Millet Seed Sensitization in a Lovebird Keeper: A Case Report

Bravo E[1], González Mahave I[2], Sola JP[1], Vidal Oribe I[2], Hernández Alfonso P[2], Peñalver-Mellado M[1]
[1]*Research and Development Department, Probelte Pharma S.L.U., Murcia, Spain*
[2]*Allergology Department, Hospital San Pedro, Logroño, Spain*

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Common millet (*Panicum miliaceum* L) is a highly nutritious cereal used for human consumption, bird seed, and ethanol production [1]. In fact, it is the sixth most consumed cereal worldwide, and more than a third of the population takes millet habitually [2,3]. However, nowadays, the grain is known in developed countries mainly as food for birds and livestock.

Millet-induced anaphylaxis was first described in 1981 by Parker et al [4]. Since then, it has been reported to induce serious anaphylactic reactions after ingestion. Millet has also been reported to be an inhalant allergen in atopic bird keepers [5,6]. Analysis of sera from bird keepers revealed that 63% had specific IgE for millet and that several bands were detected by immunoblot, the 3 most common corresponding to proteins of 36, 70, and 90 kDa [5,7]. Cross-reactivity has been detected with prolamin, a 20-kDa protein that is common in the several species of millet, as well as in other cereals belonging to the genus *Panicum* [8,9].

We present the case of 35-year-old lovebird keeper with persistent respiratory and nasal symptoms, including tearing and severe allergic rhinitis, which progressed to nasal respiratory failure and dyspnea. The specialist's diagnosis was rhinitis and asthma. The patient had previously been diagnosed with persistent rhinoconjunctivitis due to grass pollen in 2007 and was successfully treated with subcutaneous immunotherapy for 5 years. He reported not routinely consuming cereals, except for wheat occasionally and confirmed that the symptoms always worsened at his place of work.

Lovebirds mainly consume millet (almost 90% of their diet), compared with other foods such as birdseed and corn. Withdrawal of millet from the lovebird's diet led to an improvement in the patient's symptoms.

A skin prick test was performed with a standard aeroallergen battery, and the study was extended to bird feathers and cereals consumed by lovebirds.

Specific IgE (Phadia 100 UNICAP, Thermo Fisher Scientific) was subsequently measured to confirm the results obtained with the skin prick test. IgE was measured for millet, corn, birdseed, rye, barley, lipid transfer protein from peanuts and walnuts, and feathers (canary, parakeet, parrot).

The allergenic extracts used in the ELISA inhibition procedure are routinely manufactured for diagnosis apart from millet, which was prepared following the manufacturing protocol used for the remaining extracts. Grains of common millet (*P miliaceum*) (~18 g) were crushed and degreased with acetone for 4 hours (25% wt/vol). After evaporation of the acetone, extraction was carried out with phosphate-buffered saline (PBS) for 24 hours under stirring, and the extract was subsequently filtered (0.22-µm pore) for sterilization and lyophilization.

The allergenic extract was analyzed using SDS-PAGE under reducing conditions (Laemmli, 1970) and stained with Coomassie Brilliant Blue R (Sigma-Aldrich).

IgE-immunoblot was performed using corn and millet extract. Briefly, proteins separated by SDS-PAGE were transferred to a 0.2-µm nitrocellulose membrane. For immunodetection, the membrane was incubated with the patient's serum diluted 1:5 with blocking solution before being incubated with peroxidase enzyme–conjugated antihuman IgE (ε chain–specific) secondary antibody (Anti-Human IgE-Peroxidase, Sigma-Aldrich).

Cross-reactivity between allergens was assessed using the ImmunoCAP assay (Thermo Fisher Scientific). In the solid phase, common millet was confronted with several extracts, which acted as inhibitors. The ability of millet, corn, wheat, rice, and rye extracts to inhibit the binding of specific IgE to common millet was evaluated [10]. The extracts were incubated at 4°C and under continuous shaking with patient serum for 12 hours before the ELISA inhibition analyses (Phadia 100 UNICAP, Thermo Fisher Scientific).

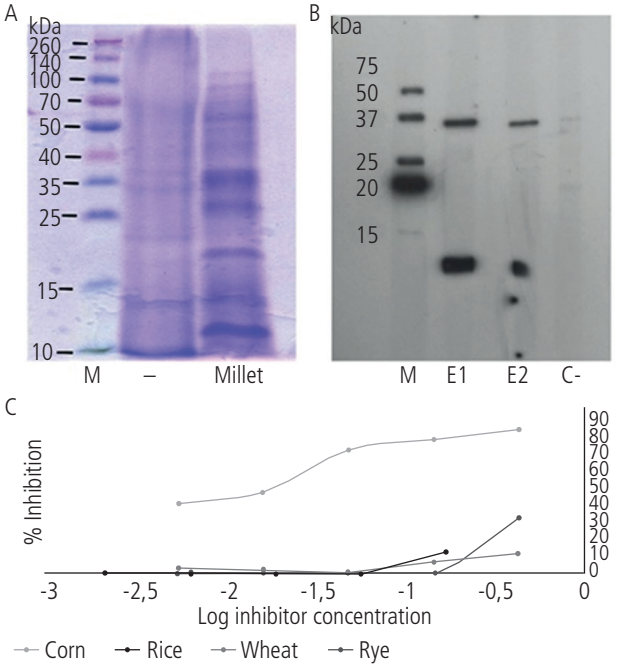

Figure. A, Protein profile of millet extracts investigated by SDS-PAGE. Extracts were analyzed under reducing conditions. Bands corresponding to main allergens are approximately 12 and 36 kDa. B, Immunoblot assay of the patient's sera at different dilutions and with a negative control (without extract). The patient presented specific IgE-binding bands with millet. E1, sera diluted 1/5 with blocking solution; E2, sera diluted 1/10 with blocking solution; C-, immunoblot without extract, negative control. C, Inhibition percentage of millet in solid phase extract (CAP) with extracts of corn, rice, wheat, and rye. High inhibition of IgE binding to corn can be observed for millet.

The millet extract and the excised bands of 12 and 36 kDa were analyzed using tandem mass spectrometry (MS/MS) coupled to high-performance liquid chromatography (HPLC) following an internal protocol based on that described by McCormark et al in 1997. Samples were proteolytically digested with trypsin, which decomposed the proteins into peptides. These were then chromatographed using reversed-phase HPLC (Waters XBridge BEH C18 HPLC column), which was coupled online with an Agilent XCT Plus Ion Trap mass spectrometer using an electrospray interface. Data were processed using LC/MSD Trap, Version 3.3 (Bruker Daltonik, GmbH) and Spectrum Mill MS Proteomics Workbench (Rev A.03.02.060B, Agilent Technologies).

The skin prick tests were positive for *Alternaria alternata* and *Cupressaceae*, thus explaining the patient's symptoms partially but not totally. Therefore, the study was extended to feathers and some cereals, revealing clearly positive results for corn, millet, and bird seed.

The only significant results for specific IgE were millet and corn, with a value of 1.17 kU_A/L and 1.47 kU_A/L, respectively. Levels detected for the other tests were very low (<0.39 kU_A/L).

After separation of the proteins present in the sample, the evaluation of the protein profile revealed a few bands of different molecular weights. Those weighing 12, 20, and 36 kDa were more intense (Figure, A).

The evaluation of the allergenic profile revealed 2 bands that reacted with the patient's serum: a 12-kDa band (higher intensity) and an approximately 36-kDa band (Figure, B).

ELISA revealed very high inhibition of millet IgE binding to corn. Values for other cereals, such as wheat, rye, and rice, were very low (Figure, C).

With excellent confidence, HPLC-MS/MS revealed coincidence with nonspecific lipid transfer protein from corn (Uniprot B6T089) in the case of the 12-kDa band and uncharacterized protein from millet (Uniprot A0A3L6RCZ2) in the case of the 36-kDa band, with homology to glucose and ribitol dehydrogenase.

We report a case of millet allergy in a bird keeper. The results of the assays enabled us to conclude that the patient was sensitized to a 12-kDa protein present in millet and corn that gives rise to cross-reactivity (as demonstrated by ELISA inhibition). The patient's dyspnea may be due to the inhalation of millet seed, since he was sensitized to 2 proteins from the millet extract (approximately 12 and 36 kDa), although this should be confirmed by nasal or bronchial provocation testing.

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Conflicts of Interest

Bravo E, Sola JP, and Peñalver-Mellado M are employees of Probelte Pharma S.L.U. The remaining authors declare that they have no conflicts of interest.

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Elena Bravo Hernández
Research and Development Department Probelte Pharma S.L.U.
S/ Antonio Belmonte Abellán, 3-7
30100 Murcia, Spain
E-mail: elenabravo@probeltepharma.es

