

What is in your pantry? Entomologic anaphylaxis

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ABSTRACT

Background: The booklouse, *Liposcelis bostrychophila*, is a potent environmental allergen clinically associated with rhinoconjunctivitis and asthma. Despite its known infestation of grain products, anaphylaxis from ingestion of this organism has, to our knowledge, not been previously reported. We present the case of a 44-year-old woman who developed anaphylaxis to ingested oats and rice shown to be contaminated with *L. bostrychophila*.

Objective: The objective was to isolate a distinct antigen from *L. bostrychophila* implicated in a case of unexplained anaphylaxis.

Methods: In vitro studies were obtained for relevant ingested materials and aeroallergens. Skin-prick testing (SPT) was performed with standard extracts, contaminated oats, fresh oats, and crushed *L. bostrychophila*. Western blots were conducted using subject and control serum to detect specific immunoglobulin E (IgE) against the grains and *L. bostrychophila* extract. Competitive inhibition immunoblotting was used to assess specificity of IgE binding.

Results: In vitro studies and SPT were notable for positive responses to dust mite and flour contaminated by *L. bostrychophila*, along with contaminated oats. Testing results for fresh oat and rice were negative. Immunoblots that used the subject's serum revealed a strongly positive band in the contaminated oat and rice extracts at 24 kD, whereas dust-mite extract yielded a single 14-kD band. Isolated *L. bostrychophila* extract also yielded a 24-kD band. Competitive inhibition experiments demonstrated that the 24-kD band in the contaminated oat extract was immunologically distinct from the 14-kD dust-mite band.

Conclusion: Our case highlights the importance of considering *L. bostrychophila* as a potential culprit for unexplained anaphylaxis due to ingested grain products. Given the ubiquitous presence of this insect, we suspect that this may be a more common problem than previously recognized.

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Anaphylaxis is the most dreaded of immediate hypersensitivity reactions. It is typically defined by multiorgan system involvement rapidly evolving in the wake of exposure to a known or suspected allergen. The exception to this clinical presentation is isolated hypotension.¹ Drugs, venoms, and foods are among the most common culprits.² Although resulting in high morbidity, anaphylaxis is rarely fatal, with an average case mortality of 0.3%.³ Perhaps the most frustrating for clinicians is the unknown or idiopathic anaphylaxis category. The incidence is undetermined and remains a diagnosis of exclusion.⁴ A subset of these mysterious events has been unraveled in previous investigations that identified mite contamination of flour as the culprit for individuals who are sensitized and who were not food allergic.

The index case, after ingestion of beignets, was reported by Erben *et al.*⁵ in 1993. There have been multiple additional reports that corroborate the anaphylactic potential of exposure to mite-contaminated flour, colloquially referred to as “pancake anaphylaxis.”^{6–9} This condition has also been categorized as oral mite anaphylaxis, with species variability linked to a geographic locale. For example, *Suidasia pontifica* and *Blomia tropicalis* were the most relevant mite species in 10 cases of oral mite anaphylaxis described in Panama City, whereas *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are more commonly reported in the United States.¹⁰

The booklouse, *Liposcelis bostrychophila*, has a worldwide distribution, infesting old books, paper, and, importantly, stored foods. The insect is brown in color and easily visualized, with an adult size of 1.0–1.3 mm. Booklice neither bite, transmit disease, nor destroy food or fabric. Under favorable conditions, however, they will rapidly proliferate, with resultant infestations viewed as unsanitary. They are thus considered “pests,” with significant economic consequences accruing from the need to discard contaminated materials.^{11,12} *L. bostrychophila* has previously been identified as a potent indoor allergen linked to nasoocular symptoms and asthma.^{12,13}

Although well recognized in studies conducted throughout the world, including in Japan, Europe, India, and China, the level of awareness concerning

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booklice or “psocids” in the United States seems to be fairly low, as reflected by the paucity of published reports.^{12–18} Specific anaphylaxis to booklice, to our knowledge, has not been previously reported in the international literature. We present a case of a 44-year-old woman who developed episodes of anaphylaxis after eating oats, as well as rice, from stored containers in her home. *L. bostrychophila* was visibly identified in flour from the same pantry. In this report, we demonstrate that sensitization to *L. bostrychophila* was the cause of recurrent anaphylaxis from ingestion of grain products contaminated with this organism.

METHODS

Our subject and control signed a consent approved by the University of California San Diego Institutional Review Board.

Case

A 44-year-old woman with a history of allergic rhinitis, mild-intermittent asthma, and childhood eczema presented for evaluation of anaphylaxis. She described making porridge with her son using steel-cut oats prepared with almond milk, chocolate chips, honey, raisins, and banana. Immediately after ingestion, she experienced itching and constriction of the throat as well as tongue swelling. Despite taking albuterol and diphenhydramine, the symptoms progressed. On arrival to urgent care, she had cutaneous erythema, angioedema, wheezing, shortness of breath, and a sense of doom. She did not have lightheadedness or abdominal pain. Treatment included intramuscular epinephrine, nebulized albuterol, diphenhydramine, and intravenous methylprednisolone, and her symptoms rapidly resolved.

Three weeks afterward, she tolerated cinnamon spice oatmeal. However, several hours later, immediately after taking ibuprofen, she had facial and tongue swelling. Diphenhydramine, epinephrine, and solumedrol were administered in the emergency department with improvement. After these events, she avoided oatmeal and nonsteroidal anti-inflammatory drugs (NSAID), including aspirin. She reintroduced wheat, almonds, banana, and chocolate into her diet without adverse reactions.

Our subject subsequently developed abdominal pain, nausea, tongue tingling, and general malaise within minutes after eating a bowl of rice. She immediately took diphenhydramine, and her symptoms resolved within a few hours. Our subject was asked to bring the original oats and rice containers to the clinic. She also brought a container of flour stored in the same pantry in which she observed small crawling insects. The three containers were named “bad oats,” “bad flour,” and “bad rice.” The oat container



Figure 1. Image of *Liposcelis bostrychophila* isolated from our subject's contaminated flour sample.

was found to be expired for 2 years. The flour and rice were not stored in their original containers, and the expiration dates were unknown. The presence of small insects in the bad flour was confirmed; however, no insects were seen in the bad rice or bad oats by visual or low-power microscopic inspection.

Insect Identification and Extract Formulation

The insects in the bad flour were isolated by using a microspatula. Several insects were placed in alcohol and sent to author E.L.M. for identification. The insects were positively identified by their appearance as *L. bostrychophila*, a species of booklouse known to infest stored grain (Fig. 1). Additional *L. bostrychophila* from the bad flour were crushed with a mortar and pestle and extracted in phosphate buffered saline (PBS) solution. Extracts were made from the bad oats, fresh oats, bad rice, and fresh rice by crushing them individually with a mortar and pestle, then extracting soluble materials in PBS solution. Fresh oat and fresh rice extracts were made by using newly purchased samples. The fresh oats were the same brand and packaging as the bad oats. The fresh rice was the same type of rice as the old rice.

Testing for Immediate Hypersensitivity

Skin-prick testing (SPT) by using bad oat extract, fresh oat extract, commercial oat extract (ALK Abello, Post Falls, ID), crushed booklouse isolated from the flour, dust-mite mix (ALK Abello), and histamine and saline solution controls was performed on the subject and on a nonallergic control according to standard technique with a UniTest[®]PC (pain control) device from Lincoln Diagnostics, Inc, Decatur, IL.¹⁹ *In vitro* studies for relevant allergens were done in a commercial laboratory by ImmunoCap ISAC, scale 0–VI (ARUP Laboratories, Salt Lake City, UT).

Tryptase Level

Baseline and reaction tryptase levels (normal range, <10.9 ng/mL) were measured by a commercial laboratory (ARUP Laboratories).

Graded Oral Aspirin Challenge

An unblinded oral challenge was performed with 40.5 mg, 81 mg, 162 mg, and 325 mg of aspirin at hourly intervals as per standard operating procedures.

Immunoblotting

Sera from the subject and the control were used for immunoblotting to detect immunoglobulin E (IgE) antibody against the bad oat extract, fresh oat extract, bad rice extract, fresh rice extract, crude *L. bostrycho-phila* extract, and mixed dust-mite extract. Immunoblotting was done by using slot blots or sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE). Western blotting used precast gels (Any kD Mini-PROTEAN TGX; Bio-Rad BioRad Laboratories, Hercules, CA). Nitrocellulose (slot blot) or polyvinylidene difluoride (PVDF) membranes (Western blot) were probed by using the subject's or the control's serum at a 1:100 dilution incubated overnight at 4°C. Bound IgE was detected by using a 1:100,000 dilution of a commercial horseradish peroxidase conjugated anti-IgE antibody (mouse monoclonal [B3102E8] anti-human IgE fragment crystallizable (Fc) (horseradish peroxidase) ab99806; Abcam, Cambridge, MA) and measuring chemiluminescence using a charge-coupled device (CCD) camera-based system (ChemiDoc XRS+ System; BioRad Laboratories). For competitive inhibition immunoblots, the sera were preincubated overnight at 4°C with the extract or PBS solution control and then used for immunoblotting.

RESULTS

Routine Clinical Laboratory Testing

Tryptase was not measured at the time of the reactions. Baseline tryptase obtained at the time of allergy evaluation was normal (4.5 ng/mL). *In vitro* specific IgE testing results were negative for oats and showed a level II for wheat, which was clinically well tolerated. Results for tree nuts and rice were also negative, and there was a low-level positive result for sesame, which was also tolerated. The aeroallergen panel investigation of specific IgE *in vitro* was diffusely positive, including for cat (level III), horse (level II), dog (level II), *Aspergillus* (level II), grass (level II), mesquite (level III), and olive (level II). Dust mite had the most pronounced sensitization (level V), with *Dermatophagoides pteronyssinus* greater than *Dermatophagoides farinae*. The total IgE value was elevated at 1460 IU/mL.

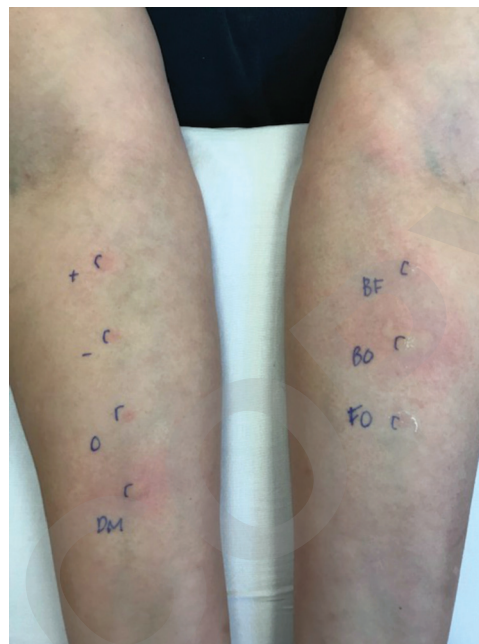


Figure 2. Skin-prick testing results, showing oat extract (O), dust mite (DM), booklouse isolated from the flour (BF), bad oats (BO), fresh oats (FO), histamine (+), and normal saline solution (–) in the subject. The image was taken 15 minutes after prick testing with the samples.

Aspirin and Acetaminophen Challenge

Aspirin challenge was discontinued after development of periorbital angioedema 30 minutes after a dose of 162 mg. Our subject was treated with diphenhydramine, epinephrine, and solumedrol, with resolution. The tryptase level was normal (4.8 ng/mL) during the reaction. She subsequently tolerated graded oral challenge to acetaminophen up to 320 mg.

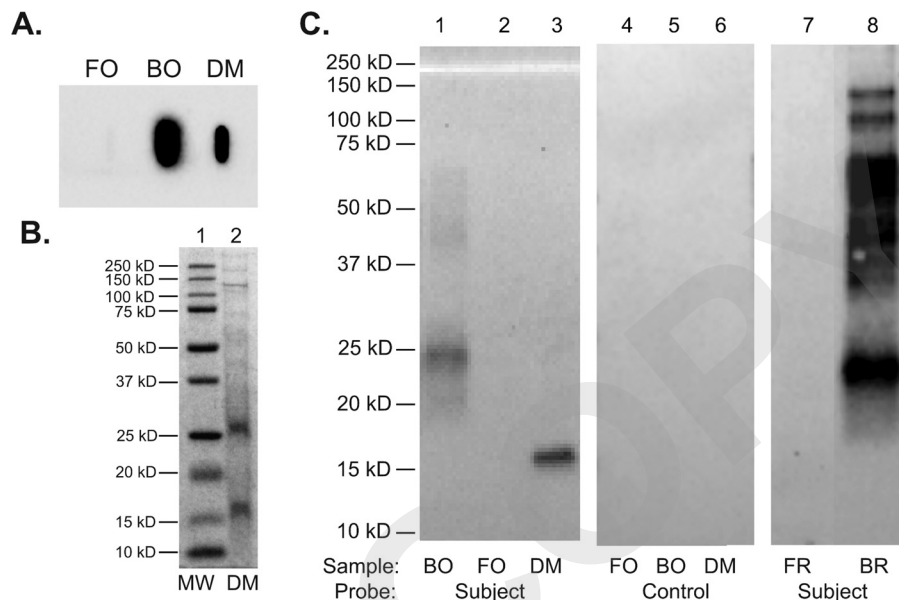
SPT

SPT results for commercial and fresh oat extract were negative; however, the subject strongly reacted to the bad oats, with a 22-mm wheal and 45-mm flare. The SPT was repeated in the subject and in a normal control using the bad oats, crushed booklouse from the flour, fresh oats (of the identical brand), commercial oat extract, and mixed dust mite. A repeated SPT confirmed the sensitivity to bad oats and also showed sensitivity to the booklouse and dust-mite extract (Fig. 2). SPT results were negative to fresh oats and commercial oats. Identical SPT results performed on a control were negative except for the histamine control (results not shown).

Identification of the Allergen in the Bad Oats and Bad Rice

To exclude an irritant response as the cause of the positive skin testing reactions, we determined whether there was specific binding of IgE to the bad oat extract

Figure 3. Characterization of the major allergen in bad oats and bad rice. (Panel A) Slot blot, using fresh oat (FO), bad oat (BO) and dust-mite (DM) extracts probed by using serum from the subject, followed by detection of specific immunoglobulin E (IgE) binding. (Panel B) Dust-mite extract separated on sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie blue, showing major dust-mite bands at 26 kDa and 16 kDa. (Panel C) Immunoblot of FO, BO DM, fresh rice (FR), and bad rice (BR) extracts. Lanes 1–3 and lanes 7 and 8 were probed with the subject's serum, lanes 4–6 were probed with the control's serum.



by slot blotting. Bad oat extract, fresh oat extract, and dust-mite extract were spotted on nitrocellulose membranes and probed by using serum from the subject. As shown in Fig. 3A, the subject's serum showed specific IgE binding to the bad oats and dust mite, but not to the new oats.

To further characterize whether the offending allergen(s) in the bad oats and bad rice could be the result of contamination with dust mites, we analyzed the molecular weight of the bands recognized by the subject's IgE. On a Coomassie-stained gel, the dust-mite extract contained two major bands, at apparent molecular weights of 16 kDa and 26 kDa (Fig. 3B). When tested with immunoblotting, the subject's serum showed specific IgE binding to an ~24-kDa band in the bad oats (Fig. 3C, lane 1). In contrast, the subject's serum did not show any specific IgE binding to fresh oats (Fig. 3C, lane 2) and recognized a 16-kDa band in the dust-mite extract (Fig. 3C, lane 3). The control's plasma was negative to fresh oats, bad oats, and dust-mite extracts (Fig. 3C, lanes 4–6). The subject's serum also recognized a 24-kDa protein in the bad rice extract but did not show any IgE reactivity to the fresh rice extract (Fig. 3C, lanes 7 and 8).

Identifying the *L. bostrychophila* Allergen

Because the band recognized by the subject's IgE in the bad oats and bad rice was distinct from the band recognized in the dust-mite extract, we assessed whether the *L. bostrychophila* that was contaminating the flour could be the potential allergen. A gel was loaded with bad oat extract, dust-mite extract, and *L. bostrychophila* extract, which was then immunoblotted by using the subject's serum to assess specific IgE binding. The subject's serum again showed reactivity to a 24-kDa band in the bad oats as well as a 16-kDa

band in the dust-mite extract (Fig. 4A, lanes 1 and 2, respectively). The *L. bostrychophila* extract showed a prominent band at 24 kDa (Fig. 4A, lane 3), similar to the bad oats (Fig. 4A, lane 1).

Because the *L. bostrychophila* extract appeared similar to the allergen in the bad oats and bad rice, we used competitive inhibition to further explore the possibility that the reactions to the bad oats and bad rice were caused by contamination by *L. bostrychophila* (Fig. 4B). When the subject serum was preincubated with PBS solution then immunoblotted, the same 24-kDa and 16-kDa bands were seen (Fig. 4B, lanes 1 and 2, respectively). However, when the subject's serum was preincubated with dust-mite extract and then immunoblotted, the bad oat band at 24 kDa was still present, whereas the 16-kDa band disappeared (Fig. 4B, lanes 3 and 4, respectively). Furthermore, when the subject's serum was preincubated with bad oat extract and then immunoblotted, the bad oat band at 24 kDa disappeared, whereas the 16-kDa band was still present (Fig. 4B, lanes 5 and 6, respectively).

DISCUSSION

Our subject provided an illustrative case study for approaching "unexplained anaphylaxis." The events followed ingestion of stored grain products that she had previously tolerated. Given her history of mite sensitivity, our initial suspicion centered on mite contamination, as previously documented.^{5–9} After the second anaphylactic event, our attention with regard to the identity of the primary allergen was redirected to the flour-contaminating insect. We identified the organism as *L. bostrychophila*, the most common species of booklouse, which belongs to the order Psocoptera, and is widely distributed in kitchens, granaries, and flour

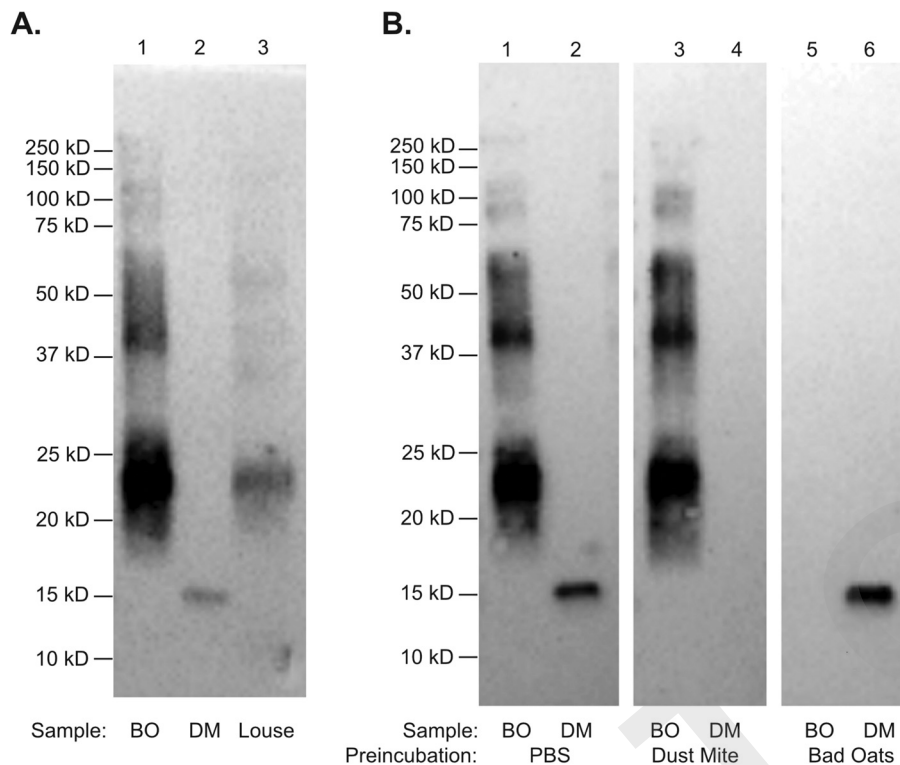


Figure 4. Identification of the major allergen in the bad oats. (Panel A) Immunoblot of bad oat (BO), dust-mite (DM), and *Liposcelis bostrychophila* (Louse) extracts probed with the subject's serum, followed by detection of specific immunoglobulin E (IgE) binding. (Panel B) Competitive inhibition immunoblot of BO and DM extracts probed with the subject's serum, followed by detection of specific IgE binding. Lanes 1 and 2, subject plasma preincubated with phosphate buffered saline (PBS) solution; lanes 3 and 4, subject plasma preincubated with dust-mite extract; lanes 5 and 6, subject plasma preincubated with BO extract.

mills throughout the world, and commonly infests stored food. Local populations of *L. bostrychophila* can grow rapidly from a single female and can account for the majority of insects in dust samples, with a frequency of up to 90%.¹³ Although known to provoke respiratory symptoms in subjects with allergy, we presented the first report, to our knowledge, of anaphylaxis to this particular insect.²⁰

Dust-mite antigens from *D. pteronyssinus* and *D. farinae* have been well characterized. The mite allergens are labeled according to their chronological order of purification and homology to allergens already identified. From 80% to 90% of patients with mite allergy react to the major group 1 or 2 allergens (Der p1, Der p2, Der f1, and Der f2). Der p2 and Der f2 have a 15 kDa lipid-binding protein, which corresponds to the ~16-kDa band visualized on our Western blot.²⁰ A 26 kDa protein has been isolated from *L. bostrychophila*, designated Lip b 1, which seems to be a novel allergen.^{15,21} Whether Lip b1 cross-reacts with other arthropod allergens, to our knowledge, has not been previously studied. Molecular cloning of complementary DNAs (cDNAs) encoding Lip b1 identified two variants that share an 87% identity. It has further been confirmed that this allergen is an independent marker for unique sensitization to *L. bostrychophila*.

Ishibashi *et al.*²¹ demonstrated reactivity to sera from patients with asthma and sensitized with booklice. Der p1 and Der f1 have cysteine protease bands of 24 and 27 kDa, respectively. Our subject's plasma contained IgE that reacted with a 24-kDa band found in the bad oats

and bad rice. Competitive inhibition showed that this band was identical to the 24-kDa band identified in the *L. bostrychophila* extract. Together with the lack of a 14-kDa band, this demonstrated that the immunodominant allergen in the bad oats and bad rice was not dust mite but rather *L. bostrychophila*. Although we did not observe any intact booklice in the bad oats or bad rice, these containers were sitting next to the contaminated flour in the same pantry. Notably, the contaminated material was able to elicit an anaphylactic reaction despite heat exposure during the preparation of the rice and oatmeal.

We were intrigued by our subject's reactivity to aspirin and NSAIDs. A subset of patients who are aspirin intolerant have been described with periorbital angioedema related to aspirin and NSAIDs.²² These individuals were found to be generally younger and with a history of atopy. In a previous report of patients with anaphylaxis after ingestion of mite-contaminated wheat flour, 87% were found to react to aspirin challenge.⁷ This was not able to be explained by any contamination of the flour with aspirin. Although hypotheses have been proposed,²³ the association between the arthropod-induced anaphylaxis and periorbital angioedematous reactions to aspirin remains unexplained.

CONCLUSION

For patients with non-food allergies who present with anaphylaxis to ingested materials, it is important to consider the booklouse *L. bostrychophila*. Further

research is needed to determine the prevalence of sensitization to *L. bostrychophila* among populations in the United States. Such investigations are currently limited by the absence of a commercial extract for *L. bostrychophila* for testing. Our case underscores the importance of asking patients to bring in substances believed to be linked to an allergic reaction for inspection and direct testing to assist in diagnosis of potential culprits. Clinically, it may also be worthwhile to test patients with entomologic anaphylaxis for aspirin and/or NSAID sensitivity.

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