

Collection of House Dust for Analysis of Mite Allergens

Allergen-Reducing Effect of a Self-Administered Procedure

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To assess allergen-reducing effect of dust collection from mattresses, patients were asked to vacuum clean the entire surface of their mattresses for 5 min with their household cleaners at specified intervals ranging from 1-21 days. Ten patients performed four collections, nine patients only two. Amounts of dust, concentrations and amounts of major allergens from *Dermatophagoides pteronyssinus* (Dp42), *Dermatophagoides microceras* (Dm6), and *Dermatophagoides farinae* (Df6) were determined. The first sampling caused a statistically significant reduction in the absolute amounts of allergens in a following sample. The same tendency was seen in dust weight but not in concentrations of allergens. At intervals of 1 or 3 weeks no consistent changes could be registered. Differences were small and good reproducibility of the sampling and analysing procedure could be assumed. Since the self-administered procedure is much cheaper and easier to handle than sampling done by technicians with special equipment, it can be recommended for sequential analysis of allergen exposure.

Key words: allergen analysis; *Dermatophagoides* species; dust sampling; enzyme immunoassay/ELISA; house dust mites; reproducibility.

Accepted for publication 11 March 1986

Examination for different allergenic components in dust is a useful tool in clinical allergological work. It can be used at home or workplace to reveal hidden allergenic exposures when an allergy has been verified. Furthermore, dust examinations can be valuable in controlling the effects of different allergen-reducing measures, i.e. exclusion of animals or changes in indoor climate. Animal dander and hair on clothing, carpets, or furniture, could trigger allergic symptoms without the patients being aware of any exposure. In house dust allergy dust analyses are often of even greater importance since it is the only means of verifying an allergen contact.

Recent progress in allergen purification and characterization (4, 7) has led to the development of specific assays, such as radioim-

munoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), for measurement of house dust mite allergen content in dust (9, 10). The existence of strongly immunogenic, species-specific epitopes on the excrement major allergens (Dp42 = P1 of *Dermatophagoides pteronyssinus* and analogues Df6 and Dm6 of *Dermatophagoides farinae* and *Dermatophagoides microceras*) has allowed production of immunoabsorbed rabbit antibodies recognizing each single species (8). Thus, quantitative information can be obtained from each dust sample regarding major allergen content and species composition.

The aim of the present study was to investigate the effect of repeated vacuuming on the load of house dust mite allergens and removable dust in beds.

MATERIAL AND METHODS

Nineteen house dust-allergic patients with previously demonstrated mite fauna in their beds participated in the study. Patients were requested to collect dust samples with their own vacuum cleaners at different intervals during the months of December and January. By written instruction the participants were requested to install new dust bags in their cleaners and to vacuum their bed cover, mattress and covering mattress for exactly 5 min. As soon as possible the dust bag was mailed to the investigators and kept at -18°C until analysis.

Ten patients were instructed to collect dust four times at intervals of 1 day, 1 week and 3 weeks (Group I), and nine patients performed dust collections twice at an interval of 3 weeks (Group II). A variety of vacuum cleaners and bags were used, but each patient maintained the same kind of equipment throughout the study.

The dust bags were sliced upon and the recovered dust content of each bag was weighed. Dust preparation and ELISA procedure for measurement of major allergens Dp42, Df6, and Dm6 of the three *Dermatophagoides* species: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Dermatophagoides microceras*, is described elsewhere (8). Briefly, extraction took place 1:5 (w/v) in 0.125 M ammoniumhydrogencarbonate. One hundred μl dust extracts and dilutions thereof (1:10, 1:100 and 1:1000) were transferred to wells in polystyrene microtiter trays, previously coated with affinity-purified, monospecific rabbit antibodies to the major allergens Dp42, Df6, or Dm6. After overnight incubation and repeated washings, horse radish peroxidase-conjugated rabbit antibodies to allergens Dp42, Df6, or Dm6, rendered species-specific by sepharose column immunoabsorption, were added. After a 1 h incubation period and repeated washings, substrate (hydrogenperoxide/orthophenylene-diamine) was added to each well and reaction carried out for 15 min in darkness. Reading of the absorption at 492 nm took place 5 min after addition of 1.0 M sulphuric acid. Standard response curves were constructed from refer-

ence whole mite culture extract solutions of each species with major allergen concentrations in the range 1.2–600 ng/ml. Assay sensitivity was 8, 3, and 30 ng/ml for allergens Dp42, Df6 and Dm6, respectively.

The second bag from each patient was examined more closely in order to determine the amount of allergen retained on the bag wall after removing dust. A 2 cm broad band was cut out from top to bottom of the bag. The mite allergens were extracted from this band by 3 ml 0.125 ammoniumhydrogencarbonate for 3 h at 20°C and measurement performed by ELISA as mentioned above. Total allergen amount left on the bag wall was estimated from the measured surface area of the entire bag and of the paper band analysed.

Statistics

Paired t-test was used in comparison within the two groups of dust collections, and differences between the groups were evaluated by t-test. To reduce the risk of obtaining mass-significance when analysing the same data set in several ways, only differences resulting in P -values < 0.01 were regarded as significant. To approximate to normal distribution, log values of allergen concentrations and allergen amounts were used in data analysis. Regression analysis was performed on data from each participant in Group I, and relative changes in dust load and allergen contents with 95% confidence limits were calculated for the samples collected at a 3-week interval.

RESULTS

Concentrations of major allergens measured in the dust samples varied from < 15 ng/g dust (detection limit) to 300,000 ng/g, with *D. pteronyssinus* as the dominating species, followed by *D. microceras* and *D. farinae* (Fig. 1). When major allergens were expressed as total weight in samples, the Dp dominated again (Fig. 2). Mixed populations were found in all homes except one, where only *D. pteronyssinus* allergen was recorded. All possible combinations of the presence of two species in the same mattress were observed

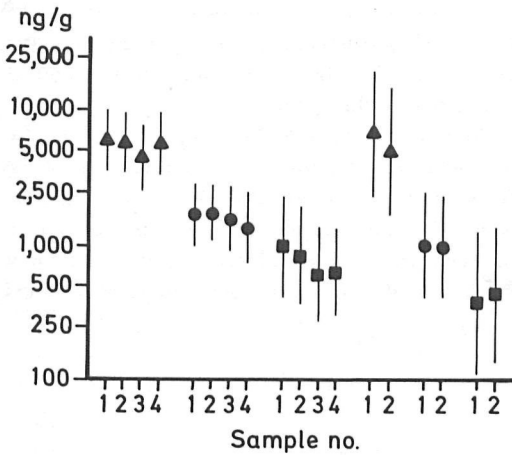


Fig. 1. Concentrations in bed dust of major allergens from three *Dermatophagoides* species (mean $\pm$ SEM). Four or two samples (Group I and Group II) from each bed analysed at different intervals (see text). $\blacktriangle$ *D. pteronyssinus*, Dp42. $\bullet$ *D. microceras*, Dm6. $\blacksquare$ *D. farinae*, Df6.

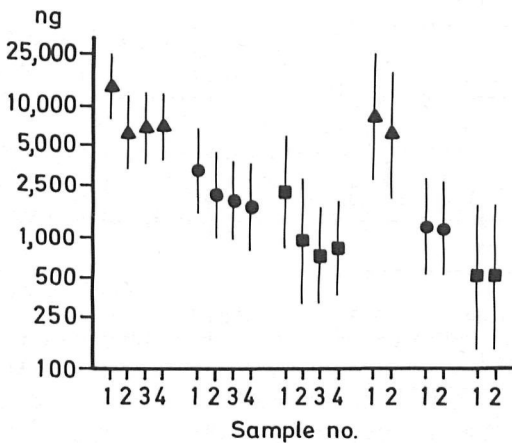


Fig. 2. Total weight of major allergens from three *Dermatophagoides* species in bed dust (mean $\pm$ SEM). Four or two samples (Group I and Group II) from each bed analysed at different intervals (see text). $\blacktriangle$ *D. pteronyssinus*, Dp42. $\bullet$ *D. microceras*, Dm6. $\blacksquare$ *D. farinae*, Df6.

(Dp + Df, Dp + Dm and Df + Dm). In 11 of 21 mattresses all three species were recorded, although in very different concentrations. The species distribution in each home was constant during the study period. The amount of re-

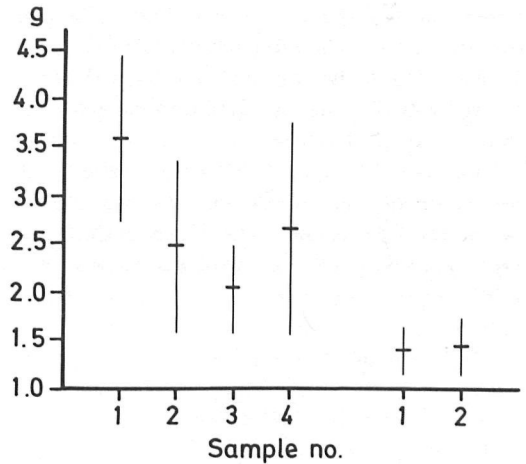


Fig. 3. Amounts of bed dust (mean $\pm$ SEM). Four or two samples (Group I and Group II) from each bed analysed at different intervals (see text).

movable dust in dust bags ranged from 0.25 to 8.6 g with mean values of 1.4 to 3.6 g at different times of sampling (Fig. 3).

With an interval of 1 day between two samplings an insignificant reduction in dust weight of the second sample was observed (Table 1). Mean change was 31 % (95 % confidence interval: -70 % to +8 %). At 1 week from the two initial samplings, the collectable amount of dust was reduced significantly compared with the initial value (with 95 % confidence interval of -14 % to -72 %). A subsequent interval of 3 weeks increased the dust amount to values not significantly different from the initial (95 % confidence interval: -78 % to +26 %).

There was a slight tendency towards a reduction in concentration of the three major allergens subsequent to the sequential dust collections (Table 1). Statistical significance was obtained only in two of the 18 changes analysed (Df6 and Dp42 following two samplings).

One dust collection significantly reduced the collectable amount of allergen the following day for Dp42 and Df6. No statistically significant differences were observed in the total amounts of major allergens which could be removed at subsequent intervals of 7 or 21 days (Tables 1, 2).

The initial dust weight in Group II was much

lower than in the first group. The allergen-reducing effect of the first sampling in Group I could not be demonstrated for Group II - differing by having the sampling interval extended from 1 day to 3 weeks.

The two groups were comparable with respect to age of houses and cleaning habits, but mattresses tended to be older in Group I, mean age being 8.2 years versus 3.9 years ($P = 0.04$).

Regression analyses performed on data sets from each participant in Group I did not disclose correlation between (log) concentration of allergen and dust amount.

After emptying the dust bags 5-90% of the allergens were retained on the paper walls of the bags (Fig. 4). Generally, small amounts of removable dust implied a higher percentage of allergen on the bag wall (correlation coefficient $r: -0.7, P < 0.01$). The percentage of retained

Table 1

Mean values of dust weight, concentrations of three house dust mite major allergens, and amounts of allergens in sequential samples from two groups of patients. Means are calculated from samples actually containing the allergen

Dust sample No.	Group I				Group II	
	1	2	3	4	1	2
Dust weight (g)	3.6 — 0 — — ** — — 0 —	2.5 — 0 —	2.0 — 0 —	2.7 — 0 —	1.4 — 0 —	1.4 — 0 —
Dp42 (mg/g)	5.9 — 0 — — ** — — 0 —	5.7 — 0 — — * —	4.4 — 0 —	5.7 — 0 —	6.8 — 0 —	5.0 — * —
Dm6 (mg/g)	1.7 — 0 — — 0 — — 0 —	1.7 — 0 —	1.6 — 0 —	1.3 — 0 —	1.0 — 0 —	1.0 — 0 —
Df6 (mg/g)	1.0 — * — — ** — — 0 —	0.8 — 0 — — * —	0.6 — 0 —	0.6 — 0 —	0.4 — 0 —	0.4 — 0 —
Dp42 (mg)	14.0 — *** — — *** — — ** —	6.3 — 0 —	6.8 — 0 —	7.0 — 0 —	8.2 — * —	6.0 — 0 —
Dm6 (mg)	3.3 — * — — * — — 0 —	2.1 — 0 —	1.9 — 0 —	1.7 — 0 —	1.2 — 0 —	1.2 — 0 —
Df6 (mg)	2.2 — ** — — *** — — * —	0.9 — 0 —	0.7 — 0 —	0.8 — 0 —	0.5 — 0 —	0.5 — 0 —

0: $P \geq 0.05$; * $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$.

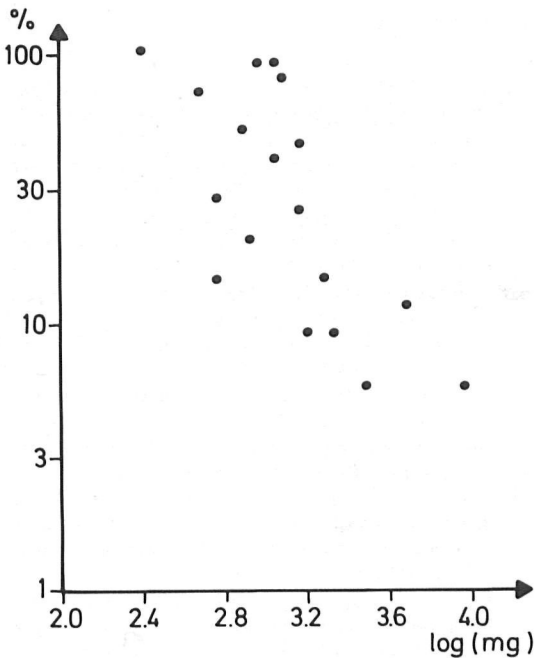


Fig. 4. Relative amount of mite allergen left on wall of dust bag and weight of corresponding dust sample.

allergens was not correlated to either concentration of allergens ($r = -0.2$, $P > 0.05$) or area of the dust bag ($r = -0.3$, $P > 0.05$).

DISCUSSION

Following the initial cleaning effect, very small changes were found with the sampling interval of 1 week, thus an interval of 3 weeks could be expected to eliminate any cleaning effect of the previous sampling. Therefore changes during the 3-week period could be used to assess reproducibility of the dust collection and analysing procedure. Very limited differences could be registered during this

period (Table 2) and, subsequently, reproducibility seems to be fully acceptable.

Several methods for dust sampling have been described. The majority deal with material for microscopic identification and counting of whole mites. In such cases a cautious method, not injuring the animals, would be preferable, and special brushing or suction equipment has been advised (1, 2, 11). Dust for chemical analysis does not have to contain undamaged mites, and, subsequently, as confirmed by the present study, it can be collected with less caution. Patients managed to get almost identical results from repeated samplings with their own vacuum cleaners and very little instruction. Thus, technicians or costly special equipment for this kind of dust collection seems unnecessary.

The present study appears to indicate that vacuum cleaning for 5 min of mattresses of a certain age can reduce the allergen contents. The reduction of 34–58% is comparable to the fall in number of whole mites of about 70% found in other studies (3, 6). Contrary to whole mites, which seem to be removed less easily than dust (3), we found the concentrations of allergens in the dust samples to be fairly constant, probably because allergens, more than whole mites, resemble the other constituents of mattress dust. In the present study further vacuuming removed fairly constant amounts of allergen with little relation to the cleaning interval. It could be speculated that the total load of allergens, especially in the mattresses, was probably very large compared with the amounts removed by vacuuming of the surfaces. Intervals of 1 week or more between such vigorous cleaning efforts would therefore in old mattresses be insufficient to give clinically significant reductions in allergens. Whether or not mite population in newer mattresses could be halted has to be further investigated.

Table 2

Relative changes with 95% confidence limits based on samples collected by all participants at 3-week interval

Allergen	<i>D. pteronyssinus</i>	<i>D. microceras</i>	<i>D. farinae</i>
Conc. %	-4 (-43 to +63)	-14 (-49 to +47)	+6 (-63 to +201)
Amount %	-14 (-53 to -56)	-10 (-54 to +77)	+6 (-62 to +200)

Considerable amounts of allergens could be extracted from the paper walls of the various dust bags. The trapping of allergen by the paper fibers could be explained by the nature of the allergenic material. The diameter of the fecal pellets containing high concentrations of allergen can be as small as 10 μm (10), which allows these particles or their fragments to pass into the paper wall of the dust bag and probably even through the wall. The variability in amount of retained allergen is probably influenced by the use of many different dust bags and vacuum cleaners. Such factors would be eliminated when focusing on the change in allergen exposure of one individual, provided identical equipment is used.

From a clinical point of view it could be argued whether the amount of allergen or the allergen concentration would be the most relevant parameter to monitor. In theory, the allergen exposure would parallel the weight of allergen in dust samples collected in identical ways. Nevertheless, by choosing the allergen concentration one would to some extent eliminate the errors caused by variations in sampling procedures of the individuals (i.e. different times of suction) or different degrees of emptying dust bags before analysis. Neither in the present nor in other studies (5) did the amount of dust collected by standardized sampling influence the allergen concentration. Therefore one would expect the mite allergens and textile fibers plus human dander – the major contents of mattress dust – to be removed in the same proportion during the sampling procedure. Thus, for practical reasons, the allergen concentration seems the preferable parameter for this kind of study.

ACKNOWLEDGEMENT

Statistical assistance was received from the Danish Medical Research Council.

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