

Association of House Dust Allergen Concentrations With Residential Conditions in City and in Rural Houses

Aleksandra Wardzyńska, MD,¹ Barbara Majkowska-Wojciechowska, MSc, PhD,¹ Jolanta Pełka, MD,² Leszek Korzon, MD,³ Magdalena Kaczała, MD,¹ Marzanna Jarzębska, MSc,¹ Tomasz Gwardys, MD,¹ and Marek L. Kowalski, MD, PhD¹

Background: The aim of the study was to evaluate the relationship between house dust mite, cat and dog allergen levels with household characteristics in the houses of children living in urban and rural areas in central Poland.

Methods: Dust samples were collected from 141 urban and 191 rural houses. Der f1 + Der p1, Can f1, and Fel d1 levels were measured and associated with residential conditions and atopy-related health outcomes assessed by clinical examination and skin prick testing.

Results: Concentrations of mite allergens were lower, and cat and dog allergen levels were higher in urban houses. Fel d1 and Can f1 levels depended on the presence of a respective animal in the house. In urban houses, Der p1 + Der f1 concentration was lower in households with central heating, whereas Can f1 concentration was related to building age. Multivariate analyses revealed that the concentrations of house dust mite and dog allergens were associated with relative humidity, number of people in the household, and the presence of a dog at home. There was no significant association between allergen level and sensitization or atopic diseases.

Conclusions: Concentrations of indoor allergens in urban and rural houses differ significantly, and residential conditions associated with allergen levels seem to be different in both environments.

Key Words: allergen concentration, house dust mite, cat allergen, dog allergen, allergy, residential conditions

(*WAO Journal* 2012; 5:22–27)

An increase in the prevalence of allergic diseases such as atopic asthma and allergic rhinitis and conjunctivitis affect dwellers of urbanized areas more than those of rural ones. Studies conducted in various countries have suggested that growing up in a rural environment may lead to the development of fewer allergies.^{1–5} The rural environment seems to be favorable for the development of the immunological system, particularly at the stage of its maturation. Putative protective

factors associated with the rural environment include the presence of bacterial endotoxins,⁶ early contact with farm animals, and the consumption of nonprocessed food, including nonpasteurized milk.^{1,7}

The high prevalence of sensitization to house dust allergens in the urban environment has been reported in several countries,⁸ including Poland.⁵ In some studies, the exposure to house dust allergens was shown to be the relevant factor for the presence of mite-specific as well as cat- and dog-specific IgE in atopic individuals.^{9,10} Variations in the levels of allergens have been associated with worsening of symptoms and remission periods in the course of allergic diseases.^{11–14} However, there are few studies comparing house dust allergen concentration in rural and urban environments with well-defined residential conditions and a documented prevalence of allergic sensitization and symptoms in children.

The aim of our study was to evaluate the exposure to inhalant indoor allergens in 2 different communities and to determine the relationship between residential conditions and concentrations of allergens in houses of children living in urban and rural areas in central Poland.

METHODS

Participants

Out of 404 junior-school children (12–16 years old) recruited from schools situated in the center of Łódź (an industrial city in central Poland) and 2 schools from the rural community, characterized in a previous study,⁵ dust samples were taken from 141 urban and 191 rural houses.

The majority (n = 152) of rural children lived on traditional farms with farmyards, whereas 32 children lived in non-farm houses, and only 7 children lived in multifamily buildings. Most rural houses were built with bricks or hollow blocks. The houses of urban children were mainly apartment buildings built of brick (n = 72) and of concrete blocks (n = 45). The remaining children (n = 24) lived in single-family houses.

Questionnaires

The questionnaire administered to parents included questions related to residential characteristics and the presence of animals in their houses. The data on, among other things, humidity, temperature, numbers of domestic animals,

From the ¹Department of Immunology, Rheumatology and Allergy, Medical University of Lodz, Lodz, Poland; ²Allergy Center 'Medyk', Wielun, Poland; ³Allergy Center, Słupia, Poland.

This study was supported by National Research Committee grant No 3PO5D 050 22.

Correspondence to: Marek L. Kowalski, MD, PhD, Department of Immunology, Rheumatology and Allergy, Chair of Clinical Immunology and Microbiology, Medical University of Lodz, Pomorska 251, 92-213 Lodz, Poland. Telephone: 00 484 2675 7309. Fax: 00 484 2678 2292. E-mail: marek.kowalski@csk.umed.lodz.pl.

Copyright © 2012 by World Allergy Organization

and types of vacuum cleaner used in houses were collected or verified by a trained technician during the taking of dust samples.

Assessment of Allergic Status of Children

The methodology of clinical assessment and skin testing was described before.⁵ In brief, a panel of skin tests (Allergopharma, Reinbek, Germany) was performed, which included the following allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, rabbit, hamster, guinea pig, rat, swine, birch, grass mix, mugwort, plantain, *Alternaria tenuis*, and *Cladosporium herbarum*. A wheal of ≥ 3 mm in diameter was considered as a positive result.

The total level of IgE antibodies and specific IgE against *D. pteronyssinus* mite allergens were measured in the serum by the UniCap system (Pharmacia Company, Uppsala, Sweden). The diagnosis of allergic disease (or its lack) was made by an allergologist, based on clinical examination and the results of positive skin test (SPT).

Collection of Dust Samples and Measurement of Allergen Concentrations

Samples of dust were collected from November 2003 to April 2004 (apart from in January 2004). Samples collected at the end of November 2003, in December 2003, and in February 2004 were classified as winter samples, whereas samples collected at the end of March and in April 2004 were classified as spring samples.

In each house, 3 dust samples were collected by vacuum cleaner with a dust-collecting device ("Micron Magic" Hepa Filtration; Kirby Company, Cleveland, OH): 2 from a mattress and 1 from a carpet in the child's bedroom. The first vacuum cleaning of mattresses lasted for 2 minutes and second for 5 minutes. Because of the absence of differences in allergen concentrations between samples 1 and 2 collected from the mattress (data not shown), the data were averaged.

The carpet in the child's bedroom was vacuum cleaned for 2 minutes (in the case of no carpet being present, a sample was collected from an armchair and a sofa or other piece of upholstered furniture in the child's bedroom). Allergens were extracted from dust according to the immunoassay manufacturer manual. Briefly, the dust samples were taken and after collection, immediately frozen at -20°C . Dust was sieved and suspended in PBS-T (0.05% Tween-20 in phosphate-buffered saline). After this, the samples were resuspended using a vortex mixer and rocked for 2 hours at room temperature. After centrifugation, the supernatant was stored at -20°C . All dust samples were collected and then extracted by the same person. Concentrations of mite (Der p1 + Der f1), cat (Fel d1), and dog allergens (Can f1) in dust were assessed by enzyme-linked immunosorbent assay (INDOOR Biotechnologies, Charlottesville, VA.), and results were expressed in microgram per gram of dust ($\mu\text{g/g}$). Temperature and relative humidity measurement was taken only once during dust sample collection.

Ethics

Informed consent was obtained from parents and caregivers of all participants. The study was approved by the local ethics committee.

Statistical Analysis

The differences between allergen concentrations were analyzed by the Mann-Whitney *U* test. Allergen concentrations, humidity, and temperature were expressed as medians with 25th to 75th percentiles. Relations between temperature, relative humidity and allergen concentrations were analyzed by linear Pearson correlation. Odds ratios with 95% confidence intervals were calculated for allergen sensitization and allergic diseases. Before multivariate analysis, the allergen data was log transformed, and principal component analysis was performed to reduce the number of variables. After transformation, the concentrations of allergens were compared using independent samples *t* test. Multivariate logistic regression was used to determine the association between allergen concentrations and housing characteristics. Discriminant function analysis was used to determine which variables discriminate urban and rural environments. $P < 0.05$ was regarded as significant. Statistica 6.0 PL software (StatSoft, Inc., Tulsa, OK) was used for all statistical analyses.

RESULTS

Allergen Concentrations in Rural and Urban Houses

The concentration of mite allergens in samples collected from mattresses were higher [median (25–75 percentiles): 1.29 $\mu\text{g/g}$ (0.86–1.95) vs. 0.18 $\mu\text{g/g}$ (0.09–0.57); $P < 0.001$], as well as from carpets [1.41 $\mu\text{g/g}$ (0.85–2.19) vs. 0.13 $\mu\text{g/g}$ (0.07–0.41); $P < 0.001$] in the houses of children living in the country than in the city. The concentrations of dog allergens were lower in mattresses from rural houses than in those from urban houses [0.35 $\mu\text{g/g}$ (0.22–0.66) vs. 2.44 $\mu\text{g/g}$ (1.28–5.11) $P < 0.001$] and lower in carpets [0.27 $\mu\text{g/g}$ (0.19–0.46) vs. 2.35 $\mu\text{g/g}$ (0.81–4.15); $P < 0.001$]. The median level of cat allergens in mattresses from rural homes were significantly lower than in those from urban houses [0.43 $\mu\text{g/g}$ (0.28–0.64) vs. 0.61 $\mu\text{g/g}$ (0.27–0.84); $P = 0.002$], whereas in the sample collected from carpets, the median concentrations of cat allergens were higher in case of rural children [0.45 $\mu\text{g/g}$ (0.24–0.78) vs. 0.32 $\mu\text{g/g}$ (0.12–0.75); $P = 0.016$]. In mattresses in urban houses, the concentrations of mite, dog, and cat allergens were higher than in carpets (for all $P < 0.001$). In rural houses, only concentrations of dog allergens in samples from mattresses were higher than in samples from the carpet ($P < 0.001$).

Allergen Concentrations and Presence of Animals

In the houses of urban children, where animals were kept (cats were present in 12 houses, dogs in 57 houses), the concentrations of allergens were higher than the concentrations

of allergens in houses without a respective animal (Table 1). In rural houses, only the median concentration of cat allergens in the sample from mattresses in houses with a cat ($n = 23$) was higher than in houses without a cat ($n = 168$). In rural houses, there were no differences in the median level of allergens, regardless of having ($n = 13$) or not having ($n = 176$) a dog. The concentrations of mite allergens in both environments were not related to the presence of an animal (data not shown).

Influence of Temperature, Relative Humidity of the Air, and Seasons of Collecting Samples on Allergen Concentrations

At the time of dust sample collection, the temperature of the air in children's bedrooms in the country was lower 19°C [$18\text{--}20^{\circ}\text{C}$] vs. 20°C [$19\text{--}21^{\circ}\text{C}$]; $P < 0.001$), and relative humidity of the air in bedrooms was significantly higher (67% [$63\text{--}70\%$] vs. 48% [$42.5\text{--}55.5\%$]; $P < 0.001$) than those of the air in bedrooms in the city.

A negative correlation was found between the concentrations of dog allergens in mattresses in the country and the relative humidity of the air in the child's bedroom ($r = -0.17$; $P = 0.019$).

The concentrations of mite allergens in the sample from carpets in the urban houses decreased with increases in temperature ($r = -0.25$; $P = 0.015$), whereas in the houses of rural children, a weak positive correlation was observed between the dog allergen concentrations from mattresses and temperature ($r = 0.18$; $P = 0.017$).

Allergen Concentrations and Residential Conditions

For urban children living in old buildings ($n = 72$), the median concentrations of dog allergens were lower [mattresses: $1.99 \mu\text{g/g}$ ($0.87\text{--}3.91$) vs. $3.18 \mu\text{g/g}$ ($1.88\text{--}5.47$),

$P = 0.007$; carpets: $1.82 \mu\text{g/g}$ ($0.73\text{--}3.47$) vs. $2.87 \mu\text{g/g}$ ($1\text{--}4.51$), $P = 0.042$].

Urban houses with the central heating ($n = 84$) had lower concentrations of mite allergen in the mattresses and the carpet than houses heated in another way ($n = 42$) [mattresses: $0.14 \mu\text{g/g}$ ($0.09\text{--}0.34$) vs. $0.39 \mu\text{g/g}$ ($0.13\text{--}1.64$); $P < 0.003$, carpets: $0.1 \mu\text{g/g}$ ($0.07\text{--}0.2$) vs. $0.39 \mu\text{g/g}$ ($0.09\text{--}1.31$); $P = 0.003$].

Multivariate Analysis

Allergen concentrations were log transformed and then transformed using principal component analysis to reduce the number of variables. The first component positively correlated with Can f1 and negatively with Der p1 and Der f1, and the second principal component was positively associated with Fel d 1. Factor loadings of the first component clearly differentiate the variability of allergens between rural and urban areas ($t^0 = 18.99$; $df = 221$; $P < 0.0001$). Hence, a higher concentration of dog allergens and lower level of mite allergens were present in the city. An inverse relationship was observed in the rural houses. In addition, the concentration of allergens in samples taken from mattresses and carpets was significantly correlated (Fig. 1). The second component did not differ between urban and rural houses ($t^0 = 1.28$; $df = 221$; $P = 0.2$). Multivariate logistic regression was conducted to estimate the association between allergen concentrations in both environments and housing characteristics. In multivariate analysis, only relative humidity, number of people in the household, and the presence of a dog at home were associated with the first component (for all $P < 0.001$). The second component was not related to residential conditions.

The discriminant analysis was performed to select housing characteristics potentially associated with the concentrations of allergens, which differentiate urban and rural houses. The mean canonical variable was -1.12 for the rural area and 2.72 for the urban area, which shows a discrimination between rural and urban housing conditions. The most

TABLE 1. Concentration of Cat and Dog Allergens in Urban and Rural Houses With and Without Respective Domestic Animals

| Allergen | Concentration of Allergen in House With Respective Animal ($\mu\text{g/g}$) | Concentration of Allergen in House Without Respective Animal ($\mu\text{g/g}$) | <i>P</i> |
|---------------|---|--|----------|
| Urban | | | |
| Cat (Fel d 1) | | | |
| Mattresses | 0.8 (0.65–1.07) | 0.59 (0.26–0.83) | 0.04 |
| Carpets | 1.18 (0.69–1.47) | 0.3 (0.1–0.7) | <0.001 |
| Dog (Can f1) | | | |
| Mattresses | 5 (3.25–7.07) | 1.55 (0.85–2.44) | <0.001 |
| Carpets | 3.95 (2.72–5.25) | 1.01 (0.59–2.14) | <0.001 |
| Rural | | | |
| Cat (Fel d 1) | | | |
| Mattresses | 0.62 (0.42–0.77) | 0.42 (0.26–0.61) | 0.006 |
| Carpets | 0.43 (0.28–0.72) | 0.46 (0.22–0.78) | 0.94 |
| Dog (Can f1) | | | |
| Mattresses | 0.55 (0.17–2.47) | 0.35 (0.22–0.64) | 0.23 |
| Carpets | 0.37 (0.25–1.53) | 0.27 (0.19–0.45) | 0.10 |

Results are expressed as medians and 25th to 75th percentiles.

| Levels of allergens in samples | PC I | PC II |
|--------------------------------|--------|-------|
| Derp1 + Derf1 mattresses | -0.749 | 0.244 |
| Derp1 + Derf1 carpets | -0.819 | 0.110 |
| Feld 1 mattresses | 0.169 | 0.795 |
| Feld1 carpets | -0.105 | 0.775 |
| Canf1 mattresses | 0.775 | 0.316 |
| Canf1 carpets | 0.748 | 0.317 |

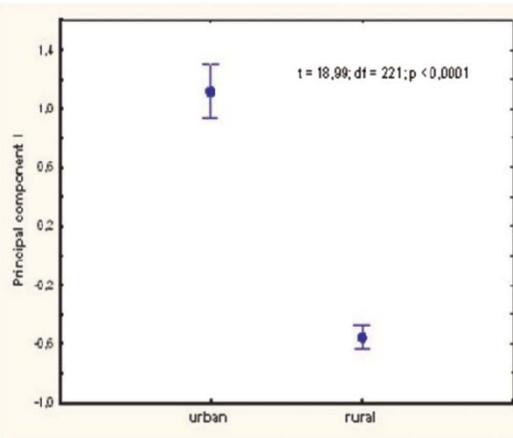


FIGURE 1. The table presents the factor loadings, which characterizes association of allergen concentrations with the first component. PC I, first principal component; PC II, second principal component. The figure beside shows comparison of the first component between urban and rural houses. Student *t* test was used for comparison; ranges indicate 95% confidence intervals.

significant factors for environmental discrimination were relative humidity, number of people in the household, the type of vacuum cleaner (with or without disposable dust bag), the presence of a dog at home, and the age of the buildings (Table 2) (for all *P* < 0.001).

Indoor Allergen Concentrations and Allergy in Children

The prevalence of sensitization to house mites, cat, and dog allergens and the prevalence of atopy (defined as sensitization to at least one of tested allergens) were significantly higher among urban than among rural children (Table 3). Living in a city was a significant risk factor for the presence of allergic diseases and allergic sensitization.

The concentrations of mite and dog allergens were similar in allergic and nonallergic children in both environments. However, cat allergen concentrations in mattresses and carpets in children with allergic rhinitis were significantly higher as compared with those of healthy children [mattresses: 0.71 µg/g (0.46–0.93) vs. 0.55 µg/g (0.19–0.8); *P* = 0.015, carpets: 0.46 µg/g (0.18–0.96) vs. 0.25 µg/g (0.09–0.69); *P* = 0.027]. The presence of SPTs to mites, cat, or dog allergens was not associated with concentrations of their respective allergens.

No correlation was observed between the level of specific IgE against Der p1 or total IgE in children and the concentrations of mite allergens in the investigated houses.

DISCUSSION

In our study, different concentrations of cat, dust mite, and dog allergens were found in rural and urban houses. All these levels were low in relation to the house dust allergen concentrations reported in other countries. This could be due to an unidentified technical error connected with sample collection or storage but might be related to the climatic conditions in Poland because similar concentrations of Der p, ¹⁵ and Der p1 and Der f1 ¹⁶ were found in 2 different studies conducted in Poland. The higher level of mite allergens in rural than in urban houses has been reported in studies from different regions of Europe: Sweden, ¹⁷ New Zealand, ⁷ and in the ALEX study conducted in Germany, Austria and Switzerland. ⁶ In our study, the most plausible explanation for the differences between mite allergen concentration in rural and urban houses seemed to be related to building characteristics. The majority of rural children lived in single-family detached houses, whereas the urban children in the study lived in multifamily buildings. The type of building seems to have a vital impact on indoor relative humidity, creating favorable conditions for the development of mites in single-family buildings. ¹⁸ Our observations are in line with other studies showing higher levels of mite allergens in single-family detached houses as compared with multifamily buildings. ^{19,20} In addition, other factors like age of the building, type of ventilation, age of mattresses or carpets, and floor coverings may contribute to an indoor microclimate that is favorable for the

TABLE 2. Summary of Discriminant Function Analysis Between Housing Characteristics in Urban and Rural Houses

| Housing Characteristics | Standardized Coefficient | Correlation Between Square Root and Housing Characteristics | <i>P</i> |
|-----------------------------------|--------------------------|---|----------|
| Relative humidity | -0.707 | -0.613 | <0.001 |
| Number of people in the household | -0.423 | -0.404 | <0.001 |
| Age of the building | -0.387 | -0.278 | <0.001 |
| Presence of dog in the house | 0.394 | 0.392 | <0.001 |
| Type of vacuum cleaner | 0.402 | 0.333 | <0.001 |

The negative value of standardized coefficient indicates the greater influence of housing condition in the rural area, whereas positive value indicates the greater influence of housing condition in urban area.

TABLE 3. Prevalence of Asthma, Rhinitis, and Allergic Sensitization in Urban and Rural Children

| | Urban, n = 141, n (%) | Rural, n = 191, n (%) | P | Odds Ratio (95% Confidence Interval) |
|--------------------------------------|-----------------------|-----------------------|--------|--------------------------------------|
| Asthma | 22 (15.6) | 3 (1.57) | <0.001 | 11.58 (3.75–39.75) |
| Allergic rhinitis and conjunctivitis | 55 (39) | 21 (10.99) | <0.001 | 5.18 (2.93–9.14) |
| SPT+ Der pl | 49 (34.75) | 29 (15.18) | <0.001 | 2.98 (1.76–5.04) |
| SPT+ Der fl | 49 (34.75) | 18 (9.42) | <0.001 | 5.11 (2.81–9.32) |
| SPT+ cat | 34 (24.11) | 7 (3.66) | <0.001 | 8.35 (3.57–19.56) |
| SPT+ dog | 27 (19.45) | 3 (1.57) | <0.001 | 14.84 (4.38–50.28) |

SPT+, positive skin prick test.

development and proliferation of mites.^{21–23} Interestingly, association of house dust mite allergen concentrations with indoor housing conditions were found only in urban but not in rural houses. This observation may result from less variability in residential conditions in rural area because most children lived in single-family houses and only few in multifamily buildings. Alternatively, it cannot exclude that other housing conditions not assessed in our study were associated with the variability of allergen concentrations in rural houses.

The most significant factor affecting cat and dog allergen levels in the house dust seems to be the presence of a pet in the house.⁹ In our study, room temperature, relative humidity, and seasons of the year have not been associated with pet allergen levels. Some studies suggest a potential influence of the periodical growth and loss of fur on the level of animal allergens in houses.^{9,24} The concentrations of cat and dog allergens in urban, but not rural, houses with an animal were higher than in case of children not having neither a cat nor a dog.^{25,26} This discrepancy between rural and urban houses could be explained by different habits of keeping these animals: in the country, animals are kept mostly outdoors, whereas the opposite situation was observed in the city. The fact that detectable levels of cat and dog allergens were found in both rural and urban houses without animals can be explained by an easy transmission of these allergens on clothes or other objects. Such way of transferring Can f1 and Fel d1 allergens is probably responsible for the presence of pet allergens in public transportation, cinemas, hotels, schools, and public institutions.^{27,28}

Although the essential role of exposure to indoor allergens in the process of allergic sensitization and development of allergy symptoms has been documented, its relationship with allergen concentration and timing of exposure is not well understood. In our study, there was no clear association between the current concentrations of allergens in house dust and the presence of allergic sensitization. However, only a single measurement of allergen concentration was performed at the time of clinical assessment, and it is not clear to what extent it may reflect exposure to allergens earlier in life, when the sensitization process was likely to occur. Furthermore, the presence of factors modulating the immune response in the environment (ie, endotoxins and β -glucans) seem to be equally important for the development of allergic sensitization and symptoms, questioning the predictive value of allergen concentration measurement.^{29,30}

To conclude, our study documented differences in concentrations of mite, cat, and dog animal allergens in the house dust in the homes of children from rural and urban environments. We also identified factors related to the indoor environment that have an influence on allergen levels and should be considered while preventive measures are taken.

ACKNOWLEDGMENTS

The authors thank the principals and teachers of participating schools and Lodz, Mierzyce, and Slupia for their assistance. They also thank allergologists Dr M. Czarnecka and Dr B. Kubicka–Kozik for their support and Dr Z. Wojciechowski for statistical analysis of data.

REFERENCES

- Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, et al. Early life exposure to farming environment is essential for protection against the development of asthma and allergy: a cross-sectional survey. *Lancet*. 2001;358:1129–1133.
- Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin Exp Allergy*. 2002;57:1094–1096.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy*. 2000;30:187–193.
- Sozanska B, Macneill SJ, Kajderowicz-Kowalik M, Danielewicz H, Wheatley M, et al. Atopy and asthma in rural Poland: a paradigm for the emergence of childhood respiratory allergies in Europe. *Allergy*. 2007;62:394–400.
- Majkowska-Wojciechowska B, Pełka J, Korzon L, Kozłowska A, Kaczała M, et al. Prevalence of allergy, patterns of allergic sensitization and allergy risk factors in rural and urban children. *Allergy*. 2007;62:1044–1050.
- Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med*. 2002;347:869–877.
- Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy*. 2002;57:1171–1179.
- Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. European Community Respiratory Health Survey I. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. *Allergy*. 2007;62:301–309.
- Parvaneh S, Kronqvist M, Johansson E, van Hage-Hamsten M. Exposure to an abundance of cat (Fel d 1) and dog (Can f 1) allergens in Swedish farming households. *Allergy*. 1999;54:229–234.
- Carlsten C, Dimich-Ward H, Becker AB, Ferguson A, Chan HW, DyBuncio A, Chan-Yeung M. Indoor allergen exposure, sensitization,

- and development of asthma in a high-risk birth cohort. *Pediatr Allergy Immunol.* 2010;21:740–746.
11. Sporik R, Squillace SP, Ingram JM, Rakes G, Honsinger RW, Platts-Mills TA. Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. *Thorax.* 1999;54:675–680.
 12. Lau S, Illi S, Platts-Mills TA, Riposo D, Nickel R, et al. Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood—report of the German Multicentre Allergy Study (MAS 90). *Allergy.* 2005;60:766–773.
 13. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med.* 1990;323:502–507.
 14. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of pediatric asthma severity with exposure to common household dust allergens. *Environ Res.* 2009;109:768–774.
 15. Kowalski ML, Majkowska-Wojciechowska B, Grzegorzczak J. Stężenie alergenów roztoczy kurzu domowego Der p I w mieszkaniach łódzkich. *Alergia Astma Immunol.* 1996;1:141–146.
 16. Jedrychowski W, Mauger U, Zembala M, Perzanowski MS, Hajto B, et al. Risk of wheezing associated with house dust mite allergen and indoor air quality among three year old children. Kraków inner city study. *Int J Occup Med Environ Health.* 2007;20:117–126.
 17. Radon K, Schottky A, Garz S, Koops F, Szadkowski D, et al. Distribution of dust-mite allergens (Lep d 2, Der p 1, Der f 1, Der 2) in pig-farming environments and sensitization of the respective farmers. *Allergy.* 2000;55:219–225.
 18. Wickman M, Nordvall SL, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. *Allergy Clin Immunol.* 1991;88:89–95.
 19. Chew GL, Higgins KM, Gold DR, Muilenberg ML, Burge HA. Monthly measurements of indoor allergens and the influence of housing type in a northeastern US city. *Allergy.* 1999;54:1058–1066.
 20. van Strien RT, Gehring U, Belanger K, Triche E, Gent J, Bracken MB, Leaderer BP. The influence of air conditioning, humidity, temperature and other household characteristics on mite allergen concentrations in the northeastern United States. *Allergy.* 2004;59:645–652.
 21. Mührshahi S, Marks G, Vanlaar C, Tovey E, Peat J. Predictors of high house dust mite allergen concentrations in residential homes in Sydney. *Allergy.* 2002;57:137–142.
 22. Chan-Yeung M, Becker A, Lam J, Dimich-Ward H, Ferguson A, et al. House dust mite allergen levels in two cities in Canada: effects of season, humidity, city and home characteristics. *Clin Exp Allergy.* 1995;25:240–246.
 23. Van Strien RT, Verhoeff AP, Brunekreef B, Van Wijnen JH. Mite antigen in house dust: relationship with different housing characteristics in the Netherlands. *Clin Exp Allergy.* 1994;24:843–853.
 24. Munir AK, Björkstén B, Einarsson R, Schou C, Ekstrand-Tobin A, Warner A, Kjellman NI. Cat (Fel d 1), dog (Can f 1), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. *Allergy.* 1994;49:508–516.
 25. Heinrich J, Bedada GB, Zock JP, et al. Cat allergen level: its determinants and relationship to specific IgE to cat across European centers. *Allergy Clin Immunol.* 2006;118:674–681.
 26. Fahlbusch B, Gehring U, Richter K, et al. INGA-Study Group. Indoor factors and Genetics in Asthma. Predictors of cat allergen (Fel d 1) in house dust of German homes with/without cats. *J Investig Allergol Clin Immunol.* 2002;12:12–20.
 27. Custovic A, Green R, Taggart SC, et al. Domestic allergens in public places. II: Dog (Can f1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings. *Clin Exp Allergy.* 1996;26:1246–1252.
 28. Custovic A, Fletcher A, Pickering CA, et al. Domestic allergens in public places III: house dust mite, cat, dog and cockroach allergens in British hospitals. *Clin Exp Allergy.* 1998;28:53–59.
 29. Chan-Yeung M, Manfreda J, Dimich-Ward H, et al. Mite and cat allergen levels in homes and severity of asthma. *Am J Respir Crit Care Med.* 1995;152:1805–1811.
 30. Schram-Bijkerk D, Doekes G, Boeve M, et al. Nonlinear relations between house dust mite allergen levels and mite sensitization in farm and nonfarm children. *Allergy.* 2006;61:640–647.