

Indoor allergen levels in Guangzhou city, southern China

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To cite this article: Zhang C, Gjesing B, Lai X, Li J, Spangfort MD, Zhong N. Indoor allergen levels in Guangzhou city, southern China. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02465.x.

Keywords

cockroach; exposure; house dust mite; indoor allergens; risk factors.

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Accepted for publication 9 July 2010

DOI:10.1111/j.1398-9995.2010.02465.x

Edited by: Hans-Uwe Simon

Abstract

Background: High levels of sensitization to house dust mites have been observed in Chinese allergic patients. This study has measured levels and distributions of mite and cockroach allergens in household dust in Guangzhou. Influences of home characteristics and seasonal changes on allergen levels were also investigated.

Methods: Dust samples were collected from bedding and living room from households in Guangzhou. Major allergens from *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras*, *Blomia tropicalis* and cockroach allergens were measured by ELISA. Home characteristics were obtained from a questionnaire.

Results: Four hundred and four dust samples were collected from 107 homes during October 2006 to November 2007. House dust mite allergen levels were detectable in 99% of the bedding samples. Der f 1 levels were significantly higher than Der p 1 levels. High levels of mite allergens (> 10 µg/g) were observed in 88% of all the bedding samples. Cockroach allergens were detected in 93% of households and were higher in living room samples than in bedding samples. Blo t 5 and Der m 1 could not be detected in the dust samples. Having fabric furniture was a predictor of high allergen levels. Der f 1 levels were higher in summer time than in winter time. Cockroach allergens were higher in winter time than in summer time.

Conclusion: In Guangzhou, Der f 1 is the predominant mite allergen in dust with very high levels in bedding. Cockroach allergens are also common.

House dust mites are very important sources of indoor allergens worldwide, including China. Exposure and sensitization to house dust mite allergens have been associated with development of asthma and other allergic diseases (1, 2). Recently, China Alliance of Research on Respiratory Allergic Disease (CARRAD) showed that the prevalence of positive skin responses (SPT) among more than 6000 patients with asthma and/or allergic rhinitis from China were highest for *Dermatophagoides farinae*, *D. pteronyssinus*, *Blomia tropicalis* and cockroach species *Periplaneta americana* and *Blattella germanica* (3). The SPT prevalence ($N = 668$) for Guangzhou city were 64% for *D. farinae* and *D. pteronyssinus*, 43% for *B. tropicalis*, 18% for *P. americana* and 10% for *B. germanica*.

Allergen exposure can be indicated by the number of mites or the levels of their allergens detected in household dust. Mite fauna has been investigated in Guangzhou city showing that *D. pteronyssinus* and *D. farinae* are most frequently found (4, 5), but there is no study that has measured indoor allergens in dust samples from this city.

The aim of this study was to determine the levels and distribution of mite and cockroach indoor allergens in household

dust from bedding and living room in Guangzhou city. In addition, the influences of home characteristics and seasonal changes on the allergen levels were investigated.

Material and methods

Subjects

The homes of 107 subjects were included in this study (62 subjects were allergic outpatients visiting Guangzhou Institute for Respiratory Diseases and 45 were subjects with no clinical history or symptoms of allergy). All subjects lived in Guangzhou city, southern China. Written informed consent was obtained from participants or their parents.

Dust collection

Two dust samples per visit, one from beddings and one from living room, were collected in each home on two occasions with 6 months interval. Subjects had been living in their homes for at least 1 year before dust collection. Participants were asked not to replace their bedclothes for 2 weeks prior

to dust collection. Dust was sampled by a trained technician using a hand-held 1200 W vacuum cleaner equipped with a filter trap (ALK-Abelló, Hørsholm, Denmark). Areas vacuumed included the following: (1) sheet, pillowcase, pillow, quilt and mattress from bedding; (2) furniture, carpet, miscellaneous such as stuffy toys and cushions from living room. The amount of collected dust was 0.2–1.3 gram per sample.

Dust extraction and allergen analysis

Large particles were manually removed from the dust samples. The weight of the fine dust and filter paper was recorded, and the sample extracted 1:15 (dust to buffer w/v) in 0.125 M $\text{NH}_4(\text{HCO}_3)$ for 2 h at room temperature by gentle shaking. The extract was filtered through a 0.22 μm filter. The filtered extract solution was stored at -20°C until analysis.

Allergen content in dust extracts was measured by six ELISA. Four used monoclonal antibodies against Der p 1, Der f 1, Der m 1 (ALK-Abelló) and Blo t 5 (INDOOR Biotechnologies, Charlottesville, VA, USA), and two used rabbit polyclonal antibodies against semi-purified Bla g 1 and Per a 1 (ALK-Abelló). Detection limits were 1 ng/ml for Der p 1; 2 ng/ml for Der f 1 and Der m 1; 0.3 ng/ml for Blo t 5; 0.08 AU/ml for Bla g 1 and Per a 1. The units for dust measurements were micrograms per gram of dust ($\mu\text{g/g}$) for the mite allergens and arbitrary units per gram of dust (AU/g) for cockroach allergens. The results for cockroach allergens were combined by calculation of the sum of the AU/g dust of Bla g 1 and Per a 1, as the two ELISA had a large degree of cross-reactivity ($>25\%$). The mite ELISA had very low cross-reactivity ($<0.001\%$). Dust samples with nondetectable levels of allergens were assigned a value of half the detection limit for the specific allergen.

Questionnaire

A questionnaire about the home environment and subjects' data was administered by a trained technician at the first home visit. Information was collected about the structure of home (type, size, years of living, number of rooms), home environment (number of people and smokers, use of air conditioning, type of kitchen, type of furniture, material of quilt, pillow and mattress) and environment factors related to allergens (presence of cats and dogs).

Outdoor temperature and relative humidity in Guangzhou city

Guangzhou city is located at $23^\circ16'\text{N}/113^\circ23'\text{E}$, belonging to the subtropical monsoon area of southern China. The population was approximately 10 million people in 2007. Monthly mean values for outdoor temperature and relative humidity in Guangzhou city were obtained from Guangdong Meteorological Administration.

Statistical analysis

Data analysis was performed with SPSS® version 13.0 for Windows (SPSS Inc. Chicago, IL) for questionnaire data.

Simple and multivariate logistic regression was utilized to analyze the effect of various housing characteristics on the risk of high allergen levels ($>10 \mu\text{g/g}$ for house dust mite group 1 allergen and $>8 \text{ AU/g}$ for cockroach allergen). Results were expressed as odds ratios (OR) and 95% confidence intervals (CIs). As the distribution of allergen levels was skewed, results were expressed as medians and interquartile ranges. The Spearman rank-order correlation was used to test possible association between variables. The Mann-Whitney test was used to compare the allergen levels between groups using GraphPad Prism version 5.013. A P -value <0.05 was regarded as statistically significant.

Results

Levels and distributions of indoor allergens in bedding and living room

A total of 107 homes were visited twice with a half-year interval collecting a total of 404 dust samples (two samples per visit). The first visit was carried out from October 2006 to June 2007. The second visit was between May and November 2007. Ten subjects withdrew from the study before second visit because of moving of home.

Table 1 shows the measured results as μg Der p 1 or Der f 1 per gram dust in bedding samples and in living room samples. Cockroach allergen results are given as arbitrary units, AU per gram dust.

Median values of Der f 1 in bedding samples collected during the first and second visit were 42 and 45 $\mu\text{g/g}$, respectively. The corresponding median values for Der p 1 were 0.78 and 0.30 $\mu\text{g/g}$ dust.

Cockroach allergen levels were higher in samples from living room: 19 and 4.4 AU/g at first and second visit, respectively, compared to corresponding bedding samples; 0.81 and 2.1 AU/g dust. Levels of Der m 1 and Blo t 5 were all mainly below the lower limit of detection and are not included in Table 1.

Very few dust samples from bedding (1%) had levels of Der p 1 or Der f 1 below the lower limit of detection, whereas high levels of Der p 1 + Der f 1 ($>10 \mu\text{g/g}$) was observed in 88% of all the bedding dust samples. Der p 1

Table 1 Allergen levels in the dust samples

	Median values per gram dust (Inter Quartile Ranges)					
	Der f 1 $\mu\text{g/g}$		Der p 1 $\mu\text{g/g}$		Cockroach AU/g	
	Bedding	Living room	Bedding	Living room	Bedding	Living room
1st visit ($n = 107$)*	42 (80)	1.0 (7.3)	0.78 (4.0)	0.02 (0.16)	0.81 (3.3)	19 (90)
2nd visit ($n = 97$)	45 (81)	1.1 (15)	0.30 (3.6)	0.03 (0.18)	2.1 (10)	4.4 (27)

*Because of sample collection problems during the first visit, living room ($N = 103$).

and Der f 1 could not be detected in 38% and 13% of dust samples from living room, respectively. Cockroach allergen levels could not be detected in 46% of dust samples from bedding and 29% of dust samples from living room. Only 7% of the households did not have at least one of the four dust samples positive for either cockroach species.

Figure 1 shows histograms of distribution of the $\mu\text{g/g}$ dust allergen levels of Der f 1, Der p 1 and the combined AU/g dust values of cockroach allergens, grouped into low, medium, high and very high levels for the two visits. High levels of Der f 1 allergens, $10\text{--}100\ \mu\text{g/g}$, were detected in 52% and 60% of bedding samples during the first and second visit, respectively, and very high levels, $100\text{--}1000\ \mu\text{g/g}$, were detected in 24% and 28%, respectively. The majority of samples containing Der p 1 were found to be in the low level group, $\leq 1\ \mu\text{g/g}$.

Der p 1 and Der f 1 allergen levels were significantly higher in bedding samples when compared to living room samples ($P < 0.0001$). All Der f 1 levels in bedding and living room, respectively, were significantly higher than the

Der p 1 levels ($P < 0.0001$). Cockroach allergen levels in living room samples were significantly higher than in bedding samples ($P < 0.0001$).

There was a positive correlation between Der f 1 levels from bedding and living room, and similar for Der p 1 levels (Spearman's $r = 0.303$ and 0.425 , respectively, $P < 0.0001$). No correlation was found between levels of Der p 1 and Der f 1 in bedding. There was a positive correlation between cockroach allergen levels from bedding and living room (Spearman's $r = 0.416$, $P < 0.0001$).

Seasonal variation of indoor allergens

Average outdoor temperature and relative humidity for each sampling month from November 2006 to November 2007 is shown in Fig. 2 panel A. The values of temperature and relative humidity ranged from 14°C in January to 31°C in July and 55% in November 2007 to 81% in March. The mean temperature and relative humidity of the first visit (October 2006 to June 2007) were 21°C and 71% and the second visit

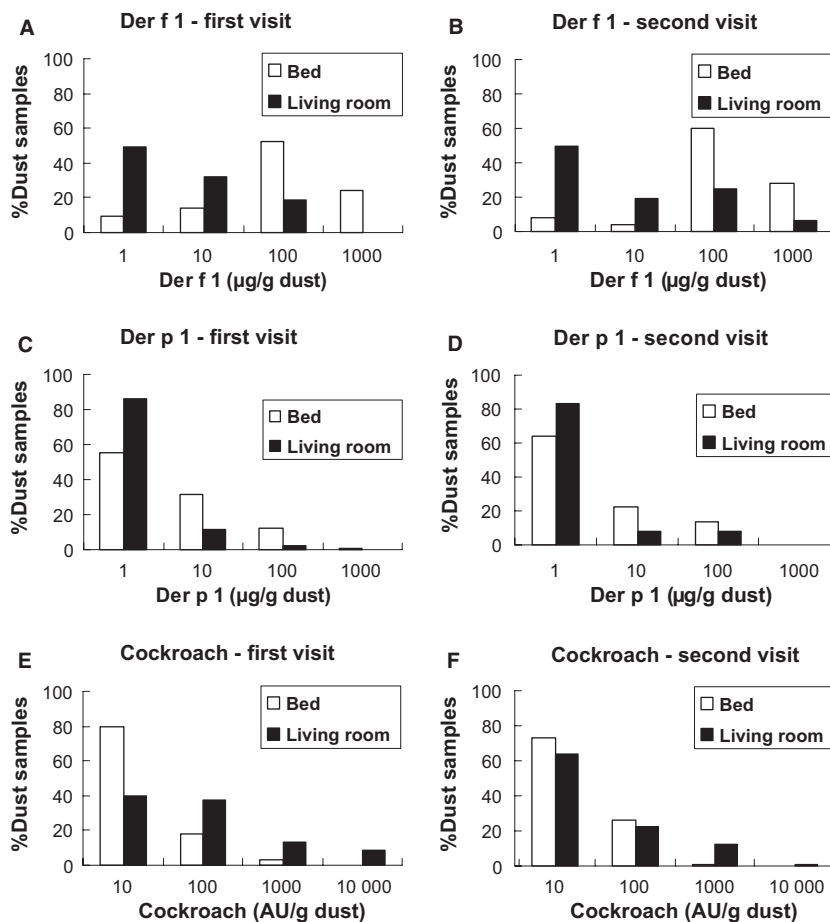


Figure 1 Distribution of allergen levels in bedding and living room dust samples from first and second visit. (A,B) Der f 1 levels ($\mu\text{g/g}$ dust) at low (≤ 1), medium ($1\text{--}10$), high ($10\text{--}100$) and very high levels ($100\text{--}1000$). (C,D) Der p 1 levels ($\mu\text{g/g}$ dust) at

low (≤ 1), medium ($1\text{--}10$), high ($10\text{--}100$) and very high levels ($100\text{--}1000$). (E,F) Cockroach allergen levels (AU/g dust) at low (≤ 10), medium ($10\text{--}100$), high ($100\text{--}1000$) and very high levels ($1000\text{--}10000$).

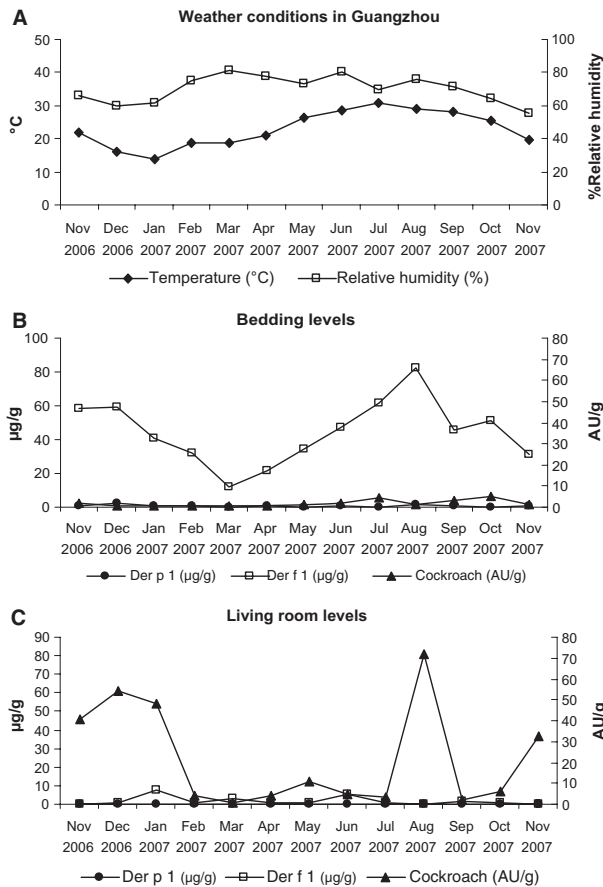


Figure 2 Climate conditions and allergen levels during collection period. (A) Weather conditions in the collection period. (B) Median levels of dust allergens in bedding each month. (C) Median levels of dust allergens in living room each month. (Note the high level of Cockroach in living room August 2007 is likely to be a random variation, as there are only four samples that month.)

(May to November 2007) were 27°C and 69%. Although dust collection started in October 2006, only 4 dust samples were collected in October 2006 and are therefore excluded from the figure.

Figure 2, panel B and C, shows the median values per month of Der p 1, Der f 1 and cockroach allergen levels in dust samples from bedding and living room. There is a clear tendency of Der f 1 in bedding to vary with the season, see Fig. 2B with minimum in March and maximum in August and for the cockroach allergen levels in living room to increase during winter with low temperature and lowest humidity (Fig. 2C). The high value for cockroach allergens in August 2007 is likely to be a random variation, as the neighboring months had low values. Der p 1 levels are too low to detect any possible seasonal influence on allergen levels.

Effect of housing characteristics on indoor allergen levels

A total of 107 homes were visited. Many characteristics were assessed by the questionnaire. Using fabric furniture and not

using leather furniture was the only identified risk factor for high Der f 1 and Der p 1 levels in living room (both $P = 0.004$). Years living in home, using air conditioning or not and material of quilt, pillow and mattress had no influence on Der p 1 or Der f 1 levels from bedding. Other factors such as presence of cats and dogs, number of people or smokers living in home had no effect on the measured indoor allergen levels.

Discussion

Although the amount of mite allergens in the Guangzhou dust samples covered a 10,000-fold range from less than 0.01 to more than 100 µg allergen per g dust, 88% of dust samples from bedding showed levels of Der p 1 + Der f 1 above 10 µg/g. Allergen concentration at this level is considered a risk factor for acute asthmatic attack and represents allergen levels at which most mite allergic patients will experience symptoms (2). The high levels of mite allergens are most likely a reflection of the year round humidity of > 55% in Guangzhou and are likely to be the cause of the high mite SPT prevalence of 64% found in Guangzhou outpatients (3).

We found a much higher level of Der f 1 compared to Der p 1 in the Guangzhou dust samples. The higher prevalence of *D. farinae* compared to *D. pteronyssinus* is not only dependent on relative humidity but on differences in the biology of the two species and temperature influences on reproduction and survival are also important factors (6). Our finding is in agreement with Lian et al. (7) who found higher levels of Der f 1 in extracts from Guangzhou air condition filters. In contrast, the study of Lai et al. (4) identified and counted double as many *D. pteronyssinus* as *D. farinae* mites in Guangzhou household dust, and similarly, Chen et al. (5) reported double as many *D. pteronyssinus* as *D. farinae* mites in school dormitory dust from Guangzhou. These results might not necessarily be opposed to each other, as some studies have shown a variable correlation between number of mites and levels of allergens (2, 7). There is a very high degree of homology between the allergens from *D. pteronyssinus* and *D. farinae* (8). It is therefore likely that the exposure seen from the subject's immune system will be very similar whether the exposure is mainly *D. pteronyssinus* or mainly *D. farinae*.

Dust samples collected from bedding had much higher levels of mite allergens compared to living room samples in agreement with previous studies from other parts of the world, for review see (6). We measured high amounts Der f 1 in bed samples with median values of 45 µg/g dust and 28% of samples containing between 100 and 1000 µg Der f 1/g dust. Terra et al. (9) found similar levels in Uberara in Brazil and Halken et al. (10) reported about 10 µg/g Der p 1 + Der f 1 in samples collected from beds in Denmark. Sun et al. (11) measured Der p 1 levels of 3 µg/g in bed samples from Hongkong, a city in close vicinity to Guangzhou; however, they did not report Der f 1 results. Huus et al. (12) investigated dust collected as a mixture from several sampling sites in about 1000 homes of asthmatic children distributed in eight different cities across North America. They found highly variable levels of Der p 1 and Der f 1 in these cities

ranging from 0% to >30% having levels in the high group (>10 µg/g) for either Der p 1 or Der f 1. Macan et al. (13) found more Der p 1 than Der f 1 on three locations in Croatia but at levels much lower than our results. Chew et al. (14) collected dust from infant beds, the floor in infant bedrooms, sitting room and kitchen. Their results showed that samples from bedroom floor had higher levels of allergen compared to bed samples, as 25% of the floor samples had Der f 1 > 10 µg/g. However, 40% of the beds did not yield enough dust for analysis, partly because of mattress plastic encasing, which might have biased their results toward lower allergen levels in beds.

Cockroach allergens Bla g 1 and/or Per a 1 could be detected in at least one of the dust samples from 93% of the investigated Guangzhou households. Cockroach allergens were more common in living room samples (71%) than in bed samples (54%). We have reported cockroach allergen levels in arbitrary units, as the two cockroach ELISAs used in this study showed a very high degree of cross-reactivity between different cockroach species and low specificity for group 1 allergens. The quantitative results for cockroach allergens reported here is therefore difficult to compare with other published findings. Chew et al. (14) found around 60% of their dust samples had Bla g 1 < 0.025 U/g. They also reported that living in an apartment increased the risk of high Bla g 1 levels compared with living in a house. All Guangzhou households in our study were apartments. Huss et al. (12) also measured cockroach allergen levels and reported their results as positive or not measurable. They found very few households (2–22%) in North America with Bla g 1 positive dust. The differences between these studies and ours could be because of the degree of infestation of the households being investigated and/or because of differences in sensitivity and specificity of allergen detection.

The cockroach levels in the sitting room showed some seasonal variation with a peak during the winter period, November to January. This indicates that the cockroaches tended to move more into houses as the outdoor temperature decreased in winter. The level of Der f 1 in bedding shows a decrease in early spring with a maximum in late summer. This does not in a simple way correlate with the weather conditions. The levels of Der p 1 and the levels of Der f 1 in sitting room and cockroach level in bedding did not show fluctuations during the year. This could be because of variations among the dust samples in addition to the low allergen levels observed.

We could not detect any Blo t 5 reactivity in the dust samples. This is in agreement with Chen et al. (5), as they could not identify any *B. tropicalis* mites in the dust from Guangzhou school dormitory. The apparent absence of *B. tropicalis*

allergens is somewhat puzzling, because SPT results from an allergic patient population in Guangzhou showed a 43% prevalence of *B. tropicalis* sensitization (3). One explanation could be that the Blo t 5 ELISA used in this study is highly *B. tropicalis* specific, whereas the SPT results might be because of IgE cross-reactivity toward other storage mite species giving rise to a positive SPT reaction that might not necessarily originate from *B. tropicalis* (15).

We applied a rather extensive questionnaire to obtain information about the household conditions to identify some characteristics that correlated with allergen dust levels. However, only the presence of fabric furniture correlated with increased allergen levels in the sitting room. This is in agreement with that fabric furniture and bedding provide an environment favoring growth and propagation of mites (6). It has been shown by Spertini et al. (16) that mite allergen levels in the home can be decreased by controlling indoor humidity. From the questionnaire, we found that although most household made use of air conditioners, there was no correlation between the availability of air conditioners and the level of allergen in the dusts. This could be because of that air conditioners in Guangzhou are used to cool the air temporarily and locally and not constantly in the whole home. If it would be technically and economically feasible to control the humidity in homes of Guangzhou, it may reduce allergens levels and exposure. Halken et al. (10) showed that by using special mattress and pillow encasing, they could reduce the exposure of house dust mite allergen from the beds and decrease medicine consumption of the patients. Our study was not designed to investigate effect of intervention, but given the very high levels of allergen in bed dust, it is likely that interventions would significantly reduce allergen levels.

Conclusion

In Guangzhou city, Der f 1 is the most predominant mite allergen in dust with a very high proportion of bedding dust samples having more than 10 µg/g. Dust samples containing > 100 µg Der f 1 per gram dust are also very frequent. Presence of cockroach allergens in the household dust is common. Climate parameters have influence on the house dust mite and cockroach allergen levels. Of the housing characteristics only presence of fabric furniture correlated with high allergen level in dust.

Acknowledgment

The authors wish to thank Nili Wei and Tiantian Liu for assisting with dust sampling, questionnaire collection and the ELISA measurements.

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