

## **Millet: A “Gluten-free” and “healthy” cereal with the potential to induce anaphylaxis**

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Millet is a small, rounded cereal from the Poaceae family. It has been consumed since 2700 BC in China [1], being currently cultivated in India, Africa, and China. In recent years, due to “gluten-free” and “healthy” diets, its consumption has increased in developed countries. It has high protein and fiber content, and is used as bird food in developed countries and in biscuits, drinks, weaning foods, and beer production [2]. Since 1981, when Parker et al. reported the first case of anaphylaxis due to millet consumption [3], different cases have been reported. Until now, a few allergens have been identified, and the cross-reactivity between millet and other cereals is not uncommon [1, 4-10].

We present a 43-year-old woman who began with a pharyngeal foreign body sensation, ocular itching, tearing, eyelid and pinna edema, nasal bleeding, flushing and palmoplantar itching with progressive body generalization after eating boiled millet seeds. She was admitted into the emergency room where no objective change in vital signs were addressed and intravenous methylprednisolone and dexchlorpheniramine were administered, although intramuscular adrenaline could also have worked as a treatment option of the anaphylaxis symptomatology. The symptoms subsided after a few hours.

She was referred to our Allergology outpatient consultation, where she also reported constant, predominantly nocturnal cough for several months. She mentioned having two seed-eating budgerigars that freely flew around the house. They were fed bird food that contained millet, among other seeds.

A Prick-to-Prick test (PP) was performed with bird food provided by the patient, budgerigar feathers, raw and boiled millet. All showed positive results. Also, intense positive reactions were observed in the skin prick test (SPT) to grass pollen, rice, and corn. Besides, immunoglobulin E (IgE) antibodies were detected in ImmunoCAP®

against grass rye, barley, rice, corn and hazelnut and budgerigar feathers. (See online repository).

Based on the positive PP to millet seeds, we performed a raw and boiled millet seed extract. A Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE) with raw and boiled millet seeds extracts was performed for protein bands determination. After the SDS-PAGE, immunoblotting with the patient's serum was conducted with both raw and boiled millet seeds extracts obtaining a unique binding band of approximately 36 kDa in the boiled millet seed extract (Figure 1). Possibly, heat could help express epitopes that would not be as available otherwise. The 36kDa band was excised from the gel of the boiled millet seed extract SDS-PAGE, and then it was digested and analyzed by Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight (MALDI-TOF) mass spectrometry (MALDI-TOF MS) at the Proteomics Department of Complutense University of Madrid. Protein identification by Peptide Mass Fingerprint (PMF) was carried out using a Uniprot DB ([www.uniprot.org](http://www.uniprot.org)) with taxonomy restriction to Viridiplantae obtaining a 55 % homology with a Globulin 1S, corresponding to a cupin. In order to test possible cross reactivity between millet and grass pollen proteins, 5 individuals with typical grass (Gramineae) pollen allergy were investigated for IgE positivity against millet performing immunoblotting with boiled millet extract. Two of these individuals recognized several bands. We cannot assure that the bands recognized by these are not the same as our patient since her serum was not included in this blotting. One of the patients had been a bird keeper in the past, (Lane 3, Online Repository Figure 1A). The rest did not show any recognition of millet proteins in accordance to Bohle et al. [1] findings (Online Repository Figure 1A). We also performed immunoblotting in order to test IgE reactivity to grass pollen of our patient and the other 5 grass pollen allergic individuals (Online Repository Figure 1B), showing the expected recognizing patterns only in grass allergic patient's serum.

To explore the origin of the cough, we carried out a basal spirometry, bronchodilatation and fractional exhaled nitric oxide with normal result, unspecific bronchial hyperresponsiveness was assessed with metacholine challenge obtaining a negative result, (the patient had not been exposed to the birds nor the bird-food in the past three

months before testing). This was followed by a specific inhalation challenge test with raw millet inducing an allergic reaction (See online Repository).

This is the first case to describe a 36 kDa IgE binding band identified as a Globulin-1S in millet seeds. In the literature available to date, this Globulin-1S could be the responsible allergen recognized [1,5,8]. In a recent publication by Bravo et al. two bands of 12 and 36 kDa were recognized by their patient, who suffered asthma and rhinitis due to millet seeds [10] (See online Repository).

Inhalation is currently theorized as a sensitization pathway, based on the observation that most cases published present bird-keepers with anaphylaxis at first millet consumption [1, 4-7]. Besides, sensitization to millet is long-lasting despite not being exposed to birds for years. Hemmer et al. described 9 patients with allergy to millet after its consumption and 8 were current or former bird-keepers [6]. On the other hand, cross-reactivity with other cereals has been described, although its clinical relevance is controversial [1,4-9]. This patient tolerated corn, rice, wheat, rye and barley after anaphylaxis due to millet ingestion. Cross reactivity with grass pollen has been discarded in previous articles and we supported those findings using the boiled millet extract (Online repository Figure 1A).

Cupins are storage proteins, previously identified, not only in millet, but also in corn, different species of wheat, and fruits such as pineapple and flowers such as orchids, among others.

In conclusion, to our knowledge this is the first case in which a globulin-1S is identified as a possible millet allergen. We have observed other bands that could be the same as the ones described previously [10]. We believe that an increase in “healthy” and “gluten-free diets” could enhance the incidence of millet sensitization, being this the second reported case of symptoms after millet inhalation and ingestion [4], and millet a potentially dangerous food in terms of allergic reactions, which is why we encourage to perform a sensitization screening in atopic bird keeper patients.

**Conflicts of interest**

J.S. reports having served as a consultant to Thermofisher, MEDA, Novartis, Sanofi, Leti, Faes Farma, Mundipharma, and GSK; having been paid lecture fees by Novartis, GSK, Stallergenes, Leti, and Faes Pharma; as well as having received grant support for research from Thermofisher, Sanofi, and ALK. V.d.P reports having served as a consultant to Astra Zeneca and GSK and having been paid lecture fees by both, B.S. declares having received a grant or contract contract by CIBER of respiratory disease, M.V. declares having been paid lecture fees by GSK. The other authors declare no conflicts of interest.

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## LEGEND TO FIGURES

Figure 1: IgE immunoblot with raw and boiled millet seeds extract of the 43-year-old women. Online Repository Figure 1 A): Recognition of millet extract by 5 grass pollen allergic patients and two controls. As it can be observed the patient had low intensity of recognition possibly due to sIgE degradation caused by freeze-thaw cycles of the sample. Figure 1 B) Recognition of grass extract by the same grass allergic patients as figure 1A and by the case patient. Each lane number corresponds to the same patient's serum in both sub-figures A and B. MK: molecular markers (kDa); Net: Buffer without serum; C: serum sample from non-allergic donor. P: serum sample from the 43-year-old allergic patient. Arrow represents IgE-band recognized by the patient.

