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The control of house dust mite allergens in rugs

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Used rugs were vacuum cleaned, "wet cleaned," "shampooed," or heated in an autoclave. The effects on the live-mite population, on the allergen (Dermatophagoides pteronyssinus, Der p I and Der p II; and Dermatophagoides farinae, Der f II) content, and on the suitability of the rugs as a habitat for house dust mites was studied. Autoclaving was most effective in all three aspects. The cleaning methods were quite inefficient for killing the mites, but a significant reduction of allergens and deterioration of the habitat quality was established. "Wet cleaning" appears particularly promising. (J ALLERGY CLIN IMMUNOL 1990;86:808-14.)

HDMs are an important source of allergens inside houses. Mattresses, stuffed furniture, and carpets are suitable breeding places for these creatures. Trials have demonstrated repeatedly that it is very difficult

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Abbreviations used

HDM: House dust mite
 Der p I: Major allergen (P1) from *Dermatophagoides pteronyssinus*
 Der p II: Major allergen from *D. pteronyssinus*
 Der f II: Major allergen from *D. farinae*
 ANOVA: Analysis of variance

to remove HDM allergens from a fixed wall-to-wall carpet.¹⁻⁴ Therefore, it could be advisable for allergic patients to replace their carpet by a smooth type of floor covering, which can be kept clean more easily. Rugs that may be used to further decorate the home

TABLE I. Overview of the carpets

Carpet No.	Material (W/S)	Thickness (mm)	Weight (kg/m ²)	Age (yr)	Li/br	Mites/gm of dust
1	W	7	1.7	>10	Li annex, br	2
2	W	10	1.6	>4	Li	1
3	W	20	4	25	Br	64
4	S	8	1.6	2	Br	8
5	W	6	2.7	50-70	Li	350
6	W	8	1.7	15	Br	380

W, wool; S, synthetic; Li, living room; Br, bedroom.

are also dust collectors and should be kept clean. Measures to prevent the accumulation of HDM allergens in rugs are studied in the present investigation. Three questions are addressed: (1) Are the mites killed or removed? (2) Are the allergens eliminated or destroyed? (3) Is the habitat rendered unsuitable for HDMs, for instance through the elimination of their food?

MATERIAL AND METHODS

Old carpets were obtained through advertisements in local newspapers in and around Amsterdam. Only those carpets were accepted that had been on the floor of a living room or a bedroom until just before the purchase. Details are provided in Table I. First, a small dust sample was obtained with a vacuum cleaner. The numbers of mites in the carpets were established with the flotation method.⁵ Most specimens were incomplete and probably dead at the time of collection. *Dermatophagoides pteronyssinus* was the dominant species, except in carpets 2 and 6, in which mites belonging to the *D. farinae* group were more numerous. Other species (*Tarsonemidae*, *Euroglyphus* sp, and *Cheyletus* sp) were found in small numbers.

Each carpet was cut into four equal parts, A, B, C, and D. From each part, 15 fragments of 13 cm by 5 cm were cut to be used as control samples. Thereafter, one of the following cleaning treatments was applied: (1) vacuum cleaning: A Reactor vacuum cleaner (HEMA bv, Amsterdam, The Netherlands) with a small nozzle was used; both sides were vacuumed for an equal length of time. (2) "wet cleaning": For this method a machine was used, made by Moor. A nonionic detergent, Tapisannass (Vorwerk & Co., Teppichwerke, Hameln, West Germany), was applied. The basic ingredients are (1) alkyl ether of polyethylene, polypropylene glycol (70% to 90%), and (2) alkyl ethers of polyethylene glycol (10% to 30%). The water content is as much as 10%. One percent of scented oil is added. One part of the mixture is dissolved in 100 parts of water before use. During approximately 1 minute, the solution is rubbed into the carpet by rotating brushes, and then the detergents are washed out with plenty of water. Only cold water is applied. Subsequently, the carpet passes through a mangle, which removes about 75% of the water. The carpets are hung to

dry overnight in an aired room that is heated to about 25° C. (3) "Shampooing": A soap solution was introduced with a hand-held appliance and sucked up through a separate part of the nozzle. Subsequently, the same procedure was followed with clean water. Again, cold water was used only. Details on the composition of the detergents used were not revealed by the cleaning company involved. (4) Autoclaving: In an autoclave, the material was heated to 110° C during 10 minutes. Including the warming up and cooling off of the autoclave, the procedure took almost 1 hour.

After the treatment, fragments of the carpet, 13 cm by 5 cm, were cut out, adjacent to the holes left by removal of the control samples. Thus, paired comparison of the observations on treated and untreated samples is possible. From each section, A, B, C, and D, five sample pairs were used to determine the number of live mites. Five pairs were used to determine allergen content and five pairs were used to test the habitat quality.

The number of live mites was determined by the "heat-escape method."⁶ Pieces of carpet, with adhesive tape stuck to the upper side, were placed on a slide warmer. The temperature of the slide warmer ranged from 30° to 50° C but was the same for material from the same carpet and adjusted to the thickness of it. After 1 hour, the mites that were stuck to the adhesive tape were counted under a dissecting microscope. The yield was from 50% to 100%.

Extracts were made by overnight vertical rotation of 11 gm of carpet material in 35 ml of water containing 5% sodium benzoate as a preservative (pH ~ 8). Two assays were performed to quantify the concentrations of HDM allergens in these extracts. In one assay, the concentration of *Der p* I was determined, and in another assay, the amount of *Der p* II and/or cross-reacting *Der f* II was measured. *Der p* I is found in the feces of *D. pteronyssinus*. The group II allergens occur mainly in the bodies of *D. pteronyssinus* (*Der p* II) and *D. farinae* (*Der f* II).⁷⁻⁹

The *Der p* I concentration was measured by an inhibition assay analogous to the procedure described by Chapman and Platts-Mills.¹⁰ A mixture of 0.05 ml of extract and 0.05 ml of diluted rabbit anti-*Der p* I was incubated for 2 hours. Subsequently, 0.5 ml of protein A-Sepharose (1 mg/ml) in phosphate-buffered saline containing bovine serum albumin and Tween 20 and 0.05 ml of ¹²⁵I-labeled *Der p* I were added and incubated overnight under vertical rotation at room temperature. After centrifugation and washings of the Sephar-

TABLE II. Number of resident mites in treated and untreated carpet fragments

Treatment	Carpet, part	Total* (range)†		Sign-test, one-sided $\alpha = 0.05$
		Untreated	Treated	
Vacuuming (12 min/m ²)	1,B	4 (0-2)	3 (0-3)	NS (N = 35)
	1,D	2 (0-1)	4 (0-3)	
	2,B	0	0	
	3,B	10 (0-6)	13 (0-6)	
	4,B	0	0	
	5,B	0	0	
	6,B	10 (0-5)	21 (3-7)	
Vacuuming (25 min/m ²)	2,D	0	0	NS (N = 25)
	3,D	57 (0-36)	107 (0-79)	
	4,D	2 (0-2)	0	
	5,D	0	0	
	6,D	47 (2-19)	21 (2-7)	
"Wet cleaning"	1,A	0	0	NS (N = 35)
	1,C	2 (0-1)	1 (0-1)	
	2,A	0	0	
	3,A	38 (1-16)	29 (1-14)	
	4,A	0	0	
	5,A	0	0	
	6,A	57 (0-28)	46 (6-13)	
"Shampooing"	4,C	1 (0-1)	1 (0-1)	NS (N = 10)
	5,C	0	0	

NS, Not significant; N, number of pairs of fragments.

Five pairs of fragments were taken from each carpet part. Of each pair, one fragment was treated and one fragment was not treated.

*The total is the number of mites in the five fragments, either treated or untreated, from one carpet part.

†The range is in the number of mites per fragment.

ose, radioactivity bound to the solid phase was counted. The *Der p* I concentration was taken from a standard dilution curve of a *D. pteronyssinus* reference extract. The *Der p* II/*Der f* II concentration was measured by a binding assay. Monoclonal anti-*Der p* II was coupled to CNBr-activated Sepharose (0.1 ml of ascites per 100 mg of Sepharose). A mixture of 0.05 ml of extract and 0.05 ml of a human serum containing IgE antibodies to *Der p* II was incubated for 2 hours. After adding 0.5 ml of the monoclonal anti-*Der p* II Sepharose in phosphate-buffered saline containing bovine serum albumin and Tween 20 (1 mg of Sepharose per milliliter), incubation was continued overnight under vertical rotation. Thereafter, the Sepharose was centrifuged and washed, and 0.05 ml of ¹²⁵I-labeled anti-IgE was added. Incubation was continued for another day, and radioactivity bound to the Sepharose was counted. Again, the *Der p* II/*Der f* II concentration was read on a standard curve of the reference extract.

Immune reagents were provided by the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service in Amsterdam and were the same as reagents used by van der Zee et al. (see reference 24).

To test the suitability of the carpet as a habitat for HDMs, the resident mite population was killed by a cold treatment,

48 hours at -25° C. Thousands of *D. farinae* and *D. pteronyssinus* from a culture were treated similarly and none survived. The survival of cold-resistant resident strains or species cannot be completely excluded. Eighty cultured *D. farinae* were placed on each fragment, wrapped in a dust bag, and stored in a climatic room at 25° C and 75% relative humidity. After 3 to 7 weeks, the number of live mites was established by the heat-escape method.

The culture of *D. farinae* was obtained from HAL laboratories in Haarlem, The Netherlands. A modification of the heat-escape method was used to transfer the mites. Mite culture was introduced into a block of open porous polyurethane foam, measuring 1.5 cm by 5 cm by 13 cm. This was covered first by a thin layer of clean foam (0.5 to 0.7 cm), then a glass plate, and finally a piece of wet filter paper for cooling. After approximately 30 minutes on a slide warmer at 50° C, large numbers of mites will walk on the underside of the glass plate and can be easily transferred with a small brush.

Statistics

The logarithms of the 125 *Der p* I values and of the 96 *Der p* II/*Der f* II values exhibited an approximately normal distribution around their group means. ANOVA and Stu-

TABLE III. Reduction of allergen concentrations

Treatment	Carpet, part	<i>Der p</i> I			<i>Der p</i> II/ <i>Der f</i> II		
		N	% Red (x ± SD)	<i>p</i> (<i>t</i> test, one-sided)	N (x ± SD)	% Red	<i>p</i> (<i>t</i> test, one-sided)
Vacuuming (12 min/m ²)	1,B	5	43 ± 15	*	4	22 ± 13	†
	3,B	5	-18 ± 67	NS	5	-4 ± 22	NS
	5,B	5	24 ± 44	‡	4	28 ± 16	†
	6,B	5	32 ± 17	†	4	11 ± 6	†
Vacuuming (25 min/m ²)	3,D	5	37 ± 26	†	3	12 ± 9	‡
	5,D	5	52 ± 25	†	4	36 ± 28	‡
	6,D	5	6 ± 53	NS	4	-0.4 ± 15	NS
"Wet cleaning"	1,A	5	50 ± 6	§	4	28 ± 15	†
	3,A	5	37 ± 29	†	3	40 ± 26	‡
	5,A	5	45 ± 34	†	3	38 ± 40	NS
	6,A	5	41 ± 20	†	4	23 ± 6	§
"Shampooing"	5,C	5	24 ± 21	†	3	23 ± 13	‡
Autoclave	3,C	2	>97	—	2	>44	—
	6,C	3	>70	—	3	>51	—

% Red $(1 - X_c/X_e) \times 100$, where X_e = concentration in treated fragment and X_c = concentration in control fragment; *N*, number of replicate pairs of fragments; $x \pm SD$, average $\pm$ standard deviation; NS, not significant

*0.001 < *p* < 0.01.

†0.01 < *p* < 0.05.

‡0.05 < *p* < 0.10.

§*p* < 0.001.

dent's *t* tests were applied to the log-transformed allergen concentrations. In other cases, nonparametric statistical techniques were used.

RESULTS

Killing and removal of live mites

No live mites were found in carpets 2 and 5, although carpet 5 contained many dead mites (Tables I and II). In carpets 3 and 6, with many live mites, the numbers per sample varied greatly, indicating that the mites were distributed in a more or less clustered way. This hampers the detection of differences between treated and untreated samples. Undoubtedly, the autoclaved samples contained no live mites. No effort was made to check this assumption. In all other cases, there were no statistically significant differences between the cleaned samples and the untreated control samples (Table II; Sign-test, $\alpha = 0.05$, one-sided).

Removal and destruction of allergens

Results of allergen measurements are presented in Table III. Extracts of carpets 2 and 4 contained no detectable amounts of the allergens *Der p* I and *Der p* II/*Der f* II. In the remaining four carpets, the *Der*

p II/*Der f* II concentrations of some samples were below or near the detection limit. These samples were omitted.

After autoclaving the carpets, no allergen was detectable. An estimate of the minimal allergen reduction was calculated, based on the allergen content of the control fragments and on the detection limit (Table III). It is quite possible that the actual reduction is 100% or nearly that reduction. In the other treatments, the reduction is incomplete. Undoubtedly, some allergen is removed with every cleaning effort. The negative reduction that was recorded after vacuuming carpet 3 (12 minutes per square meter) indicates that only a very small fraction was removed. This may be accounted for by the thickness of this carpet.

The four parts of a carpet may contain significantly different amounts of allergen (ANOVA: *Der p* I, *p* < 0.01; *Der p* II/*Der f* II, *p* = 0.32). This lack of homogeneity complicates a comparison of the different cleaning methods. Systematic differences between the groups of reduction percentages associated with each cleaning method can be interpreted, unambiguously, as a difference in cleaning efficiency. However, with the appropriate nonparametric statistical techniques, these differences can be, at best, weakly sig-

TABLE IV. Population development in treated and untreated carpet fragments

Treatment	Carpet, part	Total mites		Treated vs untreated*	Comparison of treatments†
		Untreated fragment	Treated fragment		
Vacuuming (12 min/m ²)	1,B	518	417	$p < 0.001$ (N = 35)	a
	1,D	883	90		
	2,B	33	42		
	3,B	14	5		
	4,B	243	82		
	5,B	107	26		
	6,B	903	717		
	2,D	285	61	$p \approx 0.015$ (N = 25)	a
	3,D	20	6		
	4,D	137	52		
	5, D	92	7		
	6, D	743	676		
"Wet cleaning"	1, A	278	15	$p < 0.001$ (N = 35)	b
	1, C	1053	24		
	2, A	77	5		
	3, A	36	0		
	4, A	104	15		
	5, A	350	150		
	6, A	799	222		
"Shampooing"	4, C	527	148	$0.05 < p < 0.1$ (N = 10)	
	5, C	32	51		
Autoclave	2, C	78	2	$p \approx 0.001$ (N = 15)	b
	2, C	67	6		
	6, C	725	16		

N, Number of pairs of fragments.

Numbers of mites were determined 3 to 7 weeks after the introduction of 80 *D. farinae* (See text for explanation).

*Wilcoxon signed-ranks test, one-sided.

†Mann-Whitney U test, one-sided, comparing the "effect" of each treatment, where the "effect" is measured as total number of mites in treated fragments/total number of mites in untreated fragments. The sample size is the number of carpets for each treatment. Different symbols (a and b) indicate significant differences ($\alpha = 0.05$).

nificant. Thus, autoclaving tends to be more effective than "wet cleaning" (Mann-Whitney U test: *Der p* I, $0.01 < p < 0.1$, but *Der p* II/*Der f* II, $p > 0.1$, two-sided) and more effective than 12 minutes per square meter of vacuuming (Mann-Whitney U test: *Der p* I, $0.01 < p < 0.1$; *Der p* II/*Der f* II, $0.01 < p < 0.1$, two-sided). "Wet cleaning" tends to be more effective than 12 minutes per square meter of vacuuming (Mann-Whitney U test: *Der p* I, $0.01 < p < 0.1$; *Der p* II/*Der f* II, $0.01 < p < 0.1$, two-sided). All other differences were even less pronounced ($p > 0.10$). Larger samples, that is, more carpets, would be needed to establish the significance of the differences in efficiency between the cleaning methods.

Habitat destruction

Five sample pairs from each carpet section, provided with equal numbers of mites, were incubated

for 3, 4, 5, 6, and 7 weeks, respectively. The numbers of mites that were recovered are listed in Table IV. In carpet 1, the shortest incubation time yielded systematically lower numbers of mites in the control fragments (Friedman's test, $p = 0.01$). In the other carpets, no correlation between mite numbers and incubation time can be discerned.

In regard to the population development in the untreated fragments, large differences can be observed among the six carpets. The mites did well in carpets 1 and 2, although these carpets contained very few mites originally (Table I). In contrast, the mites barely survived in carpet 3, which originally contained many live and dead mites. The four parts of one rug produced significantly different numbers of mites in carpets 4 and 5 (Kruskal-Wallis one-way ANOVA, $0.01 < p < 0.05$).

Differences between the treated and untreated sam-

ples are highly significant in most cases (Table IV). Even if some of the resident mites survived the cold treatment, it is unlikely that these mites had a significant impact on the results, since their numbers are generally very small in comparison with the 80 introduced mites. The effect of vacuum cleaning is statistically significant. On average, the numbers of mites recovered from the vacuumed fragments is roughly half the number recovered from the control samples. Vacuuming for as long as 25 minutes per square meter was not significantly more effective than vacuuming for only half that time (12 minutes per square meter). Autoclaving and "wet cleaning" appear to be the most effective treatments for the destruction of the habitat for HDMs.

The effect of "wet cleaning" is rather variable. The population development was virtually halted in carpets 1 through 4 and strongly suppressed in carpets 5 and 6. The difference in efficacy is probably due to differences between carpets, although there is no apparent correlation with the thickness of the carpet (Table I). The effect of autoclaving was surprising. Only 24 live mites were recovered from the 15 treated samples, which is 3% of the number from the control samples. A possible explanation is that the nutritive value of the food in the carpet is diminished through the destruction of vitamins.^{11, 12} "Shampooing" appears to be effective, but the number of data is small.

DISCUSSION

Autoclaving appears to be the superior way to reduce HDM allergen levels. Autoclaving kills all mites, destroys allergens, and renders the habitat unsuitable for the mites. In connection with this last aspect, there is an important question still unanswered. How soon is the habitat suitability restored? If the habitat destruction is indeed based on the conversion of vitamins, then it may be restored through fungus growth,^{11, 12} which could be very quick. The practical usefulness of autoclaving is also doubtful. An appropriate autoclave is not available to most subjects. Materials, such as wool, will shrink severely. The method could be valuable, however, for the perfection of a controlled trial to evaluate, convincingly, the medical importance of sanitation. In The Netherlands, a number of hospitals possess autoclaves large enough to contain beds, furniture, and carpets. It combines a radical allergen reduction with the possibility of a double-blind, experimental setup.

"Wet cleaning" also effected the habitat suitability quite strongly. Acaricidal effects of shampoo residues could be involved. However, strong contact toxicity is very unlikely because so many of the resident mites survived the initial exposure to the relatively strong

soap solution. More subtle effects of the residues on reproduction, egg hatchability, or toxicity on ingestion cannot be ruled out. However, as in the case of vacuum cleaning, it appears more likely that the effect is largely due to the removal of food for HDMs. Perhaps a still better result can be achieved by passing the carpet through the machine more than one time. Substrate for fungus growth will also be removed, at least partially. Yet, the long-term effect of "wet cleaning" requires an investigation. An important aspect would be the timing of it. In winter, when the house is heated, the growth of HDM populations is limited by unfavorable physical circumstances, especially relative humidity.¹³⁻¹⁶ It is plausible that food will accumulate during this period. Removal of most of the food in early spring may well prevent a population explosion, as it often occurs in the early summer.^{1, 17-21} The removal of allergens is far from complete. However, if the growth of mite populations can be suppressed, the natural decline of allergen levels is a matter of weeks²² or perhaps months.²³

Vacuum cleaning resulted in a significant reduction of allergen content and habitat quality. Both effects will help to slow down the accumulation of HDM allergens. However, the intensity of vacuuming in these experiments was extreme. The practical value of vacuuming must be rather limited. This conclusion was also reached in connection with the cleaning of fixed wall-to-wall carpets. However, it is likely that vacuum cleaning of rugs is more effective because the air flow can go right through the carpet and because both sides of the carpet can be vacuumed. The long-term effect of regular vacuum cleaning of rugs deserves an investigation.

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