

Study of the effects of vacuuming on the concentration of dust mite antigen and endotoxin

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Background: Dust mite antigens are major sources of allergens in house dust and together with endotoxin, a proinflammatory component of gram negative bacteria also found in house dust, are important causes of tissue injury involved in the pathogenesis and severity of allergic diseases, eg, asthma.

Objective: To determine the effectiveness of vacuuming in reducing the quantity of dust mite antigens, *Dermatophagoides pteronyssinus* (Der p1 and Der p2) and endotoxin using a quantitative ELISA assay and to correlate results with those obtained using a qualitative rapid dipstick method for Der p2.

Methods: Four specimens of house dust were collected using a Kirby Model G5 vacuum cleaner with a Micron Magic Filtration system from an approximately 54" x 18" standardized area of rug from each of 20 homes at 4 time intervals over a 6-week period, ie, a baseline specimen #1 at 0 week; specimen #2 at 1 week; specimen #3 at 5 weeks (1 month after specimen #2); specimen #4 at 6 weeks (1 week after specimen #3). Three intervals were compared, ie, period 1–2 (1 week), period 2–3 (1 month), and period 3 to 4 (1 week). The concentrations of Der p1 and Der p2 were determined in dust samples using a standard ELISA assay and the concentration of endotoxin was detected using a limulus amebocyte lysate assay. Concentrations of Der p2, determined by the standard ELISA assay, were compared to those in the same samples determined by a rapid dipstick method.

Results: A wide range of values for total weight of unprocessed dust (0.3 to 59 g, $\bar{X}$ = 8.7) and finely sieved dust (0.1 to 19 g, $\bar{X}$ = 3) from all specimens were found. In finely sieved dust specimens the mean concentrations of Der p1, Der p2 and endotoxin were 775, 1310, and 3836 ng/g of dust, respectively. Following weekly vacuuming there was an increase in concentration of Der p1, Der p2, and endotoxin in 20%, 35%, and 63% of the houses, respectively, compared to in monthly vacuuming in which increases were seen in 65%, 50%, and 63% of the houses, respectively. In contrast, there was a decrease in concentration of Der p1, Der p2 and endotoxin with weekly vacuuming in 43%, 60%, and 37% of the houses respectively versus in monthly vacuuming in 15%, 35%, and 37% of the houses respectively. A correlation coefficient of 0.7 was found for the concentration of Der p2 in 37/40 samples tested detected using the ELISA method compared with rapid dipstick assay.

Conclusion: The results of this study support the effectiveness of vacuuming on the reduction of dust mite antigens (Der p1 and Der p2) ie, Der p2 > Der p1. This reduction was more pronounced with weekly compared with monthly vacuuming. No reduction in the concentration of endotoxin was found. A good correlation was found between results obtained by ELISA and rapid dipstick assay for Der p2.

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INTRODUCTION

Dust mite antigens comprise major sources of allergens in house dust^{1,4,7,11} and together with endotoxin, a proin-

flammatory component of gram negative bacteria also found in house dust, constitute major initiators not only of allergic disease but also of tissue injury implicated in asthma and its severity.^{4–6,8,9,10,12,13} Furthermore, several studies have demonstrated a direct relationship between the concentrations of allergen and endotoxin in house dust and the severity of asthma.^{8,11–13} Based upon these observations, in clinical practice, reduction of these environmental allergens by a variety of methods are commonly recommended as part of the management of asthma. Although these methods of reduction include regular vacuuming there are relatively few studies that have rigorously evaluated the effectiveness of vacuuming in reducing allergens. A recent report by Rønborg et al evaluating the effect of two different types of vacuum cleaners on airborne Fel d 1 levels did not address their efficacy in total allergen reduction.¹⁴ The present study was done to determine the effectiveness of vacuuming on reducing mite allergen and endotoxin in dust and to compare a standard ELISA with a rapid dipstick method to assay for Der p2. The purpose of the present preliminary study is to first establish the feasibility of the approach employed, then to proceed with a more definitive study.

METHODS

Four specimens of house dust were collected using a Kirby Model G5 vacuum cleaner with a Micron Magic Filtration system from an approximately 54" x 18" standardized area from each of 20 homes at 4 time intervals over a 6-week period, ie, a baseline specimen #1 at 0 week; specimen #2 at 1 week; specimen #3 at 5 weeks (1 month after specimen #2); specimen #4 at 6 weeks (1 week after specimen #3). Three in-

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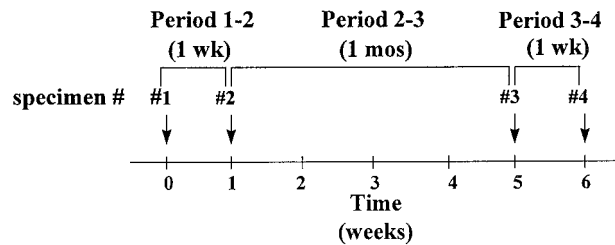


Figure 1. A schematic representation of the study period in which four specimens of house dust were collected using a Kirby Model G5 vacuum cleaner with a Micron Magic Filtration system from an approximately 54" x 18" standardized area from each of 20 homes at 4 time intervals over a 6-week (wk) period, ie, a baseline specimen #1 at 0 wk; specimen #2 at 1 wk; specimen #3 at 5 wks [1 month (mos) after specimen #2]; specimen #4 at 6 wks (1 wk after specimen #3). Three intervals were compared, ie, period 1 to 2 (1 wk), period 2 to 3 (1 mos), and period 3 to 4 (1 wk).

tervals were compared, ie, period 1–2 (1 week), period 2–3 (1 month), and period 3–4 (1 week) (Fig 1). The study was done in the metropolitan Washington, DC area during the last week of September through the first week of November in homes and apartments all of which employed air conditioning having mean temperatures of 70 to 75°F with relative humidity ~30% to 50%. There was considerable variation in the types and numbers of occupants in our study households as well as in their personal habits, eg, single occupants versus families with young children and frequency of vacuuming. Over the study period, a total of 80 samples were collected from which the dust mite antigens and endotoxin were extracted. The methodology, materials, and reagents for the dust mite antigen extraction procedure, standardized ELISA, and the qualitative rapid dipstick method were kindly provided by Dr M D Chapman and are published elsewhere.^{1,2} Briefly, the total weight of dust obtained from vacuuming was determined by weighing the Kirby Micron Magic Filtration system vacuum cleaner bag, following which the dust was removed from the bag and put through a 45-mesh screen sieve. The sieved dust was reweighed and a sample was suspended in phosphate buffered saline pH 7.4 (PBS), containing 0.05% Tween 20 (PBS-T), to achieve a final concentration of 50 mg/mL for extraction. The dust suspension was vigorously vortexed followed by a 2-hour end-over-end rotation to extract

the antigen, after which the particulate material was removed by centrifugation at 1000 *g* for 20 minutes at 4°C. The supernatant was removed and stored at –20°C until analysis using a standard ELISA method.¹ Additional studies were performed to determine the concentration of endotoxin in the dust samples using the method of Williams³ employing a Limulus Amebocyte Lysate (LAL), QCL-1000 Test Kit from BioWhittaker. An extraction procedure similar to that used for the dust mite study was employed for determination of endotoxin using pyrogen-free water as the diluent instead of PBS.

An additional set of experiments performed to compare the results obtained by ELISA for the concentration of Der p2 with those obtained by a new, more rapid diagnostic test employing a dipstick method.² Dust samples that had antigen concentrations above 0.5 µg/g dust, the lowest detectable level for the dipstick, were tested by the dipstick method. Briefly, serial dilutions of specimens were performed to obtain optimal concentrations for measurement by the dipstick method and the results were compared with those obtained using known standards. Der p2 antigen concentrations were assessed in the samples simultaneously by ELISA.

The concentrations of dust mite antigens and endotoxin were determined using multiple dilutions of samples to determine optimal dilutions for assay. The results were obtained from the

mean of duplicate samples from replicate assays.

Analysis of Data

Data analysis was performed by standard statistical methods using Microsoft Excel. The results of the changes in dust mite antigen or endotoxin concentration during the various periods were determined from the log of the ratio of the concentration(s) of antigen or endotoxin per gram dust from each specimen at the end/the start of the period. Samples with undetectable levels of antigen or endotoxin were assigned the lowest values of detection, ie, 40 ng/g dust, when using log determinations of ratios, in order to calculate changes in levels of antigen or endotoxin during the various periods. This permitted the comparison of data between homes regardless of the great variation in both the total amount of dust as well as dust mite antigen and endotoxin from house to house. The data were analyzed by measuring changes in concentrations of dust mite antigen and endotoxin over the test periods, ie, 1–2, 2–3, and 3–4 as follows:

$$\begin{aligned} &\log (\text{concentration of specimen \#2}) \\ &\quad \div \text{concentration of specimen \#1}) \\ &\quad = \text{period 1–2} \\ &\log (\text{concentration of specimen \#3}) \\ &\quad \div \text{concentration of specimen \#2}) \\ &\quad = \text{period 2–3} \\ &\log (\text{concentration of specimen \#4}) \\ &\quad \div \text{concentration of specimen \#3}) \\ &\quad = \text{period 3–4} \end{aligned}$$

Statistical analysis of the changes in total amount and concentration of dust mite antigen and endotoxin between periods 1–2, 2–3, and 3–4 were performed employing the Student's *t* test. The similarity between the concentrations of Der p1 and Der p2 in the individual homes was compared by determining a correlation coefficient. In addition, the results of the concentra-

tions of Der p2 obtained by ELISA were compared with those obtained with a rapid dipstick method by determining a correlation coefficient.

RESULTS

The weight of the finely sieved dust in the 80 specimens ranged from 0.1 to 19 g ($\bar{X} = 3$ g). Since there was no significant impact of vacuuming on the total amount of dust recovered, the results are expressed either as the total amount or concentration of specific dust mite antigen or endotoxin. Mean concentrations of dust mite antigens, Der p1, Der p2, and endotoxin in these specimens were 775, 1310, and 3836 ng/g of fine sieved dust, respectively. Although the results of the analysis of the dust samples for dust mite allergens and endotoxin revealed that these were not detectable in all four samples from each house they were detectable in the four timed collection periods at following frequencies: 40% (8/20), 40% (8/20), 75% (15/20), and 60% (12/20) of the samples for Der p1; 80% (16/20), 70% (14/20), 90% (18/20), and 85% (17/20) for Der p2; and 11/11 (100%), 20/20 (100%), 19/19 (100%) and 20/20 (100%) for endotoxin detectable levels. Two of the 20 houses had undetectable levels of Der p1 in all four samples and one of these also had undetectable levels of Der p2. Further, 12 of 20 houses had undetectable levels of Der P1 in their first sample and 10 of these remained undetectable in the second sample, ie, no change in concentration of Der p1 in period 1–2 (Fig 1). The highest detection period for both Der p1 and Der p2 dust mite antigens was after the month interval in sample #3, ie, 75% and 90%, respectively. Further, 63/80 (79%) samples collected had a higher concentration (ng/g of dust) of Der p2 allergen than Der p1. In contrast, only 11/80 (13%) of the samples had higher concentrations of Der p1 than Der p2. In addition, in 10/20 homes, there was a correlation in the change in concentration in the four samples of Der p1 with the change in concentration of Der p2 in the same four samples taken from each home, ie, correlation coefficient

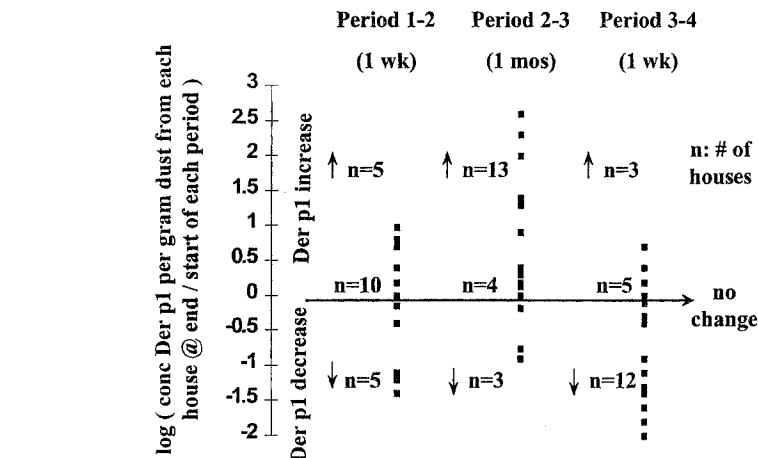


Figure 2. Change in concentration of Der p1 in house dust with different intervals between vacuuming for three consecutive study periods for 20 houses. Results are expressed as the log of the ratio of concentrations of Der p1 antigen in house dust from the end of each study period divided by the concentration at the start of that period.

cient > 0.7 . A similar correlation was detected with endotoxin, but in only 4/20 for Der p1 and 2/20 for Der p2 homes. The mean total amount of antigen for Der p1 ($\bar{X} \mu\text{g} \pm \text{SD}$) obtained at the end of a week interval between vacuuming (period 1–2 and period 3–4) was 0.7 ± 1.5 and 1.1 ± 2.3 , respectively. The mean total amount of Der p2 for these periods was 3.0 ± 2.6 and 2.3 ± 2.5 , respectively. In contrast, the mean total amount of antigen

for Der p1 and Der p2 obtained at the end of a month interval between vacuuming (period 2–3 was 7.2 ± 19 and 12.7 ± 36 , respectively).

The effects of vacuuming on the concentration of allergens in the dust from the 20 homes are shown in Figures 2 and 3. There was an increase in the concentration of Der p1 and Der p2 in 5/20 and 7/20 houses, respectively, in period 1–2, with a 1-week interval between samples. Further, no change

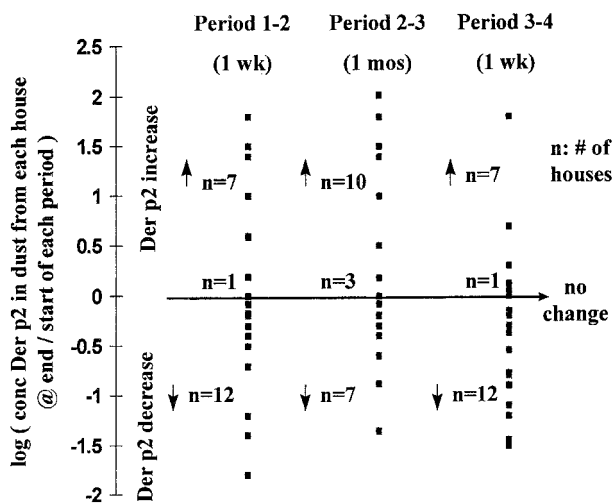


Figure 3. Change in concentration of Der p2 in house dust with different intervals between vacuuming for three consecutive study periods for 20 houses. Results are expressed as the log of the ratio of concentrations of Der p2 antigen in house dust from the end of each study period divided by the concentration at the start of that period.

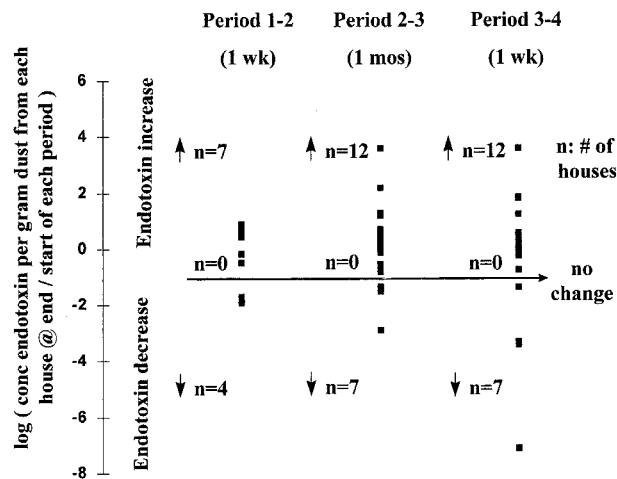


Figure 4. Change in concentration of endotoxin in house dust with different intervals between vacuuming for three consecutive study periods for 20 houses. Results are expressed as the log of the ratio of concentrations of endotoxin in house dust from the end of each study period divided by the concentration at the start of that period.

was detected at this time in the concentration of these antigens during this interval in 10/20 and 1/20 of the houses but a decrease in the antigen concentrations were detected in 5/20 and 12/20 of the houses for Der p1 and Der p2, respectively. The mean concentrations of Der p1 and Der p2 ($\bar{X}$ $\mu\text{g/g}$ of dust $\pm$ SD) in this period, with a 1-week interval between samples, was 0.12 ± 2.3 and 0.9 ± 0.9 , respectively. In period 2–3, which had a month interval between the collection of specimens, there was a greater increase in antigen concentration in 13/20 and 10/20 houses in Der p1 and Der p2, respectively. The mean concentrations of Der p1 and Der p2 ($\bar{X}$ $\mu\text{g/g}$ of dust $\pm$ SD) in this period was 2.4 ± 5.9 and 3.8 ± 9.2 , respectively. In contrast, in the final period 3–4, which had a week interval between

specimen collection, there was a predominant decrease in antigen concentration in 12/20 houses for both Der p1 and Der p2. Mean concentrations of Der p1 and Der p2 ($\bar{X}$ $\mu\text{g/g}$ of dust $\pm$ SD) in this period was 0.24 ± 0.4 and 1.0 ± 0.9 , respectively. The concentration of Der p1 in period 2–3, which had a month interval between specimen collection, was significantly greater than that detected in either weekly collection periods, 1–2 or 3–4, ($P \leq .05$).

The effects of vacuuming on the concentration of endotoxin appear in Figure 4. There appears to be no differences related to the interval between specimens but an increase was detected in a greater number of houses after periods, 2–3 and 3–4 compared with period 1–2. A great variation in the total amount and concentration of

endotoxin was detected between houses particularly in the later two periods. The mean concentration of endotoxin ($\bar{X}$ $\mu\text{g/g}$ of dust $\pm$ SD) in specimens recovered at the end of periods 1–2 (wk), 2–3 (mos), and 3–4 (wk) was 5 ± 11 , $451 \pm 1,534$ and 102 ± 446 , respectively.

Table 1 shows that the greatest number of houses, ie, 43% and 60%, had a reduction in both Der p1 and Der p2, respectively, when weekly vacuuming was done. In contrast, an increase in 65% and 50% of the houses for the concentration of Der p1 and Der p2, respectively, was detected when monthly vacuuming was done. The effects of vacuuming at a monthly interval, as determined by log of the ratio of concentration at end/start of the period, resulted in the recovery of significantly more Der p1 and Der p2 antigen compared with vacuuming at weekly intervals ($P \leq .05$). There was no significant difference in the concentration of endotoxin in specimens collected at weekly compared with that collected at a monthly interval, ($P > .05$). An increase in endotoxin concentration was seen in 63% of the homes after the first weekly vacuuming interval as well as after both the 1-month interval and the final weekly interval.

The results of the dipstick assays, in general, paralleled the results obtained with ELISA (Fig 5 a and b). Although there were some discrepancies between the two assay systems because of the quantitative nature of the dipstick assay. A good correlation was seen comparing the results of using both assay systems to analyze the concentration of dust mite antigen, the results of the two assays had a correlation coefficient of ≥ 0.7 .

DISCUSSION

Although previous studies have reported temporal or seasonal variations in mite allergen content¹⁵ or concentration,¹⁶ the present study is unique in evaluating the impact of vacuuming on mite antigen and endotoxin concentrations. The results of this study suggest that frequent vacuuming has a significant beneficial impact on the reduction

Table 1. Effects of Weekly Versus Monthly Vacuuming on % of Houses Showing Change in Concentration of Der p1, Der p2, and Endotoxin in House Dust

Change in Concentration	Vacuuming Interval	Der p1	Der p2	Endotoxin
Increase	Weekly	20%	35%	63%
	Monthly	65%	50%	63%
Decrease	Weekly	43%	60%	37%
	Monthly	15%	35%	37%
No change	Weekly	37%	5%	0%
	Monthly	20%	15%	0%

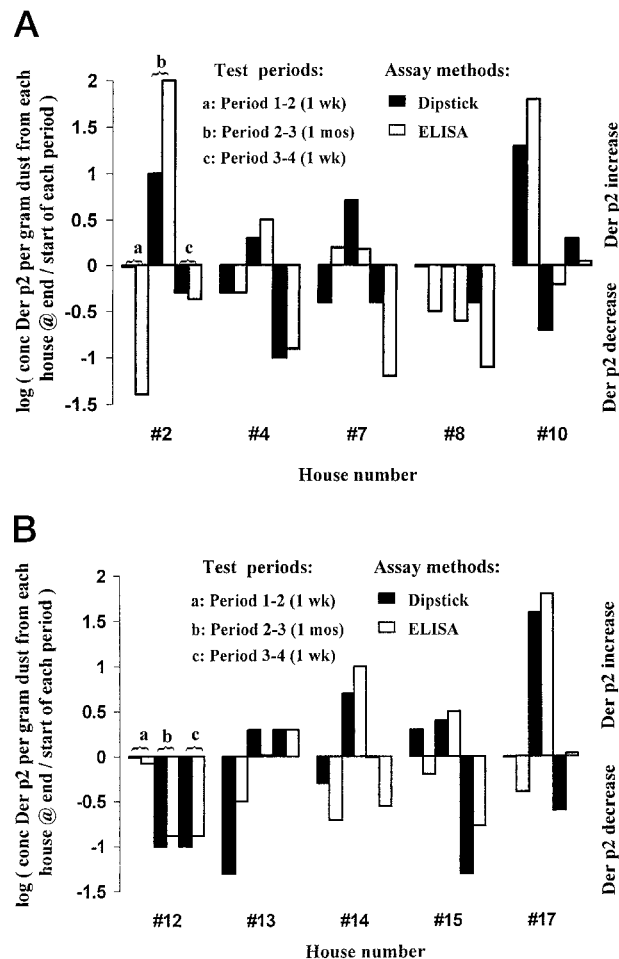


Figure 5a and b. Comparison of results obtained by ELISA assay compared with the dipstick method for the determination of the effect of vacuuming on the concentration of Der p2 in house dust for three study periods for 10 houses. Results are expressed as the log of the ratio of concentrations of Der p2 antigen in house dust from the end of each study period divided by the concentration at the start of that period.

of the dust mite antigens, Der p1 and Der p2. Weekly vacuuming was significantly better than monthly vacuuming. In contrast, the effects of vacuuming on the reduction of endotoxin concentration was less clear and suggested more complex effect(s). Both weekly and monthly vacuuming periods resulted in an increase in the concentration of endotoxin in the majority of homes. Several of the homes had very high concentrations of endotoxin in the later two periods (Fig 4). One explanation for this observation may derive from differences in size of dust mites and their fecal pellets compared with endotoxin (from intact or disrupted bacteria), ie, 140 μm , 25 μm

versus 1 μm , respectively. Alternatively, these differences could be related to changing microenvironment(s) and growth of bacteria from which endotoxin is derived. Vacuuming may stimulate bacterial growth by releasing organisms from the base of the rug, thus providing more nutrients and oxygen for growth and metabolism. These changes in endotoxin concentrations, however, appear somewhat heterogeneous and require more samples to draw definitive conclusions. The combined presence of dust mite antigen and endotoxin in house dust have prompted additional research questions. It is important to consider the immunologic role of endotoxin both as

an adjuvant promoting the immunogenicity of Der p1 and p2 antigens as well as its inherent inflammatory promoting effects. It would be of interest to evaluate the effects of vacuuming on the dust mite antigen and endotoxin reduction following longer periods of vacuuming.

A second objective of the present study was to compare the results obtained by qualitative ELISA with those by a dipstick assay of Der p2. Of particular significance in this study are the results of the correlation of the ELISA method with the rapid dipstick assay of Der p2. The positive correlation seen in specimens evaluated as well as the simplicity and ease of performance of the rapid dipstick method is highly supportive of its utility in home monitoring of dust mite antigenic load.

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