In summary, parasitic infestation is not the leading cause of eosinophilia in the United States, but it can occur. Awareness of parasites and their life cycles is imperative in the evaluation of eosinophilia and may spare patients the cost and risk of testing and immunosuppressive therapy.

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1. Segarra-Newnham M. Manifestation, diagnosis, and treatment of *Strongyloides stercoralis* infection. *Ann Pharmacother*. 2007;41:1992–2001.
2. Concha R, Harrington W, Rogers AI. Intestinal *Strongyloidiasis*: recognition, management, and determinants of outcomes. *J Clin Gastroeneterol*. 2005;39:203–211.
3. Siddiqui AA, Berk SL. Diagnosis of *Strongyloides stercoralis* infection. *Clin Infect Dis*. 2001;33:1040–1047.
4. Moore T. Eosinophilia in the returning traveler. *Infect Dis Clin North Am*. 1998; 12:503–521.
5. Fardet L. Severe strongyloidiasis in corticosteroid-treated patients: case series and literature review. *J Infect*. 2007;54:18–27.
6. Thompson BF, Fry LC, Wells CD, et al. The spectrum of GI strongyloidiasis: an endoscopic-pathology study. *Gastrointest Endosc*. 2004;59:906–910.
7. Igual-Adell R, Oltra-Alcaraz C, Soler-Company E, Sanchez-Sanchez P, Marogo-Oyana J, Rodriguez-Calabuig D. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. *Expert Opin Pharmacother*. 2004;5: 2615–2619.
8. Muennig P, Pallin D, Challah C, Khan K. The cost-effectiveness of ivermectin vs. albendazole in the presumptive treatment of strongyloidiasis in immigrants to the United States. *Epidemiol Infect*. 2004;132:1055–1063.
9. Drugs for parasitic infections. *Med Lett Drugs Ther.* 2008;50:262.
10. Klion A. Approach to the therapy of hypereosinophilic syndromes. *Immunol Allergy Clin North Am*. 2007;27:551–560.
11. Nutman TB. Evaluation and differential diagnosis of marked, persistent eosinophilia. *Immunol Allergy Clin North Am*. 2007;27:529–549.

IDENTIFICATION OF A NOVEL 17-kDa PROTEIN AS A FERRET ALLERGEN

Domestic ferret (*Mustela putorius furo*) is a mammal from the Mustelidae family. It is the third most common uncaged pet in North America after dogs and cats. In Europe, its popularity is progressively increasing, and it is also becoming a common pet. The role of cats and dogs as a cause of allergy is well known. However, ferrets are not so widely studied as a source of relevant allergens.

A 29-year-old man had a 3-year history of mild perennial asthma. He reported aggravation of these symptoms in the previous 2 years, coinciding with the entry of 2 ferrets into his house.

After obtaining informed consent, skin prick tests (SPTs) to common commercial aeroallergens were performed, with positive reactions to grass pollens, dust mites, molds, and epithelium from cat, dog, horse, and rabbit. Samples of ferret hair and ferret urine (obtained from the bedding material) were homogenized in phosphate-buffered saline, and, after centrifugation, dialysis, and further lyophilization, protein extracts were obtained. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)[1] after Coomassie staining showed protein bands ranging from 14 to 66 kDa in both types of ferret extracts (FEs). Ferret urine immunoblotting revealed IgE-binding proteins of 59, 34, 25, and 17 kDa in reducing conditions (with 2-mercaptoethanol), whereas in nonreducing conditions (without 2-mercaptoethanol), bands of 42, 23, 18, 16, and 15 kDa were detected (Fig 1A). The SDS-PAGE immunoblotting was also performed with extracts from dog dander; cat, horse, and rabbit epithelium; and urine from cat, rat, squirrel, and hamster. A 19-kDa IgE-binding band was revealed with extracts from dog dander, cat epithelium, and hamster urine. An enzyme allergosorbent test inhibition assay using commercial dog dander extract as solid phase showed 40% of IgE-binding inhibition when male urine FE was used as inhibitor at 10 mg/mL. The SDS-PAGE immunoblotting inhibition assays with male ferret urine as inhibitor and dog and cat epithelium extracts in solid phase showed total IgE-binding inhibition on 19- and 39-kDa bands, respectively.

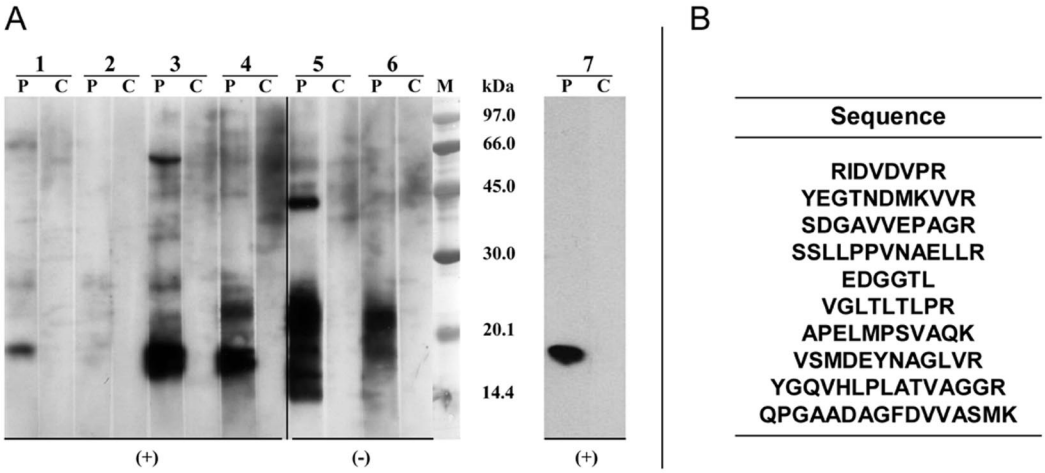

Figure 1. A, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting results. Lane 1 shows male ferret hair; lane 2, female ferret hair; lanes 3 and 5, male ferret urine; lanes 4 and 6, female ferret urine; and lane 7, 17-kDa purified protein from male ferret urine. P indicates patient serum; C, control serum (pool of sera from nonatopic subjects); M, molecular mass marker; +, sample with 2-mercaptoethanol; and −, sample without 2-mercaptoethanol. B, Peptide information for the 17-kDa protein.

We focused this study on the 17-kDa protein because it was the ferret urine protein with the highest IgE-binding capacity. This band was visualized in 2-dimensional immunoblots to specify the proteins contained. The isoelectric point was 5, detected using the 2-dimensional immunoblotting technique. The 17-kDa band was analyzed by means of matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MS) and liquid chromatography electrospray ionization tandem MS following the method of Gallego-Delgado et al.[2] The analysis of the resulting peptides using MS or MS/MS did not correspond to any previously reported allergens compared with the databases (Fig 1B). To purify the 17-kDa protein, male ferret urine was dialyzed against 20-mmol/L Tris-hydrochloride, pH 7.4, loaded on a Mono Q (GE Healthcare). The allergen was eluted with a 1M sodium chloride lineal gradient. The SPT was performed with this purified allergen with positive results. Immunoblotting with the patient's serum was performed with this purified protein (Fig 1A). Basophil activation assays using the 17-kDa purified protein were performed with the patient's heparinized whole blood (BASOTEST; ORPEGEN Pharma, Heidelberg, Germany), with concentrations ranging from 0.001 to 50 μg/mL, obtaining positive results according to the manufacturer.

Pets are usually a source of potent allergens and are permanent inhabitants of houses. Ferrets are related to minks, skunks, weasels, otters, and badgers, but they are the only domestic species in the Mustelidae family. Few cases of allergy to ferret have been reported. The capacity of these animals as a relevant source of allergens has not been extensively studied; Codina et al[3] demonstrated the presence of 4 allergens in male ferret urine (103, 81, 28.8, and 14.8 kDa) and 1 in ferret pelt (81 kDa). IgE-binding bands with similar molecular weight have been described from other mammal sources.[3,4] Nugent et al[5] described a 66-kDa protein with in vitro IgE-binding capacity from ferret hair and urine, suggesting that albumin is the relevant allergen.

In this study, clinical history and detection of specific IgE antibodies against FEs suggested an IgE-mediated allergic reaction. Immunoblotting revealed IgE reactivity with 59-, 34-, 25-, and 17-kDa proteins. Inhibition assays showed cross-reactivity between extracts from male ferret urine and dog dander and cat epithelium. The MS assays performed with the 17-kDa purified protein did not match with previously reported sequences. The positive results obtained with purified 17-kDa ferret protein, SPT, and basophil activation assay demonstrated the allergenic nature of this protein.

We presented a case of allergy to ferret involving a 17-kDa allergenic protein that has not been previously reported. Because ferret is becoming a common pet and its allergenic nature has been proved, this report should be taken into account in future diagnostic and therapeutic research in ferret allergy.

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1. Laemmli UK. Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature*. 1970;227:680–685.
2. Gallego-Delgado J, Lazaro A, Osende JI, et al. Comparison of the protein profile of established and regressed hypertension-induced left ventricular hypertrophy. *J Proteome Res*. 2006;5:404–413.
3. Codina R, Reichmuth D, Lockey RF, Jaen C. Ferret allergy. *J Allergy Clin Immunol*. 2001;107:927.
4. Gordon S, Tree RD, Newman Taylor AJ. Analysis of rat serum allergens. *J Allergy Clin Immunol*. 1997;99:716–717.
5. Nugent JS, Whisman B, Hagan LL. Ferret allergy: identification of serum specific IgE to albumin with crossreactivity to cat. *J Allergy Clin Immunol*. 2003;111(suppl 2):S324.

POMEGRANATE (*PUNICA GRANATUM*) ALLERGY: CLINICAL AND IMMUNOLOGICAL FINDINGS

Pomegranate is the fruit of the pomegranate tree (*Punica granatum*), a dicotyledon angiosperm plant of the Lythraceae family. The tree flowers in May and June, and its fruits mature in September and October. The pulp of the fruit consists of a cluster of fleshy, reddish violet seeds. Pomegranate fruits are commonly consumed in the Mediterranean areas, both raw and in processed forms, such as juice and flavorings. They have been infrequently reported to cause immediate hypersensitivity after ingestion.[1,2] Pomegranate has already been reported to contain a 29-kDa protein and a 9- to 12-kDa protein, and characterization of the latter has confirmed that it is a lipid transfer protein (LTP).[3,4] LTPs have been identified as major allergens in fruits belonging to the Rosaceae family. The wide distribution and the highly conserved structure of these proteins in various plant species confer these polypeptides a role as plant panallergens. In turn, LTPs are recognized as being responsible for immunological cross-reactivity among fruits, nuts, and/or pollens.[5]

To our knowledge, we present the first case series of patients with allergic symptoms that developed after eating fresh pomegranate fruit. The patients included the following: (1) a 19-year-old man who complained of 2 episodes of angioedema of the upper lips and tongue, which appeared a few minutes after ingestion of the fresh fruit; (2) a 13-year-old boy who had an episode of widespread urticaria and angioedema of the lips after ingestion of the fresh fruit; (3) a 31-year-old woman who complained of an episode of urticaria 20 minutes after ingestion of the fresh fruit; and (4) a 23-year-old woman who complained of an episode of angioedema of the face

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