Indoor Allergens and Microbial Bio-Contaminants in Homes of Asthmatic Children in Central Taiwan

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INTRODUCTION

Nowadays, about 90% of our lives are spent indoors, and it is believed that exposure to indoor bio-contaminants plays a significant role in allergic diseases and asthma. Of interest over the years has been the role of indoor allergens from house dust mites, domestic animals, and cockroaches and its significance to allergic diseases. Lately, indoor microbial products from bacteria and fungi have also been shown to play a significant role in allergic diseases.

Studies have shown that sensitization to house dust mite allergens is strongly associated with asthma in children and adults (1, 2), that the development of atopy and asthma follows early childhood exposure (3), strict avoidance to house dust mite allergens can reverse asthma symptoms (4, 5), and that there is a dose-response relation between house dust mite allergen exposure and the severity of asthma in house dust mite sensitized children (6). However, as with many environmental exposures, individual responses to exposure and avoidance will be influenced by a genotypic variation (7).

Epidemiological studies have suggested that micro-organisms and their components in the indoor environment, particularly bacterial endotoxin and fungal beta-glucan, may play a role in the prevalence of asthma and may account for the frequently observed association between home dampness and prevalence and severity of respiratory symptoms (8). Micro-organisms are suspected to play a role not only as producers of IgE-inducing allergens, but also as a source of various agents that may induce non-immunogenic inflammatory reactions that may account for the occurrence of adverse respiratory effects (9, 10). However, exposure to high levels of microbial bio-contaminants in infancy may be protective for the development of asthma and allergic diseases in childhood (11), which is supportive of the “hygiene hypothesis” that postulates that the increase in allergic diseases may be due to a concomitant reduced infectious stimuli exposure resulting in a shift to a Th2 profile (12).

The prevalence of allergic diseases has increased in Taiwan. Among 6- to 8-year-old children in Changwa County the prevalence of wheezing, doctor-diagnosed asthma, rhinitis, and eczema was 5.0%, 7.0%, 24.6%, and 18.0%, respectively (13). The prevalence of symptoms of asthma, rhinitis, and eczema increased by 37%, 51%, and 193%, respectively, over a 7-year period in 13- to 14-year-old children in Taipei (14). The authors concluded that the increasing prevalence of allergic diseases is a significant burden on public health systems in Taiwan.

To date, there have only been a few studies that have measured house dust mite allergen or endotoxin in the home environment in Taiwan. To our knowledge, no such studies have been done in central Taiwan, nor has beta-glucan been studied in the indoor environment in Taiwan. This study determined indoor levels of house dust mite and cockroach allergens, endotoxin, and beta-glucan in homes of asthmatic children in a major city in central Taiwan. The primary aim was to determine base-line levels of house dust mite allergens and avoidance will be influenced by a genotypic variation (7).

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and microbial bio-contaminants in central Taiwan for future allergen avoidance and reduction studies.

**Material and Methods**

A total of 120 allergic asthmatic children 5 to 14 years of age (71 males) were randomly selected from the pediatric database at Changhua Christian Hospital, Changhua City, Taiwan. All children had a measurable level of specific immunoglobulin E (IgE) to Dermatophagoides pteronyssinus ranging from 0.1 KU/L to >100 KU/L. Permission to collect indoor dust samples was obtained from the children’s parents who provided written informed consent. The study was approved by the Changhua Christian Hospital’s Ethics Committee.

Dust samples were obtained from the mattress and primary pillow of each child’s bed. Sixty-seven children slept on a dual-sided mattress (conventional side and bamboo side); therefore, separate dust samples were collected from both sides. Dust samples were also obtained from the kitchen floor of each child’s home. Dust samples were collected by trained nurses using a Life’s Good (LG Electronics, Seoul, Korea) vacuum cleaner (1,100 Watts) with a 25-µm nylon mesh bag fitted into the vacuum cleaner’s inlet hose using the same protocol that has been used in many studies on the indoor environment by the Wellington Asthma Research Group (15). The whole mattress was vacuumed for 2 minutes, kitchen floors were vacuumed for 2 minutes over a 1-m² central area, while pillows were vacuumed for 1 minute on each side, a total of 2 minutes. Dust samples were collected in the winter period between October and December.

Total dust weights were recorded and dust samples were placed into screw-top centrifuge tubes and stored at −20°C until extraction. For beta-glucan analysis, 5 mg of fine dust was removed and extracted with 5 mL of 0.3 M NaOH. For allergens and endotoxin analysis a two-step extraction protocol was followed. First, the rest of the dust sample was extracted with pyrogen-free water containing 0.05% Tween-20. The extraction volume depended on the total dust weight, more aliquated supernatant removed from the 1st extraction step. After centrifugation, supernatants were stored at −20°C for subsequent analysis of selected indoor allergens.

Double-monoclonal antibody enzyme-linked immunosorbent assays (ELISAs) using commercially available kit sets (Indoor Biotechnologies, Charlottesville, VA, USA) were used to measure allergens from house dust mites (Der p 1, Der f 1 and Blo t 5) in the supernatants from mattresses and pillows, while a monoclonal/polyclonal sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure cockroach allergen (Bl a 1) in the supernatants from kitchens, as in our previous studies (16, 17). The lower limits for detection (LLODs) are 1.0 mU/g of dust for Bl a 1 and 0.025 µg/g of dust for Der p 1, Der f 1, and Blo t 5.

Beta-glucan levels in the dust extract supernatants were assayed at a 1:1000 dilution with a modified Limulus amoebocyte lysate kinetic assay specific for beta-glucan (Glucatell; Cape Cod Inc, Falmouth, MA, USA) as in our previous studies (18). Endotoxin levels were assayed at a 1:500 dilution with a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) method (Kinetic QCL; Bio Whittaker, USA) at 37°C as in our previous studies (19).

Geometric means with 95% confidence intervals (95% CI) were calculated for house dust mite allergens, endotoxin and beta-glucan. Values below the LLOD were assigned the limit of detection. The exact Wilcoxon test for non-parametric paired data was used for comparison using “R” version 2.7.1. This version computes exact conditional p values using a shift algorithm for both tied and untied samples.

**Results**

Two of the children did not use pillows, thus results are from 118 pillows. A mean pillow dust weight of 0.1364 g (95% CI: 0.1079–0.1650) was obtained. Geometric means with 95% CI of house dust mite allergen levels from pillows are shown in Table 1. Der p 1 was detected in 113 (95.8%) of pillows, Der f 1 in 97 (82.2%), and Blo t 5 in 11 (9.3%). The highest levels recorded for Der p 1, Der f 1, and Blo t 5 were 66.2 µg/g, 39.1 µg/g, and 1.5 µg/g, respectively.

For the 120 mattresses (conventional side) a mean dust weight of 1.3585 g (95% CI: 1.1766–1.5403) was obtained. Geometric means with 95% CI of house dust mite allergen levels, endotoxin, and beta-glucan from mattresses are shown in Table 2. Der p 1 was detected in 110 (95.8%) mattresses, Der f 1 in 98 (83.1%), and Blo t 5 in 97 (82.2%). The highest levels recorded for Der p 1, Der f 1 and Blo t 5 were 37.4 µg/g, 43.2 µg/g, and 7.3 µg/g, respectively. Endotoxin and beta-glucan were detected in all mattresses within a range of 12.1 EU/mg to 3,803 EU/mg and 1.1 µg/g to 504.6 µg/g, respectively.

Sixty-seven children slept on mattresses that had a conventional mattress side and the other side made of bamboo. Comparison of allergen levels, endotoxin, and beta-glucan between paired mattresses sides are shown in Table 3. The bamboo side had significantly lower levels of Der p 1, Blo t 5, and endotoxin. Der f 1 levels were generally lower from the bamboo side but just failed to reach statistical significance, while beta-glucan levels were similar and not significantly different.

There was no association between the number of people sharing the same bed (median: 2; range 1–5), gender, age of the child, or age of the house with any of the measured parameters on pillows or mattresses. Neither was there any significant association between the child’s specific IgE to

<table>
<thead>
<tr>
<th>Table 1.—Levels of house dust mite allergens from 118 pillows.</th>
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<tr>
<td>Geometric mean (95% CI)</td>
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<tr>
<td>Der p 1 µg/g dust</td>
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<tr>
<td>Der f 1 µg/g dust</td>
</tr>
<tr>
<td>Blo t 5 µg/g dust</td>
</tr>
<tr>
<td>Total house dust mite allergen µg/g dust</td>
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</tbody>
</table>
D. pteronyssinus and Der p 1 levels on their pillow or mattress.

One hundred five kitchens (87.5%) had detectable Bla g 1 levels ranging from 0.01 U/g to 15.7 U/g with a geometric mean of 0.61 U/g (95% CI: 0.43–0.85). Bla g 1 levels were not associated with age of the house (< 1 yr; 1–5 yr; > 5 yr), which floor the dwelling was on (1–8), or whether cockroaches had recently been sighted in the kitchen.

**DISCUSSION**

This study has shown that allergens Der p 1, Der f 1, and Blo t 5 from the house dust mites *D. pteronyssinus*, *D. farinae*, and *Blomia tropicalis* are present in bedding of asthmatic children in central Taiwan. A previous study from southern Taiwan has also shown similar levels of Der p 1 from bedding (21). To our knowledge, there have been no studies that have measured either Der f 1 or Blo t 5 allergens in the indoor environment in Taiwan despite studies showing the presence of *D. farinae* and *B. tropicalis* in Taiwan (22) and demonstration of specific IgE sensitization to Der f 1 and Blo t 5 in Taiwanese children (23), including from the same region as our study (24).

Blo t 5 was detected in only 9.3% of pillows but was present in the majority of mattresses (82.2%). The levels of Blo t 5 from mattresses in our study (geometric mean: 0.14 µg/g) are much lower than those in New Zealand (24). Comparatively, in our study no Blo t 5 levels were 9.3% of pillows exceeding this level were 16.1% for Der p 1 and 9.3% for Der f 1. Correspondingly, 5.0% and 9.2% of mattresses had Der p 1 and Der f 1 levels, respectively, above 10 µg/g.

In most studies of house dust mite allergen exposure Der p 1 is the most common allergen measured. In countries such as New Zealand and Australia, where *D. pteronyssinus* is the dominant house dust mite, measuring Der p 1 gives a good exposure assessment. However, in areas where more than one type of house dust mite is present, exposure assessment needs to take into account allergens of all species. To our knowledge, no studies of house dust mite allergen exposure in Taiwan have measured Der f 1 despite the known presence of *D. farinae*. In our study there were nine mattresses and eight pillows with Der f 1 levels greater than 10 µg/g, yet corresponding Der p 1 levels were below this cut-off point. Indeed, one mattress and one pillow had Der f 1 levels of 20.4 µg/g and 26.0 µg/g, respectively, yet Der p 1 was undetectable. If we had only measured Der p 1, underestimation of significant house dust mite allergen exposure would have occurred.

Endotoxin is a component of the outer cell membrane of gram-negative bacteria. Endotoxin is ubiquitous in the indoor environment, and exposure to increased levels of endotoxin is associated with increased asthma symptoms in atopic asthmatics (28). Recent research has indicated that exposure to endotoxin in living rooms is inversely related to asthma symptoms (wheeze) in children (29). Indoor endotoxin has previously been assessed in homes of asthmatic children in Taiwan (21). Although we cannot directly compare our results with that study owing to differences in endotoxin extraction and measurement, we can compare results with those from New Zealand as endotoxin measurements were done in the same laboratory by identical methods. Endotoxin levels in central Taiwan were about threefold higher than in New Zealand (19, 20). One hypothesized reason for this is the higher occupancy in beds in Taiwan, but we could not find any association between the number of occupants sharing the bed and endotoxin levels. However, a recent study has demonstrated a fivefold difference in indoor endotoxin levels between six centers in five countries (29).

Beta-glucan is a non-allergic component of the fungal cell wall and many higher plants. Exposure to beta-glucan has various respiratory effects, including peak flow variability (10). To date, only a few studies have measured beta-glucan in the indoor home environment, and to our knowledge, our study is the first to determine indoor beta-glucan levels in Taiwan. Because the same laboratory measured the beta-glucan levels in this study, we are able to compare results to those in New Zealand. Mattress beta-glucan levels in central Taiwan were about three times lower than found in New Zealand (18). As with endotoxin, between-country differences in beta-glucan levels have previously been demonstrated (30).

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**Table 2**.—Levels of house dust mite allergens, endotoxin, and B-glucan from mattresses, and cockroach allergen from kitchens.

<table>
<thead>
<tr>
<th>Geometric mean (95% CI)</th>
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<tbody>
<tr>
<td>Der p 1 µg/g dust</td>
</tr>
<tr>
<td>Der f 1 µg/g dust</td>
</tr>
<tr>
<td>Blo t 5 µg/g dust</td>
</tr>
<tr>
<td>Total house dust mite allergen µg/g dust</td>
</tr>
<tr>
<td>Endotoxin EU/mg dust</td>
</tr>
<tr>
<td>Beta-glucan µg/g dust</td>
</tr>
<tr>
<td>Bla g 1 U/g dust</td>
</tr>
</tbody>
</table>

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**Table 3**.—Levels of house dust mite allergens, endotoxin, and B-glucan from 67 double-sided mattresses.

<table>
<thead>
<tr>
<th>Bamboo side</th>
<th>Conventional side</th>
<th>p*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Der p 1 µg/g dust</td>
<td>0.38 (0.25–0.58)</td>
<td>0.77 (0.51–1.17)</td>
<td>0.010</td>
</tr>
<tr>
<td>Der f 1 µg/g dust</td>
<td>0.24 (0.14–0.44)</td>
<td>0.55 (0.30–1.01)</td>
<td>0.058</td>
</tr>
<tr>
<td>Blo t 5 µg/g dust</td>
<td>0.024 (0.007–0.033)</td>
<td>0.165 (0.115–0.237)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total HDM allergen</td>
<td>1.35 (0.95–1.91)</td>
<td>3.50 (2.65–4.62)</td>
<td>0.0002</td>
</tr>
<tr>
<td>µg/g dust</td>
<td>27.7 (22.4–34.8)</td>
<td>25.3 (21.9–29.8)</td>
<td>0.97</td>
</tr>
<tr>
<td>Mattress dust weight g</td>
<td>2.02 (1.44–2.60)</td>
<td>1.58 (1.31–1.84)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Results are geometric means (95% CI), * Wilcoxon paired test, † mean (95% CI).
A unique feature of bedding available in Taiwan is the double-sided mattress with one side being inner sprung while the other side is made of bamboo. The bamboo side is normally used in spring and summer with the inner sprung mattress side in autumn and winter. In our study, 67 of the 120 homes had this double-sided bed; thus, we were able to compare allergen, endotoxin, and β-glucan levels from both sides. The bamboo side had significantly lower levels of Der p 1, Blt 5, and endotoxin. Der f 1 levels were lower from the bamboo side but just failed to reach statistical significance; beta-glucan levels were similar and not significantly different. As the study was conducted during the winter season when all the children slept on the conventional mattress side we cannot exclude the possibility that lower house dust mite allergens and endotoxin levels were due to the bamboo side not being slept on. If the bamboo side is also lower for allergen and endotoxin during the summer when the children sleep on that side it may be preferable for asthmatic children.

Exposure to cockroach allergen is associated with an increase in cockroach sensitization and with asthma symptoms. Kitchen Bla g 1 levels exceeding 1 U/g have been associated with cockroach sensitization in asthmatic children (31), whereas Bla g 1 levels exceeding 2 U/g increase the risk of wheezing in infants (32). Although levels of Bla g 1 in our study were generally low (geometric mean: 0.61 U/g), 58 kitchens returned Bla g 1 levels of >1 U/g of which 27 had levels >2 U/g. A recent study found that leaks, exposed food, and dirty pots were associated with high kitchen Bla g 1 levels above 8 U/g (33). We did not collect information of kitchen characteristics, but only four kitchens in our study exceeded that level; the highest Bla g 1 level was 15.7 U/g.

A limitation of our study is that we only measured allergens and microbial bio-contaminants in homes of asthmatic children. Levels of these might be lower in non-asthma households as parents of asthmatic children might practice allergen avoidance, such as the use of mattress barrier covers and other cleaning measures. However, in our study only nine of the children’s beds had a protective allergen cover over the mattress and excluding these results from the data analysis did not significantly change the geometric mean levels of the measured parameters (data not shown).

Dust samples were collected in the winter season. If collected in other seasons we may have seen changes in allergen and microbial bio-contaminants as previous studies have shown slightly higher Der p 1 levels in autumn (15) and lower temperatures are associated with lower beta-glucan levels (34). However, we have not previously found that endotoxin levels differed significantly between seasons (20). Dust collection techniques and analytical methods of allergen measurements may have affected the results of our study. For instance, large between-laboratory variability in allergen measurement has been demonstrated (34,35), and different dust samplers have been used in the many reported indoor environment allergen studies. The method for allergen measurement in this study has been extensively used in previous-reported studies from our research group (36) and thus we were able to compare levels in central Taiwan to those in New Zealand. Within-batch variation in our laboratory has been <10% over 15 years and, as reported by Pate et al., the majority of laboratories are able to measure allergen levels with the required precision and accuracy (34).

Also, we have used the same dust collection technique for all of our studies, thus reducing variability and making comparisons between this study and previous studies in New Zealand valid. Recently Sercombe et al. evaluated various dust sampling devices for home allergen determination (36). Using a nylon sock attachment as reference method, three other dust sampling devices showed consistent results with the reference device, which is the same sampling device used in all of our studies.

Correlations between settled dust substances and airborne exposure to these are generally weak or only slightly correlated. However, Custovic et al. have shown that disease activity and severity of asthma in house dust mite sensitized patients were related to bedding Der p 1 levels in the dust reservoir (37).

In conclusion, although house dust mite allergen levels in central Taiwan were generally low, there were a number of pillows and mattresses that had levels of greater than 10 µg/g, high enough to induce asthma symptoms. As we were able to detect allergens from three different house dust mite species, it would be prudent to measure all three allergens to give a more accurate index of house dust mite allergen exposure in central Taiwan. Mattresses made of bamboo had significantly lower levels of all three house dust mite allergens, and endotoxin and may be the preferred side to sleep on for asthmatic children if no other allergen avoidance measures are used. We now have data on house dust mite allergens and microbial bio-contaminants from central Taiwan that will be helpful in developing avoidance strategies for asthmatic children.

Acknowledgment

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References


