

Childhood asthma and exposure to indoor allergens: low mite levels are associated with sensitivity

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The prevalence and level of sensitivity to indoor allergens were studied in relation to current exposure at home in 124 children with perennial asthma living in three climatic zones of Sweden. The house dust mite (HDM) allergen levels were higher in the South than in the North ($p < 0.001$), while cat and dog allergen levels tended to be higher in the North than the South (n.s.). Thirty-four percent of the children were sensitive to the HDM *Der matophagoides pteronyssinus*, as determined by IgE antibodies in vitro, 27% were sensitive to *D. farinae*, 57% to cat and 55% to dog. Sensitivity to HDM was significantly more prevalent in Southern, than in Central and Northern Sweden ($p = 0.001$) where the children were more often sensitive to pets (cat $p = 0.005$, dog $p = 0.002$). A significant association between the concentration of *Der p* I and *Der f* I in the house dust and both the prevalence of sensitivity to HDM and the IgE antibody levels against mites was found even at concentrations well below the commonly suggested risk level for sensitisation of 2 $\mu\text{g/g}$ dust. No relationship was found between pet allergen concentration in the home dust and sensitivity to pets, possibly because of exposure outside home, e. g. in schools and meeting places for leisure activities. Similarly, there was no consistent association between the level of mite or pet allergen exposure at home and asthma severity as judged by symptom and medication score. The study indicates that there is no threshold value for sensitisation to mite allergens in asthmatic children, and therefore, dust allergen levels at home should be kept as low as possible in homes of children at risk for asthma.

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Up to 90% of asthmatic children and teenagers are sensitised to aeroallergens (1). The main indoor allergens are derived from house dust mites (HDM) and cat and dog dander, but the levels at which sensitisation takes place in predisposed individuals and the levels eliciting symptoms in those who are sensitive are not well established (2, 3). For HDM allergens, 2 $\mu\text{g/g}$ dust has been assumed a risk level for sensitisation against the two major allergens of house dust mites, *Der p* I and *Der f* I. The level for causing acute attacks of asthma was said to be 10 $\mu\text{g/g}$ (4, 5). These suggestions were, however, based on epidemiological studies performed only in certain climatic zones. The general validity of these levels is not known, nor is there sufficient information with regard to other allergens than house dust mites. In fact, Price et al. suggested a lower threshold (0.5 $\mu\text{g/g}$) for sensitisation based on results of a

retrospective study in infants (6). In Southern Germany (Freiburg), Kuehr et al. (7) found that mite allergen concentrations at home lower than 2 mg/g were associated with sensitisation to mites in subjects previously sensitised to other allergens. In contrast, for children who were not sensitised to aeroallergens at the beginning of the study, much higher mite allergen levels were associated with sensitisation. In a later study, the same authors found no relationship between the levels of mite allergens at home and respiratory symptoms of sensitised subjects (8). This raises the question whether there is a difference in early and late appearing sensitisation and whether a current exposure to HDM allergens is necessary to maintain sensitivity. Furthermore, the validity of the suggested threshold values for allergens from cats and dogs needs to be assessed (1, 3).

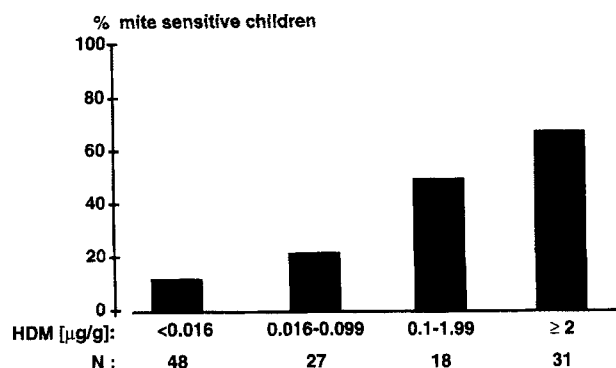


Fig 1. Frequency of sensitivity (i.e. RAST class 1 or more) to any house dust mite in 124 children with asthma in relation to the highest total mite allergen concentration [µg/g] in the house dust. The p value for the trend was <0.001 (Mann Whitney).

The objective of this study was to compare home levels of indoor sedimented allergens with current symptomatology, medication, and frequency and level of sensitivity (IgE antibodies) among asthmatic children with moderate to severe symptoms, living in three climatic zones in Sweden. This is part of a hypothesis generating assessment of associations between the home environment and bronchial asthma in childhood.

Patients and methods

Study population

Children, 4-18 years old, with perennial asthma severe enough to motivate continuous anti-inflammatory medication (sodium cromoglycate and/or inhaled steroids) attending the Paediatric Allergy outpatient clinics at the hospitals in Helsingborg (N=46), Linköping (N=37) and Umeå (N=41) were invited to participate. Helsingborg in Southern Sweden has a maritime climate similar to other coastal regions of North Western Europe. Linköping is an inland town in South Central Sweden with sometimes cold winters, while Umeå, situated 300 km south of the Arctic Circle, has cold and dry winters.

The study comprised 75 boys and 49 girls, with a mean age of 11.2 years (median 11 years). Asthmatic children with and without confirmed allergy to airborne allergens were included. All the children had been resident for at least three years in their present home.

The children and their parents answered a mailed questionnaire about the indoor environment, e.g. regarding the presence and frequency of condensation on the window panes and other moisture problems, indoor smell, wall to wall carpeting, smoking habits of family members, and past or present occurrence of pets in the house.

A structured interview regarding the frequency and severity of symptoms, impairment of physical function and present medication was done by a trained nurse. An asthma score was calculated based on the frequency of symptoms, severe enough to impair physical functions, and current medication, as described by Croner et al. (8). Briefly, the number of days/nights of physical impairment in a year and the use of drugs (inhaled β_2 agonist, oral β_2 agonist or theophylline, disodium cromoglycate and inhaled steroids) were estimated by the family. The highest possible score was 5.2. For inclusion, the asthma score at the last visit to the allergy clinic should have been 3 or more.

Dust samples

The dust was collected and handled as described by Munir et al. (9). Briefly, three dust samples were obtained by the families using their own vacuum cleaners. No cleaning was done for at least seven days before sampling. The mattress of the child's bed was vacuumed for 2 minutes, then the bedroom floor and corners of the bedroom (2 min.). Sample 2 was obtained in the living room. The time recommended for a sofa was 2 minutes. The third sample was obtained in the kitchen, bathroom and laundry room with special attention to the corners and around pipes. Determination of the allergen levels in sieved fine dust was done by ELISA using monoclonal antibodies. The allergen levels were expressed as µg/g fine dust. The detection limit of the ELISA for the assessment of *Der p I*, *Der f I* and *Fel d I* was 0.016 µg/g dust and for *Can f I* 0.06 µg/g dust. The dust samples from three homes were too small to allow determination of *Can f I*. For the HDM allergens, the "maximal mite allergen load" in a house, i.e. the highest sum of *Der p I* and *Der f I* in a location, was calculated. The dust allergen levels were then stratified into classes, i.e.: < 0.016 µg/g (not detectable), 0.016-0.099 µg/g, 0.1-1.99 µg/g, 2-9.99 µg/g, and >10 µg/g, the two latter representing suggested cut off points, and 0.1 µg/g was chosen on a log scale as a point of division in the lower range of allergen concentrations. Both species specific exposure and maximal total mite allergen exposure were compared with current sensitivity.

IgE antibodies

Circulating IgE antibodies were determined using CAP RAST (Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions. The levels were expressed in kU/l and semi-quantitatively as RAST classes I-VI as suggested by the manufacturer. RAST class I or higher indicated sensitivity.

Statistics

Statistical analysis was made with χ^2 -analysis between categorical variables. The estimation of confidence intervals for proportions was based on the binomial distribution (11) and not the normalised approximation (12, 13) since many of the proportions were based on small numbers, or the proportion values were often far from 0.5. Similarly, an assessment of the difference between proportions was based on the normalised proportions when $N \geq 30$, or Fishers exact test for $N < 30$ (12). The distributions of the IgE antibody levels and allergen content in the dust samples were skewed, decimal log transformation only partially normalising the distribution. These measurements were therefore analysed by non parametric tests, *i.e.* Wilcoxon's rank sum test and Mann Whitney U test for two group comparisons, and Kruskal Wallis test for differences of more than two groups. Analysis of sensitivity associated with the dust concentration of allergens was performed by Mann Whitney's test on the ranked concentrations (12). Correlation between continuous variables was assessed using linear regression analysis, or Spearman's rank correlation, *rs*. The PC version of Minitab statistical software (Mtb 8-s) was used.

Ethical consideration

Informed consent was obtained by the parents before the children were included in the study. Approval was granted by the Linköping University Institutional Review Board.

Results

Mite allergens and sensitivity

The mite allergens *Der p* I and/or *Der f* I were detected in 76 of 124 homes (61%). In the South (Helsingborg) mite allergens were detected in all but 2 of 46 homes (96%), as compared to 23 of 37 (62%) in Linköping and 9 of 41 (22%) in Umeå in the North.

The levels of the two allergens varied from below the detection limit up to 73 $\mu\text{g/g}$, with a median value of 0.035 $\mu\text{g/g}$. The level of *Der f* I was higher than the level of *Der p* I in 82% of the houses. The allergen levels were higher in the bed rooms than in the living room in 77%. Concentrations above 2 $\mu\text{g/g}$ dust were found in 31 of 76 (41%) houses with measurable levels. The levels were also significantly higher in Helsingborg than in Linköping and Umeå, the median values in bedroom dust being 1 $\mu\text{g/g}$, 0.02 $\mu\text{g/g}$ and below detection limit, respectively, $p < 0.001$ by Kruskal Wallis.

Serum IgE antibodies against *D. pteronyssinus* were detected in 42 of 124 (34%) children and against *D. farinae* in 33 (27%). Of the reactions to *D. pteronyssinus*, 60% were in the RAST classes III-VI as compared to 82% of the reactions to *D. farinae*. Mite sensitivity was more prevalent in Helsingborg (54%), than in the Central and Northern parts of Sweden (22%), $p = 0.001$.

The proportion of children sensitive to HDM increased with higher levels of HDM allergens in the dust (Fig. 1). A non parametric assessment for this trend (11), *i.e.* of the ranked dust concentrations on sensitivity as category (Mann Whitney), was highly

Table 1. Circulating IgE antibody levels to the house dust mites *D. Pteronyssinus* (section A) and *D. Farinae* (section B) expressed as RAST classes in 124 asthmatic children in relation to current maximal HDM allergen exposure, *i.e.* the highest sum of *Der p* I and *Der f* I allergen in the home, expressed as μg allergen/g fine dust ($p < 0.001$ for section A and B, respectively).

		Maximal HDM allergen level (<i>Der p</i> I + <i>Der f</i> I) [µg/g] at home					
		<0.016	0.016-0.099	0.1-1.99	2-9.99	>10	Total
A.							
IgE to <i>D. pt.</i>							
RAST class							
0		42	21	9	5	5	82
1-2		5	3	2	1	2	13
3-6		1	3	7	4	14	29
Total		48	27	18	10	21	124
B.							
IgE to <i>D. f.</i>							
RAST class							
0		47	22	11	6	5	91
1-2		1	4	0	0	1	6
3-6		0	1	7	4	15	27
Total		48	27	18	10	21	124

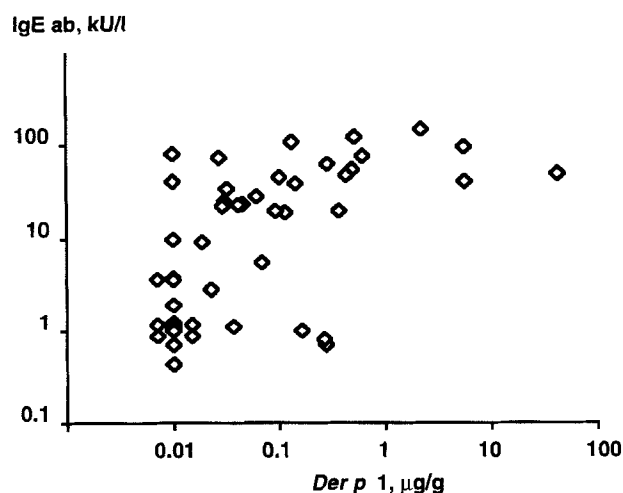


Fig 2. Circulating IgE antibodies (kU/l) to the house dust mite *Dermatophagoides pteronyssinus* in relation to the concentration of *Der p 1* [$\mu\text{g/g}$] in bed room dust in the homes of 42 children with asthma, sensitised to *Dermatophagoides pteronyssinus* ($p < 0.001$).

significant ($p < 0.001$). There were 45 houses with HDM allergen concentrations between $0.016 \mu\text{g/g}$ and $2 \mu\text{g/g}$. Fifteen of the children living there were sensitised against HDM. Employing the Mann Whitney test on this subset revealed a significant relationship ($p = 0.025$), indicating that the level was associated with sensitivity also in this low range of allergen concentrations.

The levels of either of the HDM allergens or their sum in the dust samples were strongly associated with the presence of circulating IgE antibodies against *D. pteronyssinus* and *D. farinae* in the children, $p < 0.001$ according to Kruskal Wallis. In the 48 houses without any detectable HDM allergens in the dust lived 42 children who were not sensitive to HDM (88%), while in the 21 houses in which the mite allergen levels were above $10 \mu\text{g/g}$, 16 children were sensitive to HDM (76%) (Table 1). There was also a relationship between the magnitude of the IgE antibody response against house dust mites and current exposure to HDM allergens, as demonstrated by the relation between sensitivity and exposure to *Dermatophagoides pteronyssinus* allergen ($p < 0.001$ whether using statistics for a linear or non-linear relationship) (Fig. 2). Spearman's rank correlation (r_s) showed significant correlations ($p < 0.001$) whether relating IgE to *Dermatophagoides pteronyssinus* to *Der p 1* concentrations in the bedroom (0.57) or living room (0.53) dust. There were also considerable correlations between the concentrations of the two types of HDM allergens and between HDM allergen concentrations in various locations in the homes (r_s values between 0.60 and 0.96).

There was, however, no significant relationship between disease severity expressed as asthma score and either sensitivity to HDM or mite allergen levels at home.

Cat and dog allergens and sensitivity

Fel d 1 was detected in the dust from all homes, in all but one of the living room samples and in 93% of the bedrooms. The levels were higher in the living room than in the bedroom in most houses ($p < 0.01$, Mann Whitney). The highest level was $27.9 \mu\text{g/g}$ and the median level for all houses was $0.63 \mu\text{g/g}$ when taking the highest value for each house. Seven homes had a cat and these had the highest cat levels (14). The maximal concentrations of *Fel d 1* in the homes of children avoiding direct contacts with cats (no cats at present or in the past, avoiding to visit friends and relatives with cats, $N = 51$) were normally distributed after log transformation, the maximum value being $5.3 \mu\text{g/g}$.

There was a non-significant trend towards higher cat allergen levels in Umeå than in Helsingborg. In the homes where all contacts with cats were avoided, the cat allergen concentrations were almost the same in the three regions. The proportion of families avoiding cats varied between 33% (Umeå) and 46% (Helsingborg; n.s.).

Circulating IgE antibodies against cat were detected in 71 of 124 children (57%). The levels were in RAST classes III-V in 76% of them. The prevalence of sensitivity to cat was higher in Umeå (73%), as compared to Linköping (62%) and Helsingborg (39%; $p = 0.005$). The antibody levels against cat were also higher in Umeå than in Helsingborg ($p = 0.001$, Kruskal Wallis).

The range of *Can f 1* varied from <0.06 to $866 \mu\text{g/g}$, with a median value of $0.8 \mu\text{g/g}$ in 121 homes. Three households had a dog and the median level in them was $300 \mu\text{g/g}$ (14). The highest levels were found in the living rooms in 62% ($p < 0.01$ Mann Whitney) while 25% had the maximal levels in the bedrooms and 13% in the kitchens and/or bathrooms. The distribution of *Can f 1* was extremely skewed with the majority of measurements in the lower range. The levels in the homes where no family member had known contacts with dogs ($N = 30$) came close to a normal distribution after logarithmic transformation. The highest level in these was $11.4 \mu\text{g/g}$. The dog allergen levels were similar in the three centres. Dog avoidance was reported by 17% (Umeå) to 30% (Helsingborg) of the families (n.s.).

IgE antibodies against dog were found in 68/124 (55%). The levels were in RAST class III-VI in 66% of them. The prevalence of dog sensitivity was 73% in Umeå, 54% in Linköping and 39% in Helsingborg, $p = 0.006$ by χ^2 . The IgE antibody levels to dog

varied in a similar fashion between the geographical areas, $p=0.001$ by Kruskal Wallis.

There was no correlation between the pet allergen level in house dust and sensitivity to either cat or dog. Asthma scores were however weakly associated with sensitivity to dog. The median score among the non-sensitised children was 2.3 (95% CI 2.1-2.7, Wilcoxon's sign rank sum test) and the median score of the sensitised children was 3.0 (95% CI 2.6-3.2). The difference was significant ($p=0.011$, Mann Whitney). Similarly, the children with sensitivity to cat tended to have a higher asthma score than those without antibodies against cat ($p=0.077$, Mann Whitney). *Fel d I* concentrations were marginally lower in the homes of patients with symptom scores above the median ($=3$), as compared to the patients with lower scores ($p=0.05$, Mann Whitney).

Discussion

A concentration of 2 μg HDM allergen/g dust has been assumed to be a risk level for sensitivity and 10 μg HDM allergen/g dust for precipitation of acute attacks of asthma (5). This would indicate that a reduction of the allergen concentration to below 2 $\mu\text{g/g}$ dust in the houses of asthma prone children would be sufficient. In our study, 50% of the children with perennial asthma and sensitivity to HDM were exposed to HDM allergen levels at home well below this level. We found a significant association between mite allergen concentrations and the prevalence of mite sensitivity starting at very low levels, as well as increasing antibody levels with increasing concentrations of HDM in the dust. This was true even when only taking homes with HDM allergen levels $<2 \mu\text{g/g}$ into account. It was also true in all the three study centres.

We confirmed that the highest HDM allergen concentrations are usually found in the bedroom dust. The relationship between the level of sensitivity measured as IgE antibodies to HDM and the level of HDM allergen was present whether we compared sensitivity with separate or combined HDM allergen levels. The source of the dust sample also had little effect on the strength of the relationship. Our data suggest an effect on current prevalence of sensitivity of HDM allergen concentrations near the detection limit of the ELISA test used. The relationship between allergen levels and RAST classes yields support for such an effect.

It could be argued that HDM sensitisation could have occurred earlier in the children currently exposed to low HDM allergen levels, or at another site. They had all, however, been living in their present homes for at least three years with only short vacation trips to possibly mite infested re-

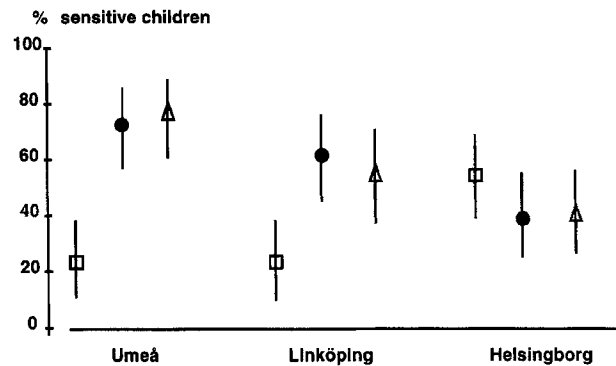


Fig 3. The prevalence of sensitivity (RAST class 1 or more) to house dust mites (HDM, indicated by open boxes), cat (closed circles) and dog (open triangles) in three regions. The 95% confidence intervals are based on the binomial distribution.

gions. There is some evidence that mite allergen levels are stable over time within a home (6, 8). HDM allergen levels seem to be low in schools and day care centres in Linköping (15, 16), as well as in schools in Norway (17). So, even if our results do not unequivocally prove that mite allergen levels $<0.5 \mu\text{g/g}$ fine sedimented dust suffice to induce sensitisation, they strongly support that these low levels may be important for continued sensitivity - thus they may be important for the prognosis of the disease. The importance of mite allergen levels lower than 2 $\mu\text{g/g}$ dust has previously been suggested by Price et al. (6) and Verhoeff et al. (18).

The highest allergen levels were not always found in the bed rooms, but sometimes in the living rooms. Possibly, local patches of humidity, for example in upholstered furniture, or in a large carpet, overlying a poorly insulated floor, can create a micro climate which is favourable to house dust mites. Families with asthmatic children are generally given advice regarding the cleaning of bedclothes, mattresses, pillows, etc. in the bed room. Upholstered furniture, cushions and blankets in the living room may be forgotten or more difficult to clean. A conclusion from our study is that other mite allergen sources than mattress dust should also be considered.

As expected (19, 20), sensitisation against HDM was more common (54%) in the South of Sweden (Helsingborg), where the climate is temperate and humid like on the European Continent, than in the areas with inland or subarctic climate (22%). House dust mites depend on a high humidity to thrive, and normally the population diminishes during the heating season in the winter in temperate climates (21), but bad housing with moisture and ventilation problems may stimulate the growth of mites also in a cold climate (22).

The incidence of pollen sensitivity is higher in the Northern countries, such as Sweden, than in central

European countries and the British Isles (23), but the pollen counts are much lower. Similarly, despite higher pollen concentrations in rural areas, positive skin prick tests are more common in children living in urban compared to nearby rural areas (24). It is tempting to suggest that factors related to the climate or pollution in an area, or unknown "life style factors" may facilitate sensitisation, even at low allergen levels (25).

The lack of association between the asthma score and sensitivity to HDM in our subjects may possibly be due to selection bias as some of the non-sensitised subjects had the highest disease scores. The lack of association between the score and the concentration of HDM allergens would appear to be in contrast to the findings by Ehnert et al. (26). A possible explanation could be anti-mite measures taken by the families (18), or that the levels of HDM allergens in our study were relatively low. Our findings are supported by a very recent report from Germany in which the allergen levels were similar in the homes of sensitised children with and without symptoms (7).

The questionnaires indicated that housing problems like moisture and wall to wall carpeting were associated with a high asthma score *per se* (10). This is in accordance with the association of home dampness and a high risk of developing asthma found in a study in the Netherlands (27).

The study confirms that pet allergens are the major indoor allergens in Scandinavia (28). Sensitivity to cat and dog was more common in the North although the *Fel d I* and *Can f I* levels in home dust were not significantly higher in the Northern than in the Southern parts of Sweden. Allergens like *Fel d I* and *Can f I* can, however, be passively transmitted, e.g. by clothes or hair, to homes (29), schools, or day care centres (30, 31). Most of the families in our study did not allow pets at home. Dybendal et al. found that homes of school children without pets had allergens derived from cats and dogs in the dust in concentrations similar to our data, while the levels found in the children's schools were about ten-fold higher (31). The slightly lower cat allergen levels in the homes of children with a high symptom score may depend on elimination efforts by the families.

In conclusion, even low levels of mite allergens appeared to affect the prevalence and level of sensitivity to HDM in children with asthma. There was no clear threshold level of mite allergens in house dust for sensitivity to mite allergens, nor for cat and dog allergens, and hence it appears important to keep the levels at home as low as possible. Other localisations than mattress and bedroom dust may be relevant sources of mite exposure at home.

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