

Mite (*Der p 1*, *Der f 1*) and cat (*Fel d 1*) allergens in the homes of babies with a family history of allergy

Munir AKM, Einarsson R, Kjellman N-IM, Björkstén B. Mite (*Der p 1*, *Der f 1*) and cat (*Fel d 1*) allergens in the homes of babies with a family history of allergy.

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Carpet and floor dust samples were collected in four different seasons, from 39 Swedish homes of babies with a family history of allergy. House-dust mite (*Der p 1*, *Der f 1*) and cat (*Fel d 1*) allergen contents were determined by mab ELISA, and the levels were related to various environmental factors. Both mite and cat antigens were detected in 94% of the samples and in all homes, but the levels were low (*Der p 1*, range 15 ng–1944 ng/g fine dust; *Der f 1*, range 14 ng–264 ng/g of fine dust; *Fel d 1*, range 16 ng–3120 ng/g fine dust). Mite-allergen levels were significantly higher ($P < 0.001$) in floor dust than in carpets, and *D. pteronyssinus* predominated. In contrast, the levels of cat antigen were significantly ($P < 0.05$) higher in carpets than in floor dust. There was no clear relation between mite-allergen levels and type of house, except that the higher values were found in homes with dampness problems. Cat-allergen levels were higher than total mite-allergen content, and the highest levels were found in homes with a cat ($P < 0.05$). Rather high concentrations of cat allergen were also found in homes without a cat, which may explain why cat sensitization is so common in Sweden. As the prevalence of house-dust mite sensitivity is increasing in Swedish children, and as the individual patient threshold for eliciting symptoms varies, we suggest that sensitization may possibly occur at a lower exposure level than generally accepted as risk level for sensitization (2 µg/g dust).

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Indoor climate and early exposure to allergens appear to be important for sensitization to inhalant allergens and development of bronchial asthma (4, 17, 20, 26). The predominant sensitizers in Swedish asthmatic children are furred pet animals, causing sensitization twice as often as house-dust mites (6, 9, 11).

Several studies have shown that mites (3, 24) and cats (29) are the sources of the predominant indoor allergens. The numbers of the most common mites in house dust, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are influenced by various environmental factors, e.g. climate (temperature, humidity) and season (2, 17, 24), altitude (19, 23), age of the house (12), living habits (21), and damp housing conditions (5, 13). It is also well known that mite concentrations are low in countries with a cold climate and high altitude. In a previous Scandinavian study from 1979 (22), Pyroglyphidae dust mites were identified by microscopy in only 1.5% of dwellings. More recent studies using the ELISA tech-

nique (18, 28), however, have suggested that the prevalence is increasing. There are several reasons for this apparent increase. As a result of the modern technology used in building houses and energy conservation measures (insulated houses), the indoor microclimate has become favorable for the growth of mites, and, accordingly, their allergens appear to be increasing in house dust in climates where they used to be rare.

The purpose of the present study was to analyze the content of major mite and cat allergens in the dust from homes of newborn infants collected in four different seasons. This was done as a part of a prospective study of the influence of mite- and cat-allergen exposure on patient sensitization to mites and cat, and development of allergic diseases in babies with a family history of allergy and hence a high risk of allergic disease. We also assessed the importance of some conditions in the homes that could influence the allergen levels in house dust.

Material and methods

Home environment

Carpet and floor dust samples were collected from 39 houses in Linköping during all four seasons, i.e. in spring (March), summer (June), autumn (October), and winter (December). The houses were selected as they were the homes of newborn children of two allergic parents. The children were born in 1989 in the University Hospital, and participate in an ongoing, long-term, prospective study of the role of early exposure to house-dust allergens in sensitization and development of allergic disease.

The parents of the children were asked to respond to a questionnaire regarding the conditions of the house, the presence of furred pets (cats), smoking habits, and other factors which may influence indoor allergen concentrations and development of atopic disease. In all, seven parameters were selected (Table 1). The indices of damp housing conditions included persistent presence of visible damp spots in the house (either on walls, floors or the ceiling), visible mold growth in the dwelling, and water condensation on double glass windows during the winter season. We also asked about unpleasant smells in the home recognized by dwellers or visitors. Houses with the presence of at least one of the first three parameters, with or without smells in the house, were considered damp houses. Only one of the houses fulfilled all the criteria for dampness.

Information on daily temperature and humidity of the outdoor air was obtained from the State Meteorological Department in Norrköping, Sweden. The

average temperatures in 1989 in this region were 4.2°C in March, 15.3°C in June, 7.7°C in October, and -1.3°C in December. Mean outdoor relative humidity was about 77%, 58%, 82%, and 82% in March, June, October, and December, respectively.

Collection of house dust

Samples were collected from two different sites in the homes by the parents, using their own vacuum cleaners. A fresh disposable paper bag was placed in the vacuum cleaner before collection of each sample. Dust sampling was standardized by time and area. The floor under and in front of the bed and the largest and most used carpet (not in the kitchen or bedroom) were vacuumed for 2 min/m². Parents were advised not to clean their houses for 3 days prior to collection of the dust samples.

Dust processing and extracts

Bags were cut longitudinally and the specimens were passed through a sieve with a porosity of 300 µm to obtain the fine dust. In all, 287 dust samples (out of an ideal number of 312) were available for analysis.

Aqueous extracts of house dust samples were prepared, with an extraction ratio of 1:10 (w/v), by dissolving the dust sample in phosphate-buffered saline (PBS) (pH 7.6) during constant rotation for 24 h at 4°C. The solution was clarified by centrifugation twice at 3500 rpm (average) for a total of 20 min. The extracts were stored frozen at -20°C in three separate aliquots until analyzed to avoid repeated freezing and thawing.

Allergen determination

ELISA (enzyme-linked immunosorbent assay) test kits for assaying *Der p 1*, *Der f 1*, and *Fel d 1*, which contained monoclonal antibodies against *Der p 1* (clone 5H8 C12 D9), *Der f 1* (clone 6A8 B10 D12), *Fel d 1* (clone 6F9 A4-H1), a reference sample of *Der p 1*/*Der f 1* (2500 ng/ml), and monoclonal biotinylated second antibodies for *Der p 1*/*Der f 1* (clone 4C1 B8 3F8) and *Fel d 1* (clone 3E4 C4-C10), were obtained from Drs Chapman and Platts-Mills (Virginia, USA). A reference sample for *Fel d 1* (10.5 u/ml) was obtained from the Office of Biologics (Bethesda, MD, USA). Peroxidase-labeled streptavidin and the substrate ABTS (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) were purchased from Sigma, St Louis, MO, USA.

The ELISA assay was performed as described previously (14). The interassay variation for repeated assays was 18%, 13.3%, and 14% and the intra-assay variation was approximately 6%, 9%, and 6.5% for *Der p 1*, *Der f 1*, and *Fel d 1*, respectively.

Table 1. Relation between certain environmental conditions and total levels of mite (*Der p 1* and *Der f 1*) allergens in floor dust and cat (*Fel d 1*) allergen in carpet dust. Peak seasonal values were obtained in October or December. * $P < 0.05$. All other comparisons were nonsignificant

Environmental factors	No. of houses	Geometric mean (± 1 sd) of peak seasonal value (ng/g)
Dampness in home	19	236 (120-463)
No dampness	20	272 (138-535)
Houses in city	20	226 (125-411)
Houses in rural area	19	285 (136-597)
Old houses (>20 yr)	17	248 (124-497)
New houses (<20 yr)	22	258 (132-504)
Private (bungalows) houses	24	267 (133-533)
Flats and apartment buildings	15	234 (122-452)
Wooden houses	20	282 (167-586)
Houses other than wood	19	226 (124-413)
Houses with smokers	10	292 (188-454)
Houses with no smokers	29	240 (115-505)
Cat at home	6	782 (283-2163)*
Cat living with neighbor	13	356 (124-1024)
No cat at home or with neighbor	20	220 (72-670)*

The lower quantitation level of our study was 1.5 ng/ml for *Der p 1*, 1.4 ng/ml for *Der f 1*, and 1.6 ng/ml for *Fel d 1*. Values below this level were given a value of 1 ng/ml in the calculations, corresponding to 10 ng/g of fine dust which approached an absorbance reading above the blank.

Statistical analysis

The ELISA results were transformed into decimal logarithms, thus obtaining a normal distribution of the values. Student's t-test and factorial ANOVA techniques were utilized to test the level of significance between variables. Simple correlation technique/multiple regression analysis was employed to test a possible association between variables and environmental parameters.

The study was approved by the Ethics Committee for Human Research at the University Hospital of Linköping, and oral or written informed consent was obtained from all participants.

Results

Mite allergen was detected in 94% of the samples and in at least one sample from each house. The amount of allergens varied over the year. Although all houses had detectable mite allergens on at least one occasion, the levels never exceeded 2 µg/g dust. The mite-allergen content was more than 100 ng/g in only 34% of the samples, and 10 samples contained between 500 ng/g and 1944 ng/g of fine dust (Figs 1 and 2).

The levels of mite allergens were significantly higher ($P < 0.001$) in floor than in carpet dust (Figs 1 and 2). Both species were detected in all houses in

at least one season. The mean value of *D. pteronyssinus* (*Der p 1*) was 255 ng/g and of *D. farinae* (*Der f 1*) 89 ng/g ($P < 0.01$). There was a significant correlation between the two allergens in floor dust (*Der p 1*/*Der f 1*: $r = 0.71$; $P < 0.001$), but not in carpet dust ($r = 0.16$). The contribution of *D. farinae* was 25% of the total mite-allergen content. The total mite-allergen levels in samples were used for assessing the role of house conditions and seasonal variations.

The mite-allergen content was higher in floor samples in October and December ($P < 0.001$) as compared to March and June (Figs 1 and 2).

The amount of mite allergens in carpet dust but not in floor dust tended to be higher in damp and tight houses, as compared to dry and well-ventilated buildings. This observation was supported by the finding that the house fulfilling all the criteria of dampness contained the highest amount of mite allergen in carpets, i.e. 1944 ng/g fine dust. Eight of the 10 samples with mite allergen content between 0.5 and 1.9 µg were collected from houses with dampness.

No appreciable difference was observed between mite-allergen content and the other environmental parameters and housing conditions listed in Table 1.

Cat allergen was also detected in 94% of samples and in all homes. The amount of the major cat allergen *Fel d 1* was generally higher ($P < 0.001$) than that for mite allergen in all sites, with a mean value of 459 ng/g dust (Table 1). In 20% of the samples the *Fel d 1* allergen content was more than 500 ng/g (Fig. 3). In 18 of these samples the levels were between 1 µg and 3.1 µg/g of fine dust. The allergen levels were higher in carpets ($P < 0.05$) than on floors (carpets vs floor, 347/74 ng/g).

The concentration of the major cat allergen *Fel d 1* in both carpets and on floors was higher than that

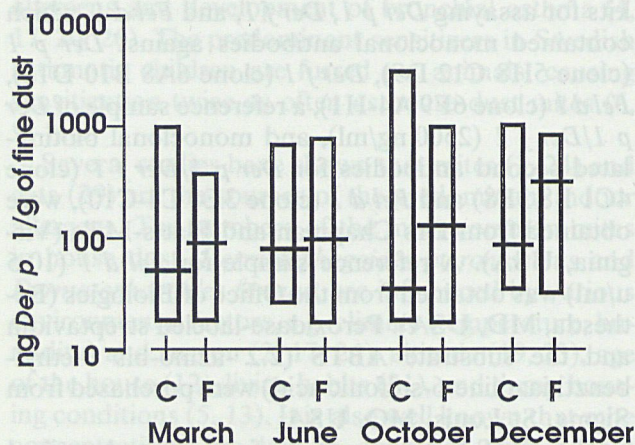


Fig. 1. Levels of *Der p 1* in carpets and floor dust sampled over 1 year in homes of 39 babies at high risk of development of allergy. The samples comprised carpet (C) and floor dust (F). The levels are given in ng antigen/g fine dust and the median values and ranges are shown.

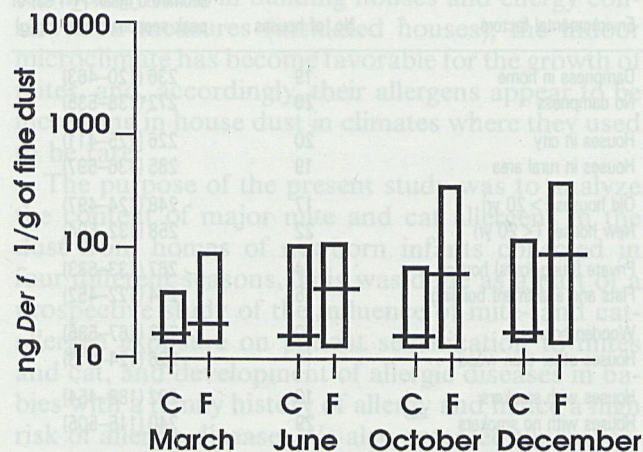


Fig. 2. Levels of *Der f 1* in carpets and floor dust sampled over 1 year in homes of 39 babies at high risk of development of allergy. Explanations as in Fig. 1.

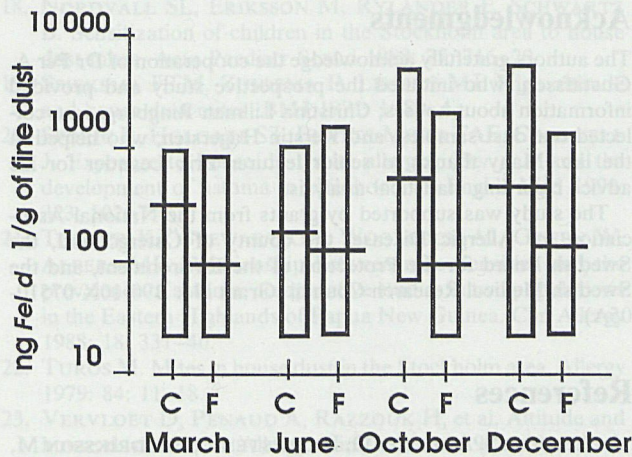


Fig. 3. Levels of *Fel d 1* in carpets and floor dust samples over 1 year in homes of 39 babies at high risk of development of allergy. Explanations as in Fig. 1.

of *Der p 1* and *Der f 1* in most of the samples throughout the year. *Fel d 1* allergen levels were significantly higher ($P < 0.01$) in October and December than in March and June.

The cat-allergen levels were significantly ($P < 0.02$) higher in dust from carpets in damp houses than in dry and well-ventilated homes. There was a correlation ($r = 0.4$; $P < 0.05$) between presence or absence of dampness and the amount of *Fel d 1* allergen.

The highest *Fel d 1* levels were recorded (mean value 782 ng/g dust) in houses with a cat ($P < 0.05$), while intermediate levels (mean value 356 ng/g dust) were found in homes with no cat but where a neighbor had a cat (Table 1). There was a correlation ($r = 0.4$; $P = 0.05$) between the presence or absence of a cat at home and the amount of *Fel d 1* allergen, and a significant correlation ($r = 0.5$; $P < 0.05$) with total amount of mite allergens.

Discussion

The study confirms that the amount of major mite allergens *Der p 1* and *Der f 1* is low in house dust collected in central Sweden. In a recent case-control study in the Stockholm area, which has a similar climate, similarly low levels of mite allergens were reported (28).

The prevalence of mite species varies between countries, and even in the same country it may vary between regions. In the Stockholm area Wickman et al. (28) have shown that *D. microceras* was the predominant mite species. We could not analyze the levels of *D. microceras* in our study, but of the two analyzed species of *Dermatophagoides*, *D. pteronyssinus* overshadowed *D. farinae*. This is similar to other studies from the same part of the world (10, 16) in which *D. pteronyssinus* was found to be the predominant species.

The seasonal peak levels of mite allergens occurred in late autumn and winter. This agrees with findings by Murray et al. in Canada (17), who showed that house-dust-mite-induced respiratory symptoms were more prominent in November and December than at other times of the year. At that time of the year mites are dead and disintegrated to dust which can easily become airborne and more readily sensitize the respiratory tract and elicit symptoms than intact mites.

Dampness in the house is a major indoor environmental factor for an increased number of mite allergens (5). The house with the worst dampness problems in our study also contained the highest amount (1944 ng/g) of mite allergens, and seven samples from houses with less pronounced dampness problems contained more than 500 ng/g. This indicates that modern energy-saving measures which tend to increase indoor humidity can, at least to some extent, explain the increasing prevalence of mite sensitization in the Nordic countries (18, 28).

In the predisposed infant, the likelihood that allergic disease will develop is influenced by allergen exposure during the first months of life (4, 15, 20, 26) and by various non-specific environmental factors, including parental smoking (1). Wahn et al. (25) in their study in Germany reported higher mite-growth in coal-heated homes. In our study, mite-allergen concentrations tended to be higher in houses with smokers.

The major cat allergen *Fel d 1* was detected in all homes, even in those where there had supposedly not been a cat for years. This allergen is not easy to eliminate from an indoor environment (30), and the allergen may be introduced into the house by an occasional visit of a cat or from the clothes of visitors keeping cats. This possibility can be supported by a study of dust in several Swedish schools (Munir et al., Allergens in school dust, Submitted), where horizontal surfaces of chairs, which contained more *Fel d 1* allergen than tables and floors, were supposedly contaminated by *Fel d 1* from the clothes of teachers and children keeping cats, as the total amount of cat allergen was related to the number of teachers and students with cats at home.

Significantly higher amounts of *Fel d 1* in the winter are most likely because cats spend more time indoors during the winter and windows and doors are closed.

Cat antigens were remarkably ubiquitous, and as the total mite-allergen content was lower than that of *Fel d 1*, we assume that the problems caused by cat allergen are of greater significance than those of mite allergens in this region. This agrees with previous findings by our group (9), as sensitization to cat was the most common finding in asthmatic children, being present in 63%, while mite sensitization was found in 25% of the same children.

Current notions are that the risk level of mite-allergen exposure for both sensitization and the development of asthma is equivalent to 2 µg *Der p 1*/g of dust (27). Risk levels for cat allergen *Fel d 1* sensitization have not yet been identified, but Gelber et al. (8) recently reported that 8 µg of *Fel d 1*/g of fine dust can trigger asthma in cat-sensitive patients. We selected newborn babies with a biparental history of allergy, i.e. at high risk for allergy, and analyzed dust samples from their homes four times during their first year of life. As the prevalence of mite sensitization is rapidly increasing in our catchment area and as the levels of mite allergens in house dust are still relatively low, our data may indicate that levels below 2 µg/g dust can be enough to sensitize those who are especially prone to allergy. Environmental adjuvant factors may affect the threshold levels of allergen.

The sensitivity of allergic patients to a certain allergen may differ by 4 powers of 10, i.e. 10 000 times (7). This is true for all common types of allergens. Hence, sensitized individuals will react with symptoms over a broad range of concentrations of allergen in the environmental dust. There may very well be a similarly wide range in the level of exposure needed for sensitization. The relation between exposure to an allergen and subsequent development of sensitization and allergic disease may vary between individuals and in the same individual over time. Therefore, the proposed level of sensitization probably may not be true for all ages, individuals, geographical areas, and climatic conditions.

In a previous study in a Stockholm area with a similar climate to ours, no significant mite-allergen levels were found (22), but, more recently, Nordvall et al. (18) reported an increasing rate of sensitization to house-dust mite, and a relation to dampness in the houses. Further, Wickman et al. (28) showed that the major mite-allergen levels were below 1 µg/g dust in the homes of house-dust-mite-sensitive children.

In conclusion, the major dust-mite antigens *Der p 1*/*Der f 1* and cat antigen *Fel d 1* were detected at least once in all investigated homes. Among the mites *D. pteronyssinus* was predominant. The mite-allergen levels in the homes of babies with a family history of allergy were, however, far below those proposed as sensitizing. Modern construction patterns of buildings and energy-saving measures can create an indoor climate favoring mite growth and/or mite sensitization, and this may explain why children are increasingly becoming sensitized to house-dust mites in Sweden. As the individual patient threshold for symptomatic asthma varies, sensitization may possibly occur at an exposure level below 2 µg/g dust in a cold climate. The levels of cat antigen were higher, and this would explain the fact that allergy to pets is more common than mite allergy in this region.

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