HOUSE DUST MITE ALLERGENS IN SAUDI ARABIA: REGIONAL VARIATIONS AND IMMUNE RESPONSE

Abdulrahman S. Al-Frayh, MD, FACAAI; Syed M. Hasnain, PhD, FACAAI; Mohammad O. Gad-El-Rab, MD, FACAAI; Bente Schwartz, MSc; Khalid Al-Mobairek, MD; Sultan T. Al-Sedairy, PhD

In order to assess the causative extrinsic allergic factor(s) in school-age children diagnosed as having bronchial asthma and allergic rhinitis, and to qualitatively and quantitatively evaluate the presence of house dust mites (HDMs) in the homes of these children in Saudi Arabia, a study analyzing mite contents in 165 samples collected from patients' indoor environment was conducted. The dust samples were collected from four regions of Saudi Arabia, showing variation in their geography and climate. Immunochemical assays were performed using ALK reagents by ELISA technique. A total of 462 children were also tested using skin prick test (SPT) method for IgE-mediated reactions to HDMs. The samples from the Central dry region revealed a very low amount of the potent house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*, the two dominant species in various parts of the world). The samples from the Southern mountainous region contained a very high concentration of *Der p* I (84,000 ng/g of dust), while the Western coastal region showed a high concentration of *Der f* I (up to 22,000 ng/g). The mid-Western agricultural region did not exhibit any significant level of either *Der p* I or *Der f* I. The maximum level of *D. pteronyssinus* detected in the Central dry region was 106 ng/g of dust. The data exhibit both qualitative and quantitative variations of HDMs in the three regions and may be attributed to variation in geography and climate, particularly humidity of the regions, which vary significantly. Riyadh in the Central region is considered to have low humidity (<40%), while humidity in the Western coastal region, Jeddah, and the Southern region of Abha is comparatively higher, which helps house dust mites thrive. SPT results in these regions with house dust mite allergens (in addition to other common inhalant allergens) also revealed a considerable number of IgE-mediated reactions, consistent with the frequency of house dust mites in the region. Though more data are being accumulated on the subject to conduct a statistical comparison and more skin tests are underway in the Southern region, the study suggests the presence of at least two HDMs as well as qualitative diversity and quantitative variation of house dust mites in Saudi Arabia. The study also indicates, with a considerable number of IgE-mediated reactions, the possible influence of mites in the allergic manifestations of many patients, which is not only common, but increasing in parts of the country. *Ann Saudi Med 1997;17(2):156-160.*

The species of house dust mites (HDMs), *Dermatophagoides pteronyssinus* and *D. farinae*, as well as other animal-origin allergens such as airborne pollen, fungal spores, cat dander and saliva, and cockroach fecal particles, have been implicated as a cause of respiratory allergies in humans. Both species of mites are known to be prevalent indoors and are considered to be potent allergens in many parts of the world, especially in humid climates.

The role of mites (family Pyroglyphidae) as an important source of house dust allergens was established several years ago.[1,2] Since then, contribution of HDMs to the clinical symptoms of bronchial asthma, allergic rhinitis, and atopic dermatitis, has been fully studied and documented.[3-5] Physiochemical and antigenic properties of mites have also been studied using immunochemical, molecular and immunological technique and the allergens involved have been characterized.[6]

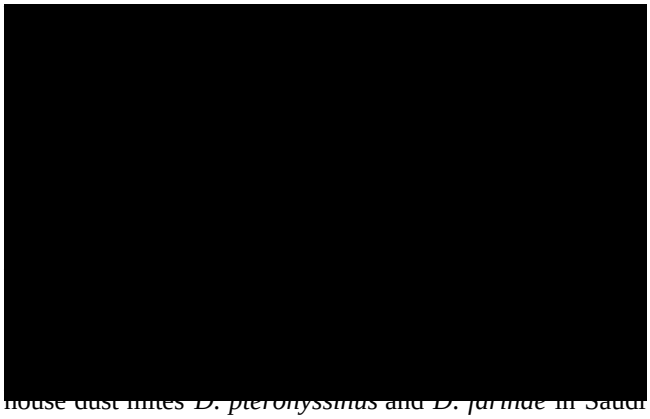
Pyroglyphidae mites usually account for 90% of the mites in houses and though there are several species known to exist, four species, namely *D. pteronyssinus*, *D. farinae*, *D. microceras* and *Euroglyphus maynei*, are dominant all over the world. Another species of mite which can occur in houses include storage mites (family Acaridae); several species of storage mites are also known to exist.

Saudi Arabia is a fast developing country situated in the middle of the Arabian Peninsula. Health care facilities, including hygiene, in the Kingdom, are considered to be excellent. According to our recent prevalence study,[7] bronchial asthma emerged to be a common disease in children. About 10% of children nationally suffer from

From the Departments of Pediatrics (Drs. Al-Frayh, Al-Mobairek), Pathology (Dr. Gad-El-Rab), College of Medicine and KKUH, Riyadh, Biological and Medical Research (Drs. Hasnain, Al-Sedairy), King Faisal Specialist Hospital and Research Centre, Riyadh, and ALK Laboratories (Ms. Schwartz), Horsholm, Denmark.

Address reprint requests and correspondence to Dr. Al-Frayh: Department of Pediatrics, College of Medicine and KKUH, P.O. Box 2925, Riyadh 11461, Saudi Arabia.

Accepted for publication 28 May 1996. Received 5 December 1995.



house dust mites *D. pteronyssinus* and *D. farinae* in Saudi homes and SPT reactions in patients from different parts of the Kingdom.

Material and Methods

House Dust Samples

Mattresses and carpets are the primary source of mites in the houses. Thus, the samples were collected through the individual's own vacuum cleaner after the operation on their respective home carpets. In order to avoid contamination, samples were collected in sterile polythene bags and on "ALK filter" devices especially designed for this purpose (ALK Laboratory, Denmark). Samples were extracted individually and allergens were quantitated using specific antibodies from ALK Laboratories, Denmark by ELISA procedure.

Samples were collected from four different geographical regions in Saudi Arabia. The areas were: 1) Abha, from the Asir region in the south of Saudi Arabia. The area is surrounded by mountains and hills with a mild climate and with a relative humidity around 80% to 90%, and is termed the Southern mountainous region. 2) Jeddah, in the Western region, situated on the coast of the Red Sea. The area is a mixture of ancient and newly developed architectural settings, with a hot climate and very high humidity (90%), and is termed the Western coastal region. 3) Qassim, an agricultural area in the midwest of the country with the humidity ranging between 50% and 70%, and is termed the mid-Western agricultural region. 4) Riyadh, in the Central province, a modern city situated in the middle of the desert with a hot climate and low humidity (40%) termed as the central dry region.

ELISA Procedure

Dust Extraction: 200 mg of dust from each sample was extracted by agitation in an extraction buffer (1:10 w/v) for two hours at room temperature. The suspension was then filtered through a filter paper and through a 0.22 µm membrane filter. In some cases, the suspension was centrifuged for 15 minutes at 1000 g. Extracts were stored at –20°C until use.

Antigen Incubation: Standards: A dilution series of reference antigen of 4000 ng/mL by twofold dilution was prepared from SQ Mite Extract (ALK Laboratory, Denmark) just before use.

100 µL reference antigen solution was pipetted in the microtiter plate (96 wells), 100 µL Blank (PBS-BSA), 100 µL sample dilutions and 100 µL specificity control and left for incubation for three to 24 hours at 20°C.

100 µL of HRP-labelled antibody in a proper dilution was added in each well, incubated for one hour at 20°C and washed three times with PBS-TWEEN as above.

100 µL of substrate solution was added in each well and incubated at 20°C in darkness for exactly 15 minutes, after which the reaction was stopped by the addition of 100 µL 1 M sulfuric acid and left in darkness for half an hour. Absorbance was read by spectrophotometer at 490 nm.

A calibration curve was constructed by plotting the absorbances (in absorbance units) for each standard measurement in a semilogarithmic blot. The dust concentration (in ng/g) was obtained by multiplying the sample extract-concentration (in ng/mL) by a factor of 10.

Skin Prick Testing (SPT): A total of 462 asthmatic children, including 120 in the Central dry region, 120 in the Western coastal region, 156 in the Southern mountainous region and 66 from the mid-Western agricultural region, were skin tested with commercial extracts of indoor inhalant allergens including HDMs mixed and *Dermatophagoides farinae.* The procedure for SPT was standard, which included 1% histamine phosphate as a positive control and saline a negative control.

Results

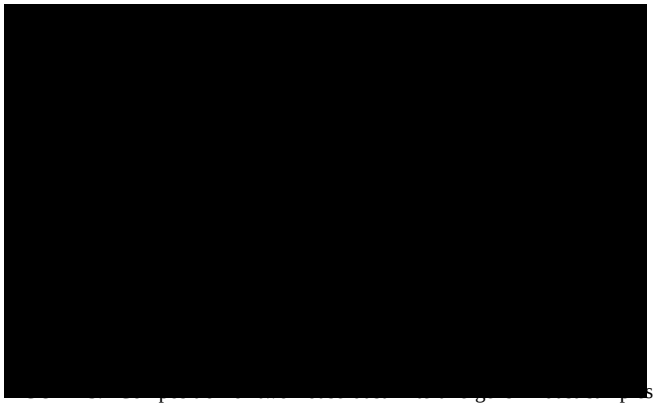
Der p I and *Der f* I contents in house dust samples from the Southern mountainous region of Abha (ABH) are presented in Figure 1. Data for *Der p* I and *Der f* I contents in samples from the mid-Western region of Qassim (Q) are presented in Figures 2 and 3.

Figure 1 shows that about 25% (10 samples) of all samples in Abha contained more than $3x10^3$ ng/g dust of *Der p* I while 10% (four samples) contained more than $10x10^3$ ng/g of dust, with two samples containing $39x10^3$ and $84x10^3$ ng/g. In the same samples, the contents of *Der f* I did not cross even 60 ng/g of dust, which is a negligible or trace amount.

In Qassim (Q) samples (Figures 2 and 3), neither *Der p* I nor *Der f* I was present in more than a trace amount. Data from the Western coastal region (Jeddah=J), presented in Figure 4, exhibited one sample ($22.9x10^3$ ng/g) of dust, while samples from the Central dry region (Riyadh=R) did not show any noticeable quantity of either

FIGURE 1. Composition of two house dust mite allergens in dust samples from asthmatic homes in the Abha region.

FIGURE 2. Composition of two house dust mite allergens in dust samples from asthmatic homes in the Qassim region.



from asthmatic homes in the Qassim region.

samples collected from Riyadh and Jeddah.

Der p I or *Der f* I.

SPT Results

Skin prick test (SPT) results of 462 patients tested are presented in Tables 1, 2 and 3. Table 1 clearly indicates that in the mountainous region, *D. pteronyssinus* gave 25.1% positive reactions compared with about 7.6% in the agricultural region. Positive reactions to *D. farinae* were proportionally low in both regions.

Because of a shortage of *D. pteronyssinus* antigen, only *D. farinae* was tested in both dry and coastal regions. However, HDM (mixed) was also tested in the dry region. Table 2 indicates a total of 12.6% positive reactions by *D. farinae* in the dry region, compared to 56.3% total positive reaction in the coastal region (Table 3).

Discussion

Epidemiologic studies from Europe and North America have proved that levels of mite exposure of 2 µg or 10 µg of group I allergens per gram (equivalent to 100 or 500 mites per gram) of dust are relevant to bronchial asthma in HDM-sensitive patients.[4,5] Quantitative evaluation of mites or mite allergens provide a valid "index of exposure," which can be used for risk evaluation in the above patients.[5] Studies have also confirmed that appropriate mite-avoidance measures in the bedroom decreases symptoms in mite-sensitive asthmatic patients,[8] and secondly, mite immunotherapy appears to be helpful in some of these patients.[2,9,10]

It is evident from our data that the two clinically important HDM species, *Dermatophagoides pteronyssinus* and *D. farinae* are present with regional diversity in Saudi homes in levels exceeding threshold values for sensitization and for acute attacks of bronchial asthma.

The date also revealed that more than 25% samples in the mountainous region of Abha contained above the threshold level of *Der p* I, with a maximum level of84,000 ng/g dust. Contrary to that, *Der f* I contents in the same samples remained far too low and did not reach even one-tenth of the sensitization level. Comparison of the data from the Qassim agricultural region (Q) reveals neither any significant presence nor major differences in *Der p* I and *Der f* I contents. In fact, there were more samples

TABLE 1. *Results of SPT reactions of asthmatic children in mountainous and agricultural regions of Saudi Arabia.*

	Southern mountainous region (Abha, n=156)		Midwestern agricultural region (Al-Qassim, n=66)	
	# of +ve pt.	% of +ve pt.	# of +ve pt.	% of +ve pt.
D. pteronyssinus	46	25.1	5	7.6
D. farinae	35	19.1	3	4.5
Cat fur	46	25.1	15	22.7
Cockroach	34	18.6	23	34.8
Sheep wool	25	13.7	7	10.6
Goat Hair	41	22.4	11	16.7

TABLE 2. *SPT-positive results of 120 asthmatic children tested in central dry region (Riyadh) of Saudi Arabia.**

Indoor allergens	Mild** (%)	Moderate (%)	Strong (%)	Total (%)
House dust mites (mixed)	12.7	9.9	8.4	31.0
D. farinae	7	2.8	2.8	12.6
Cat fur	8.4	7.1	56.3	71.8
Cockroach	15.4	14.1	9.9	39.4
Sheep wool	12.7	5.6	8.4	26.7
Dog hair	2.8	4.2	1.4	8.4
Cotton flock	8.4	15.5	14.1	38.0
Mean	9.6	8.5	14.5	32.6

Indoor allergens accounted for a mean 32.6% SPT-positive reactions in dry region (Riyadh). Other allergens included in the panel were various pollen grains and fungi. *Percentages were calculated from the number of positive reactions obtained (=71); **weal and erythema: <3 mm compared to saline control=mild; between 3-5 mm compared to saline control=moderate; >5 mm compared to saline control=strong.

from this region analyzed to see any variation. A number of samples from the dry region, Riyadh (R), and the coastal region, Jeddah (J), were also analyzed.

While the analyses of more dust samples from dry and particularly coastal regions are needed, the samples from the coastal region showed a different profile from the mountainous region. The data revealed the presence of *Der f* I in a clinically significant level in Jeddah compared to *Der p* I as recorded in Abha. The maximum level of *Der f* I recorded was 22,945 ng/g of dust, compared to a maximum of 90 ng/g of *Der p* I. The data for the dry region (Riyadh) indicate the absence or very low level of any of these potent allergenic mite contents. The maximum recorded value was 106 ng/g of *Der p* I and 26 ng/g of *Der f* I in the dry region.

HDM sensitivity is known to be frequent in a mild humid climate with mean monthly indoor relative humidity (RH) >50% for four or more months per year. The HDMs *D. pteronyssinus* and *D farinae* become dehydrated and die when they are kept at a RH <50% under controlled laboratory conditions.[11,12] Even when the optimum RH for a species is reached, the mite population does not expand immediately. A month or two elapses before the number increases.

Even the slight variation in humidity greatly influences the mite concentration. It has been documented[13] that there were six times fewer HDMs at 75% RH than at 80%. It has also been shown that below the critical equilibrium humidity (70%), the mite can no longer maintain its body and progressively loses water until it succumbs to desiccation.[14,15]

SPT results of 462 patients tested with *D. pteronyssinus* and *D. farinae* (presented in Tables 1, 2 and 3) show that up to 25.1% and 19.1% positive reactions were obtained by *D. pteronyssinus* and *D. farinae* respectively in asthmatic children in the mountainous region, while 56.3% positive reactions were obtained by *D. farinae* in coastal areas. In agriculture and dry regions, the figures were 7.6% and 12.6% respectively for *D. pteronyssinus* and *D. farinae.* However, 31% positive reactions to HDM reveal sensitization of individuals (or those already sensitized) in the dry region (Riyadh) as well. A possible explanation may be that a proportion percentage of the Riyadh population might have more exposure to a humid climate by frequent travel abroad and/or within the country. Thus, it is not inconceivable that such a population may have been sensitized outside Riyadh and/or outside the Kingdom, but not in Riyadh, where a negligible amount of both *Der p I* and *Der f I* were detected. As such, therapy specific for mites should not be considered for patients who currently live in this "dry" area and have a positive SPT (or RAST) to mite, before determining if they previously resided in a humid area, a place in which they are more likely to have acquired the sensitivity.[16] Most of the HDM-sensitive patients in Riyadh may have lived in a humid, mite-infested area within the country or abroad. In humid areas of the Kingdom, it is somewhat clear to see the variation in SPT reactivity with two mite extracts. The study supports the suggestion[3] that the cross-reactivity between *D. pteronyssinus* and *D. farinae* is not complete. Alternatively, one can agree with Barbee et al.,[17] who found that positive SPT to *D. farinae* are relatively uncommon in children living in the dry climate of Arizona. Our study is in agreement with this study, in that Riyadh children did not react to *D. farinae* while the HDMs mixed were relatively frequent, even in a "dry" climate.

Saudi Arabia is a large country with considerable vari-

TABLE 3. *SPT-positive results of 120 asthmatic children tested in Western coastal region of Saudi Arabia.**

Indoor allergens	Mild** (%(	Moderate (%)	Strong (%)	Total (%)
D. farinae	14.9	32.2	9.2	56.3
Cat fur	14.9	17.2	21.9	54.0
Cockroach	17.2	13.8	3.5	34.5
Dog hair	17.2	10.3	1.2	28.7
Cotton flock	19.5	18.4	8.0	45.9
Mean	16.7	18.4	8.8	43.9

Indoor allergens accounted for a mean 43.9% SPT-positive reactions in the coastal region. *Percentages were calculated from the total number of positive reactions obtained (=87); **weal and erythema: <3 mm compared to saline control=mild; between 3-5 mm compared to saline control=moderate; >5 mm compared to saline control=strong.

ation in geography, climate and lifestyle. Thus, the variations in the contents of dust mite species in different geographical regions can be attributed to climate and geography of the regions which, in turn, increases the risk factor for sensitive individuals in the indoor environment. In addition, in Saudi Arabia's traditional society, where people prefer to stay more indoors than outdoors (this is also because of the hot weather of the region), the impact of indoor allergens and their sensitization effects are increased.

IgE-mediated reaction to patients in the regions also clearly identify the direct impact of sensitization, resulting in higher percentages for those allergens prevalent in their respective regions. The impact of other indoor allergens, e.g., cat and cockroach, with positive reactions in the children, are subject to another detailed communication and thus are not discussed here.

The above discussion supported by our data in various regions of the Kingdom leads to the conclusion that concentrations of HDMs are influenced by geography and climate, and the humidity of the region in question, which, in turn, influences the sensitization, pattern and degree of SPT reactivity.

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