

Environmental assessment of *Dermatophagoides* mite-allergen levels in Sweden should include Der m 1

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The major allergen of *Dermatophagoides microceras*, Der m 1, as well as the allergens of *D. pteronyssinus* and *D. farinae*, Der p 1 and Der f 1, were analyzed in the homes of 111 asthmatic children in three climatic regions in Sweden. The numbers and species of mites were determined by microscopy, and circulating IgE antibodies against mites were measured. Der f 1 was the predominant house-dust-mite (HDM) allergen, Der p 1 the least often found, and Der m 1 represented 31% of the allergen load. However, in the Linköping area, Der m 1 was the major HDM allergen (58%). Mite counts and allergen levels correlated well. Current exposure to HDM allergens at home was associated with the serum IgE antibody response to HDM in the children with no threshold level. Of the children with IgE antibodies against HDM, 67% reacted to all three mites. Mite sensitization rates were marginally increased (7%) by the addition of IgE analysis of *D. microceras* to the routine analysis of IgE antibodies against *D. pteronyssinus* and *D. farinae*. Thus, Der m 1 may be an important HDM allergen and should be considered when HDM exposure data are assessed in areas with a climate like that of Sweden.

**A. Warner^{1,4}, S. Boström²,
A. K. M. Munir⁴, C. Möller³,
C. Schou⁵, N.-I. M. Kjellman⁴**

¹Department of Paediatrics, Helsingborg Hospital, Helsingborg, Sweden; ²Zoo-Tax, Swedish Museum of Natural History, Stockholm, Sweden; ³Department of Paediatrics, University Hospital, Umeå, Sweden; ⁴Department of Paediatrics, University of Linköping, Sweden; ⁵Allergologisk Laboratorium, Hørsholm, Denmark

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Dr Aina Warner
Department of Paediatrics
Helsingborg Hospital
251 87 Helsingborg
Sweden

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House-dust mites (HDM) are potent indoor allergen sources all over the world (1). The *Dermatophagoides* mites *D. pteronyssinus* and *D. farinae* have been found to proliferate in homes in all types of climates, if the indoor climate is suitable (2). Korsgaard found that adult asthmatic subjects with HDM allergy were significantly more exposed to mites in bedrooms than controls (3), and the same relationship was found for children (4). The counting of mites in house dust is time-consuming and requires expertise (5). Therefore, *D. pteronyssinus* and *D. farinae* mite allergens were characterized and used to replace the counting (6). Measuring environmental HDM by monoclonal antibodies has made it possible to conduct epidemiologic studies on the relationship between exposure to HDM and asthma (7–11). For relation of sensitization to HDM to environmental exposure, the levels of the major allergens, i.e., usually the Der 1 (or Der 1 and Der 2) allergens from all relevant mite species, must be measured.

Exposure at home to even low levels of Der p 1 and Der f 1 was associated with the presence of,

and level of IgE antibodies against, HDM in our climate (12). However, a third *Dermatophagoides* mite, *D. microceras*, morphologically very similar to *D. farinae* (5), can also be found in our region. Its main allergen, Der m 1, can also be assessed (13) and was found in homes in Sweden's temperate climate (14). Therefore, analysis of Der m 1 was undertaken in the remaining dust samples from our previous study (12).

We wanted to determine how common Der m 1 was in homes from three climatic regions in Sweden, its correlation with mite counts, its relevance for sensitization to HDM in asthmatic children, and, finally, whether the suggested threshold value for sensitization of 2 µg HDM allergen per gram dust (1) was applicable in view of these new data.

Material and methods

All patients 6–18 years of age with moderate to severe asthma in the outpatient pediatric allergy specialized clinics of the hospitals in Helsingborg,

Linköping, and Umeå were invited to the study. Helsingborg, a city on the southwestern coast of Sweden, has a maritime temperate climate; Linköping, an inland city situated in east-central Sweden, has an inland temperate climate; and Umeå in the north, situated near the Gulf of Bothnia on the east coast, has a subarctic climate. A little over 50% of the patients agreed to participate, and two siblings under 6 years were accepted in Umeå since they were already sensitized. The mean age was 11.1 years; 65 were boys aged 4–17, mean 10.9 years; and 46 were girls, aged 4–16, mean 11.9 years.

Dust samples were collected by the families in September–November 1989 from three locations of the homes:

- 1) the bedroom: the mattress was vacuumed for 2 min, and then the floor, and all corners around the walls and windowsills where insects may be found
- 2) the living room: the sofa and upholstered furniture were vacuumed for 2 min, and then the carpet and floor, and all corners of the room
- 3) the kitchen and bathroom: special care was taken to find dust from near the sink, refrigerator, larder, and toilet, as well as under the bathtub and the nooks and corners.

After the coarse dust was sieved off, a total of 126 dust samples were submitted to analysis of Der p 1 and Der f 1 by ELISA using monoclonal antibodies (15). The allergen levels were expressed as µg/g fine dust. The detection limit of the ELISA for the assessment of Der p 1 and Der f 1 was 0.016 µg/g dust. The dust samples were then kept frozen at –20°C, until Der m 1 levels were assessed in the remaining extracted dust by ELISA using monoclonal antibodies against Der m 1 in samples from 111 homes in 1996. The detection limit for the analysis was 0.02 µg/g. The cross-reactivity of the Der m 1 assay is 0.002% with Der f 1 and 0.001% with Der p 1, and the cross-reactivity of the Der f 1 assay with Der p 1 is 0.003% and with Der m 1 0.046%. The cross-reactivity of the Der p 1 assay is 0.007% with Der f 1 and 0.044% with Der m 1.

The *coarse dust* was sent for microscopic examination at Zoo-Tax (Swedish Museum of Natural History, Stockholm). From each sample, a subsample of 0.25 g dust was taken for counting and identification of mites, other arachnids, and insects. The subsample was distributed into small Petri dishes (4 cm diameter) with alcohol, and the mites were picked out under a stereomicroscope at low magnification (×25–40). Mites were transferred to lactic acid as clearing agent and incubated at 50°C for about 24 h. After clearing, they were mounted in Hoyer's medium and subsequently counted and

identified with a compound microscope (×400). Dust samples from 55 homes were analyzed by microscope.

Serum IgE antibodies against the HDM *D. pteronyssinus*, *D. farinae*, and *D. microceras* were measured by the Pharmacia CAP® System (Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions.

Statistics

For comparison of categorical data chi-square analysis, the normalized proportional formula, or Fisher's exact test was used. Even after logarithmic transformation, the distribution of Der m 1 remained skew; therefore, for continuous data, the Kruskal-Wallis test, Wilcoxon's sign test, or the Mann-Whitney *U*-test was used. For correlation between counted mites and allergen levels, single regression in log-logarithmic transformation was used. For correlation between the magnitude of the IgE response and allergen levels in the dust, Spearman's rank correlation coefficient r_s was used. The 5% level, two-tailed, except when indicated, has been accepted for significance. The χ^2_{trend} , and, alternatively, the Mann-Whitney test for trend have been applied, as described by Altman (16). Minitab statistical software Mb8s was used with its applications (17).

Ethics

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

Results

Der m 1 levels

Of the 111 homes, 37 contained measurable Der m 1 levels (33%). The maximal level found in a home containing Der m 1 ranged from 0.1 to 932 µg/g dust. In homes containing Der m 1, the median level was 0.41 µg/g, 95% CI 0.2–0.99 µg/g (Wilcoxon's sign test), and the interquartile range was 0.15–8.46 µg/g. The highest proportion of homes containing Der m 1 was found in the Linköping region (55%) and Helsingborg (37%), compared to Umeå (6%), *P* by $\chi^2 < 0.001$ (Table 1). The corresponding median levels of Der m 1 in homes containing this allergen were 0.50, 0.39, and 0.14 µg/g (NS), respectively. Der m 1 levels were higher than the sum of Der p 1 and Der f 1 in 23 (29%) of the homes containing mites, of which 18 were in the Linköping area (58%), three in

Table 1. Maximal levels of Der m 1 allergen in dust samples from three locations in homes of 111 asthmatic children in three climatic zones in Sweden (A) compared to when maximal findings of Der p 1, Der f 1, and Der m 1 were summed together (B). Difference of finding HDM infestation was significant in all three areas by normalized proportional test

	Helsingborg		Linköping		Umeå		Total	
	A	B	A	B	A	B	A	B
Below detection	24	1	17	7	33	25	74	33
<0.1 µg/g	0	4	1	8	0	5	1	17
0.1–0.99 µg/g	8	7	13	14	2	4	23	25
1–9.9 µg/g	5	8	1	3	0	0	6	11
≥10 µg/g	1	18	6	6	0	1	7	25
Sum findings	14	37	21	31	2	10	37	78
All	38		38		35		111	
P=	<0.001		0.014		0.011		<0.001	

Helsingborg, and two in Umeå (8 and 10%, respectively). Of the 59 samples containing Der m 1, 14 were from bedrooms, 28 from living rooms, and 17 from kitchen and bathrooms. The median levels in these locations were 4.8, 0.6, and 0.2 µg/g, respectively ($P=0.006$ by Kruskal-Wallis test).

Der m 1 levels together with Der p 1 and Der f 1 as part of the HDM allergen load

Of the 111 homes tested, 33 had no measurable HDM allergens. In Helsingborg, 37; in Linköping, 31; and in Umeå, 10 homes contained HDM allergens. The maximal sum of the three Der 1 allergen levels in the 78 homes with HDM allergens was 0.016–934 µg/g. The median level was 0.64 µg/g. Fig. 1 shows a box plot of the HDM levels in the

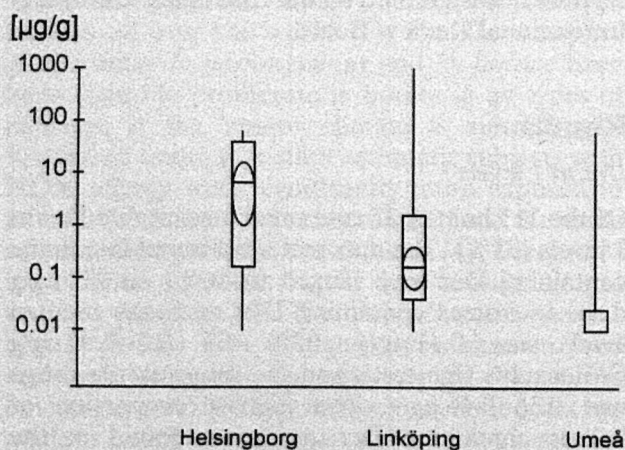


Fig. 1. Distribution of maximal levels of HDM allergens (Der p 1, Der f 1, and Der m 1) found in homes of 111 asthmatic children living in three climatic areas in Sweden. Boxes delineate middle two quartiles; semicircles, 95% CI for median by Wilcoxon's sign test. Vertical lines represent range of allergen levels; horizontal lines, median levels. Levels were significantly different between areas; $P=0.002$ between Helsingborg and Linköping, $P<0.001$ between Linköping and Umeå by Mann-Whitney U-test.

different areas. The median levels in the three areas were 6.7, 0.27, and 0.10 µg/g, respectively ($P<0.001$ by Kruskal-Wallis test). Table 1 shows an overview of the Der m 1 allergen levels and the HDM levels. In only three homes, in Helsingborg, the HDM levels were between 1 and 2 µg/g. The contribution of each Der 1 allergen to the HDM allergen load was calculated by dividing the particular Der 1 level by the sum of all three HDM allergens. Taking all study sites together, the Der m 1 part of HDM allergens was 31%; Der f 1, 53%; and Der p 1, 16%. In the Linköping area, Der m 1 represented 58% of the HDM allergens (Fig. 2). The addition of Der m 1 to the HDM allergens significantly increased the proportion of mite-infested homes in Linköping, compared to when only Der p 1 and Der f 1 allergens were assessed ($P=0.025$ by proportional test, one-sided) (Fig. 3). The assessment of Der m 1 alone, however, was a poor indicator of the HDM-allergen load in all three study areas (Table 1). In the minority (33%) of the homes with HDM allergens, the findings were of one species only. HDM-allergen levels exceeded 2 µg/g in 23/38 (61%) of the Helsingborg homes, while 9/38

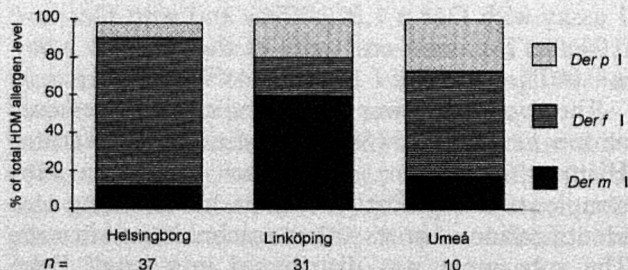


Fig. 2. Parts that each Der 1 allergen represented of total HDM-allergen load in homes of asthmatic children from three climatic areas. Each area has representation on y-axis from 0 to 1. In Helsingborg, Der f 1 represented 78% of allergen level in 37 homes; in Linköping, Der m 1 represented 58% of allergen level in 31 homes; and, in Umeå, Der f 1 represented 56% of allergen level in 10 homes.

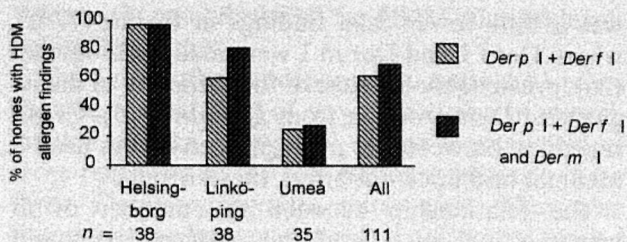


Fig. 3. Proportion of homes with HDM-allergen findings in three climatic areas of Sweden when Der p 1 and Der f 1 were measured compared with when Der m 1 was also included. Difference in Linköping was significant ($P < 0.025$, one-sided proportional test).

(24%) homes in Linköping and 1/35 (3%) in Umeå contained HDM allergens in such levels (Table 1). In the 33 homes with HDM levels of $\geq 2 \mu\text{g/g}$, Der m 1 was found in 18 (55%) homes; 10/23 in Helsingborg and 8/9 in Linköping.

Counted *Dermatophagoides* mites and Der 1 allergen levels

The rank correlation (r_s) between counted *D. pteronyssinus* mites and the level of Der p 1 in 16 homes with findings of the mite was 0.69 ($P < 0.01$). Between *D. farinae* in 21 homes with findings and the level of Der f 1, r_s was 0.76 ($P < 0.002$). Between counted *D. farinae* and *D. microceras* summed together and the level of Der f 1 and Der m 1, r_s was 0.60 ($P < 0.01$). There were only five homes with microscopic findings of *D. microceras*.

The rank correlation (r_s) between the maximal value of the three Der 1 allergens and the maximal count of *Dermatophagoides* mites in 28 homes with findings was 0.85 ($P < 0.002$). After logarithmic transformation of both variables, an almost linear relationship was found, $r_p = 0.90$ (Fig. 4). In the 19 homes with no counted HDM (40%), 13 contained no Der 1 allergens (68%), four had levels below

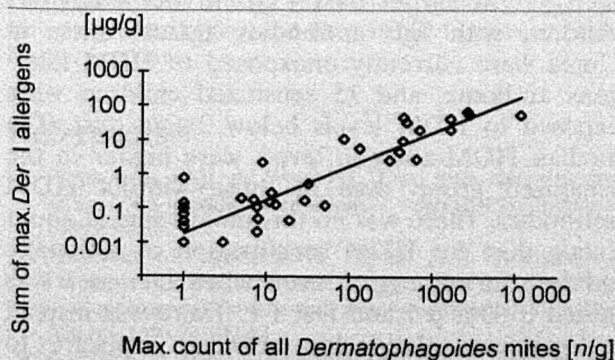


Fig. 4. Sums of Der 1 allergens from HDM *D. pteronyssinus*, *D. farinae*, and *D. microceras* in comparison with microscopic counts of three *Dermatophagoides* species in homes of 47 asthmatic children. Line represents regression line ($P < 0.001$).

0.1 $\mu\text{g/g}$, and the remaining two had 0.14 and 0.75 $\mu\text{g/g}$, respectively. In the 14 homes with no HDM allergens detected, the maximal mite count was two specimens/g dust in one home.

Serum IgE antibodies against HDM

Serum IgE antibodies against some HDM were found in 42 of the 111 children (38%). Among them, antibodies against *D. pteronyssinus* were found in 39, against *D. farinae* in 30, and against *D. microceras* in 34 children. Antibodies against all three HDM were found in 28/42 children (67%). The highest proportion of sensitized children with antibodies against all three mites were living in the south (18/20), while the proportions in the east-central region (7/12) and in the northern region (3/10) were much lower (P by $\chi^2 < 0.01$). Serum IgE antibodies against *D. pteronyssinus* alone contributed to 6/42 (14%) (95% CI: 5–29%), against only *D. microceras* to 3/42 (7%) (95% CI: 1.5–19%) of the HDM antibodies. No child had IgE antibodies against only *D. farinae*.

Serum IgE against *D. microceras* and Der m 1 allergen levels

Exposure to Der m 1 at home above the detection level was associated with IgE antibodies against *D. microceras* ($P = 0.035$, Mann–Whitney U-test).

Serum IgE against the three HDM and the three Der 1 allergen levels

There was a highly significant relationship between the HDM-allergen level and the frequency of the IgE response against HDM ($P < 0.001$ by χ^2_{trend}). The proportions of children with IgE antibodies against HDM among children living in homes with the exposure levels < 0.016 , 0.016–0.09, 0.1–0.99, 1–9.9, and $> 9.9 \mu\text{g/g}$ dust were 6/33, 5/17, 7/25, 5/11, and 19/25, respectively (Fig. 5).

Six children with serum IgE antibodies against HDM were found in the unexposed group, and all of them were from Umeå. Of the 33 children who had an exposure at home below 0.25 $\mu\text{g/g}$, nine had serum IgE antibodies against HDM, compared to 6/12 children living with exposure at home of between 0.25 and 1.99 $\mu\text{g/g}$. When these groups were allocated the scores 1, 2, and 3, χ^2_{trend} was significant ($P = 0.042$).

The Spearman rank correlation (r_s) between HDM-allergen levels (any mite, or all three mites) and the maximum serum IgE response to HDM was 0.51 ($P < 0.01$).

The 42 children with serum IgE antibodies against HDM were exposed to HDM allergens at

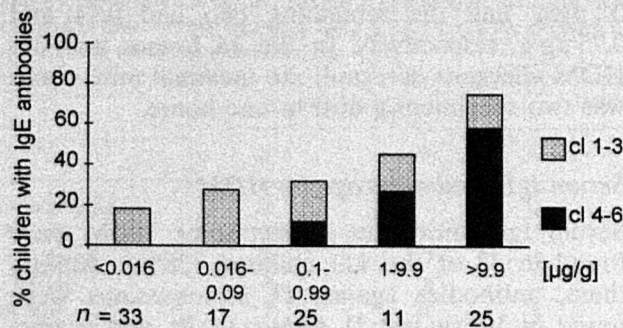


Fig. 5. Serum IgE antibodies against three HDM in 111 asthmatic children in relation to sum of three Der 1 allergen levels. Serum IgE levels were in classes 1–3 and 4–6 according to Pharmacia CAP® System.

home ranging from under the detection limit to 933 µg/g, median level 3.13 µg/g (95% CI: 0.22–19.9 µg/g), while the 69 children without any IgE antibodies against HDM had Der 1 levels at home ranging from under the detection limit to 46.7 µg/g, median 0.044 µg/g (95% CI: 0.01–0.13 µg/g).

Discussion

We found that Der m 1 is an important HDM allergen in our region. Der m 1 constituted one-third of the HDM allergen load in our samples, and in Linköping more than half of it. Wickman et al. also found that Der m 1 was more common than Der p 1 in dust samples in Stockholm, which has a climate similar to that of Linköping (14). With the addition of Der m 1 data, all HDM-sensitized children in Helsingborg and Linköping were found to be exposed to HDM allergens at home, while, in Umeå, 6/9 HDM-sensitized children were not exposed at home. Furthermore, the proportion of mite-infested homes increased significantly in Linköping (up to 82%), when the house-dust samples were measured also for Der m 1. In support of our findings, Der m 1 was the predominant HDM allergen in the homes of children with hay fever and controls in the Linköping area (18). In Helsingborg, Der f 1 surpassed Der m 1. In the Linköping area, in spite of its predominance, the measurement of Der m 1 alone could not replace the other two Der 1 allergens because a significant number of HDM-exposed children would have been missed (Table 1).

In the Umeå area, Der m 1 added modestly to the HDM-allergen load, but, as in the Helsingborg homes, only in already mite-infested houses. We found that Der p 1 was the least common HDM allergen in our samples, in contrast to the findings from the UK by Hart & Whitehead (19). The proportion of sensitized children living in homes with levels of HDM allergens of ≥ 2 µg/g dust (60%)

was a little lower than findings in Berlin (77%), where Der f 1 and Der m 1 were analyzed together (20), presumably because of the difficulty of distinguishing *D. microceras* from *D. farinae* (5). Cross-reactivity between the mite-allergen assays used is minimal and does not affect these results.

The relationship between the number of *D. pteronyssinus* mites and the antigen levels was similar to the findings of Tovey et al. (21) and Platts-Mills et al. (22). We found a good correlation between the combined mite counts of *D. farinae* and *D. microceras* and their combined allergens Der f 1 and Der m 1, as did Lind (13, 23). The fact that *D. microceras* was found in just a few samples is related to the detection limit of the microscopy rather than the specificity of the ELISA assay for Der m 1 (5). As in the previous studies, there were some homes in which allergens were found, but not mite bodies. Mite allergens are stable under simulated (24) and natural conditions (25), and may be present after the infestation of mites has ceased or diminished, or they may be passively transferred into the dust. The storage of the samples is unlikely to have affected the results, as they were kept at -20°C , a temperature which, in our experience, does not affect the activity of Der 1 allergens, in spite of their papain-like protease activity (26). Since there are geographic differences in HDM infestation (27–29), the local fauna should be generally assessed by microscopy, and the relevant allergens measured before a conclusion about the allergen exposure can be made in an area. In Sweden, this should include measurement of Der m 1 and allergens from four storage mites (Warner et al., submitted to *Allergy*).

Primary sensitization to HDM seems to take place at an early age (30–33). An amount of 2 µg/g dust has been proposed as a risk level for sensitization to the two major HDM allergens Der p 1 and Der f 1 (1, 34). However, Price et al. have suggested a lower threshold; i.e., 0.5 µg/g Der p 1 in carpet dust (35). In our study, six children with IgE antibodies against mites in Umeå were currently unexposed to HDM allergens at home, and 15 sensitized children were exposed to HDM levels below 2 µg/g dust. The median HDM-allergen levels were higher in the sensitized group than in those without HDM antibodies. There was no threshold value at about 2 µg/g dust for HDM sensitization of asthmatic children in our region even when Der m 1 was added to Der p 1 and Der f 1. There was instead a log-linear relationship between exposure to HDM allergens and the frequency as well as the level of the IgE antibody response, starting from the detection level. The trend we found by χ^2_{trend} was significant also when assessed by the Mann–

Whitney U-test ($P=0.034$) for HDM-allergen levels below $2 \mu\text{g/g}$ dust.

The IgE antibody responses to individual mites showed a great deal of cross-reactivity. In experimental settings, Lind et al. found a great deal of cross-reactivity of the IgE response between these three *Dermatophagoides* mites (36). They also found that the human serum IgE antibodies tended to react more to *D. pteronyssinus* than to the other two *Dermatophagoides* species. The presence of specific serum IgE antibodies against a particular mite species in a patient was not associated with exposure to the same mite species at home. *D. farinae* had a leading role in the HDM infestation of the homes, but not so in the antibody response. A panel for determination of IgE antibodies against all the three *Dermatophagoides* mites could be considered for diagnosing HDM sensitivity in patients, especially in areas with a relatively low HDM exposure. Adding determination of IgE antibodies against *D. microceras* to the usual tests for antibodies against *D. pteronyssinus* and *D. farinae* is predicted to increase the prevalence of HDM sensitization by 5–10% in this patient group.

In conclusion, Der m 1 is an important HDM allergen in our climate and should be assessed when the HDM allergen level is measured. The addition of serum IgE antibodies against *D. microceras*, as in a panel of the three HDM, would marginally increase the rate of sensitization to HDM. Adding analysis of Der m 1 did not change our previous impression that there is no threshold level for sensitization, but did indicate a progressively increasing risk from very low levels of exposure.

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References

- Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma – a worldwide problem. *J Allergy Clin Immunol* 1989;83:416–27.
- Wickman M, Emenius G, Egmar A-C, Axelsson G, Pershagen G. Reduced mite allergen levels in dwellings with mechanical exhaust and supply ventilation. *Clin Exp Allergy* 1994;24:109–14.
- Korsgaard J. Mite asthma and residency. A case control trial on the impact of exposure to house dust mites in dwellings. *Am Rev Respir Dis* 1983;128:231–5.
- Smith TF, Kelly LB, Heymann PW, Wilkins SR, Platts-Mills TAE. Natural exposure and serum antibodies to house dust mites of mite-allergic children with asthma in Atlanta. *J Allergy Clin Immunol* 1985;76:782–8.
- Colloff MJ, Spieksma FThM. Pictorial keys for the identification of domestic mites. *Clin Exp Allergy* 1992;22:823–30.
- Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TAE. Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p 1* and *Der f 1*, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987;80:184–94.
- Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol* 1989;83:857–82.
- Sporik R, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen of children admitted to hospital with asthma. *Clin Exp Allergy* 1993;23:740–6.
- Marks GB, Tovey ER, Toelle BG, Wachinger S, Peat JK, Woolcock AJ. Mite allergen (*Der p 1*) concentration in houses and its relation to the presence and severity of asthma in a population of Sydney schoolchildren. *J Allergy Clin Immunol* 1995;96:441–8.
- Kuehr J, Frischer T, Meinert R, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994;94:44–52.
- Kuehr J, Frischer T, Meinert R, et al. Sensitization to mite allergen is a risk factor for early and late onset of asthma and for persistence of asthmatic signs in children. *J Allergy Clin Immunol* 1995;95:655–62.
- Warner AM, Björkstén B, Munir AKM, Möller C, Schouh C, Kjellman N-IM. Childhood asthma and exposure to indoor allergens. Low levels are associated with sensitization. *Pediatr Allergy Immunol* 1996;7:61–7.
- Lind P. Enzyme-linked immunosorbent assay for determination of major excrement allergens of house dust mite species *D. pteronyssinus*, *D. farinae* and *D. microceras*. *Allergy* 1986;41:442–51.
- Wickman M, Nordvall SL, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. *J Allergy Clin Immunol* 1991;88:89–95.
- Munir AKM, Björkstén B, Einarsson R, et al. Mite allergens in relation to home conditions and sensitization of asthmatic children from three climatic areas. *Allergy* 1995;50:55–64.
- Altman D. Practical statistics for medical research. London: Chapman and Hall, 1991.
- Zehna PW. A Minitab companion with macros. Menlo Park, CA: Addison Wesley, 1992.
- Ferdousi HA, Dreborg S. Asthma, bronchial hyper-reactivity and mediator release in children with birch pollinosis. *Clin Exp Allergy* 1997;27:530–9.
- Hart BJ, Whitehead I. Ecology of house dust mites in Oxfordshire. *Clin Exp Allergy* 1990;20:203–9.
- Lau S, Falkenhörst GF, Weber A, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989;84:718–25.
- Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981;124:630–5.
- Platts-Mills TAE, Heymann PW, Chapman MD, Hayden ML, Wilkins SR. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P₁ equivalent in house dust and dust mite extracts. *J Allergy Clin Immunol* 1986;78:398–407.
- Lind P. Demonstration of close physicochemical similarity

- and partial immunochemical identity between the major allergens Dp42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). *Int Arch Allergy Appl Immunol* 1986; 79:60-5.
24. Kort HSM, Kniest FM. Four-year stability of *Der p* I in house dust under simulated domestic conditions *in vitro*. *Allergy* 1994;49:131-3.
25. De Boer R, van der Hoeven AD, Stapel SO. The decay of house dust mite allergens, *Der p* I and *Der p* II, under natural conditions. *Clin Exp Allergy* 1995;25:765-70.
26. Stewart GA, Kollinger MR, King CM, Thompson PJ. A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*. *Allergy* 1994;49: 553-60.
27. Chan-Yeung M, Becker A, Lam J, et al. House dust mite allergen levels in two cities in Canada: effects of season, humidity, city and home characteristics. *Clin Exp Allergy* 1995;25:240-6.
28. Arruda LK, Rizzo MC, Chapman MD, Fernandez-Caldas E, Platts-Mills TAE, Naspitz CK. Exposure and sensitization to dust mite allergens among asthmatic children in São Paulo, Brazil. *Clin Exp Allergy* 1991;21:433-9.
29. Dybendal T, Elsayed S. Dust from carpeted and smooth floors. VI. Allergens in homes compared with those in schools in Norway. *Allergy* 1994;49:210-16.
30. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergen in children at risk of allergic disease. *Arch Dis Child* 1985;60:727-35.
31. Warner JA, Little SA, Pollock I, Longbottom JL, Warner JO. The influence of exposure to house dust mite, cat, pollen and fungal allergens in the home on primary sensitisation in asthma. *Pediatr Allergy Immunol* 1993;1:79-86.
32. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 1993; 147:573-8.
33. Sporik R, Holgate ST, Platts-Mills TAE, 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;323:502-7.
34. Platts-Mills TAE, Ward G, Sporik R, Gelber L, Chapman M, Heymann P. Epidemiology of the relationship between exposure to indoor allergens and asthma. *Int Arch Allergy Appl Immunol* 1991;94:339-45.
35. Price JA, Pollock I, Little SA, Longbottom JL, Warner JO. Measurements of airborne mite antigen in homes of asthmatic children. *Lancet* 1990;336:895-7.
36. Lind P, Ingemann L, Brouvez M. Demonstration of species-specific sensitization to major allergens of *Dermatophagoides* species by solid phase absorption of human IgE antibodies. *Scand J Immunol* 1987;25:1-10.

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