

Prevalence and distribution of indoor allergens in Singapore

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Summary

Background and aims Immediate hypersensitivity to indoor allergens is known to be associated with allergic asthma. This study evaluated the prevalence and distribution of six indoor allergens in 956 dust samples obtained from homes, childcare centres, schools, and a hospital in tropical Singapore. Seasonality of mite allergens was also assessed.

Methods The major allergens of the *Dermatophagoides* spp. dust mites, Der p 1 and Der f 1; major cat and dog allergens, Can f 1 (dog) and Fel d 1 (cat); and cockroach, Bla g 1, were measured by specific enzyme immunoassays. Allergen levels of the storage mite, *Blomia tropicalis* (Blo t), were measured by a fluorescent allergosorbent test (FAST) inhibition assay.

Results Our results showed that homes had significantly higher concentrations and prevalence of allergens compared with the other locations, except for Bla g 1, where higher mean levels were found in schools. Within the homes, the highest concentrations of mite allergens were found in mattresses (geometric mean: 1.2 µg/g dust Der p 1; 2717 Allergen Units per gram dust [AU/g] Blo t), and carpets (1.5 µg/g Der p 1; 1620 AU/g Blo t), whilst Bla g 1 was mainly concentrated in the storerooms (geometric mean = 3.5 units/g) and kitchens (geometric mean = 5.1 units/g). The major cat and dog allergens were well distributed and not confined to homes with pets. Their highest levels were found in dust of soft furnishings, carpets and mattresses. There was an absence of significant seasonal variation in Der p 1, Der f 1 and Blo t levels in the homes over a 1 year period.

Conclusion The results indicate that compared with public places, the home constitutes a major reservoir of indoor allergens. Allergens of the storage mite, *B. tropicalis*, should be considered as a major allergenic component of dust in Singapore.

Keywords: indoor allergens, dust mites, cat, dog, cockroach, homes, childcare centres, schools, *Blomia tropicalis*

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Introduction

Immediate hypersensitivity to indoor allergens is associated with bronchial asthma in children and young adults [1]. These allergens not only trigger acute attacks of asthma, but chronic exposure is also an important cause of airway inflammation [2–4]. In Singapore, one in five schoolchildren have been identified with doctor-diagnosed asthma [5]. The

majority (>90%) of these asthmatic children are sensitized to the house dust mites, *Dermatophagoides pteronyssinus* and *D. farinae* [6], as well as the storage mite, *Blomia tropicalis* [7]. In addition, a proportion of them are also sensitized to cat (25%) and dog (35%) dander [8], and cockroach (53%) allergens [9].

The use of immunoassays to measure major indoor allergen levels is currently considered the best index for estimating indoor allergen exposure [10–14]. With most populations now spending more time indoors [15], increased indoor allergen exposure has been postulated to play a causal role in the worldwide increase in asthma prevalence [16,17]. The situation is similar in Singapore,

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as more of our population are retreating into the closed air-conditioned comforts of the office, school and home. However, a substantial proportion ($\approx 85\%$) of our population, still live in well ventilated government subsidized apartments (Singapore Census of Population 1990) [18], and the majority of our government schools are not air-conditioned. The weather in Singapore is tropical, with warm temperatures ($24\text{--}31^\circ\text{C}$) and high humidity ($64\text{--}96\%$) all year round.

In this study, an evaluation of the prevalence and distribution of commonly found indoor allergens, Der p 1, Der f 1, Bla g 1, Fel d 1, and Can f 1, in dust samples obtained from homes, schools/university, childcare centres and a hospital in Singapore was carried out by specific enzyme immunoassays. In addition, measurement of *B. tropicalis* (Blo t) allergen levels, a common mite species in the tropics [19,20], was also carried out by fluorescent allergosorbent test (FAST) inhibition assay [21]. Seasonal variations in the levels of the three dust mite allergens (Der p 1, Der f 1 and Blo t) were assessed over a 1 year period at various niches within residential homes. These data would provide important information on the prevalence and distribution of these allergens in Singapore, and therefore assist in the formulation of strategies for allergen avoidance. Our results here showed that these indoor allergens were highly prevalent in Singapore, with the most prevalent being the Der p 1 and Blo t mite allergens.

Materials and methods

Dust sample collection

A total of 956 dust samples were collected, between 1993 and 1995, from various niches within 103 residential homes of asthmatic children and volunteers, 10 schools, 10 childcare centres, a university, and a hospital. To examine if seasonal variations in dust mite allergen levels occurred in our environment, serial three-monthly samples from various niches within 20 homes over a one year period were evaluated. The selection of homes were based on the presence of detectable levels of Der p 1 and Der f 1 in at least one niche within the home at the first visit and the house owners' consent for repeated visits. A modified Kirby Classic III (Kirby Co, USA) vacuum cleaner, which was adapted with a chamber that collects dust onto a filter paper, was used for sampling. Each sample was obtained by vacuuming an area of 1 m^2 for 2 min. Cross contamination of samples was avoided by using a different filter paper each time and dust particulate in the vacuum cleaner blown out repeatedly prior to the next sample. Dust samples were stored at 4°C in grip lock bags during transportation back to the laboratory. The samples were then stored at -20°C until processed.

Allergen extraction

Dust samples were sieved through a No. 45 mesh screen ($355\text{ }\mu\text{m}$ diameter) to remove large particles and fibre. The fine dust were suspended in borate buffered saline (pH 8.0) containing 0.1% Tween 20 (BBS-T) in a proportion of 100 mg fine dust per 2 mL buffer. For samples containing less than 50 mg fine dust, 1 mL BBS-T was added. The dust suspensions were rotated end on end overnight at 4°C , supernatants harvested by centrifugation and frozen at -70°C until assayed for the allergens of interest.

Immunoassays

Sandwich enzyme immunoassays for Der p 1, Der f 1, Can f 1, Fel d 1, and Bla g 1 were carried out using monoclonal (MoAb)/polyclonal antibody systems and standards obtained from M. Chapman (Biotechnologies Pte Ltd, UK) [11–14]. 96-well microtitre plates (Immunolon II, Dynatech, USA) were coated with the respective MoAb at a concentration of $1\text{ }\mu\text{g/well}$. After washing, the plates were blocked with 1% bovine serum albumin for an hour at room temperature. This was followed by washing and incubation with 100 μL dust samples (in duplicate) at 37°C for 2 h. Standard curves were constructed using the standardized reference standards provided. Following extensive washing, incubation with the respective biotinylated second antibodies was carried out for 1.5 h at 37°C , then with streptavidin-peroxidase (Sigma S5512) at a concentration of 0.25 ng/mL for 0.5 hour at 37°C . Colour development was achieved with 100 $\mu\text{L/well}$ of 1 mM 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) in 70 mM citrate phosphate buffer (pH 4.2) with hydrogen peroxide. The reaction was stopped by adding 100 $\mu\text{L/well}$ 2 mM sodium azide, and OD read at 414 nm. The intra-assay variations were less than 5% for all assays and the interassay variations ranged between 12.3 and 18.2% (depending on the allergen). The lowest allergen concentrations detected in these assays were 0.5 ng/mL for Der p 1 and Der f 1, 0.04 mU/mL for Fel d 1 (where 1 U or unit allergen = $4\text{ }\mu\text{g}$ protein), 2 IU/mL for Can f 1 (1 IU or International Unit = 1 ng protein), and 0.01 U/mL for Bla g 1. The levels reported in this study were all converted to $\mu\text{g/g}$ fine dust for comparison purposes, except for Bla g 1 where a conversion factor was not available.

FAST inhibition assay for *Blomia tropicalis* allergen levels

Allergen levels of *B. tropicalis* were measured by FAST inhibition assay [21]. Lyophilized allergen extracts of cultured *B. tropicalis* were kindly provided by Enrique Fernandez-Caldas (Madrid, Spain). The allergens were extracted 1/50 w/v in 0.1 mol/L ammonium bicarbonate and used as the standard allergen extract for this assay.

FAST inhibition was carried out as follows. Blank FAST wells (BioWhittaker, USA) were coated with 100 μ L of allergen extract at 5 μ g/well and incubated overnight. After washing with PBS-Tween, 50 μ L of dust extract and 50 μ L of pooled sera, which contained high titers of IgE to *B. tropicalis* (FAST 4+, >17.5 IU/mL) were added to each well. Following an incubation for 1.5 h, the wells were washed, coated with anti-IgE alkaline phosphatase-labelled antibody, developed with fluorogenic substrate according to manufacturer's specifications, and read using a 96-well fluorometer (BioWhittaker, USA). The results were extrapolated to a standard curve prepared by using a positive pooled sera and serial dilutions of the *B. tropicalis* extract. Only samples with FAST inhibition between 10 and 80% (within the straight exponential line of the standard curve) were considered interpretable. Samples with FAST inhibition above 80% were further diluted and re-assayed. The standard *B. tropicalis* extract was arbitrarily assigned a concentration of 25 000 allergy units [AU] [22] and results were thus expressed as AU per gram of dust.

Environmental assessment

The principal occupant of each home was interviewed with regards to the type and age of the home, number of occupants, presence and type of pets, and the use of air-conditioning. Sanitary conditions and practices were also evaluated. Interviews regarding cleaning practices were also conducted at the childcare centres and hospital. In addition, on-site environmental parameters, such as relative humidity and temperature were measured at each visit.

Statistical analysis

Analysis was carried out using the statistical package SAS v6.08 for Windows [23]. Data were normalized by logarithmic transformation of positive samples. Comparison of allergen concentrations between location and niches was analysed using the analysis of variance (ANOVA) and seasonal variation was evaluated using ANOVA with repeated measures. An additional test for the presence of seasonality described by Kendall and Ord [24] was used. The incidence of positive samples and allergen levels above the proposed sensitization thresholds (2 μ g/g for group 1 dust mite allergens [25], 8 μ g/g Fel d 1 and 10 μ g/g Can f 1 [26,27]) were analysed using the Chi-square or Fisher's exact test.

The association between allergen levels, housing characteristics and cleaning practices was examined individually using the Mann-Whitney *U*-test (for bivariate data) and Kruskal-Wallis test (for those with three or more categories of comparison). Multiple comparisons between categories were made, where applicable, using the method described

by Siegel and Castellan [28]. Housing characteristics and cleaning practices, which showed marginally significant association ($P < 0.10$) with allergen concentrations in the univariate analysis, were then submitted to a logistic regression and analysed in a backward elimination manner with the significant level for staying in the model set at a *P*-value of less than 0.05. The housing characteristics and cleaning practices analysed as independent variables in the model included: the type and age of home, number of occupants, presence of pets, use of air-conditioning, mean temperature and relative humidity; the type and age of mattress used, frequency of mattress-cover wash, use of plastic or vinyl encasing over the mattress; the type and age of sofa, frequency of sofa cover wash; the presence and age of carpet, frequency of carpet vacuuming; and the frequency of floor sweeping and mopping.

Results

Distribution and levels of indoor allergens measured by immunoassays

Table 1 summarizes the overall distribution of the allergens studied. Der p 1 was the most prevalent allergen, with up to 88% of dust samples from homes having detectable levels of the allergen. The other allergens were detected in approximately between 44 and 63% of the samples from homes. In general, the homes had the highest concentrations of allergens compared with those of the other locations studied, except for Bla g 1, where higher mean levels were found in schools (Fig. 1). This pattern of lower allergen levels (except for Bla g 1) in locations outside the home was also evident when samples from similar niches (e.g. mattresses in homes and childcare centres) were compared (data not shown).

Within the niches in the homes, the highest concentrations of the Der p 1 allergen were found in the mattresses (geometric mean: 1.2 μ g/g) and carpets (1.5 μ g/g), with $\approx$ 28% and 36% of the samples, respectively, having Der p 1 levels above the 2 μ g/g threshold (Table 1). Bla g 1 was mainly concentrated in the storerooms (3.5 units/g) and kitchens (5.1 units/g). The highest levels of the major cat and dog allergens were found in the dust of soft furnishings which included the sofa, carpet and mattress (Table 1).

The levels of each dust mite allergen, Der p 1 and Der f 1, within different niches of the homes, showed significant correlation (e.g. bedroom floors with mattresses [$r^2 = 0.3$, $P < 0.01$], living-room sofas with carpets [$r^2 = 0.6$, $P < 0.001$], and bedroom mattresses with living-room sofas [$r^2 = 0.5$, $P < 0.001$] and carpets [$r^2 = 0.6$, $P < 0.001$]). Der p 1 levels within a particular niche tended to correlate positively with Der f 1 levels only in areas where the overall allergens levels are low.

Table 1. Overall distribution of indoor allergens

Location	Der p 1						Der f 1						Blag 1						Can f 1						Feld 1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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mean = geometric means of positive samples.

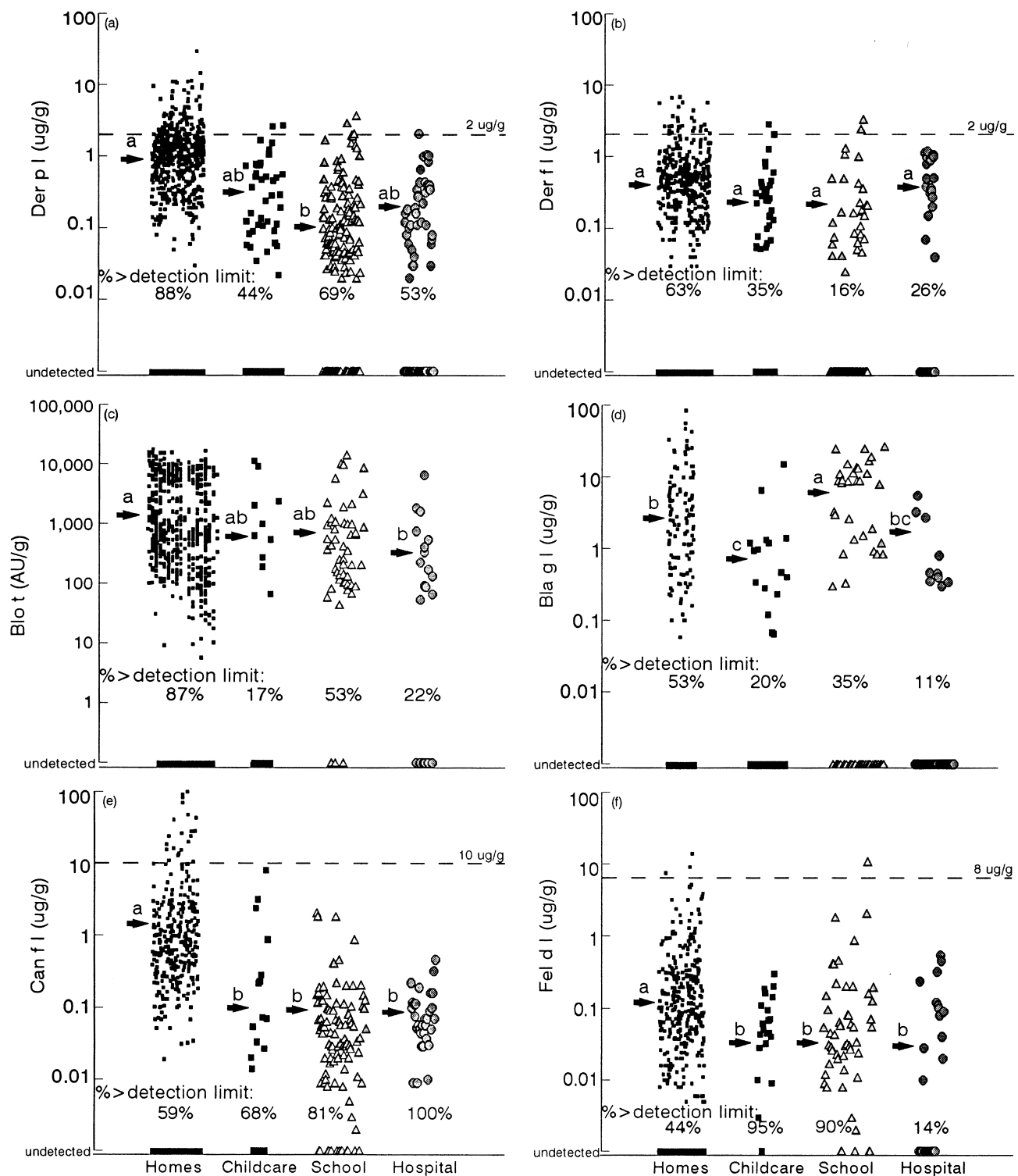


Fig. 1. Distribution of common indoor allergens, Der p 1 (a), Der f 1 (b), Blot (c), Bla g 1 (d), Can f 1 (e), and Fel d 1 (f), in residential homes (■), childcare centres (■), schools/university (▲) and a hospital (●) in Singapore. The geometric mean allergen levels are shown with an arrow (▲). Means sharing the same letter are not significantly different ($P > 0.05$). The dotted line (---) indicates the proposed threshold levels. The percentage of samples within the detectable limits of the respective assays are noted within the plots.

Table 2. Distribution of *Blomia tropicalis* allergens

Location	n	Blo t		AU/g dust	
		%			
		+ve	>50% inh	mean	max
Home	498	87	32	1017	15 472
Bedroom					
Mattress	123	97	37	2717	15 472
Floor	83	65	15	480	5561
Cupboard	48	71	25	524	1989
Living room					
Sofa	140	93	35	1225	15 251
Carpet	53	96	39	1620	13 562
Storeroom	24	92	35	587	11 387
Kitchen	27	93	25	1023	7545
Childcare	17	59	18	564	11 230
School/Uni	53	70	11	632	14 200
Hospital	22	55	10	289	6500

mean, geometric means of positive samples.

Blomia tropicalis antigen levels — FAST inhibition assay

Blomia tropicalis allergens were detected in 494/590 (84%) of the dust samples tested, ranging from samples with undetectable or low FAST inhibition capacity (below 10%) to a maximum of 15 472 AU/g (Table 3). More than 50% inhibition was detected in approximately a third of the positive samples. Higher mean levels were found in the homes (geometric mean: 1017 AU/g) compared with other locations studied. Similar to Der p 1, the highest concentrations of the Blo t allergen within the homes were found in bedroom mattresses (2717 AU/g) and carpets (1620 AU/g), with $\approx 37\%$ and 39% of the samples, respectively, with $>50\%$ FAST inhibition capability. In the same way, Blo t allergen levels at different niches within the homes showed significant correlation (e.g. bedroom floors with mattresses [$r^2=0.2$, $P<0.05$], living-room sofas with carpets [$r^2=0.6$, $P<0.001$], and bedroom mattresses with living-room sofas [$r^2=0.4$, $P<0.001$] and carpets [$r^2=0.5$, $P<0.001$]). Blo t allergen levels however, did not show any significant correlation with Der p 1 or Der f 1 levels.

Seasonal variation of Group 1 and *Blomia tropicalis* allergen levels

Serial measurements of levels of Der p 1, Der f 1 and Blo t in homes over a 1-year-period did not show statistical significant seasonal variation in these allergen levels (Fig. 2). Nevertheless, mean concentrations of Der p 1 and Der f 1

from all niches (except for Der f 1 levels on sofas) fell by $\approx 30\text{--}50\%$ from the annual average during the fourth quarter of the year (October–December) and rose (with the exception of Der p 1 levels on carpets) by $10\text{--}90\%$ depending on the niche, in the second quarter (April–June). In contrast, more than 100-fold variations in concentrations of Der p 1 and Der f 1 were found between houses within each sampling. Blo t allergen levels showed a reverse trend from that of the group 1 mite allergens, with the highest mean levels detected between October–December, and lowest in the first or second quarter (depending on the niche). There was no statistical correlation between group 1 mite allergen or Blo t concentrations with relative humidity or temperature, as these two environmental parameters remained constantly high through the year with only minimal variations. The test for seasonality [24] also did not show the presence of significant seasonal variations in all three allergen levels.

Effect of environmental factors and cleaning practises on allergen levels in homes

Since the homes had the highest levels of dust mite and cat and dog allergens compared with the other locations, the effect of housing factors on these allergen levels were evaluated in the 103 homes studied. Although Fel d 1 and Can f 1 allergens were well distributed throughout the locations studied, their levels in homes with cats ($n=3$) and dogs ($n=6$) were found to have significantly higher levels of the respective allergens than homes without pets (medians: Fel d 1: with cat = $0.10\text{ }\mu\text{g/g}$ dust, without cat = $0.01\text{ }\mu\text{g/g}$ dust, $P<0.03$; Can f 1: with dog = $0.58\text{ }\mu\text{g/g}$ dust, without dog = $0.15\text{ }\mu\text{g/g}$ dust, $P<0.01$). Table 3 summarizes the results of the analysis on the allergen levels in mattresses and sofas which were analysed by multiple logistic regression. Cleaning practices, such as frequency of changes in mattress covers, the encasing of mattresses with vinyl covers and the type of mattress had an effect on Der f 1 and Can f 1 levels in dust from mattresses. With living-room sofas, allergen levels was found to be associated with the age of sofa, number of household occupants, presence of pets (cats/dogs), the type and age of home.

Discussion

This study describes the prevalence and distribution of six common indoor allergens in Singapore. As expected of our tropical climate, the mite allergens Der p 1, Der f 1, and Blo t were the most common and prevalent allergens found. Of the group 1 allergens, Der p 1 was predominant. This was consistent with findings of previous local studies [29] and those of this region [30–33]. The lower prevalence of Der f 1 is likely to be related to the mite's preference for

Table 3. Odds ratio of significant factors (housing characteristics and cleaning practices) influencing indoor allergen levels identified by logistic regression analysis

Influencing factor vs reference category	Odds ratio (95% Confidence interval)				
	Der p 1	Der f 1	Bla g 1	Can f 1	Fel d 1
<i>Mattress</i>					
Median (reference) level	1.26 µg/g	0.25 µg/g	>0 unit/g	1.03 µg/g	0.23 µg/g
Use of plastic/vinyl covers no vs yes	NS	1.91* (1.22–2.61)	NS	–	NS
Mattress cover change monthly vs >fortnightly	–	1.86* (1.04–2.68)	–	3.26** (2.29–4.24)	–
Mattress type foam vs spring	–	–	–	1.67* (1.06–2.28)	–
<i>Sofa</i>					
Median (reference) level	0.77 µg/g	0.08 µg/g	>0 unit/g	0.79 µg/g	0.07 µg/g
Age of sofa >5 years vs ≤5 years	1.60* (1.08–2.11)	2.34** (1.67–3.01)	–	2.06* (1.37–2.75)	1.64* (1.04–2.24)
Number of occupants >4 vs ≤4	–	1.81* (1.07–2.54)	–	–	–
Presence of pets (cats/dogs) yes vs no	–	0.38* (0.03–0.84)	2.66** (1.72–3.61)	2.82** (1.89–3.76) dog only	–
House type Public flat vs house/condo	–	0.54* (0.19–0.82)	–	–	–
Age of home >10 yrs vs ≤10 yrs	–	–	2.22* (1.07–3.37)	–	–

P-values based on Chi-square analysis: * $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$.

NS — no factor (variable) remained in the backward elimination model with significant level for staying in the model set at $P < 0.05$.

lower humidity [34,35]. The correlation of Group 1 antigen levels between niches within the homes, indicate that the sampling of a single niche, preferably the soft furnishings where allergens were highest, would provide a good approximation of the allergen level of that home. In this study, Der p 1 levels were found to correlated positively with Der f 1 levels only in niches with low overall Group 1 mite allergen concentrations. This may be related to colonization of a dominant species over the other after it reaches a critical mass.

In this study, the levels of the Group 1 allergens were substantially lower than the levels reported in many tropical and subtropical regions as such as Thailand [30] and Australia [36]. Despite the use of similar immunoassays for Der p 1, the geometric mean levels in mattresses were between 5 and 40 times lower than those reported in these studies. Our levels were also lower or only marginally higher than those reported in temperate areas such as the United Kingdom [37] and Sweden [38]. These relatively

low levels of Group 1 allergens is most likely related to the correspondingly high prevalence of the storage mite, *B. tropicalis*, in the dust samples. In South America, where *B. tropicalis* is also highly prevalent, a negative correlation was obtained between *B. tropicalis* mites and Der p 1 [20]. In the South American study, the levels of Group 1 allergens were also relatively low and comparable with those of this study. Although, *B. tropicalis* mites allergen levels did not show significant correlation with Group 1 mite allergen levels in our cross-sectional data, serial measurements showed that they followed reverse trends.

The absence of seasonality in mite allergen levels is consistent with our tropical climate. Mite allergen fluctuations appear to be highly dependent on environmental temperature and humidity, and seasonal variation have been observed in regions where the climate is temperate or subtropical [39, 40]. In contrast, in the tropics [20] or in populations where the indoor environment remains

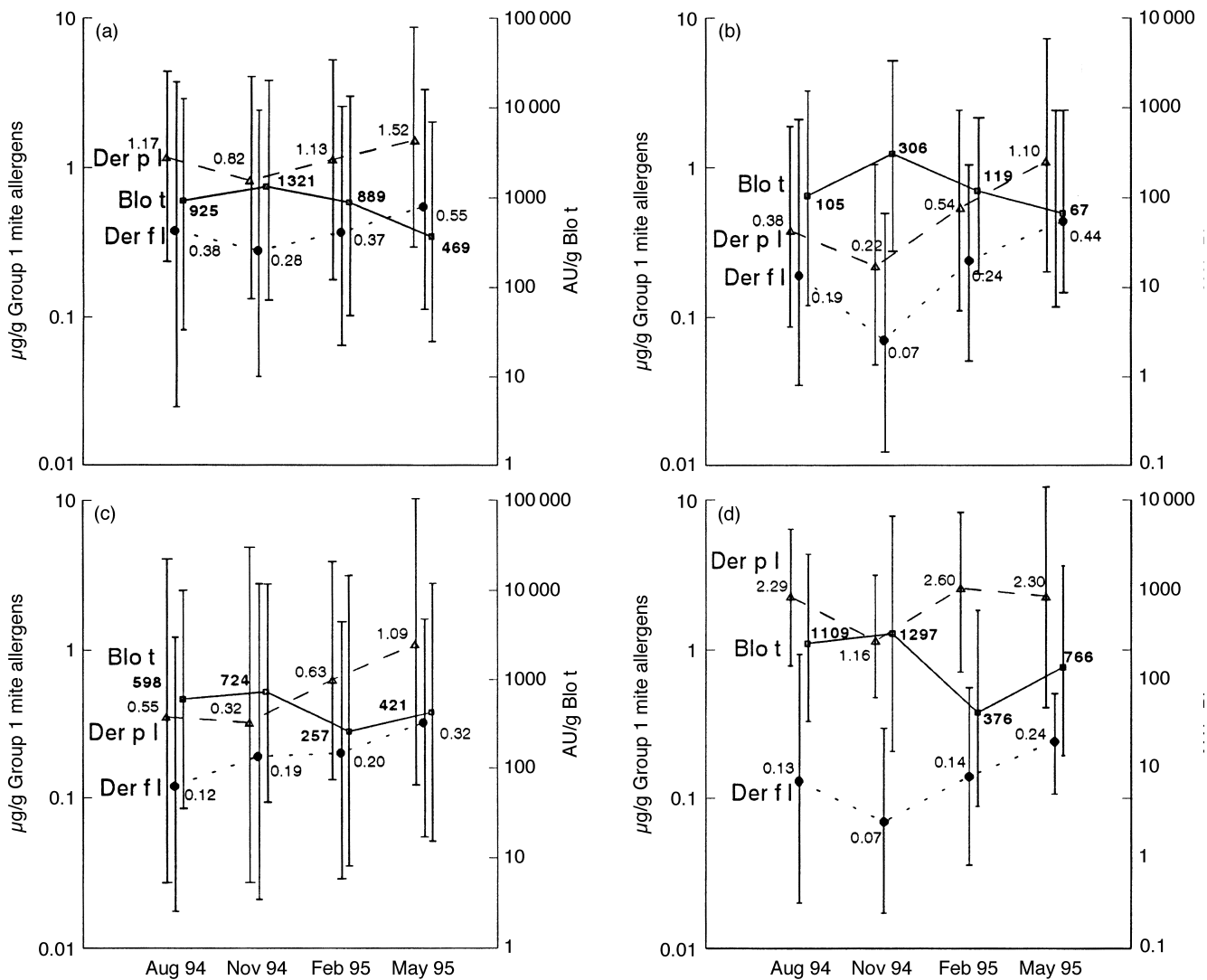


Fig. 2. Variation in Der p 1 (▲), Der f 1 (●) and *Blomia tropicalis* (■) allergen levels over one year (August, 1994–May, 1995) on mattresses (a), bedroom floor (b), living room sofas (c) and carpets (d) in residential homes in Singapore. The geometric mean levels and 95% confidence intervals of Der p 1 (— —), Der f 1 (- - -) and Blo t (—) [in bold] are indicated. The test for seasonality was found to be not significant in all cases.

relatively constant [37], seasonal variation in allergen levels were not observed.

The levels of the cockroach allergen, Bla g 1, were considerably lower than those found in the inner cities of the United States of America, where infestation may be heavy especially in the low income groups [41]. This is likely related to lower cockroach infestation in Singapore, as well as the lower numbers of the *Blattella germanica* cockroaches found locally compared to the more common *Periplaneta americana* [9]. In this study, occupants only noticed the occasional cockroach which were found mainly in kitchens and storerooms during the night. There was also no visual evidence of cockroaches in any of the locations

during the investigators visits. The higher levels of Bla g 1 in the schools compared with homes indicate that cockroach infestation is likely to be higher in these areas.

Despite the low frequency of homes with cats (1.5%) and dogs (10.5%) in Singapore homes [42], both Fel d 1 and Can f 1 were found to be widely distributed, with detectable levels of allergen in more than half of the dust samples. This phenomenon of the presence of contaminating allergens in areas without pets has been well documented [43,44]. Compared with earlier reports from the USA [45,46], the markedly lower levels of these allergens found in this study may be explained by our local habit of keeping pets outdoors, the less frequent use of carpets (19% of homes), and

the relatively well ventilated homes of the tropics. In this regard, the presence of carpets and low ventilation have been found to be important factors in increasing the accumulation of the Fel d 1 allergen [47]. Although the number of houses with cats (3/103), and dogs (6/103) in this study was small, higher levels of major cat and dog allergens were found in these homes compared with those without these pets.

The finding of higher levels of dust mite and pet allergens in residential homes compared with other indoor locations is consistent with studies in other populations [48,49]. Despite considerable human activity and the use of bedding in childcare centres and hospitals, the lower levels of allergens in these locations compared with the homes is likely to be related to the use of vinyl mattress covers and stringent cleaning measures [50]. Similarly, our data showed that homes with more stringent cleaning measures such as frequent mattress cover wash and the use of vinyl or plastic encasing may reduce allergen load.

This study reinforces the notion that although public places are contaminated with common indoor allergens, the home constitutes the major reservoir of these allergens, and should be the target of allergen avoidance measures. Although not universally found in tropical regions, Singapore, like tropical areas of South America, has a high prevalence of *B. tropicalis*. This allergen should therefore be considered as one of the major allergenic component in dust of this region.

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