

Letter to the Editor

Respiratory sensitization of a worker to the warehouse beetle *Trogoderma variabile*: An index case report

To the Editor:

IgE sensitization to the Asian ladybug beetle *Harmonia axyridis* and the black carpet beetle (*Attagenus unicolor*) in homes and to mealworm larvae from the *Tenibrio molitor* beetle in the workplace have been previously reported.¹⁻³ Since World War II, the warehouse beetle *Trogoderma variabile* (also known as the Khapra or Cabinet beetle) has been recognized to infest food warehouses and processing plants.⁴ Although it feeds on almost anything, it prefers wheat, barley, or rice, and it is very difficult to eradicate because it can slow or accelerate its reproduction cycle based on the amount of food readily available. Furthermore, it is very resistant to extreme temperature changes in the larval or adult stages. As a result, workers in these facilities inevitably have increased exposure to these insects, the byproducts of which can also enter the human food chain. Here we report an index case of a warehouse worker with specific sensitization to the warehouse beetle *T variabile*.

A 42-year-old healthy male machinist in a pet-food manufacturing plant presented with shortness of breath, chest tightness, and conjunctivitis immediately after airborne dust exposure created while cleaning a conveyor belt system. He had no history of rhinitis or asthma, although he acknowledged that previous pulmonary function test results were low for unknown reasons. He had worked at this facility for 4 years before having symptoms.

At the initial visit, his FEV₁, forced expiratory flow at 25% to 75% of vital capacity, and FEV₁/forced vital capacity were 66%, 58%, and 98% of predicted normal values, respectively, which was more consistent with restrictive lung disease. There was no improvement in FEV₁ after bronchodilator use. The patient's exhaled nitric oxide value was 33 ppb, indicating airway inflammation, and results of chest radiography were normal. Peak flow monitoring during and away from work revealed no significant variability. A baseline low FEV₁ prevented nonspecific or specific provocation.

A detailed differential diagnosis to exclude other underlying conditions presenting with restrictive lung disease, including hypersensitivity pneumonitis, connective tissue disorders, interstitial lung diseases, and structural abnormalities, was considered. Results of routine blood work, including a complete blood count with differential, complete metabolic panel, hypersensitivity pneumonitis screen, total IgE measurement, Westergren sedimentation rate, and C-reactive protein measurement, were normal. Results of skin prick tests to seasonal and perennial allergens were completely negative. Analysis of a dust sample from the workplace revealed it contained warehouse beetle (*T variabile*). A titration skin prick test (in the presence of positive histamine and negative saline controls) to warehouse beetle extracts (containing a mixture of adult insects, larvae, and exoskeletons) prepared at 1:100,000 through 1:1 wt/vol dilutions from a workplace dust sample and a commercial sample of pure warehouse larval beetles revealed wheal-and-flare responses of 9/28 mm and 7/30 mm, respectively, at a 1:100 wt/vol dilution. A titration nasal challenge to warehouse beetle dust extract prepared from the warehouse dust sample using acoustic rhinometry revealed a dramatic decrease in cross-sectional area and nasal volume after challenge with a 1:100,000 wt/vol dilution sustained

through the 1:100 wt/vol challenge dose associated with severe nasal congestion, conjunctival erythema, and serous secretions. The result of a saline control challenge was negative (Table I). A similar challenge performed on a separate day with an extract containing pure commercial warehouse beetle larvae did not elicit a reaction up to and including the dilution of 1:100 wt/vol challenge dose (data not shown). Peak expiratory flow rates before, during, and after nasal provocation did not change. Serum was obtained from the patient after signing an informed consent form approved by the University of Cincinnati Institutional Review Board for general blood draws.

Authentic warehouse beetle (*T variabile*) material was collected from vials obtained from Insects Limited, Inc (Westfield, Ind). The following samples were collected with the aid of a stereomicroscope: (1) exoskeletons, (2) adults, (3) larvae, and (4) fecal pellets. Exoskeletons were also collected from a dust sample collected from the subject's workplace. Specimens (5–30 mg) were placed in Kontes microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pa) in 20 to 60 volumes of 40 mmol/L Tris, 20 mmol/L sodium acetate, and 1 mmol/L EDTA, pH 8.3.⁵ Before homogenization, HALT Protease Inhibitor Cocktail (Pierce Biotechnology, Rockford, Ill) was added to each sample and homogenized for 2 minutes on ice by using a Kontes pellet pestle and motor (Fisher Scientific). Samples were sonicated on ice for 10 minutes, allowed to extract on ice for 1 hour, and then centrifuged for 10 minutes at 14,000g, and the supernatants were collected.

Extracts of the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* and the stored product mites *Acarus siro*, *Chortoglyphus arcuatus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* were prepared as previously reported, and cockroach extracts (*Blattella germanica* and *Periplaneta Americana*) were purchased from a commercial supplier.⁶

SDS-PAGE was performed with 4% to 15% gradient gels (Bio-Rad Laboratories, Hercules, Calif) under both reducing and nonreducing conditions, as previously described.⁵ Proteins were transferred to ProBlott membranes (Applied Biosystems, Foster City, Calif) and then probed with a 1:7.5 dilution of the patient's serum (2 mL of serum + 13 mL of buffer). IgE-binding allergens were visualized with iodine 125-labeled anti-human IgE (0.8 μ Ci per blot; DiaMed, Windham, Me) and autoradiography for varying exposure times of 1 to 8 days.

Patient serum contained specific IgE to 15 different proteins in samples prepared from warehouse beetle materials (Fig 1 and Table II). Extracts prepared from beetle larvae contained the most (10) IgE-binding proteins. Significantly, the patient showed IgE binding to the extract prepared from material collected from the workplace that was almost identical to the IgE binding to proteins in the beetle exoskeleton extract. IgE binding was not observed to dust mite, storage mite, or cockroach extract proteins (data not shown).

In-gel digestion and matrix-assisted laser desorption/ionization was performed on 2 Coomassie blue-stained samples of the 31.3-kD protein isolated from exoskeletons. Several bands indicating the presence of proteins were identified; however, database searches returned no matching results with known peptide sequences, indicating that this might represent a novel protein or proteins.

This is the first case report of a worker sensitized to warehouse beetle. Of note, atopy does not appear to be a risk factor because this worker was not sensitized to seasonal or perennial allergens.

TABLE I. Nasal provocation with an extract prepared from a warehouse dust sample containing warehouse beetle exoskeletons

Acoustic rhinometry	Cross-sectional area (cm ²)		Volume (cm)	
	Before provocation	After provocation	Before provocation	After provocation
Saline	1.21	0.97	6.61	5.48
<i>Trogoderma variabile</i> * (wt/vol)				
1-100,000	1.21	0.51	6.61	2.65
1-10,000	1.21	0.50	6.61	2.79
1-1,000	1.21	0.56	6.61	2.58
1-100	1.21	0.20	6.61	0.9

*All challenge materials were placed in the right naris under the inferior turbinate.

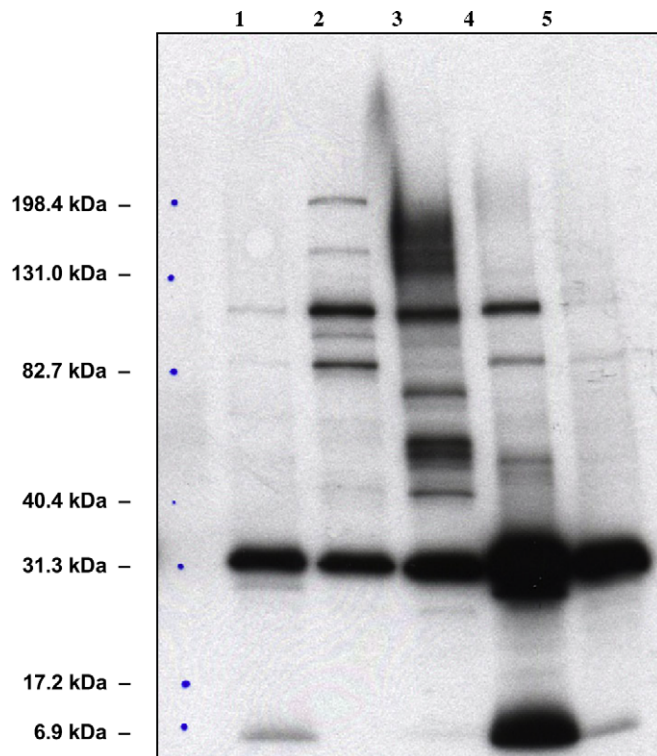


FIG 1. IgE-binding profile of the patient's serum after 4 days of autoradiography. Molecular weight markers are shown at left. Lane 1, Authentic exoskeletons; lane 2, authentic adults; lane 3, authentic larvae; lane 4, authentic feces; lane 5, exoskeletons collected from a workplace sample.

Furthermore, cross-reactivity was not observed with house dust mite, storage mite, and cockroach. Whether *T variabile* cross-reacts with other beetles in the Coleoptera order requires further investigation.

From our analysis, it appears the major sensitizing allergen in our worker was derived from beetle exoskeletons, which is supported by the presence of specific IgE to a single protein (molecular weight, 31.3 kD) present in the authentic exoskeleton extract (Figure 1, lane 1) and to a matching exoskeleton extract prepared from a workplace sample (Figure 1, lane 5). This protein was also present in authentic adults, larvae, and feces extracts (Figure 1, lanes 2-4). Matrix-assisted laser desorption/ionization analysis was unable to identify the specific protein or proteins corresponding to this band, indicating it might be a novel antigen. Further work is necessary to identify the actual allergenic protein or proteins.

The insect molting process involves digestion of old cuticle and formation and tanning of new cuticle, resulting in the secretion of many enzymes, including chitinases, proteases, and

other enzymes associated with new exoskeleton hardening. The larval instars molt multiple times during their growth phase, and these enzyme and digestion product residues can remain in the old exoskeleton material. The warehouse material we microscopically examined contained a large quantity of larval exoskeletons with few adults. Therefore it is likely the exoskeletons are the major source of allergen or allergens sensitizing this worker. This is supported by the patient's positive nasal provocation result to the warehouse dust sample and negative response to the larval body extract because beetles in the active life stage are not producing molting materials.

This worker was advised to avoid further warehouse beetle dust exposure, which required leaving the warehouse during activities resulting in dispersion of airborne dust. One year later, he continues to work at the same facility and with continued avoidance has not experienced further related health problems.

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TABLE II. Relative IgE binding intensity to proteins on immunoblots performed under reducing conditions

Band no.	Authentic exoskeletons	Authentic adults	Authentic larvae	Authentic feces	Exoskeletons from workplace sample	MW (kd)
1		+++				199.9
2			++			170.8
3		+++	++			151.2
4			++			135.8
5	++	+++	+++	+++	+	112.3
6		+++	+			98.9
7		+++		+++	+	85.8
8			+++			75.3
9			+++			59.1
10			+++			55.5
11				+++		52.5
12			+++			43.0
13	+++	+++	+++	+++	+++	31.2
14	+			+++		27.7
15	+++			+++	+++	5.1

Bands were scored as +++ if they first appeared on the film after 1 day of exposure, ++ if they appeared after 2 days of exposure, and + if they appeared after 4 days of exposure.

MW, Molecular weight.