidoglyphus destructor*, *Ty-*

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*rophagus putrescentiae*, and *Glyciphagus domesticus*), pollens, fungi, dander, and flour and green house insects (*Tenebrio molitor*, *Sitophilus granarius*, *Ephestia* sp, *Tribolium confusum*, and *Tetranychus urticae*). Ten control subjects were also tested with the cricket extracts.

#### Lung Function Tests

Flow volume spirometry and prebronchodilator and postbronchodilator tests were performed. To evaluate work-related asthma, peak expiratory flow (PEF) values were monitored 4 to 5 times a day (including measurements performed before, during, and after work) during a 2-week working period.

#### Nonspecific Bronchial Hyperreactivity to Histamine

The nonspecific bronchial hyperreactivity test was performed in accordance with the approach used by Cockcroft et al,<sup>10</sup> with some modifications. The aerosolized particles were generated by a continuous pressurized nebulizer model De Vilbiss 646 with an output of 0.28 mL/min. The result was expressed as the provocative concentration of histamine causing a 20% decrease in forced expiratory volume in 1 second (FEV<sub>1</sub>) (PC<sub>20</sub>). According to our laboratory standards, a PC<sub>20</sub> of more than 16 mg/mL was considered to be within normal limits, whereas PC<sub>20</sub> values of 16 to 1 mg/mL, 1 to 0.125 mg/mL, and less than 0.125 mg/mL indicated mild, moderate, and severe bronchial hyperresponsiveness, respectively. Nonspecific bronchial hyperreactivity was evaluated 24 hours before and after specific bronchial challenge testing (BCT).

#### Specific BCT

Specific BCT was performed with an *A domestica* complete-body extract starting with a concentration of 0.03 mg/mL. A control challenge with solvent (0.9% sodium chloride, 0.03% human serum albumin) was performed before allergen challenge. The patient inhaled the aerosolized allergen in increasing concentrations for 2 minutes at tidal volume. Spirometry was performed 10 minutes after each inhalation and 10, 15, and 30 minutes later after a positive test result (decrease of 15% of FEV<sub>1</sub> or development of respiratory symptoms). To evaluate the late bronchial response, the patient was instructed to perform PEF measurements each hour for 8 hours and a final measurement after 12 hours.

#### Specific IgE Determination

The EAST method was used to measure total IgE and specific IgE to the 3 species of crickets and to *Blattella germanica*, *Periplaneta americana*, grasshopper (*Locusta migratoria*), *Drosophila melanogaster*, fire bug (*Pirrhocoris aptera*), prawn, squid, mussel, *Ascaris* sp, *Echinococcus* sp, *Anisakis simplex*, *L destructor*, *T putrescentiae*, *Sitophilus granarius*, *Ephestia* sp, rye, barley, and oat. The same method was used to determine the specific IgE levels to purified recombinant tropomyosin from *D pteronyssinus* and to purified natural tropomyosin from *P americana*, snail (*Helix aspersa*), pine processionary caterpillar (*Thaumetopoea pityocampa*), and prawn (*Penaeus* sp). Briefly, a solid phase was obtained by coupling the extracts solution (10 mg/mL) to the 6-mm-

diameter cyanogen bromide (CNBr)-activated paper disks, and EAST was performed in accordance with the manufacturer's instructions (specific IgE EIA kit; HYTEC; HYCOR Biomedical Ltd, Edinburgh, Scotland).

#### Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a 12.5% gel according to Laemmli,<sup>11</sup> and samples were prepared under reducing (by adding  $\beta$ -mercaptoethanol) and nonreducing conditions. Separated protein bands were electrophoretically transferred to polyvinylidene fluoride and blocked with 5% defatted dry milk in 0.1% Tween-20, Tris-buffered saline for 1 hour at room temperature. Membranes were incubated overnight at 4°C with the patient's serum followed by antihuman IgE–horseradish peroxidase conjugate incubation. Specific IgE was detected by a chemoluminescence method as recommended by the manufacturer (ECL-Plus; Amersham Pharmacia Biotech, Uppsala, Sweden).

#### EAST Inhibition Assays

To evaluate cross-reactivity among the 3 species of crickets and among crickets and other arthropods, the patient's serum was first incubated with the cricket extracts or with extracts from *D pteronyssinus*, prawn, squid, and *Helianthus annuus* pollen (negative control of inhibition), and then measurement of specific IgE to crickets was conducted. The concentration of the inhibitor phase was 2 mg/mL in all cases.

## RESULTS

The SPT results were positive with whole and crushed extracts from *G campestris*, *G bimaculatus*, and *A domestica*, whereas the test results in 10 controls were negative. The results of SPTs with *Olea europaea* pollen, cat dander, storage mites (*T putrescentiae*), and *Ephestia* sp were also positive. The results of SPTs performed with extract from food for the crickets were negative.

Levels of specific IgE were positive to extracts from *G campestris* whole (2.9 kU/L) and crushed (1.9 kU/L), *G bimaculatus* whole (2.4 kU/L) and crushed (1.2 kU/L), *A domestica* whole (5.4 kU/L) and crushed (2.1 kU/L), fire bug (2.1 kU/L), grasshopper (0.5 kU/L), prawn (0.8 kU/L), and squid (0.6 kU/L); specific IgE levels to any other kind of tested extract were negative. Specific IgE levels were also negative (<0.35 kU/L) to all the tropomyosins from different origins tested (mites, insects, mollusks, and crustaceans). The total IgE level was 131 kU/L. The absolute eosinophil count in peripheral blood was 1,377 cells/mL.

Monitoring of the PEF rate showed a baseline average value of 400 L/min, which decreased to 160 L/min (40%) and was exclusively related to the feeding of the crickets but not with other activities performed on the farm. Because the patient worked at the cricket farm 7 days a week, we could not obtain PEF baseline values on a nonexposed day. Spirometry results were normal (FEV<sub>1</sub>, 2.99 L/s, 94.7%), and a bronchodilator test result was negative. The baseline hista-

mine inhalation test demonstrated bronchial hyperresponsiveness (PC<sub>20</sub>, 0.81 mg/mL).

BCT showed a dual response to *A domestica* complete-body extract (0.30 mg/mL) with an immediate decrease of FEV<sub>1</sub> (up to 17%) and a late asthmatic reaction, which was detected 6 hours after the challenge by the peak flow meter recordings with a decrease of 30% from baseline values (Fig 1). The patient complained of nasal obstruction immediately after the test. Twenty-four hours after the test, the patient showed normal spirometry values, and then an increase in nonspecific bronchial hyperresponsiveness (PC<sub>20</sub>, 0.28 mg/mL) was demonstrated.

SDS-PAGE analysis performed with the whole-body cricket extracts showed several bands between 18 and 90 kDa. IgE immunoblotting (Fig 2) revealed a similar pattern of IgE-binding bands with the 3 species of cricket extracts. The pattern, in nonreducing sample conditions (without β-mercaptoethanol), showed an intense IgE-binding band of 78 kDa and a faint band of 64 kDa, whereas in reducing conditions there were 2 intense bands of 58 kDa and 52 kDa and some others of higher molecular mass (107 kDa, 97 kDa, 80 kDa).

EAST inhibition studies showed a high degree of cross-reactivity among the 3 species of crickets (up to 86% for *A domestica* and *G campestris*). Cross-reactivity between crickets and fire bug (*P aptera*), another arthropod (Order Hemiptera, family Pyrrhocoridae), was observed, although to a lesser extent, whereas non-cross-reactivity was found between each species of crickets and prawn (crustacean), squid (mollusk), *D pteronyssinus* (mite), and *H annuus* pollen.

## DISCUSSION

This study reports the case of a female patient who experienced episodes of rhinitis and asthma related to her work on a farm, where *G campestris*, *G bimaculatus*, and *A domestica* were bred. The results indicate an IgE mechanism, as demonstrated by SPT, specific IgE determination, and BCT results to *A domestica* body extract. In addition, the monitoring of PEF during working days also demonstrated the associa-

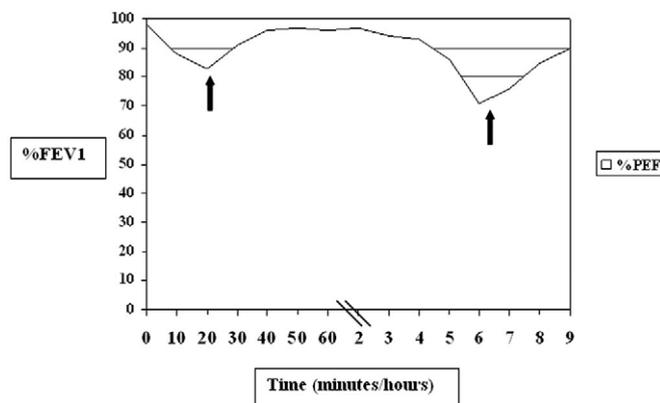


Figure 1. Monitoring of forced expiratory volume in 1 second (FEV<sub>1</sub>) and peak expiratory flow (PEF) after bronchial challenge test.

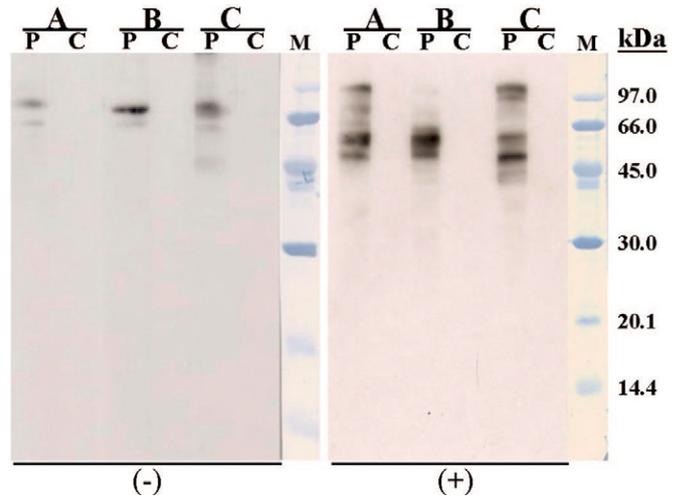


Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting. A, *Gryllus campestris* extract (whole); B, *Acheta domestica* extract (whole); C, *Gryllus bimaculatus* extract (whole). Lane P: patient serum; lane C: control serum (pool of sera from nonatopic subjects). M indicates molecular mass markers; (–), sample without β-mercaptoethanol; (+), sample with β-mercaptoethanol.

tion between her symptoms and professional activity. Furthermore, bronchial exposure to *A domestica* body extract was followed by an increase of nonspecific bronchial hyper-sensitivity.

The first case reported on specific allergy to crickets dates back to the article by Cazort and Jhonston in 1955.<sup>5</sup> The next case was reported by Crawford<sup>6</sup> in 1978 and described a 16-year-old boy with allergic rhinoconjunctivitis and asthma on exposure to crickets, which he used as fish bait. Bagenstose et al,<sup>7</sup> in 1980, described 2 patients who developed allergic rhinitis and bronchial asthma because of exposure to crickets while employed at an amphibian facility, where crickets were raised as food for frogs; these authors presented well-founded evidence of type I hypersensitivity symptoms. In 1980, Harfi<sup>8</sup> described a 16-year-old boy with eyelid edema, upper lip inflammation, headache, tears, and nasal and bronchial symptoms when using crickets as fish bait. The results of SPT and conjunctival and bronchial provocation tests were positive for cricket body extract.

In our patient, sensitization was produced by environmental exposure in the workplace. Because specific IgE levels to other arthropods such as *D pteronyssinus*, shrimp, flea, or grasshoppers was lower than to crickets and EAST inhibition assay results were negative, we can confirm that the patient was primarily sensitized to crickets and rule out that the patient's symptoms were due to sensitization to other arthropods.

For the 3 species of crickets, higher levels of specific IgE were obtained when extracts were prepared from whole bodies rather than triturated ones. This result suggests that cricket allergens are easily extractable proteins, because their presence in whole-body extracts was higher than in crushed ones.

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Tropomyosin, a muscle protein with a molecular mass of approximately 36 to 40 kDa, has been described as a relevant allergen from crustaceans, mites, insects, and mollusks and as responsible for cross-reactivity among these arthropods.<sup>12</sup> However, in our case, neither specific IgE to tropomyosin from different species nor IgE-binding band with tropomyosin molecular mass was detected. Instead, bands of 58, 52, and 107 to 80 kDa were shown. IgE binding bands of 38 to 73 kDa have been identified in the intestinal tract of *Locusta migratoria*, an Orthoptera responsible for respiratory allergy in laboratory workers.<sup>3</sup>

Our patient eliminated her exposure to crickets and was treated with inhaled long-acting bronchodilators and corticosteroids with good response. Currently, she is asymptomatic and only occasionally needs inhaled salbutamol. To summarize, we describe a case of occupational respiratory allergy to crickets where immediate-type allergy to *A domestica* was confirmed by skin tests, specific IgE tests, and BCT.

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