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DOMESTIC ALLERGENS: A STUDY OF ASTHMATIC CHILDREN IN SAUDI ARABIA

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Abstract

The levels of cockroach antigen, mouse urinary protein, and cat antigen vary greatly among Saudi households. In this study, wide ranges of concentrations of these three allergens were found among the samples. In addition, there did not appear any relationship (either positive or negative) in the levels of the three allergens, i.e. cockroach antigen did not appear to relate to the levels of mouse urinary protein or cat antigen in the samples, nor did mouse urinary protein to the levels of cat antigen.

Definite differences in the levels of the allergens were found in samples from homes of asthma children as compared to those of nonasthma children.

With respect to all three allergens, samples from homes of asthma children on the average contain much higher concentrations of all the three allergens than did samples from those homes of nonasthma children.

The estimate of the relative risk of asthma for an increase in the concentration of cat antigen of 100 ng/g dust is 1.18.

Introduction

Increasingly exposure to raised levels of allergenic material in childhood is being implicated in the development of bronchial asthma. Growing sophistication in measuring allergen levels, quantifying spores and counting dust mites has added scientific strength to associations which were previously descriptive.

Most studies have focussed on exposure to the house dust mite. Following a greater than 20 fold increase in asthma in some areas of the highlands of Papua New Guinea [1] demonstrated significantly higher mite counts in the bedding of the asthma high prevalence communities compared with those of low prevalence. Korsgaard [2] calculated the relative risk of developing asthma in Danish houses with high dust mite counts at seven times that of houses with low dust mite

levels.

Sporik *et al.* [3], in a long term longitudinal study, calculated that those children exposed to high dust mite allergen levels (*Der p*1)were 4.8 times as likely to develop asthma as children in houses with low *Der p*1 levels.

Riyadh, Saudi Arabia has a year round relative humidity between 20-40°C or less inhibiting the growth of house dust mites. This provides a mite-free environment in which to study the impact of other domestic allergens on the development of asthma.

Materials and Methods

Reagents

Allergen extracts used for immunizing rabbits and for affinity purification of antisera were obtained from ALK, Denmark (Cat Hair SQ555; *Dermatophagoides pteronyssinus*; SQ503, *Dermatophagoides farinae* SQ504) and Greer Laboratories, USA (*Blattella germanica*, *Periplaneta americanum*). Mouse urine was collected from male Balb/c mice. Bioitin-x-NHS was obtained from Calbiochem (CA), ELISA plates (Immuno 1) from Nunc (Denmark) and Streptvidin/ Biotin/HRP reagent was purchased from Amersham (UK).

Antisera

Rabbits were immunized with one 200µg dose of allergen extract in complete Freunds adjuvant followed by two doses of 100µg in incomplete Freunds adjuvant at ten day intervals.

An IgG fraction was prepared from whole serum by ammonium sulphate precipitation followed by ion-exchange chromatography and affinity purified against the immunizing antigen.

Biotinylation

Affinity purified antibody preparations were incubated with biotin at a 3:1 (biotin : IgG) molar ratio for two hours at room temperature. Following overnight dialysis against phosphate buffered saline the labelled antibodies were stored at 4°C in 50% glycerol and 0.01% sodium azide.

Patients

All patients were children attending the clinic of one of the investigators (AR Al Frayh). This was a general paediatric clinic with a special interest in asthma. The clinic is located in a hospital which covers one area of the city and is attended largely by Saudi nationals. Sixty six asthma patients were identified. Then 21 non asthma cases were selected at random as controls.

Dust Samples

Patients were requested to vacuum their bedrooms, mattresses and living rooms for 10-15 minutes using a clean vacuum bag. The vacuum bags were then sealed in a plastic bag and delivered to the clinic.

Aqueous extracts were prepared by vortexing a weighed sample of sieved dust in phosphate buffered saline (2ml per gram of dust) for 30 seconds. Extracts were then filtered and stored at -20°C.

ELISA Procedure

ELISA plates were coated overnight at 4°C with 100µl volumes of allergen extract-specific antibody diluted with carbonate buffer. After washing with PBS-Tween 20 (0.5%), 200µl volumes of 1% BSA were added to each well for two hours as a blocking agent.

Following removal of the blocking agent, dust samples diluted in PBS-Tween 20 (0.05%) were added to the wells in 50µl volumes and incubated for two hours. After washing, biotinylated allergen extract-specific antibody was added to appropriate wells for one hour.

After further washing 50µl Avidin-Biotin-HRP reagent was added to all wells and incubated for 30 minutes. 100µl of substrate (10 mg orthophenylenediamine dihydrochloride, 4µl hydrogen peroxide in 10 ml citrate/phosphate buffer pH 5.0) was added to plates after a final wash and incubated for 30 minutes at room temperature. The reaction was stopped by the addition of 50 µl 10% HCl and the absorbance measured at 490 nm. Standard curves were prepared from allergen extracts to determine the concentration of related protein in each dust sample. Results were expressed as ng specific protein/g dust.

Statistical Methods

The major purpose in this study was to investigate cockroach antigen, mouse urinary protein, and cat antigen and the relationships among the quantities of these with asthma. To this end, correlation coefficients among levels of the substances were calculated. Differences in the levels of each substance between the asthmatic and non-asthmatic children's homes were measured with the Mann-Whitney test. Multivariate analyses of differences between the two types of homes (asthmatic/nonasthmatic) were carried out through logistic regression methods. The SAS and statistical cooputer packages were used for all analysis.

Results

Domestic allergen data collected from 87 homes are presented in Fig. 1.

Cockroach antigen was detected in 56% of dust samples tested, the majority containing 100 to 10,000 ng/g dust but a few samples exceeded 10,000 ng/g. Only 15% of samples showed mouse urinary protein, most ranged from 100 to 400 ng/g. Cat antigen was detected in 69% of dust samples ranging up to 11,200 ng/g with the majority of samples showing less than 1000 ng/g.

Dust mite protein was either undetectable or present in trace amounts. Samples collected from the houses of children with asthma showed significantly higher levels of cat and cockroach antigens than dust samples from houses of non-asthmatic. These differences were statistically significant, at the $p<0.05$ level, (Mann-Whitney test)..

Fig. 2 (a, b & c) displays the data graphically. Cat protein above 500 ng/g dust was found in 19.6% dust samples from asthmatic children but only in 4.7% of samples from non asthmatics. Cockroach protein above 500 ng/g was detected in 40.9 of samples from asthmatics and in 14.7 of samples from non asthmatics.

Either cat or cockroach protein above 500 ng/g were found in 50% samples from asthma children compared with samples from 14% of non asthmatic.

Conversely cat and cockroach protein were undetected more commonly in dust samples from non-asthmatic than asthmatic children: 38% and 18% respectively for cat, and 67% and 36% for cockroach.

The relative risk of asthma being diagnosed in children from houses with cat antigen levels above 500 ng/g was 5.38 (0.52-5.6) and with cockroach above 500ng/g was 2.76 (0.7-10.89). For houses with dust samples containing either cockroach or cat antigen above 500 ng/g the asthma relative risk for children was 2.87 (0.85-9.30) and above 100 ng/g was 4.46 (1.28-15.5).

The investigation of any associations among the levels of the three substances among the 87 houses did not reveal any positive correlation. As regard to the presence (i.e. nonzero level) or absence of a substance, the following was found. For the samples from 13 houses, no cockroach antigen, mouse urinary protein and cat antigen was found. For the samples from 46 houses, more than a single substance was found. Of these samples, 8 contained all three substances.

Summary statistics on the level of some domestic allergen are presented in Table 1 (a & b).

Table 1: (a & b): present some summary statistics on the levels of cockroach antigen (Cockroach), mouse urinary protein (Mouse), and cat antigen (Cat) found in the 87 homes

(a)

	N	Mean	Median	TR Mean	ST Dev	SE Mean
Cockroach	87	1420	108	890	3232	346
Mouse	87	32.83	0.00	19.57	86.81	9.31
Cat	87	286.1	152.0	242.4	379.7	40.7

(b)

	Min	Max	Q1	Q3
Cockroach	0	16750	0	900
Mouse	0.00	380.0	0.00	0.00
Cat	0.0	1600.0*	88.0	256.0

* Transformed from 11200/ng/g to 1600 for the conveyance of logistic regression analysis which otherwise would have been difficult.

Correlation coefficients were calculated for the associations of substance presence among the three substances. The correlation between the presence of cockroach and that of mouse was 0.118, between cockroach and cat was 0.277 and between mouse and cat was 0.076. All of these coefficients are statistically non-significant [(Table 2 (a & b)].

Samples collected from the houses of children with asthma showed significantly higher levels of cat ($P=0.02$) and cockroach ($P=0.04$) antigens than dust samples from houses of non-asthmatic children. Levels of mouse urinary protein did not differ significantly ($P=0.44$) between the two groups.

The association among the levels of the three sub-

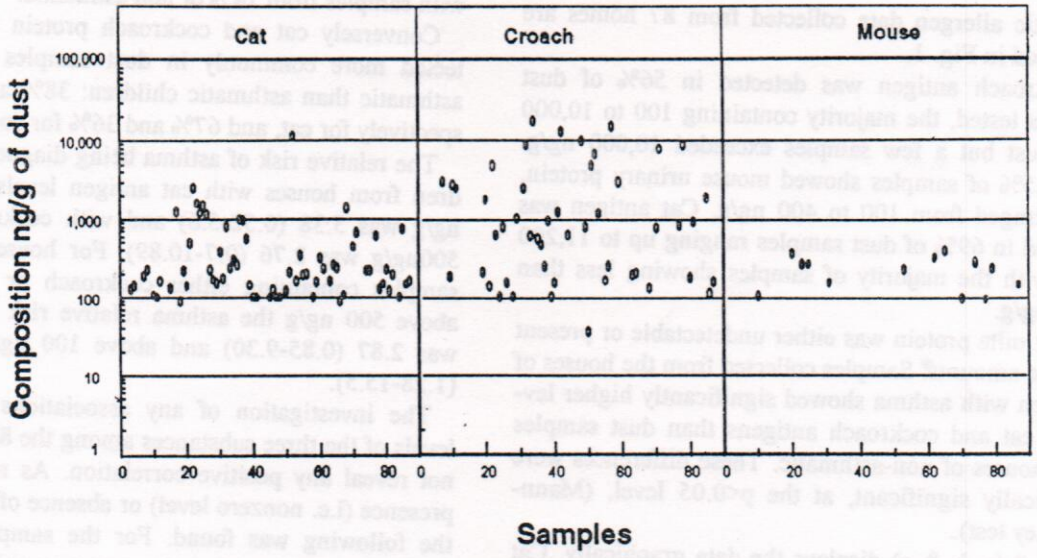

Fig. 1: Composition of domestic allergens in dust samples from asthmatic and non-asthmatic homes

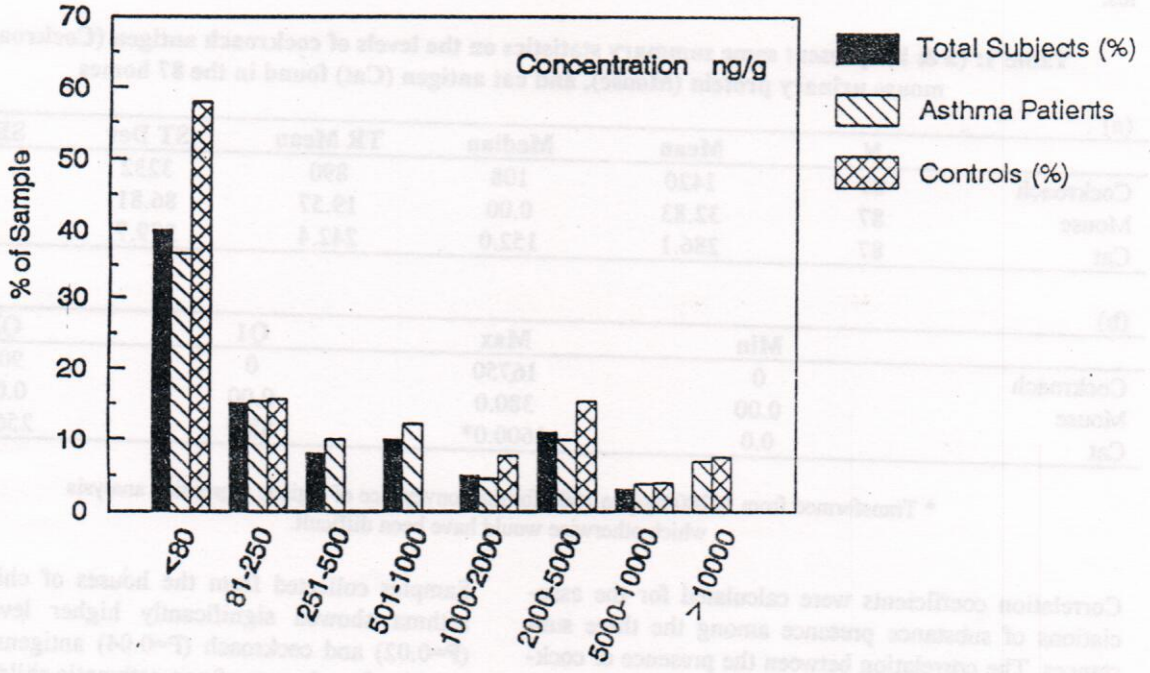

Fig. 2(a): Allergen concentrations in house dust (Cockroach)

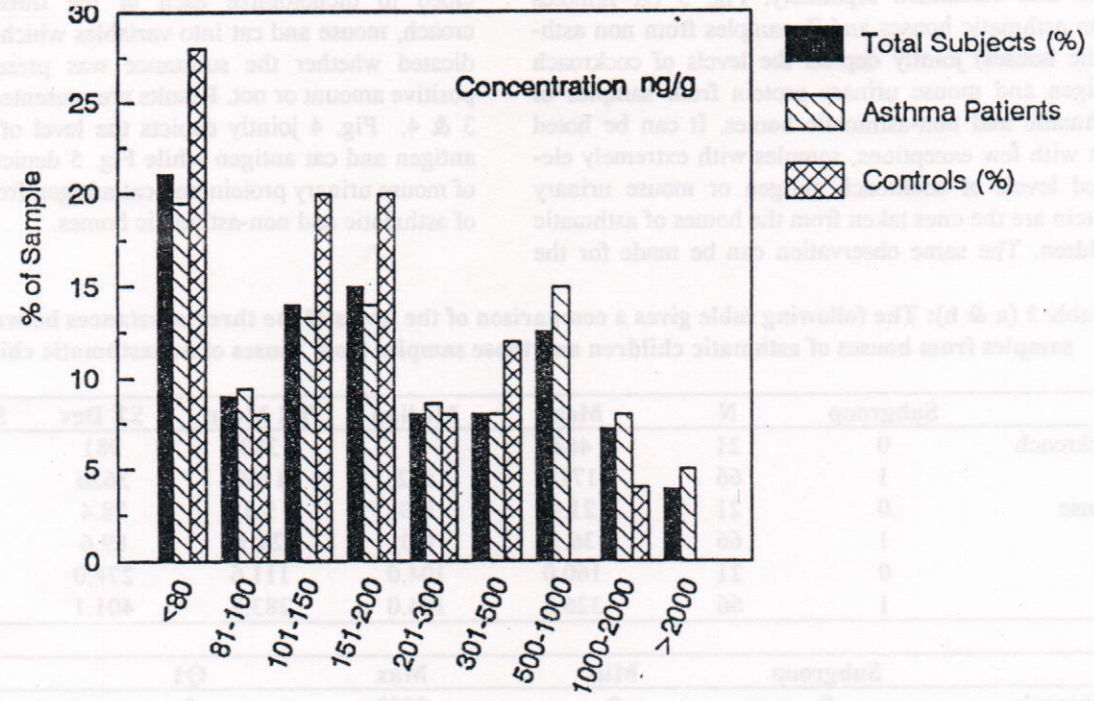

Fig. 2(b): Allergen concentrations in house dust (Cat Dander)

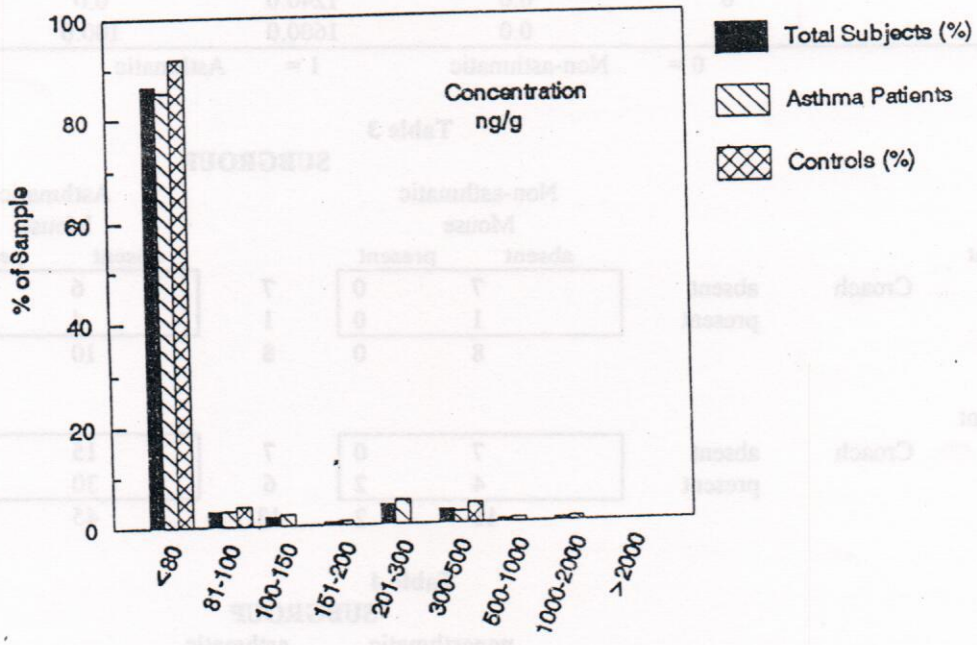

Fig. 2(c): Allergen concentration in the homes of asthmatic and control subjects (Mo use urine)

stances for the asthmatic and non-asthmatic groups were also calculated separately. Fig. 3 (A=samples from asthmatic houses and Z=samples from non asthmatic houses) jointly depicts the levels of cockroach antigen and mouse urinary protein from samples of asthmatic and non-asthmatic homes. It can be noted that with few exceptions, samples with extremely elevated levels of cockroach antigen or mouse urinary protein are the ones taken from the homes of asthmatic children. The same observation can be made for the following two figures (Fig. 4 & 5). Again, it was decided to dichotomize each of the three variables croach, mouse and cat into variables which simply indicated whether the substance was present in any positive amount or not. Results are presented in Tables 3 & 4. Fig. 4 jointly depicts the level of cockroach antigen and cat antigen while Fig. 5 depicts the level of mouse urinary protein and cat antigen from samples of asthmatic and non-asthmatic homes.

Table 2 (a & b): The following table gives a comparison of the levels of the three substances between those samples from houses of asthmatic children and those samples from houses of nonasthmatic children

(a)

	Subgroup	N	Mean	Median	TR Mean	ST Dev	SE Mean
Cockroach	0	21	484	0	383	981	214
	1	66	1718	152	1160	3626	446
Mouse	0	21	21.4	0.0	5.3	78.4	17.1
	1	66	36.5	0.0	24.1	89.6	11.0
Cat	0	21	160.0	104.0	111.6	274.0	59.8
	1	66	326.3	164.0	283.9	401.1	49.4

(b)

	Subgroup	Min	Max	Q1	Q3
Cockroach	0	0	2900	0	192
	1	0	16750	0	940
Mouse	0	0.0	350.0	0.0	0.0
	1	0.0	380.0	0.0	0.0
Cat	0	0.0	1240.0	0.0	174.0
	1	0.0	1600.0	100.0	335.0

0 = Non-asthmatic 1 = Asthmatic

Table 3

			SUBGROUP					
			Non-asthmatic Mouse			Asthmatic Mouse		
Cat absent			absent	present		absent	present	
	Croach	absent	7	0	7	6	1	7
		present	1	0	1	4	1	5
			8	0	8	10	2	12
Cat present								
	Croach	absent	7	0	7	15	3	18
		present	4	2	6	30	6	36
			11	2	13	45	9	54

Table 4

		SUBGROUP		
		nonasthmatic	asthmatic	
CAT	absent	4.83	15.17	20
	present	16.16	50.83	67
		21	66	87

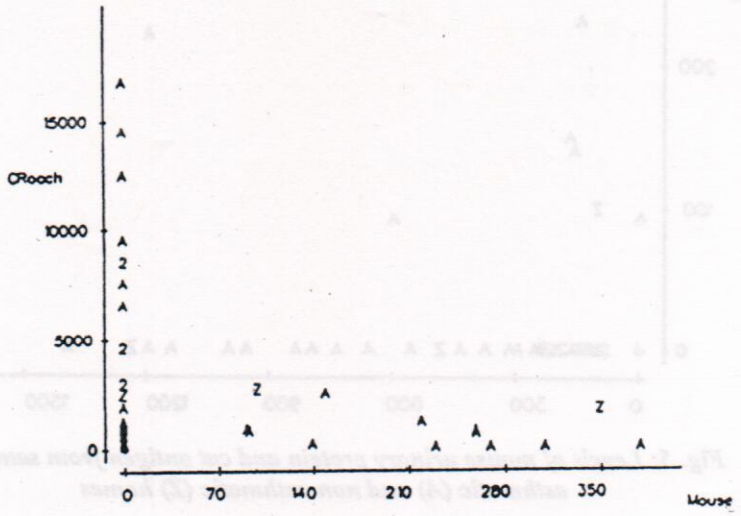

Fig. 3: Levels of cockroach antigen and mouse urinary protein from samples of asthmatic (A) and non-asthmatic (Z) homes

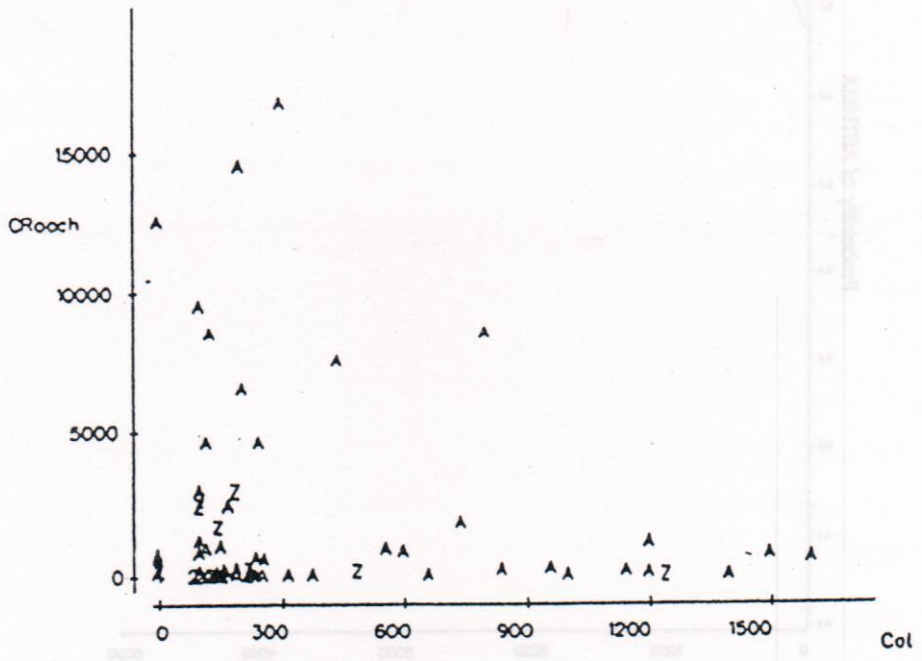

Fig.4: Levels of cockroach antigen and cat antigen from asthmatic (A) and non-asthmatic (Z) homes

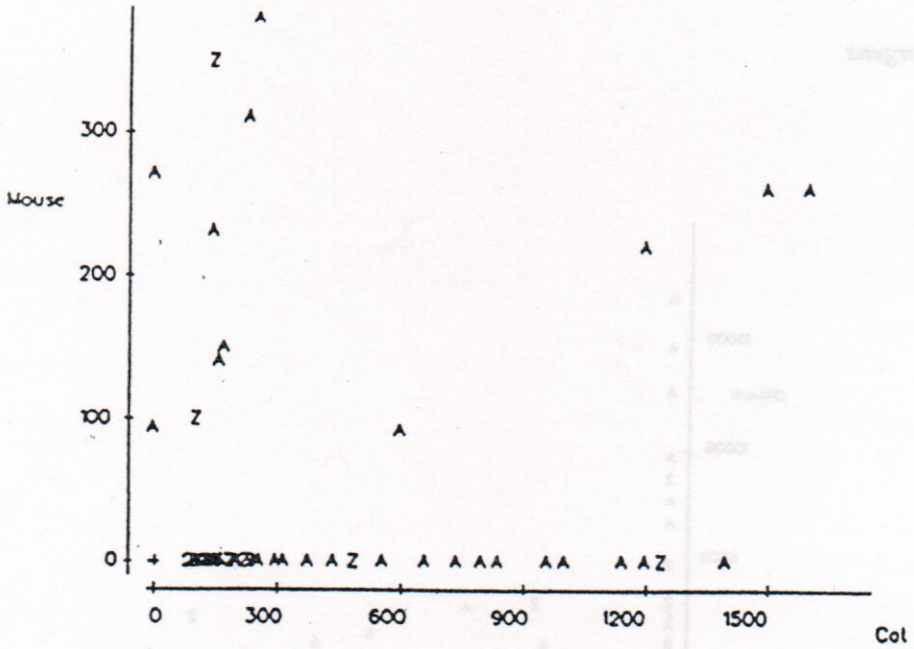

Fig. 5: Levels of mouse urinary protein and cat antigen from samples of asthmatic (A) and non-asthmatic (Z) homes

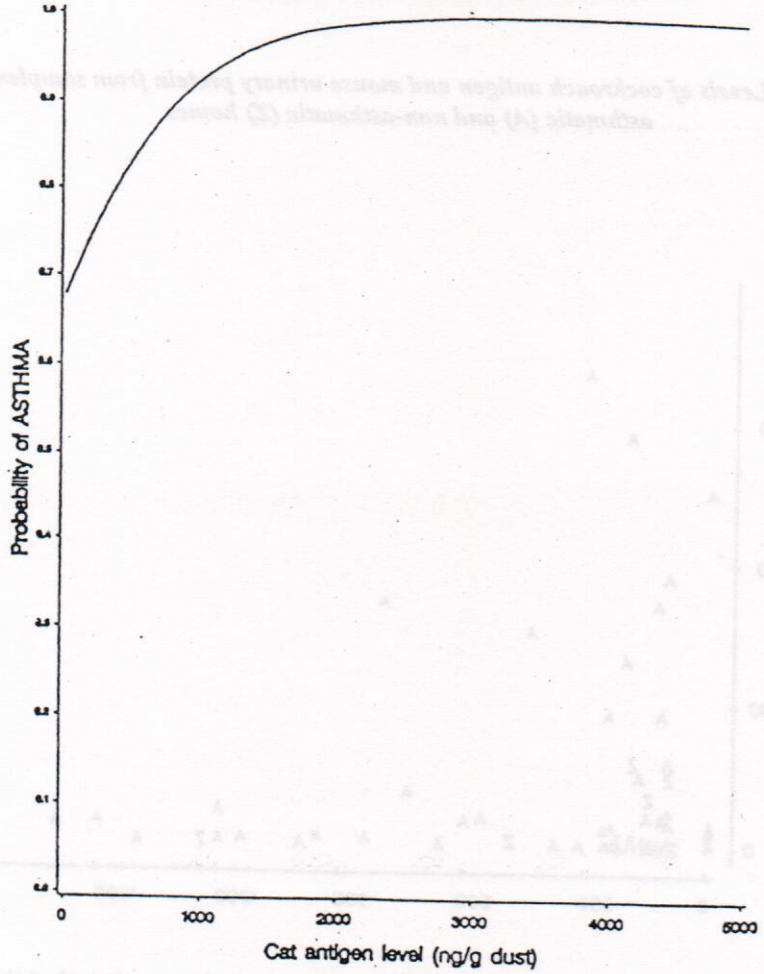

Fig.6: Estimated relationship between the probability of asthma (plotted on the ordinate) and the level of cat antigen (plotted on the abscissa)

Logistic regression of asthma on cat antigen

The logistic regression model is as follows:

$$\text{Probability of asthma} = \frac{\exp(B_0+B_1x)}{1+\exp(B_0+B_1X)}$$

In this application of logistic regression, the relevant dependent variable is the probability of asthma and the relevant independent variable is the level of cat antigen (i.e. X in the above function is the level of cat antigen in units ng/g).

The following table displays the estimates obtained.

Table 5

Term	Coefficient estimate	Standard error	95% C.I. of Exp (Coef)			
			Coef /SE	Exp (Coef)	Lower	Upper
B_1	0	0	1.62	1	1	1
B_o	0.75	0.32	2.34	2.11	1.12	3.98

The likelihood ratio test for measuring the significance of the estimate of B_1 yields a significance level of 0.046. The column labeled EXP(Coef) is an estimate of the percentage rate of increase (or decrease) in the probability of asthma per unit in the level of cat antigen. Here, the quantity equals 1.0018. That is, for every one hundred ng/g increase in the concentration of cat antigen, it can be expected that there will be an increase of 18% in the probability of asthma. The following graph displays the estimated relationship between the probability of asthma (plotted on the ordinate) and the level of cat antigen (plotted on the abscissa) (Fig. 6).

A multiple logistic regression analysis was carried out to investigate the effect of cat antigen on the probability of asthma after adjusting for any effect that cockroach and/or mouse urine might have on the probability.

The following table displays the estimates for B_1, B_2, B_3, B_4, B_5, and B_6 that were obtained through the multiple logistic regression analysis (Table 6).

Table 6: Multiple logistic regression analysis: the estimate for the co-efficient associated with the CAT term is 0.0012. This is very similar to the value of the co-efficient associated with the CAT term in the previous logistic regression when only the CAT term was included. Therefore, the independent relationship between cat antigen and the probability of asthma appears to be best estimated by that given in the first logistic regression analysis

Coefficient	Coefficient estimate	Standard error	95% C.I. of Exp (Coef)			
			Coef /SE	Exp (Coef)	Lower	Upper
B_1	0	0	0.61	1	1	1
B_o	0.01	0.02	0.73	1.01	0.98	1.06
B_3	0	0	1.07	1	1	1
B_4	<-0.0001	<0.0001	-1.44	1	1	1
B_5	<-0.0001	<0.0001	0.54	1	1	1
B_6	<-0.0001	0	0.33	1	1	1
B_7	0.53	0.35	1.51	1.7	0.84	3.42

Discussion

This study has aimed at investigating the degree to which three allergens viz. - cockroach antigen, mouse urine protein, and cat antigen, may be related to (or maybe even contributed to the cause of) asthma. It should not be forgotten, though, that this is a retrospective study, and at best it can only find associations. No cause-effect statement can be derived from a study such as this. The study is important, especially to the extent that it may lead to prospective studies to confirm any cause-effect conclusions that may be suggested by this study.

This study forms part of a nationwide survey of

bronchial asthma in three cities of the Kingdom, Riyadh in the dry central plateau, Jeddah on the Red Sea, and Dahran on the Arabian Gulf. Prevalence of asthma was calculated from results of a questionnaire answered by over 3000 Saudi school children. Wheeze within the past 12 months was recorded in 12.6% of children in Jeddah, and 11.9% of Riyadh children, but significantly fewer children in Damman, 6.5%. Results of other respiratory questions including diagnosis of asthma showed each was lowest in Dammam. [4].

The positive association between potentially allergenic proteins in household dust and the presence of asthma parallels the dust mite results of Korsgaard [5], Sporik *et al.*[6] and Ivevsen, *et al.* [7]. However, our own data now add cat protein and cockroach protein to the catalogue of environmental risk factors for atopic families. Others include exposure to cigarette smoke [8], also confirmed from our prevalence studies, and respiratory viral infection.

It could be argued that better indicators of allergen exposure would be measurements of pure allergens such as *Der p*I for dust mite and *Fel d*I for cat. But in their absence an assay for cockroach or cat protein, standardized against skin test material, provided a within-study, reproduceable measure of the presence of the relevant protein, comparable to counting dust mites.

The measurement of single, specific allergenic proteins such as *Der p1* in dust mite and *Fel d1* in cat would have enabled direct, quantitative comparisons between this and other studies. However, assays for cat hair, cockroach, dust mite and mouse urine proteins provide a meaningful, reproducible measure for the presence of specific allergens within this study.

This study reinforces the implications for atopic families that exposing their children to domestic environments with high concentrations of allergenic material such as dust mites, cats, cockroach represents a possible hazard by increasing the children's risk of developing asthma.

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