Quantitation of *Blomia tropicalis* Allergen Blo t 5 in Cereal and Cereal-Based Foods Consumed in the Nile Delta, Egypt

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Abstract. The mite *Blomia tropicalis* has significant prevalence worldwide. Blo t 5 is a major *B. tropicalis* allergen that has been associated with sensitization and allergic symptoms in many asthmatic patients. Besides house dust, contaminated foodstuffs are an important source of exposure to *B. tropicalis* allergens. In this study, a double sandwich enzyme-linked immunosorbent assay (ELISA) using mAb 4D4 and biotinylated mAb 4G9 was done to detect Blo t 5 allergen in different types of raw and processed foods, including wheat, corn, rice, bean, wheat and corn flour, cake, and rusk, which were collected from retail stores in the Nile Delta of Egypt. Out of 88 samples, 38 (43.2%) were positive for Blo t 5 allergen with no significant statistical difference in positivity according to food type. In positive samples, the Blo t 5 concentration ranged from 10 ng/g to 790 ng/g. This study showed that Blo t 5 should be considered as an important allergen that presents a risk to the Egyptian population and should become a routine allergen in the skin prick test to improve the management of allergic disease.

INTRODUCTION

Currently, there is an increase in the prevalence of allergic disease that estimated by 20% of the world populations.^{1,2} Allergic disease is caused by an immunological reaction to an allergen, which is a protein or glycoprotein that induces specific immunoglobulin E (IgE) antibodies in atopic individuals, and is associated with clinical evidence of an immediate hypersensitivity response.^{3,4} Mites represent a major source of allergens, which cause about 50% of such allergic manifestations.⁵ Although the main source of mite allergens is believed to be exposure to indoor house dust mites, there is increasing evidence pointing to oral ingestion of mites as a source of allergen exposure.^{6,7} *Blomia tropicalis*, a member of the Echimyopodidae family, is a common mite species found not only in tropical and subtropical regions but also in temperate regions.^{8,9} This mite has been found in house dust from homes in Hong Kong, Brazil, Venezuela, Columbia, Taiwan, Malaysia, Spain, Egypt, and the United States.¹⁰ In addition, many studies reported the presence of *B. tropicalis* in different types of foodstuffs.^{11–14} Exposure to mite allergens may occur via inhalation of house dust or ingestion of contaminated raw or processed food.^{7,11} Many allergens have been recognized in *B. tropicalis* extract,⁸ the most important of which is the species-specific 14-KDa Blo t 5,^{15,16} to which up to 92% of allergic patients are sensitized.¹⁷ In this work, we surveyed a variety of foodstuffs sold and consumed in the Nile Delta, Egypt, for the presence of *B. tropicalis* Blo t 5 allergen to highlight the potential risk of exposure to that allergen in the Nile Delta.

METHODS

Sample collection. The samples were collected from retail stores and bakeries in five cities from three adjacent governorates located in the center of the Nile Delta in Egypt, namely Benha, Toukh, Qalyub (Qalyubia governorate), Berket Alsaba (Monufia governorate), and Tanta (Gharbia governorate). The climate in the study area is subtropical (approximate latitude 30° north, longitude 31° east). In the retail stores and bakeries,

the grains and products are kept in open containers and on shelves exposed to the external environment. Samples were kept in closed plastic bags until examination. Eighty-eight samples were collected and included the most commonly used categories of dry food (57 wheat, 16 corn, 9 rice, and 6 bean samples). The wheat samples were classified into four categories: 11 whole wheat, 21 wheat flour, 10 uncooked foods (cake mix and pasta), and 15 cooked foods (rusk and cake). The corn samples included nine whole corn samples and seven corn flour samples.

All large-sized samples were properly ground and then 100 mg were added to 1 mL phosphate buffered saline (pH 7.4) and mixed vigorously for 2 hours on a shaker. The mixture was centrifuged for 10 minutes at 3,000 rpm and the supernatant was collected and kept at 4°C until used.

Enzyme-linked immunosorbent assay for detection of Blo t 5 allergen. A double sandwich enzyme-linked immunosorbent assay (ELISA) was done using mAb 4D4 and biotinylated anti Blo t 5 mAb 4G9 according to the manufacturer's instructions (Indoor Biotechnologies Inc., Charlottesville, VA, Product code: EL-BT5). Both antibodies have been reported to bind to a species-specific epitope on the *B. tropicalis* allergen, Blo t 5. A double dilution of the rBlo t 5 Standard (Product code: EL-BT5, Indoor Biotechnologies Inc.) was used to make a standard curve for the calculation of the Blo t 5 allergen concentration in the samples.

Statistical analysis. The collected data are presented as numbers and percentages. Data were analyzed using microstate software (Ecosoft, Inc. Company, Chicago Classic Computing, Chicago, IL); the χ^2 and the "Z" tests were the tests of significance. Two portions from one sample and two independent groups were analyzed. The acceptable level of significance in this study was $P < 0.050$ (two-sided test).

RESULTS

Our results show that *B. tropicalis* allergen Blo t 5 was detected in 43.2% of the total screened samples. The highest prevalence was found in Tanta city (63.6%), Gharbia governorate, and was statistically significant. A low prevalence was found in the three cities of Qalyubia governorate. The lowest prevalence (28.6%) was found in Benha city (Table 1). Comparison of the data from different cities showed no statistically significant differences.

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TABLE 1

Blomia tropicalis allergen (Blo t 5) prevalence in three different governorates in Nile Delta, Egypt

Area	No. of samples	ELISA for Blo t 5		P value
		Positive (%)	Negative (%)	
Qalyubia Governorate				
Benha	7	2 (28.6)	5 (71.4)	0.41
Toukh	20	6 (30)	14 (70)	0.17
Qalyub	19	7 (36.8)	12 (63.2)	0.53
Menufia Governorate				
Berket AlSaba	20	9 (45)	11 (55)	0.85
Gharbia Governorate				
Tanta	22	14 (63.6)	8 (36.4)	0.025*
Total	88	38 (43.2)	50 (56.8)	

ELISA = enzyme-linked immunosorbent assay.

*Statistically significant result.

The Blo t 5 allergen was detected in all types of cereals examined (Table 2). The highest prevalence (70%) was detected in an uncooked wheat flour mix and was not statistically significant, while the lowest ratio (14.30%) was detected in corn flour samples, and was statistically significant. No significant difference was found between the results for the different types of foodstuffs examined. The concentration of Blo t 5 in positive samples ranged from 10 to 790 ng/g, with the highest concentrations found in whole wheat and wheat flour samples (Figure 1).

DISCUSSION

Although the role of *B. tropicalis* allergens in the sensitization of atopic patients is well-established worldwide, especially in (sub) tropical and temperate countries,^{9,18–20} only two reports documented the presence of *B. tropicalis* in Egypt.^{21,22} Both reports depended on methods for counting mites that did not necessarily correlate with risk of allergen exposure.²³ Because of this, we used an immunochemical technique that has largely replaced biological methods for assessing exposure,²⁴ due to its high sensitivity and specificity.²⁵

Our results show that *B. tropicalis* Blo t 5 allergen was detected in a variety of foodstuffs sold in retail stores including wheat, corn, rice, beans, and processed foods (rusk, pasta, and pancakes). This result is contrary to that of Yi and others,²⁶ who suggested that the contamination of food with *B. tropicalis* depends on the presence of wheat in the food.

TABLE 2

Prevalence of *Blomia tropicalis* allergen (Blo t 5) in cereals and cereal-based food samples, using double sandwich ELISA

Sample type*	No. of samples	ELISA for Blo t 5 detection		P
		Positive (%)	Negative (%)	
Whole wheat	11	5 (45.45)	6 (54.55)	0.77
Wheat flour	21	12 (57.10)	9 (42.90)	0.51
Wheat flour mix (uncooked)	10	7 (70)	3 (30)	0.16
Processed wheat mix (cooked)	15	4 (26.70)	11 (73.30)	0.04†
Corn	9	2 (22.20)	7 (77.80)	0.044†
Corn flour	7	1 (14.30)	6 (85.70)	0.007†
Rice	9	5 (55.60)	4 (44.40)	0.73
Bean	6	2 (33.33)	4 (66.67)	0.39
Total	88	38 (43.20)	50 (56.80)	0.19

ELISA = enzyme-linked immunosorbent assay.

*No significant difference was found in results of different sample types.

†Statistically significant result.

However, our results are supported by other studies that reported mite contamination in corn flour, rice, and beans.^{14,27} In addition, cheese, ham, chorizo, and salami were reported to be contaminated with mites when stored for long periods at room temperature.^{13,28,29}

To our best knowledge, this is the first study reporting presence of *B. tropicalis* allergens in the Nile Delta. The high prevalence of Blo t 5 allergens reported in this study may conflict with the rare detection of *B. tropicalis* in Egypt. Nevertheless, this may be explained because Egypt is the largest importer of grain in the world. Grain is imported from many countries worldwide and could have been contaminated with mites or their fragments and feces in the country of origin. Another explanation could be that the use of the ELISA technique for detection of mite allergens instead of whole mite detection using a counting technique may have led to a poor estimate of food contamination with mite allergens, especially for cereal-based processed foodstuffs. Our data indicate that the Egyptian population is at high risk for exposure to *B. tropicalis* allergens. Similar results were reported from different areas around the world. Zhang and others³⁰ reported that *B. tropicalis* allergens were detected in 494/590 (84%) of dust samples tested in Singapore. They claim that *B. tropicalis* is the most prevalent mite identified in house dust in Singapore and the atopic population is highly sensitized to *B. tropicalis* allergens. Also, *B. tropicalis* was found in 30% of the samples collected from Florida in the United States.³¹ The medical importance of our results is emphasized by various worldwide studies that found *B. tropicalis* allergens are an important cause of allergic disease. The manifestation may be severe enough to cause oral mite anaphylaxis. A case of anaphylactic shock after eating a pancake containing *B. tropicalis* allergens was reported by Barrera and others.¹¹ Many studies reported that about 2.4–93.7% of allergic patients show presence of a specific IgE against *B. tropicalis* allergens,^{9,21,22,32–37} of which 42–98% showed a positive reaction against the Blo t 5 allergen.³⁸

This study reported presence of Blo t 5 allergen in many types of foodstuffs consumed in the Nile Delta. Until 1990, house dust inhalation was considered as the major route of exposure to mite allergens.³⁹ However, it was suggested that ingestion of contaminated food can trigger allergic manifestations in many atopic individuals.^{40–42} Some studies established that ingested mite allergens are heat stable and can still cause an allergic response after ingestion.^{6,7}

Although the recorded concentration of Blo t 5 is not very high, and the highest concentration was 790 ng/g, consumption of an amount of cereal-based food as little as 3 g may present a risk for a sensitized patient and an asthmatic attack might be induced if ≥ 25 g of contaminated food was consumed. This postulate was based on the suggestion of the Second International Meeting of Dust Mites and Asthma held in England in 1990, which indicated that exposure at a level of ≥ 2 μ g/g of dust to group 1 and 2 allergens was a risk factor for sensitization in genetically predisposed individuals, while exposure at a level ≥ 10 μ g of mite allergens per gram of dust was a risk factor for the onset of acute asthma.⁴³

This study provided limited but useful information on the contamination of cereal and cereal-based foods with *B. tropicalis* allergens that carries two risks for a population: first, the sensitization of the general population via long-term ingestion of allergen-contaminated food and second, aggravated

FIGURE 1. Concentration of Blo t 5 allergen in different positive food samples using double sandwich enzyme-linked immunosorbent assay (ELISA) (ng/g).

manifestations in patients with preexisting allergic disease.⁴⁴ Blo t 5 allergen should become a routine allergen considered in the clinical diagnosis of patients who complain of an apparent food allergy. Our result is limited by the small sample size, targeting Blo t 5 allergen only; a lack of a microscopic parasitological examination of the samples; and the study of risk factors that affect food contamination with mites in Egypt. A larger-scale study is necessary to emphasize the risk of exposure on ingestion or contact with different foodstuffs and to establish the threshold level of Blo t 5 that might trigger an allergic reaction on ingestion.

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REFERENCES

1. WHO, 2003. *Prevention of Allergy and Allergic Asthma*. WHO meeting report. WHO/MNC/CRA/03.2. Geneva, Switzerland: World Health Organization.
2. Bousquet J, Dahl R, Khaltayev N, 2007. Global alliance against chronic respiratory diseases. *Allergy* 62: 216–223.
3. Platts-Mills TA, Solomon WR, 1993. Aerobiology and inhalant allergens. Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. *Allergy: Principles and Practice*, 4th edition. St Louis, MO: Mosby-Year Book, 469–528.
4. Cromwell O, 1997. Biochemistry of allergens. Kay AB, Kaplan AP, Bousquet J, Holt PG, eds. *Allergy and Allergic Diseases*, Volume 1, 2nd edition. Oxford, United Kingdom: Blackwell Science Ltd., 797–810.
5. Arlian LG, Platts-Mills TA, 2001. The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol* 107: S406–S413.
6. Sanchez-Borges M, Capriles-Hulett A, Fernandez-Caldas E, Suarez-Chacon R, Caballero F, Castillo S, Sotillo E, 1997. Mite-contaminated foods as a cause of anaphylaxis. *J Allergy Clin Immunol* 99: 738–743.
7. Sanchez-Borges M, Suárez-Chacon R, Capriles-Hulett A, Caballero-Fonseca F, Iraola V, Fernandez-Caldas E, 2009. Pancake syndrome (oral mite anaphylaxis). *World Allergy Organ J* 2: 91–96.
8. Caraballo L, Puerta L, Martinez B, Moreno L, 1994. Identification of allergens from the mite *Blomia tropicalis*. *Clin Exp Allergy* 24: 1050–1060.
9. Juliá-Serdá G, Cabrera-Navarro P, Acosta-Fernández O, Martín-Pérez P, García-Bello MA, Antó-Boqué J, 2012. Prevalence of sensitization to *Blomia tropicalis* among young adults in a temperate climate. *J Asthma* 49: 349–354.
10. Arruda LK, Chapman MD, 1992. A review of recent immunochemical studies of *Blomia tropicalis* and *Euroglyphus maynei* allergens. *Exp Appl Acarol* 16: 129–140.
11. Barrera OM, Murgas I, Miranda RJ, 2012. First report of anaphylactic shock caused by the ingestion of mite-infested flour in Panama. *World Allergy Organ J* 5 (Suppl 2): S104.
12. Wen DC, Shyur SD, Ho CM, Chiang YC, Huang LH, Lin MT, Yang HC, Liang PH, 2005. Systemic anaphylaxis after the ingestion of pancake contaminated with the storage mite *Blomia freemani*. *Ann Allergy Asthma Immunol* 95: 612–614.
13. Armentia A, Fernandez A, Perez-Santos C, De la Fuente R, Sanchez P, Méndez J, Stolle R, 1994. Occupational allergy to mites in salty ham, chorizo and cheese. *Allergol Immunopathol (Madr)* 22: 152–154.

14. Franzolin MR, Baggio D, 2000. Mite contamination in polished rice and beans commercialized in municipal markets. *Rev Saude Publica* 34: 77–83.
15. Arruda LK, Fernandez-Caldas E, Naspitz CK, Montealegre F, Chapman MD, 1994. Molecular cloning of *Blomia tropicalis* allergen Blo t 5 and its sequence homology to *D. pteronyssinus* Der p 5. *J Allergy Clin Immunol* 93: 205.
16. Arruda LK, Vailes LD, Platts-Mills TAE, Fernandez-Caldas E, Montealegre F, Lin KL, Chua KY, Rizzo MC, Naspitz CK, Chapman MD, 1997. Sensitization to *Blomia tropicalis* in patients with asthma and identification of Blo t 5. *Am J Respir Crit Care Med* 155: 343–350.
17. Carvalho KA, de Melo-Neto OP, Magalhães FB, Ponte JCM, Felipe F, Santos M, Lima G, Cruz A, Pinheiro CS, Pontes-de-Carvalho L, Alcantara-Neves NM, 2013. *Blomia tropicalis* Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. *BMC Immunol* 14: 11.
18. Caplin J, Capriles-Hulett A, Iraola V, Pinto H, Sánchez-Borges M, de los Santos G, Fernández-Caldas E, 2009. Allergic sensitization to domestic mites in Corpus Christi, Texas. *Allergy Asthma Proc* 30: 166–170.
19. Pefura-Yone E, Kengne A, Afane-Ze E, Kuaban C, 2014. Sensitization to *Blattella germanica* among adults with asthma in Yaounde, Cameroon: a cross-sectional study. *World Allergy Organ J* 7: 22.
20. Sun B, Zheng P, Wei N, Huang H, Zeng G, 2014. Co-sensitization to silkworm moth (*Bombyx mori*) and 9 inhalant allergens among allergic patients in Guangzhou, southern China. *PLoS ONE* 9: e94776.
21. Gamal-Eddin FM, Shoker NI, 1989. The present status and ecology of house dust mites in El-Minia Governorate (Upper Egypt), as basis for future studies on environment pollution with house dust mite allergens or on vaccination with specific mite antigens. *J Egypt Soc Parasitol* 19: 332–341.
22. Kenawy MA, Awad AA, El-Shafei AM, Helmi N, Abdel-Hamid YM, El-Zohery YZA, 2012. House dust mites associated with the asthmatic patients in some houses of Cairo, A.R. Egypt. *Egypt Acad J biolog Sci* 4: 1–5.
23. Mollet JA, Vailes LD, Avner DB, Perzanowski MS, Arruda LA, Chapman MD, Platts-Mills TAE, 1997. Evaluation of German cockroach (Orthoptera: Blattellidae) allergen and seasonal variation in low-income housing. *J Med Entomol* 34: 307–311.
24. Chapman MD, Smith AM, Slunt JB, Vailes LD, Arruda LK, 1995. Immunochemical and molecular methods for defining and measuring indoor allergens: in dust and air. *Pediatr Allergy Immunol* 6: 8–12.
25. DeBoer DJ, Schreiner TA, 2001. Commercial dry dog food in the north central United States is not contaminated by *Dermatophagoides* house dust mites. *Vet Dermatol* 12: 183–187.
26. Yi FC, Chen JY, Chee KK, Chua KY, Lee BW, 2009. Dust mite infestation of flour samples. *Allergy* 64: 1788–1789.
27. Guerra Bernd LA, Arruda LK, Barros Antunes HB, 2001. Oral anaphylaxis to mites. *Allergy* 56: 83–84.
28. Llicardi G, D'Amato M, D'Amato G, 1996. Oral allergy syndrome after ingestion of salami in a subject with monosensitization to mite allergens. *J Allergy Clin Immunol* 98: 850–852.
29. Kasti GL, Codina R, Ledford DR, Lockey RF, 1998. Mite contamination of food stuffs. *J Allergy Clin Immunol* 101: 24–25.
30. Zhang L, Chew FT, Soh SY, Yi FC, Law SY, Goh DYT, Lee BW, 1997. Prevalence and distribution of indoor allergens in Singapore. *Clin Exp Allergy* 27: 876–885.
31. Fernandez-Caldas E, Fox RW, Bucholtz GA, Trudeau WL, Ledford DK, Lockey RF, 1990. House dust mite allergy in Florida. Mite survey in households of mite-sensitive individuals in Tampa, Florida. *Allergy Proc* 11: 263–267.
32. Podder S, Gupta S, Saha G, 2010. Incrimination of *Blomia tropicalis* as a potent allergen in house dust and its role in allergic asthma in Kolkata metropolis, India. *World Allergy Organ J* 3: 182–187.
33. Yong SB, Wu CC, Tzeng YC, Hung WC, Yang KD, 2013. Different profiles of allergen sensitization in different ages and geographic areas in Changhua, Taiwan. *J Microbiol Immunol Infect* 46: 295–301.
34. Larenas-Linnemann D, Michels A, Dinger H, Shah-Hosseini K, Mösges R, Arias-Cruz A, Ambriz-Moreno M, Barajas MB, Javier RC, Prado M, Moreno M, Almaraz RG, García-Cobas CY, Imperial D, Muñoz RG, Hernández-Colín D, Linares-Zapien F, Luna-Pech JA, Matta-Campos JJ, Jiménez NM, Medina-Ávalos M, Hernández AM, Maldonado AM, López DN, Nazara LJ, Sanchez ER, Ramos-López JD, Rodríguez-Pérez N, Rodríguez-Ortiz PG, 2014. Allergen sensitization linked to climate and age, not to intermittent-persistent rhinitis in a cross-sectional cohort study in the (sub) tropics. *Clin Transl Allergy* 4: 20.
35. Sun B, Zheng P, Zhang X, Huang H, Chen D, Zeng G, 2014. Prevalence of allergen sensitization among patients with allergic diseases in Guangzhou, southern China: a four-year observational study. *Multidiscip Respir Med* 9: 2.
36. Oliveira-Santos J, Motta-Francob I, Barreto D, Solé R, 2014. Asthma in adolescents—prevalence trends and associated factors in northeast Brazil. *Allergol Immunopathol (Madr)*. doi:10.1016/j.aller.2014.05.006.
37. Lâm HT, Ekerljung L, Bjerg A, VãnTyòng N, Lundbäck B, Rönmark E, 2014. Sensitization to airborne allergens among adults and its impact on allergic symptoms: a population survey in northern Vietnam. *Clin Transl Allergy* 4: 6.
38. Taketomi EA, Almeida KC, Pereira FL, Silva D, 2010. Allergens: sources, exposure and sensitization levels, diagnostic tools and immunotherapeutic applications. *JMMS* 1 (12 Special Issue): 580–588.
39. Olsen AR, 1998. Regulatory action criteria for filth and other extraneous materials II. Allergenic mites: an emerging food safety issue. *Regul Toxicol Pharmacol* 28: 190–198.
40. Erben AM, Rodriguez JL, McCullough JM, Ownby DR, 1993. Anaphylaxis after ingestion of beignets contaminated with *Dermatophagoides farinae*. *J Allergy Clin Immunol* 92: 846–849.
41. Matsumoto T, Hisano T, Hamaguchi M, Miike T, 1996. Systemic anaphylaxis after eating storage-mite-contaminated food. *Int Arch Allergy Immunol* 109: 197–200.
42. Blanco C, Quiralte J, Castillo R, Delgado J, Arteaga C, Barber D, Carrillo T, 1997. Anaphylaxis after ingestion of wheat flour contaminated with mites. *J Allergy Clin Immunol* 99: 308–313.
43. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD, 1992. Dust mite allergens and asthma: report of a Second International Workshop. *J Allergy Clin Immunol* 89: 1046–1060.
44. Arshad SH, 2010. Does exposure to indoor allergens contribute to the development of asthma and allergy? *Curr Allergy Asthma Rep* 10: 49–55.