

Do mite avoidance measures affect mite and cat airborne allergens?

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Summary

Background Effective mite allergen avoidance measures are presumed to reduce airborne allergens yet the quantity in the air is rarely measured.

Objective To monitor airborne allergen during a placebo-controlled mite allergen avoidance study.

Methods Bedrooms of 56 atopic asthmatic children were randomly allocated to hot washing and encasing covers + acaricide (active regime) or placebo treatment. Dust was collected from the mattress, bedding and carpets; airborne allergen was measured using Casella samplers and dust settling in open Petri dishes. Der p 1, Der p 2 and Fel d 1 were measured.

Results After 24 weeks of mite allergen avoidance the Casella air-samplers collected Der p 1 less frequently in active than placebo-treated bedrooms (0 vs 29%, $P < 0.05$) and Petri dishes in the active group collected less than baseline (0.2 vs 0.6 ng/day $P < 0.05$). Homes without cats had less cat allergen than cat-owning homes and when actively treated for 24 weeks showed a greater reduction ($P = 0.03$) in mattress cat allergen than the placebo group.

Conclusion Encasing covers and hot washing of bed linen reduced mite aeroallergen (and mattress cat allergen in the absence of cats). This could mean dual benefits to a patient sensitive to both mite and cat.

Keywords: airborne allergen, bedding covers, benzyl benzoate, carpets, Casella sampler, Der p 1, Der p 2, Fel d 1, house dust mite, mattress

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Introduction

The allergens of house dust mite (HDM) and the domestic cat are widely present in the home. They have been implicated in the provocation of asthma attacks. Up to 90% of asthmatic children in the UK are sensitized to HDM and 40% to allergens of cat [1]. Assessment of exposure to these allergens is important in understanding and controlling allergic disease.

A number of studies have shown that allergen avoidance measures (e.g. encasing bedding and mite killing procedures) have been effective in reducing amounts of mite

allergen collected from surfaces of bedding and carpets [1–3]. Little evidence has been presented on the effect of avoidance procedures on cat allergen (Fel d 1) surface content or on airborne HDM or cat allergens. Such measurements are particularly relevant to both the natural exposure to allergen and to attempts to reduce exposure artificially. While it has been assumed that the amount of allergen in the air is related to the amount of allergen the patient breathes, in practice it has been extremely difficult to detect mite allergen in the air in undisturbed conditions [1,4] although cat allergen is more easily detected [5]. This is probably related to the relative size of the airborne particles. Der p 1 allergen is principally associated with mite faecal particles of $\approx 20 \mu\text{m}$ diameter, Der p 2 appears to be associated with larger particles derived from the longer mite body which remain in the air for only a short period. These mite

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allergen-containing particles have been found to have different aerodynamic properties [2,5].

A series of measurements of airborne mite and cat allergens were made over the 24 weeks of a double-blind controlled trial which attempted to remove mite allergen from children's bedrooms [3]. We used two different air sampling methods — continuous air suction through a filter with assay of the trapped allergen (Casella samplers) and settled dust collected in open Petri dishes [6]. Two types of Casella sampler were used.

Materials and methods

Subjects

Seventy atopic asthmatic children with a major skin prick test response to HDM and with higher mattress Der p 1 content (median 580 ng range 100–28 000) were enrolled in the trial from 130 potential subjects and randomly allocated into active and placebo groups (35 in each group). Patient selection and randomization procedures are described in detail elsewhere [3]. Ethical permission was obtained from our (UBHT) research ethics committee and informed consent obtained from the children and parents/guardians. Sixty-two children started the study. The data were collected between September 1992 and June 1993.

All homes were naturally ventilated and all except one had two floors with the bedrooms upstairs. One home had all the rooms at ground level. Information from a self-administered questionnaire completed by the child's parent indicated that 60% of homes were more than 50 years old. Twenty-five percent of that total were more than 80 years old. Although 6% of bedrooms had no heating, 80% of homes were centrally heated and 30% had double glazing on the windows.

Avoidance or placebo measures were directed at the children's bedrooms only. There were two beds in 30% of children's bedrooms and both were treated identically; such bedrooms were either double active or double placebo. The bedroom carpet was treated either with Acarosan powder (major ingredient benzyl benzoate 5%, Crawford Chemicals, Milton Keynes, UK) or inert chalk dust. The mattress(es), duvet(s), pillow(s) and upholstered furniture were treated with Acarosan foam (benzyl benzoate 2.6%) or water spray (placebo). All items were thoroughly vacuumed using a 1000-W Medivac Microfilter vacuum cleaner (Medivac, Wilmslow, UK), Acarosan or placebo was applied, left on 24 h (the children slept elsewhere) until the items were dry and then revacuumed. After the second vacuuming, allergen exclusion covers (Intervent; cotton encasings coated on one side with polyurethane, WL Gore, Woking, UK) or cotton placebo covers were fitted to the mattress(es), duvet(s) and pillow(s) and left unopened *in situ* for 24 weeks.

The carpets were revacuumed by our therapeutic nurse with the Medivac 4 weeks after initial treatment. Families were asked to wash the bed linen weekly at 60 °C (active) or 40 °C (placebo) and to continue their normal cleaning routines. A questionnaire completed at the end of the trial showed that blinding of the families had been effective [7].

Dust sampling and allergen measurement

Dust samples were taken from the carpet, mattress, duvet and pillow of each child before treatment (baseline), and at 2, 6, 14 and 24 weeks after the start of treatment. Airborne allergen was usually measured for the 14 days prior to collecting surface samples at these time intervals using Petri dishes and Casella samplers. No Casella sampler collection was made 0–2 weeks after the start of treatment.

Surface sampling

Surface dust was collected by a blinded investigator (JW) through the sheet on the surface of the mattress cover, from the duvet cover and carpet in the bedrooms of the children. A Hoover Dustette (150 W) vacuum cleaner with a 10 micron mesh nylon filter attached behind the flattened nozzle was moved in a standard manner for a period of 2 min within a 0.25-m² template [8]. Pillows were vacuumed for 30 s within a 0.06-m² template [3]. Allergen is expressed as total surface content in nanograms.

Air sampling

Casella samplers were calibrated before use with the apparatus and method recommended by the manufacturers. The Casella air samplers (Casella, Bedford, UK) sampling at 2 L/min (PTFE filters, pore size 1.2 µm 25 mm diameter, Sartorius Ltd, Epsom, UK) was clipped to the edge of the pillow ≈ 20 cm from the sleeping child's head. The two different types of Casella samplers were randomly allocated to collect cumulative samples. Thirty-one homes used the battery-driven Casella sampler AFC123. It was switched on before the child went to bed and switched off when parents retired and the battery was then put on to charge from the mains. Parents recorded the time the Casella sampler had been running. Twenty homes used the mains-driven Casella sampler AFC124 which had an integral time recorder. It was switched on before the child went to bed and switched off shortly after rising. Pilot studies using AFC123 over one and three nights had detected Der p 1 in only a low proportion of samples. For this reason both types were used each night for a median of 12 days, range 8–14 days, to accumulate a sample of dust over this time period. The results are expressed as ng/m³ air sampled, i.e. weight per unit volume of air sampled.

Petri dishes (8.5 cm diameter, pretreated with 2% teleostean gelatine [9]) were exposed continuously for a median of 12 days, range 8–14 days; one on the floor and one at pillow height [6]. The hypothesis was that dust disturbed from items in the room during the exposure time would become airborne and later settle. Baseline dust allergen data sets were collected from 61 homes. Data are presented as ng/day.

Allergen analysis

Dermatophagoides pteronyssinus which is the major house dust mite found in Bristol [10] produces the allergens Der p 1 and Der p 2. All children's homes were analysed for both allergens. Fel d 1, produced by cats, was measured in samples from the seven cat owners and 19 other control families. Noncat-owning families (both active and placebo) from the same locality were selected as controls for each cat owner.

Dust samples from the Petri dishes and air filters were extracted in batches in 1 mL PBS containing 0.5% TWEEN 20 and 0.2% bovine serum albumin overnight. Dust samples were centrifuged (Micro-centrifuge, Quickfit Instrumentation, Stone, UK), supernatants taken and stored at -20°C for assay. Air samples on filters were sonicated for 10 s at 22 micron amplitude, filtered (Anotop 10 plus, $0.2\ \mu\text{m}$, Whatman, Bantry, UK) and stored at -20°C for assay. Der p 1, Der p 2 and Fel d 1 were measured using a two-site monoclonal antibody ELISA [11]. In our laboratory the coefficients of variation were 21% for Der p 1 and 17.1% for Der p 2 assay; the minimal detectable quantity (threshold) for the Der p 1 assay was 1 ng/mL. In all assays the technician was blinded as to whether the samples were active or placebo.

Statistical analysis

Nonparametric tests were used as the data were extremely skewed due to the large proportion of zero values, especially after treatment. The nonparametric data were expressed as medians and ranges. Spearman's rank correlation coefficient was used to investigate the relationship between surface and air sampling techniques. Mann–Whitney tests were used for comparisons between treatment groups and Wilcoxon matched pairs signed rank tests for comparisons between baseline and treatment within a group. If scores were categorical, Fisher's Exact test or McNemar test were used as appropriate. All *P*-values are two-tailed, with a *P*-value of less than 0.05 considered to indicate statistical significance. The data (*x*) were transformed to $\text{Log}_{10}(1 + x)$ in Fig. 1. Statistical analysis was performed using SPSS (Chicago, USA) for Macintosh.

Results

Sixty-two children started the trial and baseline measurements were made on them all. Treatment was incomplete in six families so all subsequent measurements were discarded, there were 26 active and 30 placebo correctly treated rooms at the start of the avoidance trial. Covers were proven without the subject's knowledge to have been removed since the previous visit in two active and two placebo homes so all subsequent measurements were discarded. There were only seven cat-owning homes and by chance six were found to be allocated to the active group.

Surface sampling results for HDM allergen have been reported in detail elsewhere [3].

Baseline

Casella sampler results

Table 1 summarizes these results.

Forty-five of the 51 homes given Casella samplers at baseline produced a convincing family record card of Casella sampler usage and an acceptable filter (27 of 31 battery Casella samplers and 18 of 20 mains Casella samplers). The most frequent cause of sample discard or loss was tampering with the filter by the child ($n = 5$). Such difficulties tended to recur in the other recording periods.

There were other unquantified sources of variation: The battery charged AFC123 Casella sampler was sometimes left running all night by mistake by the parents. Only if the battery charge lasted the night could the time the Casella sampler had run be recorded accurately. The mains Casella sampler AFC124 sometimes stopped pumping after a few nights. Changing to a clean new filter always remedied this but subsequent analysis found no HDM allergen on the filter papers ($n = 4$).

The minority of Casella sampler filters contained detectable Der p 1 allergen (40%) and far less collected Der p 2 (3/44). There was no difference between the different Casella samplers in the proportion of positive detection of Der p 1 (both $\approx 40\%$). However, when comparing only those Casella samplers that did detect Der p 1, the mains-powered Casella samplers collected lower quantities of Der p 1 per unit volume of air (median 0.2 ng/m³ range 0.2–0.98) than the battery powered Casella samplers (Der p 1 median 1.35 ng/m³ range 0.43–4.49) ($P = 0.002$) presumably because they continued to run through the still hours of the night when there was less airborne dust to collect (about 12 h nightly). The battery powered Casella samplers, although they sampled air at the same rate, were used for shorter running times only around the child going to bed or rising (about 3 h nightly). Fel d 1 was detected in air samples of four out of five cat owners' homes but none was found if there was not a resident cat.

Table 1. Positive Casella recordings before and after allergen avoidance measures.

	Week	Active (total no./%+ve)	Placebo (total no./%+ve)
Der p 1	Baseline	22/50	21/33.3
	6	14/0†	22/4.5†
	24	16/0†	17/29.4*
Der p 2	Baseline	22/9.1	21/4.8
	6	14/0	22/4.5
	24	16/0	15/0

* $P < 0.05$. Active vs placebo. Fisher's exact test. † $P < 0.05$ differs from baseline McNemar test.

Petri dishes

There was no significant difference between upper and lower Petri dishes (which were exposed for the same length of time) for all the allergens, therefore the upper and lower amounts have been added to give a total Petri dish amount. Petri dishes from 85% of the 61 homes collected Der p 1 and 76% Der p 2. Fel d 1 was found in Petri dishes in six out of seven cat owners' bedrooms but only one of 19 noncat owners' bedrooms.

Relationship between different allergens

Table 2 compares detection rates and allergen content in the 26 homes in which all three allergens were measured. Allergen collected from duvets and pillows showed the

same distribution although content was always lower than carpets and mattresses. Cat-owning homes had significantly more Fel d 1 in mattresses and carpets than noncat-owning homes, although there was no difference between these homes in HDM allergen content. As the Fel d 1 'reservoir' in the bedding and carpet of noncat owners homes was very low, it is perhaps unsurprising that very little airborne Fel d 1 was detected in these homes.

Post treatment

The significant decrease in HDM allergen levels achieved in the bedding (mattress and duvet) of the active group compared with baseline and with placebos and the lack of change in carpet allergen levels has already been reported by us [3].

Effect of active treatment on surface cat allergen

The effect of treatment depended upon whether or not a cat was present in the home. In homes without cats 24 weeks after active treatment mattresses had lower Fel d 1 content than placebo treated ($P = 0.04$) (Table 2).

Casella sampler

Casella sampler results obtained during trial procedures are given in Table 3. Only the presence of allergen is reported here as the proportion of either type of Casella sampler recording detectable dust allergen was so low after either treatment that the different Casella samplers have been analysed together. Both active and placebo groups showed a reduction in positive recordings 6 weeks after baseline. This was sustained to 24 weeks only in the actively treated group. Fewer Casella samplers detected Der p 1 in actively

Table 2. Comparison of baseline detection levels in seven homes with cats (except for Casella sampler $n = 5$) and 19 geographically matched homes without cats (except for Casella sampler $n = 15$). These are the only homes where Fel d 1 was measured.

	Surface samples				Air samples			
	Mattress		Carpet		Petri dish		Casella sampler	
	% +	Median (ng)	% +	Median (ng)	% +	Median (ng/m ³)	% +	Median (ng/day)
<i>Noncat owners</i>								
Der p 1	100	460 (34–5000)	84	170 (0–3500)	90	0.7 (0–3.1)	60	0.2 (0–4.5)
Der p 2	95	180 (0–1220)	79	122 (0–1600)	94	0.1 (0–2.3)	7	0.0 (0–0.2)
Fel d 1	56	32 (0–2240)*	53	16 (0–344)**	5	0 (0–0.1)****	0	0.0 (0–0)***
<i>Cat owners</i>								
Der p 1	100	400 (64–1300)	43	22 (0–780)	100	0.4 (0.1–3.2)	40	0.0 (0–1.9)
Der p 2	100	110 (16–1604)	57	0 (0–480)	86	0.14 (0–2.7)	20	0.0 (0–0.3)
Fel d 1	100	160 (72–1848)*	100	256 (44–1520)**	86	2.5 (0–64)****	80	0.5 (0–14.7)***

% + = % positive detection of allergen. * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ **** $P < 0.0001$.

Table 3. Effect of treatments on Fel d 1 surface content of mattress and carpets in bedrooms.

		Active			Placebo			
Week	n	%	+	Median (ng)	n	%	+	Median (ng)
<i>No cats</i>								
Mattress								
	Baseline	9	55.6	24 (0–2240)	9	55.6		40
	2	8	25.0	0 (0–104)	10	40.0		0
	6	9	11.1	0 (0–328)	10	40.0		0
	24	9	0.0	0 (0–0)*†	9	33.3		0
Carpets								
	Baseline	9	55.6	32 (0–200)	10	50.0		0
	2	9	22.2	0 (0–224)	10	20.0		0
	6	7	57.1	20 (0–1360)	10	40.0		0
	24	8	37.5	0 (0–248)	10	60.0		48
<i>Cat owners</i>								
Mattress								
	Baseline	6	100	184 (72–1920)	1	160		
	2	6	100	236 (148–560)	1	216		
	6	6	100	668 (496–2040)	1	112		
	24	5	100	592 (172–1040)†	1	116		
Carpets								
	Baseline	5	100	220 (44–1520)	1	400		
	2	5	100	240 (104–376)	1	120		
	6	5	100	512 (104–1040)	1	60		
	24	5	100	356 (64–640)	1	256		

n = no. of households. % + = % positive detection of allergens. * $P = 0.039$ Active vs placebo Mann-Whitney *U*-test. †indicates difference from baseline (Wilcoxon, $P < 0.05$).

treated homes than placebo homes after 24 weeks. ($P < 0.05$).

Petri dish

Petri dishes were successful in collecting allergen. Figure 1 illustrates the Der p 1 and Der p 2 results. There was a significant reduction in Der p 1 collected in the Petri dishes of the active group. This resembles the changes recorded in surface samples taken from the mattresses [3]. Both groups showed a reduction in Der p 2 allergen after treatment compared with baseline.

Fel d 1 content remained extremely low/undetectable in noncat owners throughout the trial. In the seven cat owners, Fel d 1 content of Petri dishes remained high irrespective of treatment.

Discussion

We detected Der p 1 in Casella samplers in 40% of baseline

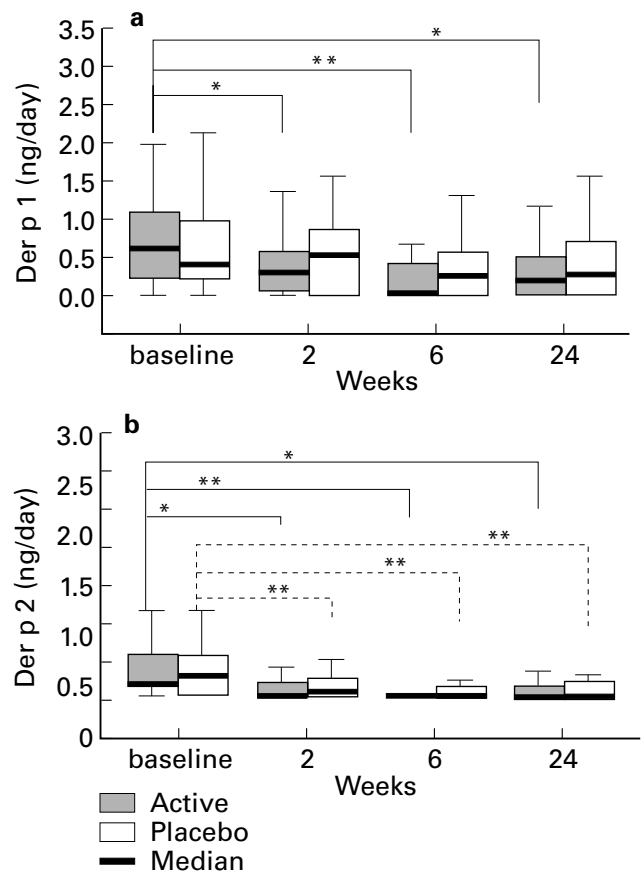


Fig. 1. (a) Der p 1 and (b) Der p 2 content of Petri dishes left open for 12 days before and after treatment in 61 homes. Active = 28, Placebo = 33. Median separates 25th and 75th percentiles (bottom and top of box). Whiskers show largest and smallest values that are not outliers. * $P < 0.05$, ** $P < 0.01$ (significant difference of treatment group from baseline Wilcoxon matched pairs). In (a) only the active treatment shows, in all cases, a significant difference.

measurements. Others using similar equipment in a similar climate did not detect any Der p 1 [4]. The difference is probably because our Casella samplers were turned on while the child was going to or getting up from bed, presumably causing some dust disturbance; the 70 children were selected because they had higher HDM content in their mattresses and a cumulative sample was collected over 12 nights. Using an air sampler with flow rates almost 10 times more powerful than our Casella samplers, HDM allergens have been found to settle very rapidly after disturbance and to be undetectable in quiet conditions [5]. Casella samplers were much less successful at collecting Der p 2 than Der p 1 (Table 3). It has been reported that the ratio of group I dust allergens to group II allergens in air during disturbance is 2.5 : 1 [5,12]. If Der p 2 is mainly associated with the heavier mite body fragments as compared with faecal particle

fragments bearing Der p 1, it may be settling out of the air quicker. The Casella samplers may have been more successful in collecting Der p 1 than Der p 2 as the suction of 2 L/min may have been sufficient to pull in more of the lighter Der p 1 particles. In contrast almost as many Petri dishes contained Der p 2 as Der p 1 although lower amounts of the former were collected.

A far greater proportion of Petri dishes collected HDM allergen compared with the Casella samplers. This could be because the Petri dishes were exposed continuously including during normal household cleaning whilst the Casella sampler collected samples only at night.

Homes with cats have been reported to have higher cat allergen in mattress [13] and air [5] than homes without cats and our findings agree with this. Fel d 1 is known to stay airborne in undisturbed conditions and this has been related to the small size of some of the suspended particles [5]. We found baseline Fel d 1 samples from mattress and carpet of *noncat* owners were very low relative to our cat owners. This is consistent with the view that low cat allergen content in mattress and carpets are not likely to produce respirable Fel d 1 in the air.

The Casella samplers and Petri dishes differ not only in effectiveness in collecting different allergens but perhaps more significantly in the proportions of the various allergens they collect. It is not known which system most closely relates to the human inhaled intake. The present results indicate the need for considerable caution in using any single system as a proxy for that intake. Swanson *et al.* [14] have reported finding correlation between mattress and air Der p 1 as we have [6]. Their measurement conditions were very different from ours — the homes all had forced air heating (unlike ours which had natural ventilation) and high volume air samplers operating at 200 L/min. Perhaps for the allergens which predominately reach the air by surface disturbance, we can anticipate a relationship between surface and air content.

The Petri dishes could be a useful alternative to Casella samplers for collection of aeroallergens. They are compared in Table 4. Petri dishes collecting settled dust provide a quiet, cheap and easy alternative method for measuring exposure to mite aero-allergen in allergen-avoidance trials. The 12-day collection period we required may make both techniques difficult for large epidemiological trials.

Effect of treatment

Der p 1 aeroallergen was reduced after allergen avoidance procedures as measured by Casella samplers and Petri dishes (Table 3 and Fig. 1). This reduction in Der p 1 aeroallergen reflects the reduction of Der p 1 we previously reported in mattress content and occurs despite carpet Der p

1 remaining unchanged [3]. This reduction produced a modest improvement in asthma symptoms, a decrease in bronchodilator use and reduced bronchial irritability in asthmatic children [7]. The results suggest that the carpet is not an important source of aeroallergen in the bedroom.

The placebo group also showed a reduction in HDM aeroallergen, especially Der p 2 collected by Petri dish when compared with baseline. This may reflect the similar change we found in HDM dust samples collected from placebo-treated mattresses, could be due to the placebo cotton covers and regular washes [3]. The initial vacuuming to remove acaricide/chalk was probably not important as it had had no effect on carpet mite content.

Cat allergen can induce asthmatic attacks in cat-sensitive children. It has been reported that reductions in airborne cat allergen, even in the presence of a cat, have been achieved by reducing furnishings, washing the cat, vacuuming and air filtration [15]. We found our HDM avoidance treatment did not reduce Fel d 1 mattress surface content in cat-owning homes. Presumably the cat was adding Fel d 1 to the environment faster than it was removed by washing and cleaning [15]. The increase in mattress Fel d 1 in cat-owning households may have been due to the cats choosing to avoid the Acarosan-treated carpet and preferring the freshly laundered bed. There is no evidence that Fel d 1 aeroallergen increased as the trial progressed but the small number of cat owners (six) could explain why no effect was detected.

At 24 weeks we found no detectable cat allergen in the mattress dust samples in the active treatment group without cats. This result was significantly lower than the placebo group and showed a significant reduction from baseline measurements. While removal of the cat is the most effective way of reducing Fel d 1 in the home [16], the concentration of allergen in the mattress can remain elevated for up to 5 years [13].

What is the reason for this reduction in mattress cat

Table 4. Comparison between Casella sampler and Petri dish.

	Casella	Petri dish
Unit Cost	Expensive (> \$500)	Cheap (\$0.17)
Noise	Can prevent sleep	Silent
Participation needed by parent/child	Min. 2× daily	Nil
Temptation for fiddling by child (lost results)	High	Low
Time recorded	Child in room	All the time
Likelihood of detecting allergen	Low	High
Close to child's nostrils	Yes	No

allergen in the noncat owners? It is not due to seasonal changes as families were studied in overlapping randomly allocated batches over 11 months, or due to vacuuming — Central, microfilter and HEPA-filter vacuum cleaners did not reduce Fel d 1 or dog allergen over a 35-day sampling period [17]. Conventional upright and canister vacuum cleaners, similar to the types used by the families of this study, were not effective in retaining cat allergen within the machine in laboratory tests [17]. It seems likely that the reduction is due to the effect of the encasing covers and/or the washing procedures. It has been demonstrated that machine washing even in cold water reduces Der p 1 concentration of bedding and if the temperature is above 55 °C the mites are killed [18]. We have already reported finding that the active treatment was associated with a dramatic decrease of surface Der p 1 on mattresses [3]. Although 22% of cat allergen particles have an aerodynamic diameter of $<2.5\text{ }\mu\text{m}$, a proportion are the same size as HDM particles [5] and could be retained within the encasing covers. The smaller particles that 'escaped' and the additions from the outside via clothes, etc. may have been controlled by regular hot washing.

Whatever the mechanism of action our active regime of using these encasing covers plus hot regular washing of bed linen may offer advantages for cat allergen control as an alternative to chemicals such as tannic acid [19] and Allersearch DMS [20] in homes without cats. Our measures could be a useful when attempting to remove all sources of cat allergen after removal of the cat but total removal of the bedding is likely to be more effective.

We conclude that mite allergen can be removed from the environment of children by effective house dust mite allergen avoidance measures (encasing covers and regular hot washes). These reduce mattress mite allergen content [3], reduce mite allergen in the air and, in noncat-owning homes, reduce the cat allergen present on the mattress.

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