

# Indoor Determinants of Endotoxin and Dust Mite Exposures in Hong Kong Homes with Asthmatic Children

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## Key Words

Chinese asthmatic children · *Dermatophagoides pteronyssinus* · Endotoxin

## Abstract

**Background:** Domestic endotoxin enhances airway inflammation and increases asthma severity in Caucasian children, but little data are published on indoor endotoxin exposure in Asian countries. This study investigated house dust endotoxin and Der p 1 levels in Hong Kong families with asthmatic children, and their effects on asthma severity. **Methods:** 115 asthmatics from a pediatric clinic underwent fractional exhaled nitric oxide (FeNO) and spirometric measurements. Home visits were then made within 2 weeks, during which parents completed the International Study of Asthma and Allergies in Childhood questionnaire. Settled dust was collected from patients' mattresses, bedroom floors and living room floors. Endotoxin and Der p 1 were measured by limulus amebocyte lysate and immunoassay, respectively. **Results:** Endotoxin was detectable in all locations from all families, whereas Der p 1 was detectable in 58–70% of indoor sites. Floors of both bedroom and living rooms had higher endotoxin but lower Der p 1 levels than mattresses ( $p < 0.001$  for both). Mattress endotoxin level correlated inversely with Der p 1 level ( $r = -0.308$ ,  $p = 0.001$ ). Household smoker, feath-

er bedding and vacuum cleaning were independent determinants of indoor endotoxin. Timing of last bedding change was associated with Der p 1 levels at all sites. Mattress endotoxin level was associated with frequency of wheezing episodes ( $p = 0.044$ ), but neither endotoxin nor Der p 1 was associated with FeNO and spirometric parameters. **Conclusions:** Domestic endotoxin levels are associated with frequency of wheezing episodes in asthmatic children but not their FeNO or spirometric measurements.

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## Introduction

Endotoxin, being a cell wall component of Gram-negative bacteria, is ubiquitously present in house dust. Endotoxin exposure has dual effects on asthma and atopy. Exposure to endotoxin early in life could be protective for the development of asthma and allergen sensitization [1]. On the other hand, many studies suggest that high house dust endotoxin levels are associated with worse asthma control, increased use of anti-asthma medications and more intense airway inflammation in patients with asthma [2–4]. Domestic burden of endotoxin was well established in both rural farms and urban cities in western countries, but there are little published data on indoor

endotoxin exposure and its determinants among Asian countries. In the United States, Thorne et al. [5] found increased house dust endotoxin to be associated with poverty, people, pets, household cleanliness and geography. Specifically, the major predictors of household endotoxin were sampling location, census division, educational attainment, presence of children, current dog ownership, resident-described problems with cockroaches, food debris, cockroach stains and evidence of smoking. On the other hand, pet keeping and the frequency of floor cleaning were associated with endotoxin levels in non-farm children from rural parts of Europe [6].

In addition to endotoxin, indoor aeroallergen exposure during early childhood has been associated with an increased risk of sensitization [7, 8]. Aeroallergen sensitization is an important component of childhood asthma [9]. For house dust mite (HDM) and cockroach allergens, there is good evidence for a dose-response relationship between exposure and sensitization and development of asthma [10, 11]. Avoidance programs starting early in life, addressing high-risk infants, have shown that sustained low levels of HDM allergens can be achieved in homes [12]. However, the clinical benefits associated with allergen avoidance are inconclusive [13–15]. Although there is a strong association between allergen sensitization and asthma, other concurrent events in early life, including viral infections and pattern of intestinal microflora, may play important roles in asthma development [16, 17]. The objectives of this study are to characterize indoor exposures of endotoxin and a major HDM allergen (Der p 1) in Hong Kong families with asthmatic children, and to assess their effects on the severity of asthma and allergy symptoms.

## Patients and Methods

### Study Population

Asthmatic children aged 5–18 years were recruited from the pediatric allergy clinic of a university teaching hospital in Hong Kong. Asthma diagnosis was made according to the American Thoracic Society guidelines [18]. The presence of aeroallergen sensitization in these children was retrieved from our case record of skin prick testing with purified extracts of *Dermatophagoides pteronyssinus*, cat dander and cockroaches (Hollister-Stier Laboratories, Spokane, Wash., USA) and measurement of specific IgE to these allergens in serum by fluorescent enzyme immunoassay (AutoCAP, Phadia AB, Uppsala, Sweden) [19, 20]. Children with  $\geq 1$  positive skin prick test or allergen-specific IgE, performed  $\leq 2$  years prior to this study, were classified as atopic. Following informed written consent, all children underwent fractional exhaled nitric oxide (FeNO) measurement followed by spirometry. Home visits were then conducted within 2 weeks. The Clinical

Research Ethics Committee of our university approved this study. Because families with asthmatics would change their home cleaning practices, this study also recruited 10 healthy hospital staff without asthma history as controls. All subjects had been free from any symptom of respiratory infection for at least 2 weeks before the study. Dust samples were collected from all patients and controls according to the same protocols described below.

### Exhaled Nitric Oxide and Spirometric Measurements

FeNO was measured at an expiratory flow rate of 50 ml/s using a chemiluminescence analyzer (NOA280i, Sievers Instruments, Boulder, Colo., USA) according to international standards [21]. These children then performed spirometry (MasterScreen, Jaeger, Würzburg, Germany) to measure forced expiratory volume in 1 second, forced vital capacity and peak expiratory flow.

### House Dust Collection and Extraction

During home visits, two research staff recorded household characteristics of the families. Parents completed the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire [22]. The presence of 'current' asthma symptoms such as frequency of wheezing episodes, exercise and speech limitation and sleep disturbance over the preceding 12 months was recorded. Similarly, 'current' household factors such as pet keeping and bedding and pillow types referred to the 12-month period before this study. In accordance with the ISAAC phase II protocol, house dust samples were then collected from three locations, namely mattress, bedroom floor and living room floor, of patients' homes using a portable vacuum cleaner (Model 4071D; Makita, Aichi, Japan) [23]. Only mattress samples were obtained from the controls. Briefly, dust collection was done on mattress after removal of all pillows, duvets, blankets and sheets by vacuuming for 2 min. For both bedroom and living room floors, dust was collected from an area as large as possible and with a maximum 4 m<sup>2</sup>. Sampling time was in proportion with the area for dust collection, depending on the type (e.g. carpeted versus smooth) of floor. Fresh dust samples were kept at  $-20^{\circ}\text{C}$  for at least 24 h to kill the live dust mites before extraction.

House dust extraction was performed according to the ISAAC protocol [23]. In brief, endotoxin and Der p 1 were extracted sequentially. Firstly, 5 ml extraction fluid [0.05% (v/v) Tween-20 pyrogen-free water] was added to the dust sample. The homogeneous suspension was incubated on a roller for 1.5 h at room temperature, and then centrifuged at 1,000 g and  $20^{\circ}\text{C}$  for 15 min. The upper 10% (500  $\mu\text{l}$ ) of the supernatant was harvested and transferred into a pyrogen-free tube and stored at  $-20^{\circ}\text{C}$  until endotoxin analysis. Following this, 500  $\mu\text{l}$  of  $\times 10$  phosphate-buffered saline was added to the remaining dust pellet. After resuspension and upside-down mixing at  $4^{\circ}\text{C}$  overnight, dust was extracted by centrifugation at 2,000 g and  $20^{\circ}\text{C}$  for 15 min. Two milliliters of supernatant was taken and stored at  $-20^{\circ}\text{C}$  for allergen analysis.

### Endotoxin and Der p 1 Measurements

House dust endotoxin was quantified in one batch using chromogenic limulus amebocyte lysate assay (QCL-1000, Cambrex Bioscience, Walkersville, Md., USA). The sensitivity of this assay was 0.1 endotoxin unit (EU)/milliliter. House dust endotoxin was expressed both as level (EU/mg dust) and load (EU/m<sup>2</sup>). Der p 1 was measured by enzyme-linked immunosorbent assay (Indoor

**Table 1.** Clinical features and lung functions of our 115 asthmatic children

| Characteristic                                                   | Result      |
|------------------------------------------------------------------|-------------|
| Age, years                                                       | 12.4 ± 3.2  |
| Male                                                             | 68 (59.1)   |
| Rhinitis ever <sup>1</sup>                                       | 102 (88.7)  |
| Eczema ever <sup>1</sup>                                         | 63 (54.8)   |
| Asthma status in preceding 12 months <sup>1</sup>                |             |
| Number of wheezing attacks                                       |             |
| None                                                             | 52 (45.2)   |
| 1–3 attacks                                                      | 40 (34.8)   |
| 4–12 attacks                                                     | 19 (16.5)   |
| >12 attacks                                                      | 4 (3.5)     |
| Sleep disturbance due to wheeze                                  |             |
| Never awakened with wheezing                                     | 78 (67.8)   |
| <1 night per week                                                | 31 (27.0)   |
| ≥1 night per week                                                | 6 (5.2)     |
| Speech limitation due to wheeze                                  | 3 (2.6)     |
| Exercise limitation due to wheeze                                |             |
| Never or occasionally                                            | 109 (94.8)  |
| 1–2 times per week                                               | 5 (4.3)     |
| ≥3 times per week                                                | 1 (0.9)     |
| Received inhaled corticosteroid treatment                        | 50 (43.5)   |
| Aeroallergen sensitization <sup>2</sup>                          |             |
| <i>D. pteronyssinus</i>                                          | 90 (78.3)   |
| Cat dander                                                       | 33 (28.7)   |
| Cockroaches                                                      | 24 (20.9)   |
| Fractional exhaled nitric oxide concentration <sup>3</sup> , ppb | 66 ± 54     |
| Spirometric parameters                                           |             |
| Forced expiratory volume in 1 second, % predicted                | 90.4 ± 15.1 |
| Forced vital capacity, % predicted                               | 99.9 ± 14.9 |
| Peak expiratory flow, % predicted                                | 86.6 ± 19.6 |

Figures are expressed either as means ± standard deviation or number with percentages in parentheses.

<sup>1</sup> Recorded by the ISAAC core questionnaire.

<sup>2</sup> Assessed by either allergen skin prick tests or the presence of allergen-specific IgE in serum.

<sup>3</sup> Technically acceptable results were found in 112 children.

Biotechnologies, Cardiff, UK) with a sensitivity of 1.6 ng/ml. Der p 1 level was expressed in micrograms per gram dust. Dust samples with undetectable endotoxin or Der p 1 were assigned values at half the respective lower detection limits in order to calculate patients' indoor exposures. A cutoff point of 10 µg/g was used to define high Der p 1 exposure [24, 25].

#### Statistical Analysis

Results were expressed in proportion or median and interquartile range. The distributions of indoor Der p 1 and endotoxin levels were skewed, so these variables were log-transformed before analysis. Numerical variables were compared between patients and controls as well as children with different asthma status

using the Student t test or ANOVA, and categorical variables were analyzed by  $\chi^2$  or Fisher's exact test. The correlation between indoor Der p 1 and endotoxin levels, FeNO and spirometric parameters were analyzed by Pearson coefficients. Home characteristics independently associated with house dust endotoxin and Der p 1 levels were analyzed by stepwise linear regression. All comparisons were made two-tailed, and  $p < 0.05$  was considered significant.

## Results

### Subjects

One hundred and fifteen (98.3%) of 117 consecutive asthmatic children seen in our clinic consented to participate. Table 1 summarizes their clinical characteristics. Ten beds from healthy laboratory volunteers were recruited as controls.

### House Dust Collection and Analysis

The median (interquartile range) weights of dust samples collected from patients' mattresses, bedroom floors and living room floors were 39.2 mg (24.6–65.9), 23.6 mg (14.7–43.9) and 28.8 mg (20.1–46.0), respectively ( $p < 0.001$  for trend). On the other hand, the amount of dust collected from patients' mattresses did not differ from that of controls [63.3 mg (36.8–116.3);  $p = 0.101$ ]. Mattress levels of endotoxin and Der p 1 did not differ either between these two groups (fig. 1). Table 2 summarizes the proportions of indoor locations with detectable endotoxin and Der p 1 in patients' families. Endotoxin was detectable in all sites from all families. Floors of both bedroom and living rooms contained significantly higher endotoxin levels than that found on mattresses ( $p < 0.001$  for both). On the other hand, mattress and bedroom floor endotoxin loads were significantly higher than that of living room floors ( $p < 0.001$  for both). Der p 1 was detectable in 58–70% of the sites. High Der p 1 exposure of  $\geq 10$  µg/g was found in 9 (7.8%) of mattresses, 1 (0.9%) of bedroom floors and none of living room floors. In total, high Der p 1 exposure at any site was detected in 10 (8.7%) families. Mattress Der p 1 level was higher than those from bedroom or living room floors. Mattress endotoxin level was inversely correlated with mattress Der p 1 ( $r = -0.308$ ,  $p = 0.001$ ), but no correlation was found between endotoxin and Der p 1 from bedroom or living room floors. Bedroom temperature was inversely correlated with endotoxin level ( $r = -0.244$ ,  $p = 0.009$ ) and endotoxin load ( $r = -0.192$ ,  $p = 0.040$ ) on mattresses but not bedroom floors, nor with indoor Der p 1 level. Bedroom humid-

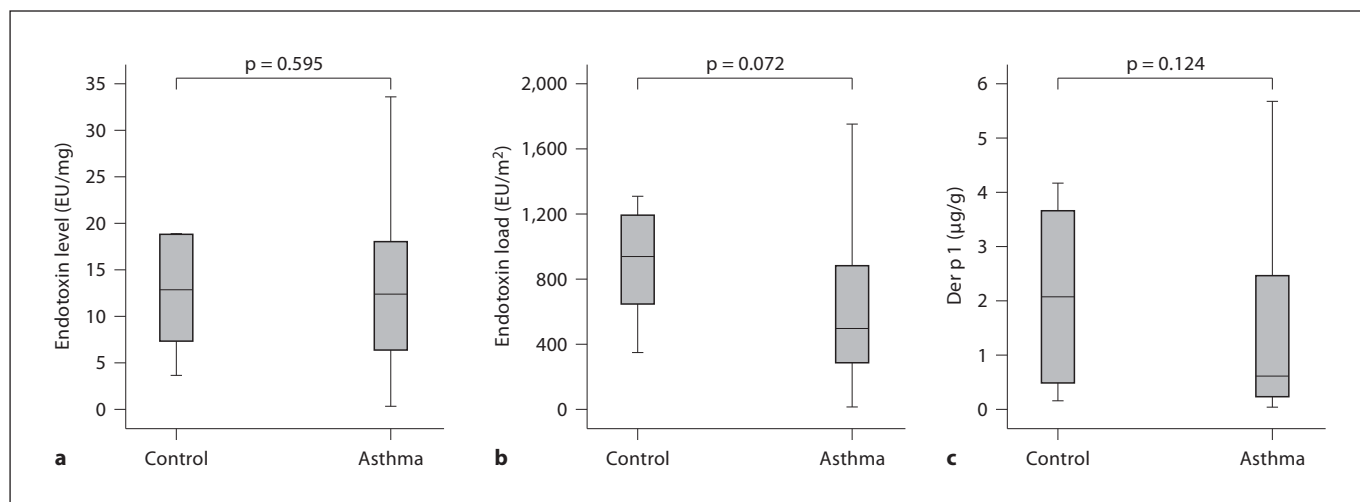


**Fig. 1.** Comparisons of endotoxin level (a), endotoxin load (b) and Der p 1 levels (c) in mattress dust samples from our asthmatic families and healthy controls.

**Table 2.** Distributions of Der p 1 and endotoxin in dust samples from different indoor sites in our 115 asthmatic children

|                                  | Mattress         | Bedroom floor    | Living room floor | p value <sup>a</sup> |
|----------------------------------|------------------|------------------|-------------------|----------------------|
| Der p 1                          |                  |                  |                   | <0.001               |
| Detectable levels, n             | 81 (70)          | 67 (58)          | 70 (61)           |                      |
| Median values, µg/g              | 0.61 (0.24–2.46) | 0.39 (0.25–0.91) | 0.32 (0.19–0.58)  |                      |
| Endotoxin level                  |                  |                  |                   | <0.001               |
| Detectable levels, n             | 115 (100)        | 115 (100)        | 115 (100)         |                      |
| Median values, EU/mg             | 12.4 (6.4–19.5)  | 24.2 (12.4–42.8) | 22.2 (14.8–36.9)  |                      |
| Endotoxin load                   |                  |                  |                   | 0.001                |
| Detectable levels, n             | 115 (100)        | 115 (100)        | 115 (100)         |                      |
| Median values, EU/m <sup>2</sup> | 497 (281–916)    | 573 (260–1,344)  | 248 (171–606)     |                      |

Figures in parentheses are percentages or interquartile ranges.

<sup>a</sup> Analyzed by ANOVA following log transformation of house dust endotoxin or Der p 1 levels.

ity was not associated with endotoxin or Der p 1 levels at any location.

#### *Domestic Determinants of House Dust Endotoxin and Der p 1 Levels*

Online supplementary tables S1 and S2 (available at [www.karger.com/doi/10.1159/000283039](http://www.karger.com/doi/10.1159/000283039)) summarize univariate analyses for the effects of different household features on indoor endotoxin and Der p 1 levels, respectively. The presence of smoking household members was associated with higher endotoxin levels on bedroom and living room floors, whereas feather bedding was associ-

ated with higher endotoxin levels on mattresses and bedroom floor. On the other hand, vacuum cleaning was associated with lower endotoxin on bedroom and living room floors. Table 3 summarizes the multivariate linear regression for factors independently associated with indoor endotoxin and Der p 1 levels. Feather bedding was associated with both higher endotoxin level ( $p = 0.023$ ) and load ( $p = 0.038$ ) on mattresses, and a trend for endotoxin on the bedroom floor. Vacuum cleaning was significantly associated with lower endotoxin load on the bedroom floor ( $p = 0.007$ ) and living room floor ( $p = 0.014$ ). Bedding last changed  $\leq 7$  days was consistently

**Table 3.** Linear regression analyses for determinants of endotoxin and Der p 1 exposures<sup>a</sup> in house dust samples

|                                                 | Mattress |       | Bedroom floor |        | Living room floor |       |
|-------------------------------------------------|----------|-------|---------------|--------|-------------------|-------|
|                                                 | $\beta$  | p     | $\beta$       | p      | $\beta$           | p     |
| <i>Endotoxin level</i>                          |          |       |               |        |                   |       |
| Household size: <50 m <sup>2</sup>              | -0.198   | 0.029 | –             | –      | –                 | –     |
| Smoker in household: yes                        | –        | –     | 0.113         | 0.230  | 0.214             | 0.021 |
| Current bedroom floor covering: carpet          | –        | –     | 0.082         | 0.385  | 0.087             | 0.350 |
| Current pillow type: cotton                     | –        | –     | -0.147        | 0.112  | –                 | –     |
| Current bedding type: feather                   | 0.207    | 0.023 | 0.174         | 0.059  | –                 | –     |
| Bedding last changed: $\leq 7$ days             | 0.249    | 0.007 | –             | –      | –                 | –     |
| Type of bedroom cleaning: vacuum cleaning       | –        | –     | -0.211        | 0.023  | -0.174            | 0.064 |
| <i>Endotoxin load</i>                           |          |       |               |        |                   |       |
| Household size: <50 m <sup>2</sup>              | –        | –     | -0.048        | 0.646  | –                 | –     |
| Lived in the home: $\geq 8$ years               | –        | –     | 0.161         | 0.071  | 0.267             | 0.003 |
| Smoker in household: yes                        | –        | –     | 0.097         | 0.290  | 0.069             | 0.454 |
| Current bedroom floor covering: carpet          | –        | –     | 0.101         | 0.262  | 0.008             | 0.928 |
| Current bedding type: feather                   | 0.194    | 0.038 | 0.151         | 0.083  | –                 | –     |
| Bedroom floor last cleaned: $\leq 2$ days       | –        | –     | -0.358        | <0.001 | -0.220            | 0.013 |
| Living room floor last cleaned: $\leq 3$ days   | –        | –     | –             | –      | -0.020            | 0.841 |
| Bedroom floor cleaning: $\leq 5$ times per week | –        | –     | -0.159        | 0.075  | –                 | –     |
| Bedding changed: $\leq 2$ times per month       | –        | –     | 0.169         | 0.061  | –                 | –     |
| Type of bedroom cleaning: vacuum cleaning       | –        | –     | -0.237        | 0.007  | -0.219            | 0.014 |
| <i>Der p 1 level</i>                            |          |       |               |        |                   |       |
| Current bedroom floor covering: carpet          | -0.164   | 0.069 | –             | –      | –                 | –     |
| Bedding last changed: $\leq 7$ days             | -0.228   | 0.013 | -0.215        | 0.022  | -0.221            | 0.017 |
| Bedroom floor cleaning: $\leq 5$ times per week | 0.237    | 0.010 | –             | –      | –                 | –     |
| Living room cleaning: $\leq 5$ times per week   | -0.066   | 0.715 | –             | –      | –                 | –     |
| Bedding change: $\leq 2$ times per month        | –        | –     | -0.150        | 0.115  | –                 | –     |

<sup>a</sup> Respective exposure variables were log-transformed before regression analyses.

associated with lower Der p 1 levels at all locations ( $p < 0.05$  for all).

#### *Asthma Status and Indoor Endotoxin and Der p 1 Levels*

Table 4 describes the relationship between allergy symptoms and mattress endotoxin exposure. Mattress dust endotoxin level was associated with the total number of wheezing episodes in the preceding year ( $p = 0.044$  for trend). Endotoxin on bedroom and living room floors was not associated with asthma or rhinitis symptoms. Among our HDM-sensitized asthmatic children, high Der p 1 exposure of  $\geq -10 \mu\text{g/g}$  at any indoor location did not show any significant relationship with asthma or rhinitis symptoms ( $p > 0.2$ ). In addition, endotoxin or Der p 1 at all three locations did not show a significant correlation with patients' FeNO or spirometric parameters (data not shown). In the subgroup of 90 children with HDM

sensitization, these objective variables did not differ between those with and without high Der p 1 exposure on mattresses ( $p > 0.25$ ).

#### **Discussion**

This is the first study on indoor endotoxin exposure among Asian children with asthma. Endotoxin was detectable in all families with asthmatic children, whereas Der p 1 was measurable in 58–70% of their indoor sites. Endotoxin load showed a significant inverse correlation with Der p 1 levels on mattresses. The presence of smokers in the household, feather bedding and vacuum cleaning were independent factors for indoor endotoxin. Timing of the last bedding change affected Der p 1 levels at all three sites. In our asthmatic children, mattress endotoxin levels were associated with the frequency of wheez-

**Table 4.** Relationship between asthma or rhinitis symptoms<sup>a</sup> and endotoxin exposures in mattress dust samples

|                                                  | Endotoxin       |                      |                         |                      |
|--------------------------------------------------|-----------------|----------------------|-------------------------|----------------------|
|                                                  | level, EU/mg    | p value <sup>b</sup> | load, EU/m <sup>2</sup> | p value <sup>b</sup> |
| Wheezing in past 12 months                       |                 |                      |                         |                      |
| Yes                                              | 12.9 (6.6–22.2) | 0.743                | 482 (287–892)           | 0.805                |
| No                                               | 12.1 (6.3–16.6) |                      | 482 (273–1013)          |                      |
| Number of wheezing attacks                       |                 |                      |                         |                      |
| None                                             | 1.1 (0.9–3.7)   | 0.044                | 281 (191–447)           | 0.551                |
| 1–3 attacks                                      | 11.3 (5.8–23.5) |                      | 471 (286–892)           |                      |
| 4–12 attacks                                     | 13.4 (8.0–18.0) |                      | 453 (221–830)           |                      |
| >12 attacks                                      | 12.3 (6.2–28.8) |                      | 511 (390–5105)          |                      |
| Sleep disturbance due to wheeze                  |                 |                      |                         |                      |
| Never woken with wheezing                        | 12.3 (6.4–24.4) | 0.700                | 400 (251–729)           | 0.831                |
| <1 night per week                                | 12.3 (6.2–18.0) |                      | 545 (331–1118)          |                      |
| ≥1 night per week                                | 12.3 (3.6–32.8) |                      | 460 (247–2440)          |                      |
| Speech limitation due to wheeze                  |                 |                      |                         |                      |
| Yes                                              | 3.2 (2.0–9.3)   | 0.061                | 331 (217–349)           | 0.206                |
| No                                               | 12.5 (6.5–20.7) |                      | 515 (280–835)           |                      |
| Exercise limitation due to wheeze                |                 |                      |                         |                      |
| Never or occasionally                            | 13.0 (5.7–19.2) | 0.593                | 475 (340–809)           | 0.459                |
| ≥Once per week                                   | 7.7 (2.6–14.3)  |                      | 218 (187–630)           |                      |
| Dry cough at night without common cold           |                 |                      |                         |                      |
| Yes                                              | 11.5 (5.4–16.4) | 0.342                | 535 (279–841)           | 0.937                |
| No                                               | 13.1 (8.7–21.3) |                      | 467 (262–1118)          |                      |
| Nose problem that interfered with daily activity |                 |                      |                         |                      |
| Not at all                                       | 12.7 (5.2–17.9) | 0.338                | 444 (190–1412)          | 0.584                |
| A little                                         | 11.9 (6.5–20.4) |                      | 533 (283–929)           |                      |
| Moderate amount                                  | 14.6 (3.9–19.2) |                      | 454 (294–806)           |                      |
| A lot                                            | 8.7 (2.4–10.6)  |                      | 224 (204–608)           |                      |

Results expressed as median with interquartile range in parentheses.

<sup>a</sup> Within preceding 12 months as recorded by the ISAAC core questionnaire.

<sup>b</sup> p for trend as analyzed by ANOVA on log-transformed endotoxin levels.

ing episodes in the past year. Neither endotoxin nor Der p 1 correlated with patients' FeNO and spirometric parameters.

Endotoxins are ubiquitously present in house dust and exert dual effects on childhood asthma and allergies. A number of studies suggested that exposure to endotoxin early in life could be protective for the development of atopic diseases [1, 26]. Increased indoor endotoxin concentrations were found in homes of farming families and in households where children had regular contact with farm animals when compared with homes of nonfarming children [1]. Endotoxin levels in house dust correlated with the presence of interferon- $\gamma$ -producing T lymphocytes in children at risk of having allergies [27]. On the other hand, endotoxin possesses strong proinflammatory properties that enhance airway inflammation. Many

studies reported house dust endotoxin levels to be associated with more severe symptoms and higher medication use in patients with asthma [2–4, 25]. Dales et al. [28] measured 5-day averaged air endotoxin in the homes of 332 Canadian children, and found that airborne endotoxin was a risk factor for respiratory illnesses during the first 2 years of life. Our findings support this hypothesis by showing that mattress endotoxin levels were higher in patients with more frequent wheezing episodes in the preceding year.

Sun et al. [24] conducted a mite indoor environment study on 70 asthmatic adults in Hong Kong. They measured Der p 1 and Blo t 5 levels in house dust samples from beds and floors, and found that Der p 1 levels were higher in bed than on the floor, whereas Blo t 5 levels were similar on these sites. High Der p 1 and Blo t 5 exposures,

exceeding 10 µg/g dust, were present in 7.2 and 17.6% of beds and 0 and 8.6% of the floors, respectively. Consistent with these findings, the present study showed that high Der p 1 exposure was detected in 7.8% of mattresses and 0.9% of bedroom or living room floors in families with asthmatic children. Furthermore, the mean mattress Der p 1 levels in our subjects (2.6 µg/g) was similar to that of the above study (3.4 µg/g) but lower than that found in another local study of asthmatic adults (8.8 µg/g) [29]. Domestic Der p 1 exposures on beds or floors were not associated with any asthma symptoms or objective variables in our children, even among those sensitized to HDM. These observations challenge the roles that HDM play in modulating asthma severity in asthmatic children.

Apart from the domestic environment, a number of studies found high levels of HDM, pet and cockroach allergens in schools and public buildings [30–33]. For example, Rullo et al. [32] reported that day care centers in Brazil were also an important source of HDM and cockroach exposures. Group 1 mite allergens >2 µg/g were found in 67% of day care and preschool samples and in 8.9% of kindergarten samples. In contrast, Bla g 1 was higher on elementary school floors than on day care center and preschool floors. Levels of Fel d 1 and Can f 1 were low in most samples. A number of studies found endotoxin exposures to be significant in day care centers and schools [32–34]. As dust samples were not collected from our patients' schools, this study might not accurately assess the harmful effects of environmental endotoxin and HDM exposures on their asthma status. Future studies that evaluate the respiratory effects of environmental endotoxin and allergens should collect and analyze settled dust samples from subjects' domestic and school environments. Technological advance also allows us to quantify the personal exposures to airborne aeroallergens [35].

Feather bedding was associated with higher endotoxin exposures from mattresses and bedroom floors in our asthmatic children, but this bedding material did not alter Der p 1 levels from these sites. Hall et al. [36] first studied endotoxin levels in dust samples collected from 8 pairs of feather and synthetic pillows. They could not find any significant difference in endotoxin levels from these pillows, but the small sample size precluded any definite conclusion. In contrast, Nam et al. [37] found 10-fold higher levels of endotoxin but similar HDM levels in buckwheat pillows, commonly used in Korea, when compared with synthetic pillows. It is postulated that the organic materials in feather may allow bacteria to grow bet-

ter, thus resulting in higher endotoxin content. Epidemiologically, early life exposure to feather quilts also protected against asthma development in a Norwegian infant cohort [38]. Larger prospective studies are needed to delineate the relationship between feather bedding, endotoxin exposure and the risk of developing asthma and atopy.

A major limitation of this study relates to the measurement of indoor aeroallergen only for *D. pteronyssinus* but not other mite species from the Pyroglyphidae family. *Euroglyphus maynei* and *D. microseras* are relevant aeroallergens in selected parts of the world [39], whereas the storage mite *Blomia tropicalis* is ubiquitous and important in semi-tropical and tropical countries [39, 40]. Hong Kong's climate is different from these tropical countries, and the sensitization rates of local patients with asthma and allergies to *B. tropicalis* are currently unknown. Nonetheless, our previous study showed that *D. pteronyssinus* sensitization accounted for atopy in virtually all patients with allergies. Thus, we believe that the measurement of *D. pteronyssinus* should provide a representative estimate on the HDM burden in our studied population. Our findings may also be weakened by the difference in the timing of indoor dust collection and assessment of asthma outcomes. While the deleterious effects of endotoxin and Der p 1 lasted for days to weeks, this study captured patients' asthma symptoms over a more prolonged period of 12 months. Thus, it might be difficult to detect any association between indoor exposures and asthma and allergy severity. Because of the cross-sectional nature of this study, we did not record the details of inhaled corticosteroid treatment in our patients over the preceding year. This problem precludes us from adjusting for this important confounder when we analyzed the relationship between indoor exposures and asthma severity over a 12-month period according to the ISAAC.

In addition to endotoxin, the severity of childhood asthma may be modulated by indoor exposures to other microbial products in moulds or Gram-positive bacteria (e.g. β-glucan, muramic acid) [41, 42]. To complicate this issue, Nathan et al. [43] found that innate immune responses of airway epithelium to HDM were mediated through β-glucan-dependent pathways. Therefore, our study was not able to characterize the exact relationship between indoor microbial exposures and childhood asthma. Future studies need to include the measurement of these newer indoor microbial factors. Another limitation relates to our method of laboratory endotoxin detection. The limulus amoebocyte lysate method can only detect intact lipopolysaccharide, whereas the newer cytokine

induction assay using a monocytic cell line is able to detect both intact and small lipopolysaccharide (<5 kDa) as well as peptidoglycan and short bacterial DNA fragments [44].

In conclusion, domestic endotoxin but not Der p 1 exposure is associated with the frequency of wheezing episodes in Hong Kong children with asthma. On the other hand, neither endotoxin nor Der p 1 exposures are associated with objective parameters of FeNO or spirometry. Indoor exposures to endotoxin and Der p 1 are influ-

enced by a number of household characteristics such as nature of bedding materials and cleaning method and frequency.

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**Table S1.** Relationship between household characteristics and practices and endotoxin levels in dust samples collected from different indoor sites

|                                             | n   | Mattress endotoxin      |                           | Bedroom floor endotoxin  |                           | Living room floor endotoxin |                           |
|---------------------------------------------|-----|-------------------------|---------------------------|--------------------------|---------------------------|-----------------------------|---------------------------|
|                                             |     | Level (EU/mg)           | Load (EU/m <sup>2</sup> ) | Level (EU/mg)            | Load (EU/m <sup>2</sup> ) | Level (EU/mg)               | Load (EU/m <sup>2</sup> ) |
| Household size: < 50 m <sup>2</sup>         | 50  | <b>11.5 (4.7-15.9)*</b> | 541 (271-841)             | 23.9 (13.4-48.5)         | <b>604 (324-1622)*</b>    | 24.6 (16.0-45.8)            | 270 (171-1123)            |
| ≥ 50 m <sup>2</sup>                         | 62  | <b>13.3 (7.2-24.1)</b>  | 459 (303-1015)            | 23.4 (10.8-39.4)         | <b>569 (158-933)</b>      | 21.1 (13.7-29.2)            | 234 (167-470)             |
| Lived in the home: ≥ 8 yrs                  | 60  | 13.4 (6.4-22.8)         | 545 (226-1013)            | 22.5 (11.8-41.9)         | <b>446 (158-1006)*</b>    | 20.8 (14.2-29.5)            | <b>212 (167-346)†</b>     |
| < 8 yrs                                     | 55  | 11.5 (6.4-16.4)         | 490 (313-898)             | 26.5 (13.8-43.1)         | <b>700 (340-1582)</b>     | 25.4 (15.9-48.1)            | <b>313 (197-1133)</b>     |
| Number in household: ≥ 5 persons            | 34  | 12.6 (6.0-27.1)         | 431 (266-1230)            | 20.5 (9.8-34.1)          | 501 (354-1267)            | 24.4 (13.5-30.2)            | 236 (180-561)             |
| < 5 persons                                 | 81  | 12.3 (6.5-17.6)         | 545 (319-824)             | 27.4 (12.5-48.8)         | 689 (227-1501)            | 21.4 (14.8-43.0)            | 251 (169-871)             |
| Current cat/dog keeping: Yes                | 10  | 12.7 (6.3-18.8)         | 483 (288-881)             | 22.4 (12.2-42.6)         | 597 (269-1317)            | 21.6 (14.7-373)             | 248 (171-599)             |
| No                                          | 105 | 10.6 (8.1-22.9)         | 622 (232-1737)            | 31.5 (15.5-58.8)         | 501 (132-1594)            | 30.2 (14.8-41.6)            | 236 (136-887)             |
| Smoker in household: Yes                    | 38  | 13.4 (8.1-20.1)         | 595 (376-1341)            | <b>27.7 (11.6-46.1)*</b> | <b>805 (254-2323)*</b>    | <b>27.5 (18.7-46.3)*</b>    | <b>249 (188-1280)*</b>    |
| No                                          | 77  | 11.4 (6.4-18.0)         | 453 (231-829)             | <b>22.1 (12.9-41.7)</b>  | <b>517 (235-981)</b>      | <b>20.1 (13.3-29.6)</b>     | <b>248 (167-465)</b>      |
| Current heating fuel: Electricity           | 55  | 13.4 (8.0-26.7)         | 533 (294-1181)            | 22.5 (13.9-36.6)         | 597 (204-1260)            | 20.8 (13.8-29.0)            | 233 (165-592)             |
| Others                                      | 60  | 12.0 (6.3-15.8)         | 475 (275-825)             | 24.9 (11.2-49.9)         | 514 (275-1614)            | 25.1 (15.5-43.4)            | 259 (179-778)             |
| Current damp spots or visible moulds: Yes   | 58  | 11.3 (5.2-20.9)         | 521 (276-1027)            | 21.6 (11.6-39.4)         | 555 (203-1286)            | 22.3 (15.9-45.9)            | 265 (199-980)             |
| No                                          | 57  | 13.1 (9.0-18.0)         | 456 (290-838)             | 27.4 (15.4-45.4)         | 573 (294-1590)            | 21.3 (13.7-35.0)            | 227 (144-483)             |
| Current bedroom floor covering: Carpet      | 4   | 12.1 (3.0-27.4)         | 421 (169-928)             | <b>9.2 (8.5-26.4)*</b>   | <b>241 (158-621)‡</b>     | <b>14.2 (9.2-19.2)§</b>     | <b>202 (171-304)§</b>     |
| Others                                      | 111 | 12.4 (6.7-18.0)         | 497 (294-916)             | <b>25.6 (13.4-43.2)</b>  | <b>597 (265-1389)</b>     | <b>23.0 (15.1-39.5)</b>     | <b>251 (171-666)</b>      |
| Current pillow type: Cotton                 | 15  | 11.0 (5.4-20.6)         | 690 (374-840)             | <b>30.1 (21.6-51.6)*</b> | 696 (414-3029)            | 28.3 (20.1-46.4)            | 489 (144-1666)            |
| Others                                      | 100 | 12.6 (6.7-19.2)         | 475 (269-989)             | <b>21.8 (10.7-41.7)</b>  | 515 (221-1285)            | 21.4 (14.3-34.2)            | 234 (171-534)             |
| Current bedding type: Feather               | 20  | <b>13.6 (8.9-27.3)*</b> | <b>749 (376-1376)*</b>    | <b>31.0 (19.5-67.8)*</b> | <b>793 (527-1377)†</b>    | 27.3 (22.1-53.3)            | 408 (249-901)             |
| Others                                      | 95  | <b>12.4 (5.5-17.3)</b>  | <b>456 (267-840)</b>      | <b>21.6 (10.3-41.9)</b>  | <b>494 (166-1286)</b>     | 21.4 (14.2-35.0)            | 227 (171-499)             |
| Duration open windows or doors: ≤ 3 hrs/day | 46  | 13.4 (9.4-20.1)         | 651 (346-967)             | 20.2 (10.3-34.2)         | 596 (156-1577)            | 18.4 (14.5-29.3)            | 222 (167-511)             |
| > 3 hrs/day                                 | 69  | 11.0 (5.5-18.0)         | 456 (270-929)             | 27.4 (15.3-47.4)         | 566 (322-1274)            | 25.1 (14.8-41.6)            | 262 (179-1013)            |
| Bedroom floor last cleaned: ≤ 2 days        | 49  | 12.6 (6.9-17.3)         | 386 (249-811)             | 21.6 (9.4-45.5)          | <b>431 (156-694)*</b>     | 20.6 (14.7-33.3)            | <b>233 (150-433)*</b>     |
| > 2 days                                    | 66  | 12.4 (6.3-19.8)         | 662 (345-1141)            | 29.4 (13.8-42.7)         | <b>795 (411-1619)</b>     | 22.3 (15.3-40.2)            | <b>268 (192-1133)</b>     |
| Living room floor last cleaned:             | 93  | 12.4 (6.7-20.1)         | 462 (288-1016)            | 24.2 (11.9-44.3)         | 508 (210-1287)            | 22.2 (14.7-39.5)            | <b>248 (169-576)*</b>     |

|                           |                 |    |                         |                |                          |                        |                          |                       |
|---------------------------|-----------------|----|-------------------------|----------------|--------------------------|------------------------|--------------------------|-----------------------|
|                           | ≤ 3 days        |    |                         |                |                          |                        |                          |                       |
|                           | > 3 days        | 22 | 12.5 (5.8-16.8)         | 670 (213-831)  | 25.5 (14.2-42.1)         | 918 (482-1809)         | 22.6 (16.2-35.5)         | <b>248 (204-782)</b>  |
| Bedding last changed:     | ≤ 7 days        | 41 | <b>14.6 (9.9-26.9)*</b> | 456 (350-821)  | 22.1 (12.2-45.7)         | 469 (145-1121)         | 23.0 (14.8-39.6)         | 233 (161-569)         |
|                           | > 7 days        | 74 | <b>11.0 (5.3-17.9)</b>  | 539 (266-1141) | 27.8 (12.3-42.6)         | 689 (388-1488)         | 21.4 (14.5-36.2)         | 258 (176-703)         |
| Bedroom floor cleaning:   | ≤ 5 times/week  | 60 | 11.7 (6.3-15.4)         | 535 (335-826)  | 22.3 (12.9-40.6)         | <b>691 (341-1553)*</b> | 21.9 (14.7-39.5)         | 281 (203-901)         |
|                           | > 5 times/week  | 53 | 13.1 (6.7-25.9)         | 414 (270-968)  | 24.2 (10.6-46.7)         | <b>463 (158-919)</b>   | 21.4 (14.7-30.9)         | 212 (163-435)         |
| Living room cleaning:     | ≤ 5 times/week  | 55 | 11.6 (6.3-15.3)         | 497 (226-830)  | 20.3 (12.4-34.7)         | 689 (325-1389)         | 21.4 (14.6-35.1)         | 275 (195-606)         |
|                           | > 5 times/week  | 59 | 13.4 (6.7-25.0)         | 453 (281-1020) | 26.8 (11.8-50.1)         | 508 (162-1289)         | 23.8 (15.4-42.3)         | 217 (167-546)         |
| Bedding change:           | ≤ 2 times/month | 64 | 12.4 (5.5-17.8)         | 556 (313-1046) | 27.1 (14.1-42.7)         | <b>744 (415-1596)‡</b> | 21.9 (14.7-36.3)         | 267 (187-675)         |
|                           | > 2 times/month | 49 | 12.6 (7.9-23.6)         | 426 (254-881)  | 21.6 (9.9-45.7)          | <b>414 (152-819)</b>   | 22.4 (14.5-39.6)         | 227 (164-517)         |
| Type of bedroom cleaning: | Vacuum cleaning | 54 | 12.5 (6.3-20.0)         | 393 (212-749)  | <b>19.6 (10.0-36.3)*</b> | <b>479 (152-805)*</b>  | <b>19.5 (11.2-26.9)*</b> | <b>227 (154-440)*</b> |
|                           | Others          | 61 | 12.3 (6.9-18.8)         | 690 (351-1252) | <b>28.8 (16.4-46.8)</b>  | <b>707 (409-1636)</b>  | <b>26.6 (15.9-43.0)</b>  | <b>275 (200-1138)</b> |
| Type of housing:          | Public          | 52 | 12.0 (5.3-17.8)         | 517 (235-844)  | 27.7 (10.5-47.5)         | 494 (259-1377)         | 22.9 (15.2-41.6)         | 243 (171-631)         |
|                           | Private         | 63 | 12.6 (8.0-21.3)         | 483 (307-1013) | 22.5 (13.6-40.9)         | 680 (260-1286)         | 22.2 (13.8-30.4)         | 248 (171-606)         |

Statistically significant results were highlighted in bold.

Results on log-transformed endotoxin variables were analyzed by Student *t* test; \*  $p < 0.05$ ; †  $p < 0.01$ ; ‡  $p < 0.005$ ; §  $p < 0.001$ .

**Table S2.** Relationship between household characteristics and practices and Der p 1 levels at the three domestic sites

| Factor                                      | n   | Der p 1 (µg/g)           |                          |                          |
|---------------------------------------------|-----|--------------------------|--------------------------|--------------------------|
|                                             |     | Mattress                 | Bedroom floor            | Living room floor        |
| Household size: < 50 m <sup>2</sup>         | 50  | 1.33 (0.27-3.20)         | 0.40 (0.27-0.91)         | 0.35 (0.19-0.71)         |
| ≥ 50 m <sup>2</sup>                         | 62  | 0.57 (0.19-2.39)         | 0.38 (0.24-0.98)         | 0.26 (0.19-0.54)         |
| Lived in the home: ≥ 8 yrs                  | 60  | 0.61 (0.24-2.12)         | 0.37 (0.19-0.91)         | 0.29 (0.19-0.56)         |
| < 8 yrs                                     | 55  | 0.63 (0.22-2.60)         | 0.40 (0.30-0.93)         | 0.33 (0.19-0.58)         |
| Number in household: ≥ 5 persons            | 34  | 1.57 (0.29-3.46)         | 0.64 (0.24-1.49)         | 0.40 (0.22-0.70)         |
| < 5 persons                                 | 81  | 0.49 (0.20-1.91)         | 0.37 (0.24-0.69)         | 0.26 (0.16-0.55)         |
| Current cat/dog keeping: Yes                | 10  | 1.11 (0.22-5.32)         | 0.76 (0.44-0.99)         | 0.27 (0.17-0.44)         |
| No                                          | 105 | 0.61 (0.24-2.46)         | 0.38 (0.24-0.90)         | 0.32 (0.19-0.58)         |
| Smoker in household: Yes                    | 38  | 0.78 (0.22-2.86)         | 0.40 (0.23-0.92)         | 0.24 (0.15-0.55)         |
| No                                          | 77  | 0.59 (0.24-2.33)         | 0.39 (0.26-0.91)         | 0.33 (0.19-0.61)         |
| Current heating fuel: Electricity           | 55  | 0.92 (0.25-2.51)         | 0.44 (0.29-1.05)         | 0.32 (0.20-0.55)         |
| Others                                      | 60  | 0.46 (0.23-2.43)         | 0.36 (0.21-0.78)         | 0.30 (0.16-0.66)         |
| Current damp spots or visible moulds: Yes   | 58  | 0.61 (0.16-2.34)         | 0.37 (0.20-0.73)         | 0.23 (0.15-0.54)         |
| No                                          | 57  | 0.63 (0.25-2.48)         | 0.49 (0.26-1.17)         | 0.35 (0.22-0.63)         |
| Current bedroom floor covering: Carpet      | 4   | <b>0.19 (0.13-0.58)*</b> | 0.39 (0.15-0.40)         | 0.14 (0.14-2.81)         |
| Others                                      | 111 | <b>0.76 (0.24-2.51)</b>  | 0.39 (0.25-0.93)         | 0.32 (0.19-0.57)         |
| Current pillow type: Cotton                 | 15  | 1.44 (0.31-2.12)         | 0.52 (0.29-1.40)         | 0.27 (0.14-0.81)         |
| Others                                      | 100 | 0.53 (0.24-2.50)         | 0.39 (0.24-0.91)         | 0.32 (0.19-0.55)         |
| Current bedding type: Feather               | 20  | 1.35 (0.25-2.28)         | 0.58 (0.28-1.14)         | 0.38 (0.22-0.61)         |
| Others                                      | 95  | 0.56 (0.22-2.51)         | 0.37 (0.23-0.91)         | 0.27 (0.17-0.57)         |
| Duration open windows or doors: ≤ 3 hrs/day | 46  | 0.92 (0.25-3.10)         | 0.36 (0.20-0.81)         | 0.23 (0.17-0.38)         |
| > 3 hrs/day                                 | 69  | 0.59 (0.23-2.13)         | 0.47 (0.28-1.07)         | 0.37 (0.19-0.66)         |
| Bedroom floor last cleaned: ≤ 2 days        | 49  | 0.46 (0.24-1.62)         | 0.37 (0.20-0.91)         | 0.32 (0.19-0.52)         |
| > 2 days                                    | 66  | 1.38 (0.20-2.66)         | 0.40 (0.27-0.97)         | 0.29 (0.16-0.63)         |
| Living room floor last cleaned: ≤ 3 days    | 93  | 0.59 (0.24-2.78)         | 0.39 (0.24-0.91)         | 0.30 (0.19-0.63)         |
| > 3 days                                    | 22  | 0.77 (0.25-2.00)         | 0.44 (0.24-1.23)         | 0.33 (0.20-0.42)         |
| Bedding last changed: ≤ 7 days              | 41  | <b>0.35 (0.17-0.76)†</b> | <b>0.34 (0.18-0.64)*</b> | <b>0.25 (0.15-0.42)*</b> |
| > 7 days                                    | 74  | <b>1.39 (0.26-3.35)</b>  | <b>0.42 (0.29-1.23)</b>  | <b>0.34 (0.21-0.73)</b>  |
| Bedroom floor cleaning: ≤ 5 times/week      | 60  | <b>1.42 (0.27-3.69)*</b> | 0.43 (0.28-1.10)         | 0.32 (0.21-0.52)         |
| > 5 times/week                              | 53  | <b>0.36 (0.16-1.59)</b>  | 0.36 (0.24-0.91)         | 0.32 (0.15-0.61)         |

|                                           |    |                          |                          |                  |
|-------------------------------------------|----|--------------------------|--------------------------|------------------|
| Living room cleaning: ≤ 5 times/week      | 55 | <b>1.28 (0.27-3.29)*</b> | 0.39 (0.25-0.91)         | 0.31 (0.22-0.48) |
| > 5 times/week                            | 59 | <b>0.36 (0.16-1.71)</b>  | 0.40 (0.25-0.93)         | 0.32 (0.15-0.65) |
| Bedding change: ≤ 2 times/month           | 64 | 1.40 (0.25-3.17)         | <b>0.44 (0.27-1.25)*</b> | 0.33 (0.20-0.63) |
| > 2 times/month                           | 49 | 0.39 (0.20-1.40)         | <b>0.37 (0.20-0.66)</b>  | 0.28 (0.17-0.54) |
| Type of bedroom cleaning: Vacuum cleaning | 54 | 0.49 (0.21-1.63)         | 0.40 (0.26-0.82)         | 0.32 (0.18-0.59) |
| Others                                    | 61 | 1.27 (0.24-3.46)         | 0.39 (0.20-1.01)         | 0.28 (0.19-0.57) |
| Type of housing: Public                   | 52 | 1.32 (0.26-2.99)         | 0.42 (0.25-0.96)         | 0.28 (0.18-0.68) |
| Private                                   | 63 | 0.50 (0.17-2.41)         | 0.37 (0.21-0.91)         | 0.33 (0.20-0.55) |

Statistically significant results were highlighted in bold.

Results on log-transformed allergen levels were analyzed by Student *t* test; \*  $p < 0.05$ ; †  $p < 0.01$ ; ‡  $p < 0.005$ ; §  $p < 0.001$ .