

Original article

Levels of house-dust-mite allergen in homes of nonallergic people in Pavia, Italy

Background: HDM distribution varies between geographic areas and may be affected by housing characteristics. We quantified Der p 1 and Der f 1 and assessed the relationships between their levels and housing characteristics in homes of nonallergic subjects.

Methods: Der f 1 and Der p 1 were measured by ELISA in dust samples from living-room floors and mattresses of 44 homes of nonallergic subjects in Pavia. Information about household characteristics was obtained by questionnaire.

Results: Der p 1 and Der f 1 concentrations ($\mu\text{g/g}$ dust, median) were 0.34 and 7.8 on mattresses, and 0.15 and 0.83 on living-room floors. Higher Der f 1 levels on mattresses were associated with synthetic pillows ($P < 0.05$), and (only when expressed as $\mu\text{g/m}^2$) with bedding washing temperature of $\leq 60^\circ\text{C}$ ($P < 0.05$). Der f 1 levels were higher on the living-room floors of homes located on lower ($\leq$ first) floors ($P < 0.05$). Good correlations were found between Der p 1 and Der f 1 expressed as $\mu\text{g/g}$ dust and $\mu\text{g/m}^2$ on both mattresses and living-room floors.

Conclusions: In homes of nonallergic subjects in northern Italy, Der f 1 exceeded the threshold for sensitization in a high proportion of mattress samples.

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Key words: *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; Der f 1; Der p 1; environment; house-dust mite; household characteristics; pillow; risk factors.

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House-dust mites (HDM) have been recognized as the main source of allergens in dust for a number of years. Exposure and sensitization to HDM have been suggested to be a major cause of asthma in most parts of the world. There is evidence of a dose-response relationship between exposure and sensitization to mite allergens or onset of allergic respiratory symptoms (1). Thus, evaluation of exposure to HDM allergens is critical to evaluate risk factors for sensitization and elicitation of symptoms in sensitized subjects. Moreover, such information helps in addressing correctly the problem of reducing exposure levels, as strongly recommended in the most recent guidelines on asthma (2).

Homes of nonallergic people may reflect exposure levels of the general population better than homes of allergic people. In fact, on the one hand, in homes of HDM-sensitized subjects, higher levels of HDM allergens have been found, and such levels could represent one of the factors provoking sensitization in these subjects (3). On the other hand, in homes of

allergic people, allergen levels may have been reduced by environmental measures recommended to sensitized subjects.

The recent availability of immunoassays with monoclonal antibodies for Der p 1 and Der f 1 has enabled many investigators to quantify exposure to HDM in epidemiologic studies. Only a few studies (4, 5) have been performed in Italy, and they have not addressed the possible relationship between home features and levels of HDM allergens.

The aim of this study was to quantify the levels of Der p 1 and Der f 1, and to assess the possible relationships with home and bed characteristics in a sample of homes in a city in northern Italy.

Material and methods

Homes

We investigated the homes of 44 subjects living in Pavia recruited from a group of subjects ($n = 71$) who

responded to an invitation, published in a local newspaper, to take part in an extensive survey of the effects of traffic exhaust on the levels of some indoor pollutants. Inclusion criteria were occupation of the present dwelling for at least 10 months/year in the last year, no known history of allergic diseases, and not having undertaken allergen avoidance measures. Selected subjects were equally distributed for age, residential area (urban and suburban), and social level (occupation, educational level).

Pavia is a historic town of 80 000 inhabitants in northern Italy. Its climate varies between seasons, with a high relative humidity all year round. In 1998, the outdoor mean relative humidity was 66% and the mean temperature 12.9°C. Winter months are relatively cold and humid (in the period November 1997–April 1998, mean monthly temperatures were 5.4°C, 1.6°C, 2.7°C, 6.8°C, and 9°C; mean monthly relative humidities were 72%, 76%, 83%, 68%, 56%, and 65%).

Questionnaires

All participants completed a self-administered questionnaire on home and bed characteristics: location of the house (urban or suburban), year of construction, area of the home, number of windows, type of heating, presence of air-conditioning, type of floor covering, presence of factors affecting the dust reservoir (carpets, upholstered furniture, stuffed toys, pelouches, wallpaper, curtains, and bookshelves), type of mattress and pillow, age of mattress, presence and type of mattress and/or pillow covers, and frequency and temperature of bedding washing. Information about number of occupants, presence of pets, occupation, and educational level of participants was also collected.

Telephone interviews were used to complete unanswered questions and clarify ambiguities.

House dust collection

Home visits took place over a period of 1 month (April 1998). Participants were instructed not to vacuum-clean their homes and not to change bedding for at least 1 week before the visit. Collection was performed as described by Dreborg et al. (6). Briefly, a trained technician collected the samples in a standardized manner by means of a dust-collection device containing a cellulose filter (ALK Laboratories, Hørsholm, Denmark) connected to a HEPA filter vacuum cleaner (1200 W). The ALK filter retains 74% of particles 0.3–0.5 µm in size, 81% of particles 0.5–1 µm, 95% of particles 1–10 µm, and 100% of larger particles. Areas of 4 m² of the living-room floors and the mattresses were vacuumed separately for 2 min for each m². The samples from the floors were taken from the central area of the room, and mattresses were vacuumed on their upper surface after removal of the bed linen. Dust collection was performed by the same technician.

Measurement of mite allergens in dust samples

The dust samples were sieved to remove large particles by means of a 300-µm sieve, and weighed. The dust was extracted together with the filter paper by agitation in dust extraction buffer (0.125 M ammonium hydrogen carbonate buffer + 0.1% sodium azide) (ALK Laboratories, Hørsholm, Denmark), in a 1:100 dust wt/v ratio for 2 h at room temperature (15–30°C). The suspension was centrifuged for 15 min at 1000 g. Supernatants were stored at –20°C until analysis. Allergen content of the supernatant was measured by ELISA, as previously described (7). Briefly, 100-µl dust extracts, references, and controls were added to the precoated wells for overnight incubation, followed by repeated washings. After a 1-h incubation with peroxidase-conjugated antibody and repeated washings, the substrate containing 1,2-phenyl-diamine dihydrochloride was added. After incubation for 15 min, the reaction was stopped by addition of sulfuric acid. The absorption at 490 nm was then immediately read. The concentrations in the samples were calculated from the standard curve. Results were expressed as both µg/g fine dust and as µg/m². The detection limit of the assay was 0.6 ng/ml.

All ELISA determinations were performed by the same technician.

Statistical analysis

The different parameters did not approach a normal distribution, even after log transformation. Results were expressed as median, 1st–3rd quartiles. The nonparametric Mann–Whitney *U*-test was used to compare Der p 1 and Der f 1 levels in the different subgroups. Correlation between variables was assessed by Spearman correlation coefficient (*rho*). Statistical analysis was performed with SPSS for Windows.

Results

All recruited subjects agreed to comply with the protocol requirements.

Der p 1 and Der f 1 were detected in all samples. The highest levels were found on mattresses (Table 1), and Der f 1 levels were generally higher than Der p 1 in most samples (Table 1). On mattresses, Der f 1 levels were significantly higher when synthetic pillows were used (12.22, 7.34–14.63 µg/g dust; 0.9, 0.43–1.35 µg/m²) instead of wool or feather pillows (7.33, 3.07–8.68 µg/g; 0.48, 0.15–0.89 µg/m²) (*P* < 0.05; *P* < 0.05) (Fig. 1). Der f 1 levels were lower when bedding was washed at temperatures of 61–90°C (7.72, 2.06–14.62 µg/g; 0.41, 0.13–0.49 µg/m²) in comparison with bedding washed at ≤ 60°C (8.24, 6.13–13.76 µg/g; 0.86, 0.44–1.24 µg/m²). The difference reached statistical significance only when levels were expressed as µg/m² (*P* < 0.05) (Fig. 2). The concentrations of Der p 1 in the group washing at

Table 1. Levels and distribution of Der p 1 and Der f 1

	Mattress	Living-room floor
Der p 1 ($\mu\text{g/g}$) (median, 1st–3rd quartile)	0.34 (0.17–0.65)	0.15 (0.11–0.21)
Der p 1 ($\mu\text{g/m}^2$) (median, 1st–3rd quartile)	0.02 (0.014–0.06)	0.006 (0.003–0.009)
Der f 1 ($\mu\text{g/g}$) (median, 1st–3rd quartile)	7.76 (5.5–13.75)	0.83 (0.33–1.94)
Der f 1 ($\mu\text{g/m}^2$) (median, 1st–3rd quartile)	0.73 (0.32–1.1)	0.03 (0.01–0.09)
Homes (<i>n</i> , %) with Der p 1 > 2 $\mu\text{g/g}$	5 (11.4)	0 (0)
> 10 $\mu\text{g/g}$	0 (0)	0 (0)
Homes (<i>n</i> , %) with Der f 1 > 2 $\mu\text{g/g}$	38 (86.4)	10 (22.7)
> 10 $\mu\text{g/g}$	17 (38.6)	0 (0)

temperatures of $>60^\circ\text{C}$ (0.35, 0.26–0.66 $\mu\text{g/g}$; 0.018, 0.009–0.034 $\mu\text{g/m}^2$) were not significantly different from those of the group washing at lower temperatures (0.32, 0.16–0.67 $\mu\text{g/g}$; 0.023, 0.015–0.086 $\mu\text{g/m}^2$), although there was a trend ($P=0.11$) to lower levels of Der p 1/ m^2 in the group washing at higher temperatures. When bedding was washed at temperatures of $>60^\circ\text{C}$, the weight of fine dust collected on mattresses was significantly lower (0.25, 0.09–0.28 g) than that of bedding washed at $\leq 60^\circ$ (0.35, 0.22–0.43 g) ($P<0.05$).

On living-room floors, Der p 1 and Der f 1 levels were generally low (Table 1). Der f 1 levels were significantly higher on the living-room floors of homes located on lower floors ($\leq$ ground and first) (1.54, 0.53–3.28 $\mu\text{g/g}$; 0.07, 0.018–0.1 $\mu\text{g/m}^2$) than those on upper floors (0.5, 0.22–1.02 $\mu\text{g/g}$; 0.014, 0.01–0.03 $\mu\text{g/m}^2$) ($P<0.05$ for $\mu\text{g/g}$ and $\mu\text{g/m}^2$) (Fig. 3).

The proportion of samples with levels of HDM allergen above the thresholds of 2 and 10 $\mu\text{g/g}$ dust is shown in Table 1.

A weak correlation was found between concentrations of Der f 1 on mattresses and in the living-room

($\rho=0.37$; $P<0.05$). A good correlation ($P<0.0001$) was found between Der p 1 and Der f 1 expressed as $\mu\text{g/g}$ and $\mu\text{g/m}^2$ on both mattresses (Fig. 4) and living-room floors (Fig. 5), except mattresses whose bedding was washed at a temperature higher than 60°C , as stated above.

No association was found between the concentration of mite allergens and the other home characteristics, nor between concentration of allergens and occupation or educational level of the participants in the study.

Discussion

In our study, we evaluated Der p 1 and Der f 1 levels in dust samples collected in homes of nonallergic subjects living in a town in northern Italy. We found HDM allergens in every home, with a predominance of Der f 1 over Der p 1. The highest levels of HDM allergens were detected on mattresses, where Der f 1 exceeded the threshold for sensitization in a high proportion of samples.

In our study, Der f 1 was found to predominate in most samples. Predominance of *Dermatophagoides farinae* had also been found in Rome, where levels of exposure were evaluated by counting mites in samples

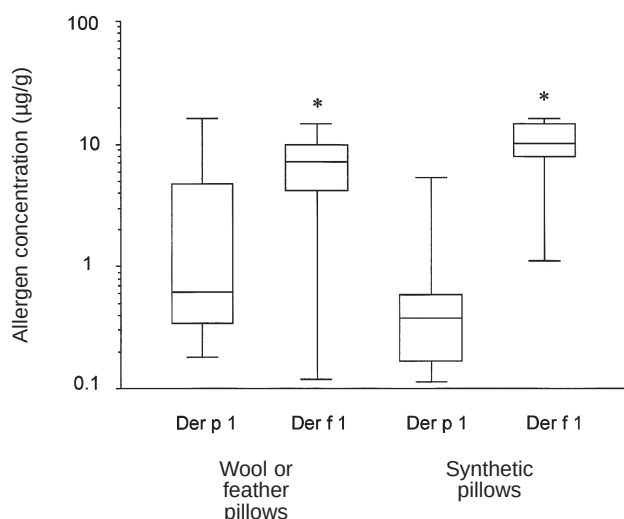


Figure 1. Der p 1 and Der f 1 concentrations on mattresses according to pillow material (* $P<0.05$).

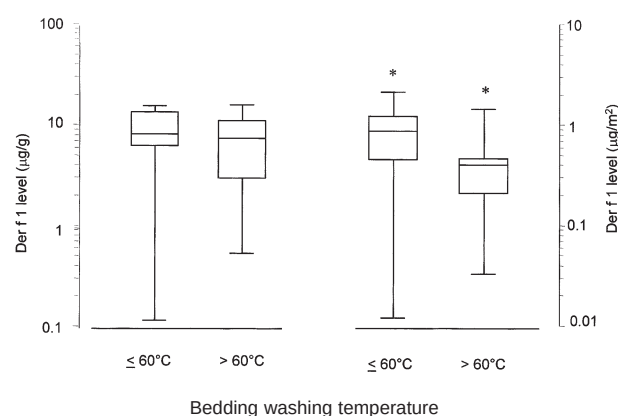


Figure 2. Der f 1 levels on mattresses according to bedding washing temperature (* $P<0.05$).

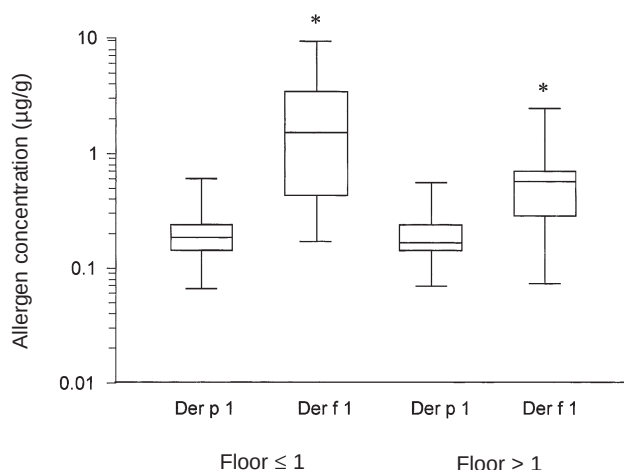


Figure 3. Der p 1 and Der f 1 concentrations on living-room floors according to floor of home (* $P < 0.05$).

from mattresses (4), and in three different regions (northern, central, and southern) of Italy, where Der f 1 was higher than Der p 1 in samples from beds and floors (5). Similar data were found in Sweden (8), Germany (7), and France (9). The predominance of Der f 1 in our sample may be related to climatic factors. In fact, it is known that *D. farinae* better survives long periods of low relative humidity (10), which may occur indoors in Pavia during the winter, when, as a consequence of the low external temperature, air is heated up to 20°C, resulting in a significant reduction of relative humidity (11). A number of studies have already measured HDM allergens in homes of nonallergic people. For mattresses, we found Der p 1 levels comparable to those

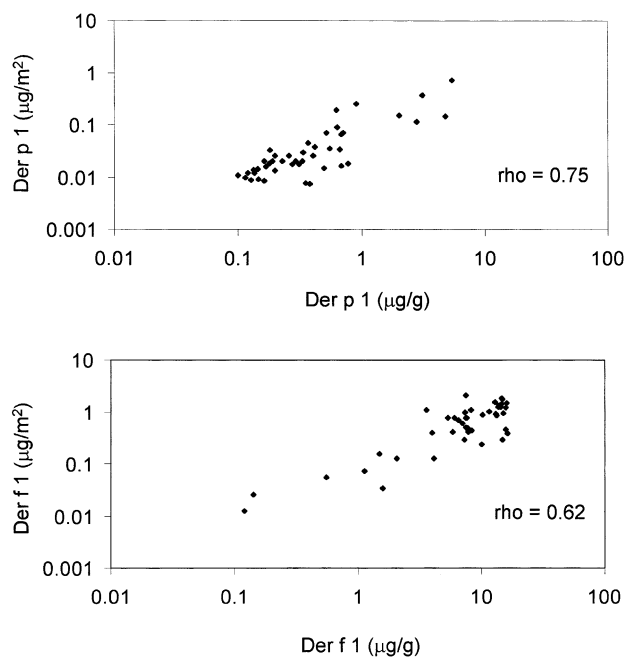


Figure 4. Correlation between Der p 1 and Der f 1 expressed as $\mu\text{g/g}$ and $\mu\text{g/m}^2$ on mattresses ($P < 0.0001$).

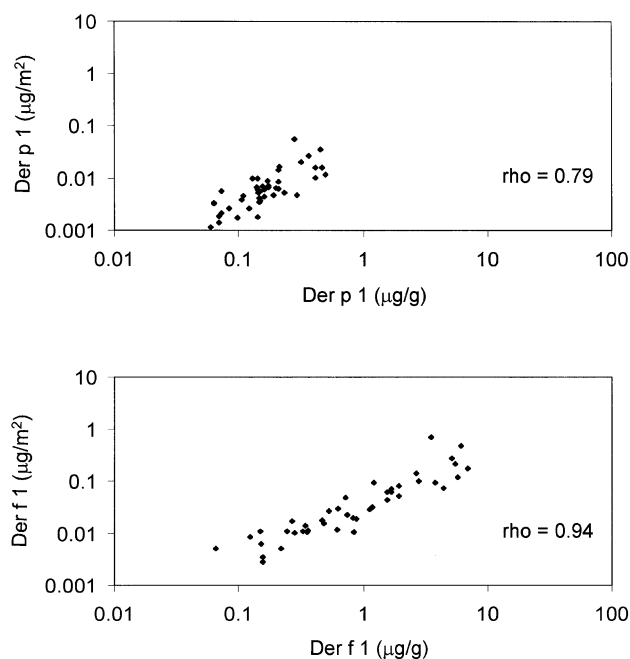


Figure 5. Correlation between Der p 1 and Der f 1 expressed as $\mu\text{g/g}$ and $\mu\text{g/m}^2$ on living-room floors ($P < 0.0001$).

detected in France (9), lower than those found in Germany (12) and Southampton (UK) (13), and higher than those detected in Norwich (UK) (14) and Denmark (15). Der f 1 levels were higher than in Germany (12), Portugal (3), and Denmark (15), but were comparable to those found in France (9). For living-room floors, Der p 1 levels found in our study were comparable to those detected in Norwich (14), but lower than those detected in Southampton (13).

Exposure to mite allergen is necessary for sensitization, and mite sensitization is a risk factor for asthma. There is evidence of a dose-response relationship between the degree of this exposure and sensitization or allergic respiratory symptoms (1); levels of 2 and 10 $\mu\text{g/g}$ of group I allergen have been proposed as the risk thresholds for sensitization and elicitation of symptoms of asthma, respectively (1), although recent works suggest that exposure to lower allergen levels is required to avoid sensitization (16). In our study, levels of Der f 1 of $> 2 \mu\text{g/g}$ on mattresses were detected in 38 (86.4%) homes, and of $> 10 \mu\text{g/g}$ in 17 (38.6%) homes, showing that a high proportion of people are exposed to levels of HDM allergens exceeding current risk thresholds.

The relatively high levels of HDM allergens found in our study could be related to the high humidity of Pavia's climate. It is well known that HDM distribution is associated with indoor humidity, which is related, in turn, to outdoor humidity (11). Indeed, the highest numbers of mites are detected in regions with high levels of outdoor humidity (17–20). Such an association has also been observed in Italy

(5). As concerns the possible effect of low outdoor winter temperatures in Pavia, we assume that the resulting low indoor humidity may not be sufficient to inhibit mite growth, at least *D. farinae* (see above). As regards levels of HDM allergen on the mattresses, we did not find any association with mattress material, as in previous studies (15, 21). By contrast, an association between pillow material and levels of HDM allergen was observed, the concentration of Der f 1 on mattresses of beds with synthetic pillows being higher than on those with wool or feather pillows. It has recently been reported that the dust collected by vacuuming polyester-filled pillows contains more Der p 1 than that from feather pillows (22, 23). The results of our study suggest that the same may happen for Der f 1. In our study, we sampled only the mattresses, and not the pillows; however, interestingly, in our study, the presence of synthetic pillows seemed to affect HDM levels on mattresses. Asthmatic patients are commonly instructed to avoid feather pillows and to replace them with those filled with synthetic materials: at present, this point is under discussion (24), and our results, which need to be confirmed, further support the need for a review of this practice.

In agreement with some reports (14, 25), and in contrast with others (26), we did not find any association between levels of HDM allergen on mattresses and the age of the mattresses. Custovic et al. (27) showed that new mattresses may become a significant reservoir of mite allergens after a short period of time (4 months): this finding can explain our results, since all mattresses of our study were older than 6 months. Der f 1 levels on mattresses expressed as $\mu\text{g}/\text{m}^2$ were related to the washing temperature of bedding; that is, the levels were lower when bedding was washed above 60°C , while no difference was found when a lower threshold was used in subgrouping (i.e., 55°C). A trend in the same direction for Der p 1 concentration per area, but not per weight of dust, was also found. McDonald et al. (28) have shown that mites are killed by water temperature of 55°C or higher. On the other hand, Luczynska et al. (14) did not find any difference in Der p 1 concentrations, expressed as allergen weight/fine dust weight, when washing at different temperatures. These different results may be accounted for by the different expressions of allergen levels; that is, number of killed mites for McDonald et al. (28) and allergen weight/fine dust weight for Luczynska et al. (14).

In our study, a significant difference in allergen concentration on mattresses with bedding washed at different temperature was found only when the results were expressed as $\mu\text{g}/\text{m}^2$. Such results could be related to the significantly lower weight of dust collected from mattresses whose bedding was washed at

temperatures of $>60^\circ\text{C}$. As all registered parameters were not different in the two subgroups, we have little explanation for our finding, which needs to be confirmed. We hypothesize that the lower weight of fine dust recovered from mattresses whose bedding was washed at higher temperatures may significantly affect the allergen weight/dust weight ratio, inducing an overestimation of allergens. Thus, our results suggest that allergen levels should be expressed both in $\mu\text{g}/\text{g}$ and in $\mu\text{g}/\text{m}^2$, as it has already been suggested in those cases when particular intervention (e.g., using acaricidal products) may affect the weight of dust (29).

As regards levels of HDM allergens on living-room floors, we found higher levels of Der f 1 in homes on lower floors, in agreement with other studies (3, 12, 14, 21, 25). This could be attributed to higher humidity on lower floors, a condition which favors mite growth.

In previous studies, high levels of HDM allergen were found to be associated with a large number of occupants in homes (3, 30, 31). We did not find such a relationship, in agreement with the observation that the number of occupants in homes may not represent a conditioning factor for mite growth, the amount of human dander shed by only one person being adequate to feed a large population of mites (11).

In agreement with Custovic et al. (29), we found a good correlation between the results of Der p 1 and Der f 1 expressed as $\mu\text{g}/\text{g}$ and $\mu\text{g}/\text{m}^2$ on both mattresses (except when bedding was washed at temperatures of $>60^\circ\text{C}$) and, particularly, living-room floors.

In conclusion, we aimed to determine levels of HDM allergens in homes of nonallergic subjects in a town in northern Italy. We found that HDM allergens were ubiquitous, with Der f 1 predominant over Der p 1. The highest allergen levels were detected on mattresses, where Der f 1 exceeded the current threshold for sensitization in a high proportion of samples. Living on lower floors may imply exposure to higher levels of HDM allergen. A good correlation was found between results expressed as μg of allergen/g of dust and μg of allergen/ m^2 , except in mattresses when bedding was washed at temperatures of $>60^\circ\text{C}$, from which dust samples of significantly lower weight were obtained. Expressing results both in $\mu\text{g}/\text{m}^2$ and in $\mu\text{g}/\text{g}$ of dust may better represent exposure, since the weight of collected dust may be affected by avoidance measures or particular treatments.

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