

House dust mites and their allergens at selected locations in the homes of house dust mite-allergic patients

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Summary

Background Knowledge of the occurrence of house dust mites (HDM) and their allergens in domestic locations is important when planning intervention.

Objective The aim of this study was to describe the distribution of HDMs and their allergens before intervention in multiple locations in the homes of newly diagnosed HDM-allergic patients with a known high Der 1 concentration in their mattress dust.

Methods Dust was collected from ten locations in the homes of eight HDM-allergic patients. Dust was analysed for allergen content with ELISAs for Der f 1, Der p 1 and Der m 1; and HDM were counted. Total allergen concentrations (μg Der 1/g dust) were expressed as the sum of Der f 1, Der p 1 and Der m 1.

Results On mattresses the median concentration was $86 \mu\text{g}$ Der 1/g dust (range 30–288) and 188 mites/g dust (range 12–1910). Der 1 exceeded $10 \mu\text{g/g}$ dust in mattresses (8/8), duvets/pillows (3/8), a bedroom carpet (1/1), a living room carpet (1/6), upholstered furniture (2/8) and a curtain (1/5). Uncarpeted floors, upholstered furniture, bookshelves and walls had significantly lower Der 1 concentration than the mattresses. The relative contribution of Der p 1, Der f 1 or Der m 1 to Der 1 was related to homes, rather than to the location. Der m 1 only occurred in minute amounts.

Conclusion For HDM intervention, our results indicate that priority should be given to the removal of allergens from mattresses, and in addition from carpets, duvets/pillows and upholstered furniture. Dust from walls, uncarpeted floors, bookshelves and curtains appear to contribute insignificantly to the domestic HDM allergen load.

Keywords allergens, Denmark, *Dermatophagoides*, dust, floor, house dust mites, housing, mattress

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Introduction

House dust mite (HDM) allergy is a major problem [1], and more evidence-based advice in HDM intervention is in demand [2]. For planning this, general knowledge about the distribution of HDMs and their allergens at different locations in the dwellings of HDM-allergic patients is necessary, but has not previously been available.

Many allergic patients suffer the burdens of a large number of restrictions and inconveniences due to secondary prevention measures suggested by varying parts of the healthcare system. Therefore, to ensure compliance it is almost as important not to give advice that is undocumented or at least made probable, as it is to give evidence-based allergen-reducing recommendations. The intervention can be claimed as documented only when HDM intervention is shown to be clinically effective in

(placebo-)controlled studies. Since only few studies of this kind are available [2], however, advice has to be based on studies on the occurrence of mites or mite allergens. Such studies can also give ideas to the planning of controlled intervention studies. HDM allergens have been shown to be very stable [3], so they must be actively removed from the homes of HDM-allergic patients; and in addition the mites have to be removed, killed or given poorer living conditions to prevent recurrence of allergen.

It is known how HDMs and their allergens are distributed on mattresses, on floors and to a lesser extent on upholstered furniture [4–11]. These studies have focused on the concentrations and not absolute amount. However, knowledge of the absolute amounts and relative distribution is often more relevant when working with HDM intervention, where priority should be given to those sites which contribute quantitatively and significantly to the total load of HDMs and their allergens in a home. Habitats for the living mites and the sites for depots of their allergens are not necessarily identical. Therefore, a description of the occurrence both for the mites and their allergens is relevant.

The aim of this study was to describe the distribution of HDMs and their allergens before intervention at multiple

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locations in the homes of newly diagnosed HDM-allergic patients with a known high Der 1 concentration in mattress dust.

Materials and methods

Selection of homes

Patients were selected from three allergy clinics in Copenhagen and were included based on the following criteria: no previous HDM intervention (newly diagnosed HDM allergy); positive skin prick test to *Dermatophagoides pteronyssinus* or *D. farinae* (> 3 mm); perennial asthma or rhinitis evaluated to be solely or partly caused by the HDM sensitization; a mattress sampling containing $> 10 \mu\text{g}$ Der 1/g dust (sampled by the patients with their own vacuum cleaner 1–3 months before the visit); having a bedroom; having a living room not used for sleeping; and use of the same mattress in the same bedroom for at least one year.

Dust sampling

Dust was collected by one of the authors (K. E. Sidenius) in the same order from as many as possible of ten predefined objects in the patient's most frequently used living room with upholstered furniture and from the patient's bedroom. In the living room, samples were taken from: uncarpeted floor (8 m^2 if possible); carpet (4 m^2 if possible); the patient's most frequently used upholstered sofa or armchair (a sofa was vacuumed for 8 min and an armchair for 4 min); and bookshelf (the top and front of the books, the shelf and other items were vacuumed for 10 min; the number of shelf metres vacuumed were noted). From the living room and the bedroom, dust from the total surface (facing the room) of the curtains was collected in a common sample. In the bedroom, samples were taken from: uncarpeted floor (8 m^2 if possible), carpet (4 m^2 if possible); all pillows and duvets; the top surface of the patient's mattress (the same as at inclusion); and wall (20 m^2 if possible) including the reverse side of pictures on this wall. The vacuumed area of uncarpeted floor and carpet in the living room was chosen to be in the proximity to the vacuumed upholstered furniture, and in the bedroom close to the bed. Uncarpeted floors, carpets, curtains, pillows and duvets were vacuumed for 1 min/m^2 ; the mattresses were vacuumed for 2 min/m^2 ; and the walls were vacuumed for $\frac{1}{2} \text{ min/m}^2$. A dust sampling device containing a cellulose filter (ALK-ABELLÓ, Hørsholm, Denmark) [12] connected to a HEPA-filter vacuum cleaner (Nilfisk-Advance UZ964 (650 W), Åmål, Sweden) was used. Dust samples were kept cool (up to three hours) and then stored at -20°C until extraction.

Allergen and mite load

Areas of uncarpeted floors, carpets and curtains were roughly measured. The number of upholstered sofas and chairs were registered and in the calculations two chairs were set to be equal to one sofa. Bookshelves were measured in running metres. The total area of the bedroom walls was defined as the sum of the four walls including doors and windows.

Based on the weight of the sample, concentration and percentage of the total object vacuumed, the total assessable amounts (load) of allergens and mites on the surface of each of the ten objects were estimated.

Processing of dust samples

After being thawed at room temperature, 0.1 g dust was stirred with 5 mL 80% lactic acid coloured with lignin pink, in an 8.5-cm circular Petri dish, providing a 1–2 mm layer of lactic acid and dust for microscopy [13]. The rest of the sample (including the filter paper) was extracted with 0.125 M ammonium hydrogen carbonate, 0.1% sodium azide [14] and 0.1 M of the protease-inhibitor ϵ -amino caproic acid which was added in a 20-mL polypropylene syringe during constant slow rotating for two hours at room temperature. The dust w/v ratio was 1 : 10, except for dust samples < 0.5 g where 5 mL buffer was used. The ooze was squeezed out of the syringe, leaving the filter and part of the coarse particles behind, and centrifuged for 20 min at 1223 g. The supernatant was filtered using a 0.8- μm acetate filter and stored at -20°C until analysis.

If the sample did contain less than 0.3 g dust, dust for mite identification was not removed before extraction. The total sample was extracted instead; subsequently syringe and filters were rinsed with distilled water. The rinsing water was removed by suction through a 0.45- μm mixed cellulose acetate and nitrate filter supported by a glass filter (Millipore®, Bedford, MA, USA). The concentrate from the filter was stirred with lactic acid, as described above, for mite quantification.

The concentration of Der p 1, Der f 1 and Der m 1 in the supernatant was individually assessed in duplicate by ELISA at ALK-ABELLÓ, Hørsholm, Denmark [15]. The detection limit, 2 ng/mL, was used for calculations when the allergen concentration was below this limit. Der 1 was calculated as the sum of Der p 1, Der f 1 and Der m 1.

After incubation at room temperature for at least one day, the mites in the lactic acid preparation were counted and identified by one of the authors (T. E. Hallas). A stereo microscope with a magnification of $\times 25$ (Olympus type SZ 4045 TR, Tokyo, Japan) was used. For the identification of mite species, the specimens were collected from the lactic acid with a needle, placed on a slide in a drop of Hoyer's medium, and examined by normal phase-contrast microscopy by $100\times$ enlargement or more (Olympus type BX 40, Tokyo, Japan).

Statistical analysis

Wilcoxon Signed Rank test was used when comparing groups, and a P -value < 0.05 was regarded as significant.

The statistical programs of SigmaStat 2.03 (SPSS Inc., Chicago, IL, USA) and Quattro Pro 8 (Corel, Ottawa, Canada) were used.

The data collection was approved by The Danish Data Protection Agency. Informed consent was obtained from the patients.

Results

Eight homes – five apartments and three one-family houses – were examined, representing the homes of five male and three female HDM-allergic patients aged 16–54 years (mean 34 years).

On mattresses the median allergen concentration was $86 \mu\text{g}$ Der 1/g dust (range 30–288) and the median mite density was 188 HDM/g dust (range 12–1910) (Table 1). This was significantly higher compared to the corresponding mattress samples

Table 1. House dust mites and house dust mite allergen (Der 1) in dust from various locations in the homes of eight HDM allergic patients

	Samples (N)	Total dust collected (g)	Der 1 (µg/g dust)	Collected Der 1 (µg/m ²)	Estimated Der 1 (µg) on total surface	Mites/g dust	Collected mites/m ²	Estimated mites on total surface
Bedroom	8	0.83 (0.34–2.70)	85.6 (30.1–288)	31.4 (10.15–383)	89.7 (15.9–689)	188 (12–1910)	77 (2–2865)	207 (4–5157)
Mattress (upper surface)	1	2.45	32.1	19.7	177	210	129	1158
Carpet	8	0.21 (0.11–0.69)*	7.2 (1.3–619)	–	2.0 (0.2–223)*	4 (0–933)*	–	4 (0–336)*
Duvets/pillows	8	0.06 (0.03–0.25)*	5.2 (0.1–91.8)*	0.0 (0.0–0.1)*	5.2 (0.1–9.8)*	129 (0–300)	0 (0–1)*	9 (0–29)*
Wall	7	0.63 (0.33–1.42)*	2.2 (0.7–4.2)*	0.2 (0.0–0.5)	2.0 (0.2–3.6)*	60 (0–167)	4 (0–19)*	64 (0–178)
Uncarpeted floor	8	0.63 (0.33–2.45)	2.4 (0.7–32.1)*	0.2 (0.0–19.7)	2.2 (0.2–177)	90 (0–210)	8 (0–129)	98 (0–1158)
Floor combined (uncarpeted or carpet)								
Living room								
Upholstered furniture	8	0.49 (0.03–0.87)	4.0 (0.7–17.0)*	–	3.4 (0.4–44.2)*	36 (0–167)*	–	10 (0–653)*
Carpet	8	2.06 (0.54–3.06)	3.2 (1.3–43.9)	1.3 (0.3–44.6)	10.3 (1.0–134)	21 (0–7)*	21 (0–82)*	64 (0–329)*
Bookshelf	8	0.08 (0.03–0.29)*	1.8 (0.5–8.7)*	–	0.3 (0.0–5.3)*	0 (0–7)*	–	0 (0–9)*
Uncarpeted floor	6	0.84 (0.20–1.22)	1.5 (0.11–4.0)*	0.1 (0.0–0.5)*	1.9 (0.4–8.1)*	5 (0–111)*	1 (0–14)*	15 (0–227)*
Bedroom and/or living room								
Curtain	5	0.09 (0.02–0.13)	3.4 (1.0–115)	0.0 (0.0–0.6)	0.2 (0.1–2.3)	8 (0–67)	0 (0–4)	1 (0–4)

Results are given as median (min–max). *showed significantly lower values $P < 0.05$ compared to the mattress in a Wilcoxon Signed Rank Test.

collected by the patients at the inclusion of the study (24 µg Der 1/g dust (range 11–114)). Only on mattresses, duvets/pillows, carpets, upholstered furniture and on one curtain, did the concentration exceed 10 µg Der 1/g dust in any of the homes (Fig. 1). On uncarpeted floors, upholstered furniture, bookshelves and walls a significantly lower Der 1 concentration than on the mattresses was found (Table 1).

Dominance of either Der p 1 or Der f 1 was observed in some of the homes, but appeared not to be related to the object vacuumed (Fig. 2). Der m 1 only contributed to the concentration of Der 1 in the small samples from curtains and bookshelves and did not add significantly to Der 1 in the mattresses, carpets and pillows/duvets (Fig. 2a).

If results from all dust samples were pooled as if it had been one sample, the ratios would be 230 ng Der p 1 per *D. pteronyssinus* mite, and 289 ng Der f 1 per *D. farinae* mite. Including only samples with at least one mite of the relevant species, the median ratios (range) were 38 (5–10231) ng Der p 1 per *D. pteronyssinus* mite ($n = 30$); and 68 (9–6665) ng Der f 1 per *D. farinae* mite ($n = 24$).

Discussion

This study describes the distribution of HDM and their allergens at selected locations in the homes of HDM-allergic patients. These homes were chosen to increase the clinical relevance of the results, and because the allergen load is usually lower in the homes of non-allergic persons [6, 16], and even lower in public places [17–22]. It is important that none of the patients had previous knowledge of their HDM allergy and thus none of them had performed any deliberate HDM intervention, before the visit, in the locations where the samples were collected. In addition, all patients were exposed to > 10 µg Der 1/g mattress dust, a concentration which is capable of causing allergic symptoms [23].

The generally accepted way to assess the HDM exposure level is to measure the concentration in (sedimented) removable dust [12], as an estimate of the amount of inhalable (airborne) allergen, which is difficult to measure in a standardized way. The total HDM load in a room or in an object, as estimated in the present study, could be of relevance too, as it sets an upper limit for the possible amounts of inhalable dust originating from the vacuumed objects. An object with high allergen concentration but only small amounts of releasable dust would tend to be clinically less relevant than objects with large amounts of dust, even if they represent lower allergen concentrations.

We found bedrooms to have the highest HDM concentrations (Fig. 1, Table 1), which is in concordance with other studies [6, 24, 25], even though some studies show living rooms to have the same or higher HDM concentrations [8–10]. Climate variations and diversities in living habits in various regions might in part explain such differences. Mattresses had, together with the only carpet placed in one of the bedrooms, the highest HDM level concerning both concentration and total assessable surface allergen/mite loads. Duvets/pillows, upholstered furniture and living room carpets showed, in some of the samples, a high allergen and mite concentration as well as a high total load. Dust from the walls, uncarpeted floors, bookshelves and curtains had only a minor influence on the HDM load due to low concentration in general as well as low amounts of dust.

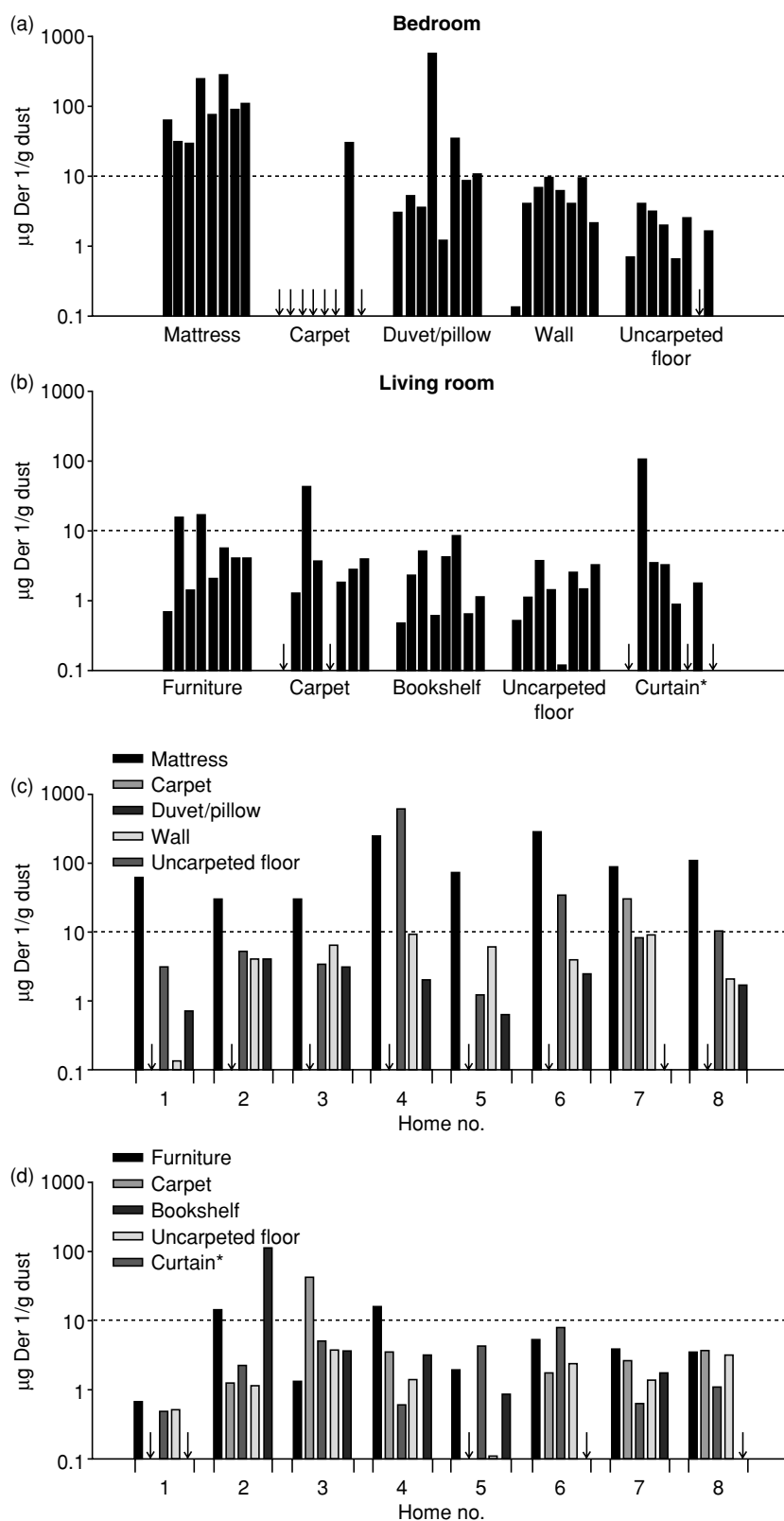


Fig. 1. Der 1 concentration in dust samples from homes of house dust mite allergic patients, sorted by object (a and b) and home (c and d). (a) and (b): results are presented from homes 1–8. Arrow indicates that the object was not present. * from bedroom and/or living room. Inserted line indicates 10 $\mu\text{g Der 1/g dust}$.

Even though the homes were less than 20 km apart, the relative dominance of the three mite species expressed by the occurrence of their major allergens Der p 1, Der f 1 and Der m 1 differed considerably. More than half of the homes were

dominated by either *D. pteronyssinus* or *D. farinae*, but homes with mixed populations were observed too, as in previous studies [25, 26]. *D. microceras* was not found by microscopy, and only small total amounts of the corresponding allergen Der m

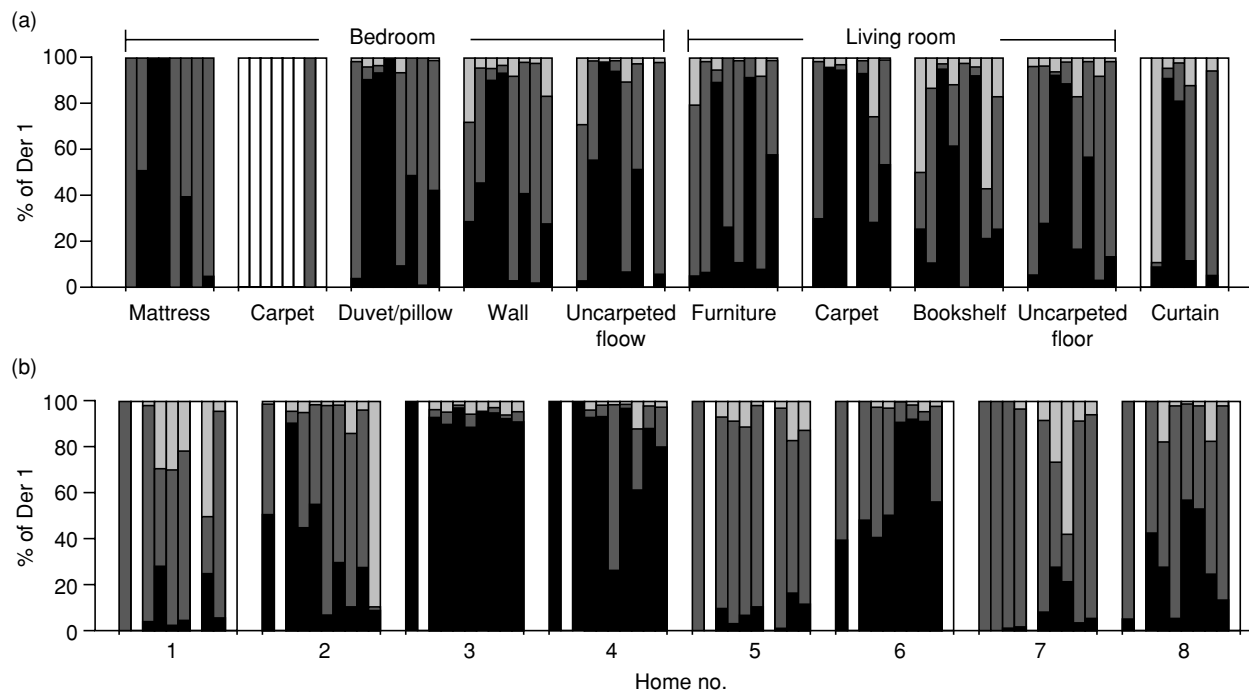


Fig. 2. Der p 1 (■), Der f 1 (■) and Der m 1 (□) in percentages of total Der 1 in dust samples from the homes of HDM-allergic patients, sorted by object (a) and by home (b). (□) indicates that the object was not present. (a): Results are presented from homes 1–8. (b): Results are presented in same order as for the objects shown in (a).

1 were observed. Consequently, at least in the Copenhagen area, it seems necessary, but also sufficient, to include the two major allergens Der p 1 and Der f 1 when estimating the HDM allergen exposure.

The various HDM species may benefit from different climatic conditions [27]; so one species could be expected to dominate in one location, such as the floor, and another to dominate elsewhere. This was not the case in our study, not even in the homes with mixed HDM populations (Fig. 2). One explanation could be that the climatic differences at various locations within one home are less pronounced, than the differences between homes. Alternatively, the majority of mites could originate and disperse from other locations than where they were found and thus the climatic conditions at these sites would determine the kind of species appearing.

The proposed equivalence of 500 mites/g dust to 10 µg Der 1/g dust [23], seems to be considerably higher than observed in the present study. We found 10-fold more allergen per mite when results from our acarological counting and allergen measurements were pooled, while the ratios in each sample varied considerably. Different results have been observed in other regions [11]. Since HDM allergens are very stable [3], the age of the mite population is a crucial factor for the relation between the amount of accumulated allergen and the number of intact mites, and a universal conversion factor cannot be expected in domestic dust.

In conclusion, when planning or performing HDM intervention, our results indicate that priority should be given to the removal of allergens from mattresses, and in addition from carpets, duvets/pillows and upholstered furniture; whereas dust from walls, uncarpeted floors, bookshelves and curtains do not seem to contribute significantly to the domestic HDM load.

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