

## Original articles

### The effect of cat removal on allergen content in household-dust samples

Robert A. Wood, MD,\* Martin D. Chapman, PhD,\*\*  
N. Franklin Adkinson, Jr., MD,\*\* and Peyton A. Eggleston, MD\*  
Baltimore, Md., and Charlottesville, Va.

*To evaluate the effect of cat removal on cat-allergen content in the home, serial house dust samples were collected from 15 homes during a 9- to 43-week period after cat removal. Samples were obtained with a hand-held vacuum cleaner, and allergen content was quantitated by a radioimmunoassay specific for the major cat allergen, Fel d I. Baseline Fel d I content ranged from 7.8 Food and Drug Administration units per gram of dust to 436.7 U/gm (median 61.2 U/gm), consistent with levels found in homes with a pet cat. Fel d I levels declined gradually in most homes, and by 20 to 24 weeks after cat removal, eight of 15 reached levels consistent with levels found in control homes without cats. In two of those homes, allergen levels fell much more rapidly after aggressive environmental control measures were undertaken. In the other seven homes, however, the decline occurred at a much slower rate, with three homes demonstrating persistent elevations in Fel d I content for 20 or more weeks. These data demonstrate that the task of allergen elimination from an indoor environment is extremely difficult, even when the source of a specific allergen can be identified and removed. (J ALLERGY CLIN IMMUNOL 1989;83:730-4.)*

Domestic cats are a common cause of allergic reactions in individuals with allergic rhinitis and asthma.<sup>1-4</sup> Since avoidance of cat allergen is the treatment of choice for such reactions, it is frequently recommended that cats be removed from the homes of cat-sensitive individuals. Trials of cat avoidance are also commonly recommended in which a cat may be temporarily removed from a home in an effort to determine its contribution to a patient's symptoms. These practices are based on the assumption that levels of cat allergen will decline after a cat is removed from

#### Abbreviations used

Fel d I: *Felis domesticus* allergen I  
RIA: Radioimmunoassay  
Mab: Monoclonal antibody  
Der p I: *Dermatophagoides pteronyssinus* allergen I  
Der f I: *Dermatophagoides farinae* allergen I  
CV: Coefficient of variation

From the \*Departments of Pediatrics, Division of Immunology, and \*\*Internal Medicine, Division of Clinical Immunology, The Johns Hopkins University School of Medicine, Baltimore, Md.; and the \*\*\*Division of Allergy and Clinical Immunology, Department of Medicine, University of Virginia, Charlottesville, Va. Supported by National Institutes of Health Grant AI-21073, by the Hospital for Consumptives of Maryland (Eudowood Fund), and the Harriet Lane Pediatric Fellowship.

Received for publication July 19, 1988.

Accepted for publication Sept. 18, 1988.

Reprint requests: Robert A. Wood, MD, The Johns Hopkins Hospital, 600 N. Wolfe St., CMSC 1103 Baltimore, MD 21205.

a home, presumably at a rapid rate. There are, however, no published data to verify this assumption. Furthermore, in three recent studies<sup>5-7</sup> it has been demonstrated that cat allergen is commonly present in settled dust, even in homes without cats, and it has been postulated that this might be due in part to residual allergen from cats that had previously lived in the home. We therefore undertook this study to determine the rate at which cat allergen declines within a home after cat removal. This was accomplished by analyzing sequential house dust samples for *Fel d I*, formerly cat allergen I,<sup>8</sup> the major cat allergen, after cat removal.

## METHODS

### Sample collection

Fifteen homes were identified in which a cat was being removed or had been recently removed. At least one cat-sensitive individual lived in seven of the homes, whereas the remainder housed nonallergic volunteers. A baseline dust sample was collected within 4 weeks of cat removal (range 0 to 28 days), and most subsequent samples were obtained at 4- to 6-week intervals (range 1 to 16 weeks). These homes have now been sampled for from 9 to 43 weeks.

All samples were collected by one of the investigators or a trained volunteer with a hand-held vacuum cleaner (Douglas Rx 365 Hand Vac, model No. 6735, Douglas Products, Walnut Ridge, Ark.). The vacuum has a disposable cloth filter that can easily be removed to facilitate dust retrieval. In each home, a carpeted area of approximately 1 m<sup>2</sup> was identified, and all samples were obtained by vacuuming this area for 5 minutes. The site for sampling was chosen as an area presumed by the home owner to be high in cat allergen. Samples were obtained from the living room or family room in 11 homes and from a bedroom in four homes.

### Dust processing

After vacuuming, the dust samples were removed from the vacuum and weighed. The samples were then sieved through a 0.3 mm brass mesh to produce fine dust. The fine dust was weighed, and a 100 mg aliquot was then extracted in 2 ml of borate-buffered saline, pH 8.0, by rotation for 2 hours. The extracts were centrifuged, and the supernatant was removed and stored at -20°C. Samples yielding <100 mg of fine dust were extracted in a proportionately smaller volume of buffer.

### Dust analysis

All samples were analyzed for the major cat allergen, *Fel d* I, with a two-site RIA, as has been described previously.<sup>5</sup> Two Mabs, specific for *Fel d* I and directed against different epitopes of *Fel d* I, were kindly provided by Dr. Robert Aalberse. A 50% saturated ammonium sulfate fraction of the first Mab (FdIB) was coupled to cyanogen bromide-activated cellulose disks (10 µg FdIB per disk). The disks were washed and placed in flat-bottomed polystyrene microtiter plates (Nunc Intermed, Roskilde, Denmark). Diluted dust extracts (0.1 ml) were then incubated with each disk for 4 hours. The disks were again washed and incubated overnight with the second anti-*Fel d* I Mab (FdIA) radiolabeled with <sup>125</sup>I (specific activity, 30 µCi/µg). The disks were then washed, dried, and counted in a gamma counter. The quantity of FdIA bound was directly related to the quantity of *Fel d* I in the sample, and values were obtained from a control curve with cat reference E3 (Office of Biologics and Research Resources, Bethesda, Md.), which contains 10.5 Food and Drug Administration units of *Fel d* I per milliliter. Doubling dilutions of this reference antigen were used to form the control curve, from 52.5 to

TABLE I. Reproducibility of RIA for *Fel d* I

	N	CV	
		Mean (%)	Range (%)
Intra-assay	12	4.5	0.9-6.7
Interassay	12	5.6	1.5-14.5
Interextract	14	10.1	1.1-17

0.8 mU of *Fel d* I per milliliter, which was run in parallel with each assay. All samples were assayed at four different dilutions, and values were interpolated from the control curve. The assay is highly specific for *Fel d* I and has a lower limit of sensitivity of 0.8 mU of *Fel d* I per milliliter (approximately 4 ng/ml).

In addition to the analysis for *Fel d* I, all samples were also analyzed for *Der p* I and *Der f* I, the major allergens of the dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, respectively. As have been described previously, these assays were also two-site monoclonal RIAs.<sup>9</sup> In brief, microtiter plates (Dynatech, Alexandria, Va.) were coated overnight with 1 µg per well of either Mab 5H8 (anti-*Der p* I) or Mab 6A8 (anti-*Der f* I) ascites in 0.1 mol/L of bicarbonate buffer, pH 9.6, at 4°C. After plates were washed, they were incubated for 1 hour with 1% bovine serum albumin and rewashed. Diluted dust extracts were then added (0.1 ml per well) and incubated for 2 hours. The plates were then washed and incubated for 2 hours with 2 ng of <sup>125</sup>I-labeled 4C1 (specific activity, 20 µCi/µg), a Mab that recognizes cross-reacting epitopes of *Der p* I or *Der f* I. All samples were assayed in duplicate at four different dilutions. Results were obtained by interpolation from a control curve constructed with doubling dilutions of *Der p* I standard (University of Virginia 86/02) or *Der f* I standard (University of Virginia 86/01)<sup>9</sup> from 250 ng/ml to 0.5 ng/ml.

## RESULTS

### Preliminary studies

Preliminary studies were undertaken to evaluate the efficiency and reproducibility of the sampling, extraction, and assay techniques. First, because of concerns that the vacuum filter might not be efficient for small particles, five homes with cats were sampled with vacuums in which a glass fiber filter with a pore size of 0.3 µm (Millipore, Bedford, Mass.) was mounted behind the cloth filter. Although *Fel d* I levels in these samples ranged from 21.4 U/gr to 87.0 U/gm, no *Fel d* I was detected on any of the glass fiber filters, documenting a filter efficiency of at least 99%.

Second, to evaluate the effects of sieving on extraction efficiency, for the first 66 dust samples a 100 mg aliquot of unseived dust was also extracted, as

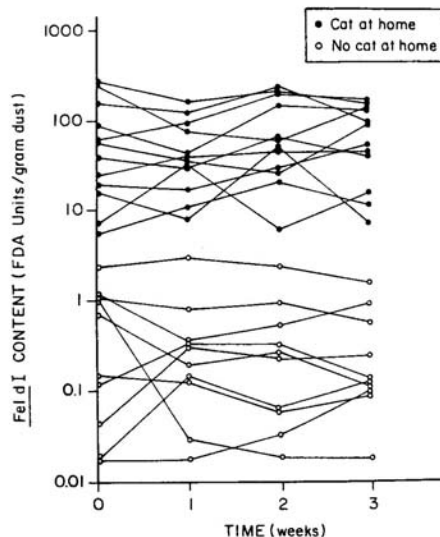


FIG. 1. *Fel d I* content of weekly dust samples obtained from homes with and without cats.

previously described. In all cases, the content of *Fel d I* in the extract of unsieved dust was less than that of the fine dust extract, with ratios ranging from 0.05 to 0.91 (median, 0.29), unsieved to sieved.

To assess assay reproducibility, 12 extracts were each divided into three aliquots and analyzed for *Fel d I* separately within the same assay. CVs ranged from 0.9% to 6.7% (mean, 4.5%) (Table I). Interassay variability was evaluated by dividing another 12 extracts into three aliquots and assaying them on different days; CVs ranged from 1.5% to 14.5% (mean, 5.6%). Finally, 14 sieved dust samples were divided into three aliquots, extracted, and assayed separately. This procedure yielded CVs of 1.1% to 17% (mean, 10.1%). Thus, both the extraction and assay techniques were highly reproducible.

We next evaluated the variability in antigen concentration in repeat house dust samples by obtaining weekly dust samples from homes both with and without cats (Fig. 1). Samples were obtained by vacuuming the same area of carpet for 5 minutes on each visit. We found a significant difference ( $p < 0.01$ ) in *Fel d I* content in homes with and without cats, with levels in homes with cats ranging from 5.6 U/gm to 282.4 U/gm ( $n = 12$ ; median, 49.2 U/gm) and levels in homes without cats ranging from undetectable to 3.2 U/gm ( $n = 10$ ; median, 0.3 U/gm). These levels are consistent with levels found in previous studies.<sup>3-7</sup> CVs for repeat samples from individual

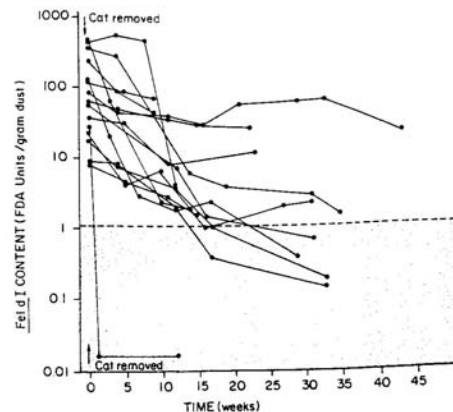


FIG. 2. *Fel d I* content of serial dust samples obtained from homes after cat removal. The dashed line (shaded below) represents the upper 95% confidence limit for *Fel d I* content in samples from the 10 control homes without cats.

homes ranged from 21% to 159% (mean, 54%). This variability was not statistically significant for any home ( $p > 0.1$ , analysis of variance).

#### Cat-removal study

In contrast to the stable levels found in repeat samples from homes containing cats, the levels of *Fel d I* in sequential dust samples from the 15 homes from which cats were removed gradually declined, as displayed in Fig. 2. Baseline *Fel d I* content, denoted for each home at time 0, ranged from 7.8 U/gm to 488 U/gm, consistent with the range found in homes with a known cat. With routine cleaning, including regular vacuuming in all homes and steam cleaning in three homes, there was a gradual decline in *Fel d I* content during a period of several months in most homes. By the twentieth week after cat removal, levels in eight of the 15 homes were within or very close to the upper 95% confidence limit of the 10 control homes without cats. Median *Fel d I* content reached that level at approximately 23 weeks after cat removal (Fig. 3).

In two homes, *Fel d I* levels declined much more rapidly. In one home, the level fell from 26.6 U/gm to undetectable during a period of 1 week. This fall coincided with the removal of all carpets and furniture from the home. In the second home, levels fell from 472 U/gm to 4.4  $\mu\text{m}/\text{gr}$  during a 4-week period. This home was noteworthy in that for the first 8 weeks of sampling, during which the home was vacant and no cleaning was performed, levels remained stable and

extremely high. Then, again after the removal of all carpets and furniture, levels declined rapidly.

In several homes the fall in cat-allergen content occurred at a much slower rate. We were unable to explain this variability in the rate of antigen decline based on differences in cleaning practices, the magnitude of the baseline level, the depth of the carpeting, or the number or breed of cats formerly living in the home.

In one home, during a 43-week period, *Fel d I* levels only fell from 82.8 U/gm to 20.6 U/gm. To investigate this home further, samples were obtained from two other sites in the home at week 43 to detect a possible reservoir of cat allergen. A sample from the basement, obtained from a workshop area where the cat had previously spent a great deal of time and which had not been as thoroughly cleaned as the rest of the home, had a *Fel d I* level of 212 U/gm. It is possible that allergen from this area had been spread throughout the home via the forced-air heating system or on the resident's shoes and clothes. Finally, we are certain that the cat was indeed removed from the home and was not simply living in this area in the basement.

#### Dust mite allergens

As a control, all samples were also analyzed for *Der p I* and *Der f I*, the major allergens of the dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, respectively. The median values for these antigens are displayed in Fig. 3, along with median *Fel d I* values, which have been grouped to include samples collected within 3 weeks of one another. Median *Fel d I* content was initially 61.2 U/gm ( $n = 15$ ) and fell to 44.7 U/gm at 4 weeks ( $n = 13$ ), to 4.2 at 10 weeks ( $n = 14$ ), to 1.8 U/gm at 15 weeks ( $n = 10$ ), and to 0.7 at 32 weeks ( $n = 7$ ). Median *Der p I* and *Der f I* levels, however, remained relatively stable, with median *Der p I* values ranging from 74 ng/gm to 190 ng/gm, and *Der f I* medians ranging from 1000 ng/gm to 1800 ng/gm. Thus, median levels of the target antigen, *Fel d I*, changed significantly after cat removal ( $p < 0.001$ , Kruskal-Wallis analysis of variance), whereas two control antigens demonstrated very little change ( $p > 0.5$ ).

#### DISCUSSION

Sensitivity to cat antigen can be demonstrated by skin tests in up to 50% of individuals with atopic disease.<sup>1-4</sup> In most cases this sensitivity is mediated by IgE antibody directed against a single cat allergen,<sup>3</sup> denoted *Fel d I*, which is produced in cat salivary glands and in sebaceous glands of cat skin.<sup>10</sup> With the recent development of sensitive and specific immu-

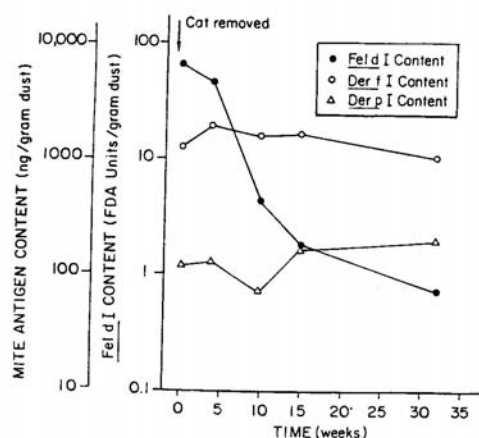


FIG. 3. Median content of *Fel d I*, *Der p I*, and *Der f I* after cat removal.

noassays for *Fel d I*, it is now possible to study the environmental distribution of this important allergen.

Clinically, although cat antigen is certainly recognized as a major perennial allergen, dust mites have traditionally been considered a far more vexing problem. Although they are visible only with a microscope, are virtually impossible to eradicate, and are capable of repopulating most areas, even if they are eradicated, it has logically been assumed that with cat allergen the only real problem is convincing the allergic patient to give up the cat. Once that hurdle could be overcome, it appeared that the problem should be resolved quickly. We have been suspicious that the situation might not be quite that simple, however, since finding in a previous study that cat allergen, like dust mite allergens, is virtually ubiquitous in Baltimore homes.<sup>7</sup>

We have now demonstrated that in most homes levels of *Fel d I* decline very slowly in settled dust after cat removal. It generally takes at least 20 weeks from the time of cat removal for these levels to fall to the levels found in homes without cats. Furthermore, in some homes levels may remain persistently elevated for even longer periods of time. Although the reasons for this variability are as yet unclear, in one home the slow rate of decline in antigen content may have been related to a significant reservoir of cat allergen in the basement.

At the other extreme, we have demonstrated in two homes that with aggressive environmental control measures, including carpet removal, it is possible to substantially reduce cat-allergen content in settled dust much more rapidly. We hope less drastic measures can be identified to accomplish effectively this task

in all homes. Unfortunately, it did appear that steam cleaning of carpets had no added benefit over regular vacuuming in the three homes in which it was used. This finding is consistent with previously studies documenting the heat stability of *Fel d 1*.<sup>8</sup>

Although no exact standards exist regarding the clinical significance of specific allergen levels in settled dust, certain guidelines have been suggested for *Fel d 1* in prior studies. Van Metre et al.<sup>11</sup> demonstrated that on exposure to the ambient air of a cat-containing room with a *Fel d 1* level of 126 U/gm, all cat-sensitive individuals who were tested developed symptoms of rhinitis and asthma within 2 hours. Subsequently, Pollart et al.<sup>12</sup> have suggested that levels >10 µg/gm (2 U/gm) are a risk factor for acute asthma attacks. This suggestion is in general agreement with our impression that the levels observed in homes without cats are rarely capable of inducing acute symptoms. However, it has also been suggested that levels as low as 2 µg/gm (0.4 U/gm), commonly observed in homes without cats, may be a risk factor sensitization to *Fel d 1*.<sup>12</sup> The possibility that exposure to low levels may lead to sensitization is also supported by data demonstrating that the incidence of cat sensitivity is no higher in patients who live with cats than in patients who do not.<sup>1</sup>

In conclusion, several important clinical points are evident from this study. First, it is clear that patients should be informed that improvement in their symptoms may not occur for a prolonged period after a cat is removed from the home. Too often patients may prematurely conclude that their cats must not have been responsible for their symptoms when they do not improve quickly after their cats are removed. Similarly, in recommending a trial of cat avoidance for a patient, any trial of less than 4 to 6 months duration may be inadequate and, in some instances, even that may not be long enough. This recommendation is very different from recommendations found in standard allergy textbooks in which trials of 3 to 6 weeks<sup>13</sup> and 2 months<sup>14</sup> are advised.

Finally, it is clear that house dust analysis is a useful tool for monitoring specific allergen content in the

allergic patient's home environment. Although further study is needed to more precisely define the clinical significance of specific allergen levels, the present study illustrates the use of this tool in evaluating both allergen exposure and the effectiveness of specific environmental control measures.

#### REFERENCES

1. Linna O. Environmental and social influences in skin test results in children. *Allergy* 1983;38:513.
2. Murray AB, Ferguson AC, Morrison BJ. The frequency and severity of cat allergy vs. dog allergy in atopic children. *J ALLERGY CLIN IMMUNOL* 1983;72:145.
3. Ohman JL, Kendall S, Lowell FC. IgE antibody to cat allergens in an allergic population. *J ALLERGY CLIN IMMUNOL* 1977;60:317.
4. Sarsfield JK, Boyle AG, Rowell EM, Moriarty SC. Pet sensitivities in asthmatic children. *Arch Dis Child* 1976;51:186.
5. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen *Fel d 1*. *J Immunology* 1988;140:812.
6. Lowenstein H, Graff-Lonnevig V, Hedlin G, et al. Immunotherapy with cat- and dog-dander extracts. III. Allergen-specific immunoglobulin responses in a 1-year double-blind placebo study. *J ALLERGY CLIN IMMUNOL* 1986;77:497-505.
7. Wood RA, Eggleston PA, Lind P, et al. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988;137:358.
8. Leitermann K, Ohman JL. Cat allergen 1: biochemical, antigenic, and allergenic properties. *J ALLERGY CLIN IMMUNOL* 1984;74:147.
9. Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TAE. Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p 1* and *Der f 1*, and quantitative analysis of the allergen content of mite and house dust extracts. *J ALLERGY CLIN IMMUNOL* 1987;80:184.
10. Bartholomé K, Kissler W, Baer H, Kopietz-Schulte E, Wahn U. Where does cat allergen 1 come from? *J ALLERGY CLIN IMMUNOL* 1985;76:503.
11. Van Metre TE Jr, Marsh DG, Adkinson NF Jr, et al. The dose of cat (*Felis domesticus*) allergen 1 (*Fel d 1*) that induces asthma. *J ALLERGY CLIN IMMUNOL* 1986;78:62.
12. Pollart SM, Chapman MD, Platt-Mills TAE. Allergen levels inside homes: risk factors for acute asthma attacks [Abstract]. *J ALLERGY CLIN IMMUNOL* 1988;82:242.
13. Jelks ML, Solomon WR. Airborne allergens. In: Kaplan AP, ed. *Allergy*. New York: Churchill Livingstone, 1985:255-320.
14. Ohman JL. Allergic reactions caused by exposure to animals. In: Lichtenstein LM, Fauci AS, eds. *Current therapy in allergy, immunology, and rheumatology*. Philadelphia: BC Decker, 1985:5-8.