

Clinical aspects of allergic disease

Cat antigen in homes with and without cats may induce allergic symptoms

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Although Fel d 1, the major cat allergen, has been found in settled dust samples from homes both with and without cats, the clinical relevance of this allergen has never been studied. In this study we measured airborne concentrations of Fel d 1 in homes both with and without cats and then attempted to relate these levels to those obtained in our experimental cat challenge model to assess their clinical significance. In baseline samples we found measurable levels of airborne Fel d 1 in all 37 homes with cats (range, 1.8 to 578 ng/m³; median, 45.9 ng/m³) and in 10 of the 40 homes without cats (for detectable samples: range, 2.8 to 88.5 ng/m³; median, 17 ng/m³). Fel d 1 was present in the settled dust of 38 of 40 homes without cats (range, 39 to 3750 ng/gm; median, 258 ng/gm), although these levels were only weakly predictive of airborne levels. Repeat samples obtained weekly from 12 homes without cats yielded measurable airborne Fel d 1 in at least one of the four samples from all homes. When compared with challenges performed in our cat room facility at low levels of airborne Fel d 1 (<500 ng/m³), these home levels are within the range capable of causing upper and lower respiratory symptoms in subjects allergic to cats. We therefore conclude that the low level cat exposure that occurs in many homes without cats is capable of inducing symptoms in some patients who are sensitive to cats. The assessment of cat exposure should not be based solely on the presence or absence of a cat in the home. (J ALLERGY CLIN IMMUNOL 1996;97:907-14.)

Key words: Cat allergen, environmental allergens, animal allergens, antigen challenge

Allergic reactions to furred pets are extremely common. Three to ten percent of the general population and 15% to 40% of atopic individuals are allergic to cats or dogs, with allergy to cats being about twice as common as allergy to dogs.¹⁻³ Over half of all homes in the United States contain at least one cat or dog, and cats are now slightly more common than dogs.⁴ Further, up to one third of individuals allergic to cats live in a home with one or more cats.⁵

Although the environmental distribution of cat allergen has been extensively studied, there is very little information about the clinical relevance of specific allergen levels.⁶⁻¹⁴ In homes with cats, levels of Fel d 1 (*Felis domesticus* allergen 1), the major cat allergen, are typically very high and it has been assumed that these levels are capable of inducing symptoms in most patients who are allergic to cats. In addition, cat allergen has been found in settled dust samples from homes without cats, as well as in every other building where it has been sought, including newly built homes, shopping malls, doctors' offices, and even hospitals.⁹⁻¹⁴ Allergen levels in these sites are typically 100 to 1000 times lower than those found in homes with cats, and the clinical significance of this low-level exposure is completely unknown. However, it has been shown that many persons who have never lived around cats become sensitized, implying that low-level exposure may at least be capable of inducing sensitization.¹⁵

In this study we measured airborne Fel d 1, a potentially more useful marker of cat exposure than settled dust, in homes both with and without

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cats. We then sought to relate these levels to those seen during challenges in our cat exposure facility and thereby assess the clinical relevance of environmental cat exposure, including the low-level exposure encountered in homes without cats.

METHODS

Home sampling methods

Homes for sampling were recruited by advertisement and word of mouth. Settled dust samples were collected by vacuuming a 1 m² area of carpeting or upholstered furniture with a hand-held vacuum cleaner (Douglas Rx 365 Hand Vac, model No. 6735; Douglas Products, Walnut Ridge, Ariz.) for 2 minutes. Filters were removed from the vacuums and stored at -4° C until processing.

Air samples were obtained with a small portable pump (Gilian Instrument Corporation, Wayne, N.J.) with flow rates of 3 to 4 L/min and a 25 mm round glass fiber filter (Millipore Corp., Bedford, Mass.) contained in a plastic cassette, which was attached to the subject's lapel. All pumps were calibrated before each use with a Buck calibrator (A.P. Buck, Orlando, Fla.), and the flow rate and sampling time were recorded to calculate the volume of air sampled. Subjects wore the pumps for 1 hour during normal household activity, but they were asked to refrain from vacuuming during the airborne sample collection. In homes with cats, subjects were also asked to refrain from directly handling their cats during the sample collection. Filters were left in the cassettes and frozen at -4° C until extraction.

Baseline air samples were obtained from 37 homes with cats and 40 homes without cats. For the homes without cats, 26 of the 40 had been occupied by the same non-cat owner for at least 3 years, and none of the homes were known to have housed a cat within the previous year. Weekly air samples were also obtained over an 8-week period in 12 cat-containing homes, and weekly paired air and dust samples were collected over a 4-week period in 12 homes without cats. Nine of the homes without cats, from which weekly samples were obtained, were selected because they had detectable airborne Fel d 1 in their baseline samples, whereas the other three were chosen at random from the baseline group. None of the homes sampled were occupied by subjects studied in the cat challenge room.

Sample extraction and Fel d 1 assay

The settled dust samples were removed from the vacuum and weighed. They 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 extracted in 2 ml of borate-buffered saline, pH 8.0, by rotation overnight. The extracts were then centrifuged, and the supernatants were removed and stored at -20° C. Samples yielding less than 100 mg of fine dust were extracted in a proportionately smaller volume of buffer.

Filters for airborne samples were left in the cassettes

and frozen at -4° C until extraction. Extraction was performed by removing the support pads from the cassettes (leaving the filter in place), adding 1.5 ml of piperazine-N, N'-bis [2-ethanesulfonic acid]-Tween-80 to the cassette, and rotating the cassette overnight at 4° C. Extracts were collected by suctioning the visible fluid from the cassette and then compressing the filter in a 3 ml syringe to express any residual fluid. Return volumes averaged 1.2 ml (range, 1.0 to 1.3 ml). Extracts were frozen at -20° C until they were assayed.

Fel d 1 was measured with a two-site monoclonal ELISA, as previously described.¹⁶ The assay has a lower limit of detection of 0.4 ng/ml for air samples, or 2 ng/m³ for typical sampling conditions at a rate of 3 to 4 L/min for 1 hour. For dust samples, the lower limit of detection is 50 ng of Fel d 1 per gram of dust.

Cat challenge

In an effort to determine the clinical significance of airborne Fel d 1 levels in homes, cat challenges were performed as part of another study, at varying airborne antigen concentrations in our cat room facility. These challenges were performed in a 13.7 m³ room containing two cats, as described previously.¹⁷ The room is furnished with a bed covered with a sheet, an upholstered chair, and a small carpet. The room's ventilation is fully adjustable. The cats are free to move about the room except during challenges, when they are placed in a wire cage. The room has been extensively characterized with regard to its ventilation, air turnover, airborne Fel d 1 levels, and airborne Fel d 1 particle size distribution.¹⁷ The room has also been studied for other pertinent allergens, including dust mite and cockroach, and those levels were undetectable.

Volunteer subjects who were allergic to cats as determined by history were recruited by advertisement and characterized by skin testing, end-point titration to cat allergen, RAST to cat allergen, and methacholine challenge. Those volunteers with documented cat sensitivity were invited to undergo a cat challenge, which involved a 1-hour exposure in the cat room. Medications were restricted before challenge as follows: antihistamines (72 hours), inhaled β -adrenergic agonists (4 hours), oral β -adrenergic agonists (short-acting, 6 hours; long-acting, 12 hours), theophylline (12 hours), nasal or inhaled cromolyn sodium (48 hours), and nasal or inhaled corticosteroids (72 hours). Immediately before challenge, the cats were put in the cage, the room ventilation was turned off, and the bedding was shaken to disturb allergen. Baseline spirometry (model DECpc, spirometer; Warren Collins Inc., Bantree, Mass.) and peak flow recordings (Mini-Wright peak flow meter; Clement Clarke International Ltd., Columbus, Ohio) were performed in an adjoining room before subjects entered the cat room. During the challenge, upper (congestion, rhinorrhea, and pruritis) and lower (chest tightness, wheezing) respiratory symptoms were graded every 2 minutes on an arbitrary scale from 0 to 3, and all coughs

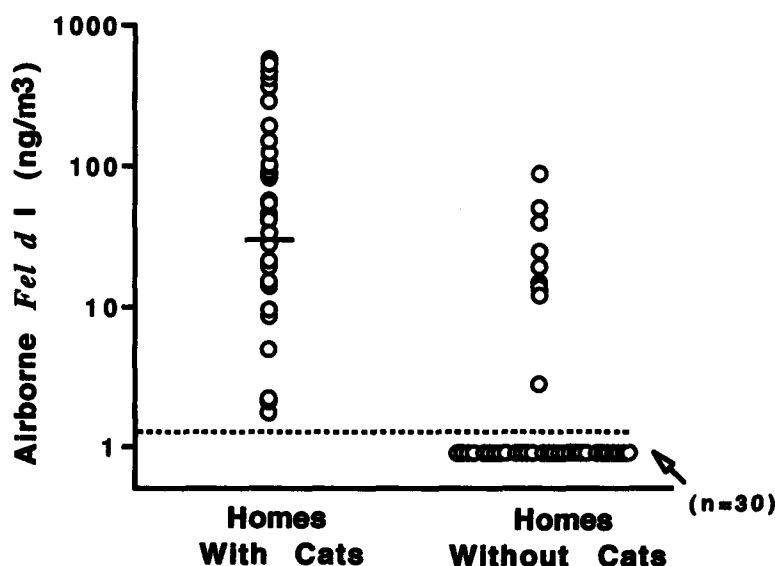


FIG. 1. Levels of airborne Fel d 1 in 37 homes with cats and 40 homes without cats. Dotted line denotes the lower limit of detection of the assay. The median level for homes with cats was 45.9 ng/m³. For homes without cats with measurable Fel d 1, the median was 17 ng/m³.

and sneezes were counted. Every 15 minutes, the subject exited to the adjoining room where spirometry was repeated. At that time, the bedding was once again shaken to maintain high airborne allergen levels. After the challenge, peak expiratory flow rates and symptom scores were monitored by the subject every 1 to 2 hours for 12 hours. Challenges were stopped before the 1-hour limit if the subject became too uncomfortable or if FEV₁ fell by more than 50%.

Airborne Fel d 1 levels were measured during challenges with the same personal samplers used in home sampling as described above. Pump flow rates were calibrated before each challenge, and the extraction and Fel d 1 assay procedures were identical to those used for the home samples.

The protocol was approved by the institutional review board of the Johns Hopkins University School of Medicine, and all subjects gave informed consent.

Statistical analysis

Spearman's correlation coefficient was used to compare paired air and dust samples. Nasal and pulmonary responses in the high- and low-level cat challenges were compared by using the Mann-Whitney U test. The variability of weekly home samples was estimated by calculating the coefficient of variation for each home after log transformation of the airborne Fel d 1 data.

RESULTS

Baseline air samples were obtained from 37 homes with cats and 40 homes without cats. As seen in Fig. 1, airborne Fel d 1 levels were higher in homes with cats than in homes without cats. Airborne Fel d 1 was detectable in all 37 homes

with cats, ranging from 1.8 to 578 ng/m³, with a median of 45.9 ng/m³. It was detectable in 10 of the 40 homes without cats, ranging from 2.8 to 88.5 ng/m³, with a median of 17 ng/m³ (in the homes with detectable allergen).

To determine whether higher airborne Fel d 1 levels in homes without cats could be predicted by Fel d 1 levels in settled dust, dust samples were also obtained from the 40 homes without cats (Fig. 2). Settled dust Fel d 1 in these 40 homes ranged from below detection in two homes to 3750 ng/gm of dust, with a median level of 258 ng/gm. Homes with detectable airborne Fel d 1 tended to have higher settled dust levels, although this correlation was not statistically significant ($r_s = 0.19, p = 0.15$). Further, there were two homes with very low settled dust Fel d 1 levels (below 100 ng/gm), which had detectable airborne Fel d 1.

To determine the variability of airborne Fel d 1 concentrations in homes, weekly samples were obtained over an 8-week period in 12 cat-containing homes and over a 4-week period in 12 homes without cats (Fig. 3). Most Fel d 1 levels in these homes with cats fell within the range of 5 to 500 ng/m³. The number of cats in each home ranged from 1 to 15, with a median of 2. Median levels of airborne Fel d 1 correlated significantly with the number of cats in the home ($r_s = 0.77, p < 0.01$).

There was substantial week-to-week variability in airborne Fel d 1 in all 12 homes, although the variability in some homes was far greater than

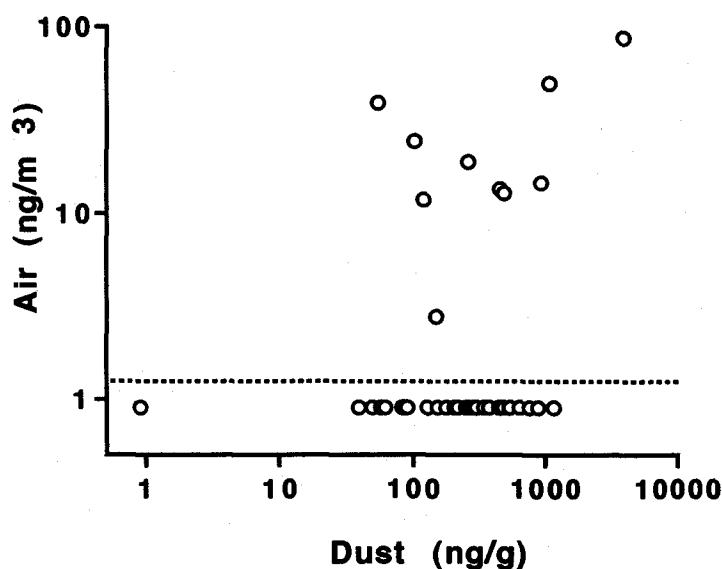


FIG. 2. Levels of Fel d 1 in paired air and dust samples collected from 40 homes without cats ($r_s = 0.17$, $p = 0.15$). Dotted line denotes the lower limit of detection of the assay for airborne samples.

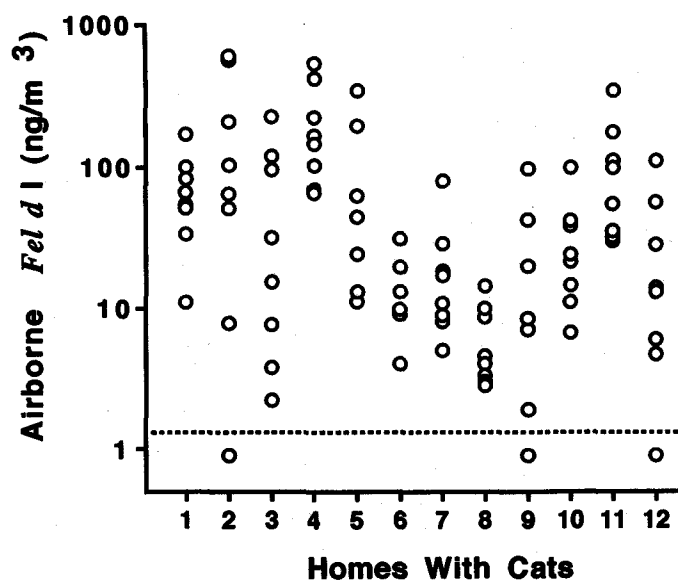


FIG. 3. Week-to-week variability of airborne Fel d 1 in 12 homes with cats sampled weekly over an 8-week period. Dotted line denotes the lower limit of detection of the assay.

that in others. For example, levels in home no. 2 ranged from below detection to 880 ng/m^3 , whereas homes no. 4 and no. 11 had generally high antigen levels and home no. 8 had consistently low antigen levels. Coefficients of variation, calculated after log-conversion of these data, ranged from 15% to 73%.

Week-to-week variability of airborne Fel d 1 was also determined in 12 homes without cats over a

4-week sampling period (Fig. 4). As noted, nine of these homes were selected because they had measurable airborne Fel d 1 in their baseline sample. Airborne Fel d 1 levels in these homes ranged from below detection to 103 ng/m^3 , or about 10 times lower than cat-containing homes. All homes had measurable Fel d 1 in at least one of their weekly air samples. Five of the 12 homes had detectable airborne allergen in three of four samples, and one

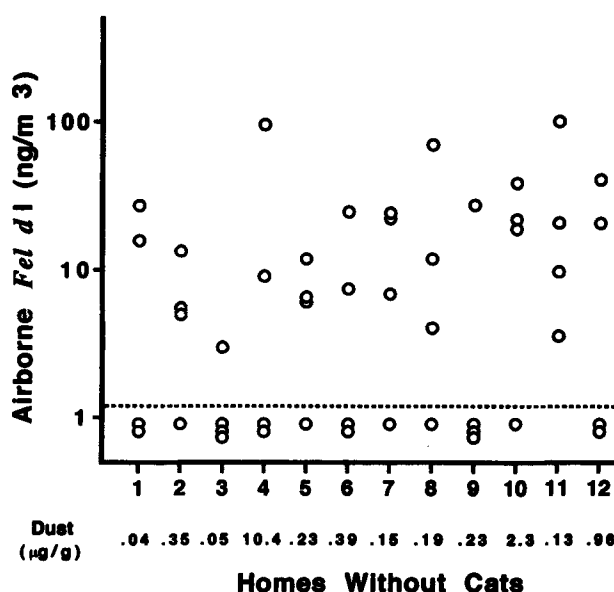


FIG. 4. Week-to-week variability of airborne Fel d 1 in 12 homes without cats sampled weekly for 4 weeks. Dotted line denotes the lower limit of detection of the assay.

had detectable allergen in all four samples. Coefficients of variation of log-converted values ranged from 50% to 321%, which was substantially greater than those observed in the cat-containing homes because of the smaller number of samples per home and the larger number of nondetectable values.

Weekly dust samples were also obtained from these homes without cats. Fel d 1 was detected in 47 of 48 samples, with concentrations ranging from 24 to 32,336 ng/gm. Median levels for individual homes ranged from 40 to 10,400 ng/gm (Fig. 4). The one home with exceptionally high settled dust Fel d 1 levels was occupied by an individual with extensive cat contact outside the home. It should be noted, however, that although this home did have one of the highest airborne Fel d 1 levels (96 ng/m³), two of its four air samples had no detectable antigen. Conversely, the home with four of four positive air samples (and the highest individual level) had very modest settled dust levels, ranging from 65 to 373 ng/gm.

To assess the clinical relevance of home airborne Fel d 1 levels, we compared these levels to those capable of causing symptoms in our cat challenge model. A total of 63 challenges were performed and categorized as either high-level or low-level, defined as an airborne Fel d 1 level of greater than or less than 500 ng/m³. The cutoff of 500 ng/m³ was chosen because levels below that are most consistent with those encountered in home

environments. There were 23 low-level and 40 high-level challenges. Airborne Fel d 1 levels in low-level challenges ranged from 13.1 to 448 ng/m³, with a median of 156 ng/m³, whereas levels in high-level challenges ranged from 622 to 31,381 ng/m³, with a median of 1729 ng/m³. Seven challenges were performed at Fel d 1 levels less than 100 ng/m³; these have been identified in the figures because of their particular relevance to homes without cats.

Upper respiratory symptom scores were averaged over the 1-hour challenge to calculate a mean score for each subject. Median symptom scores were 0.8 for the low-level challenge group and 1.2 for the high-level group (Fig. 5). Although there was a trend toward increased symptoms at higher Fel d 1 levels, these differences were not statistically significant ($p = 0.10$). In the seven challenges performed at levels less than 100 ng/m³, mean symptom scores ranged from 0.1 to 1.2, with a median symptom score equal to that of the entire low-level group (0.8).

Percent fall in FEV₁ was also compared between low-level and high-level challenges (Fig. 6). The median percent decrease in FEV₁ was 10% and 20.5% for the low-level and high-level challenges, respectively. These differences were significant, with a p value of 0.01. Notably, in the seven challenges with a Fel d 1 level less than 100 ng/m³, the median FEV₁ change was 15%, and three subjects had a fall in FEV₁ of greater than 25%. The FEV₁

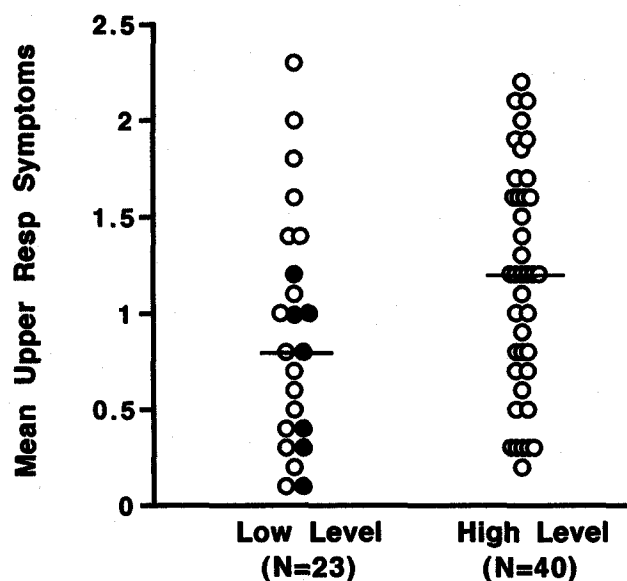


FIG. 5. Mean upper respiratory symptoms for challenges performed at low levels of Fel d 1 (<500 ng/m³) and high levels of Fel d 1 (>500 ng/m³). Solid circles represent challenges performed at Fel d 1 levels less than 100 ng/m³.

changes in these subjects were 26%, 36%, and 39% at Fel d 1 levels of 72.3, 82.1, and 54.1 ng/m³, respectively.

DISCUSSION

Although a great deal is known about the environmental distribution of cat allergen, remarkably little is known about its clinical significance. Levels of Fel d 1 in settled dust, which may be capable of inducing sensitization or disease, have been proposed but never substantiated.¹⁸ One area of particular confusion has been the low level of allergen that is commonly found in homes and other buildings that have never housed cats. In this study we have shown that Fel d 1 can also be detected in the air of many homes without cats and that similar airborne allergen levels are capable of causing significant symptoms in some patients allergic to cats.

Several prior studies have documented the presence of cat allergen in homes and buildings without cats.⁶⁻¹³ This allergen is presumed to be present because of passive transport by individuals who have been in contact with cats. In some homes it may also be the result of prior cat habitation, although in our sample 70% of the homes had been inhabited by the same person (a non-cat owner) for at least 3 years. We have now expanded on these previous studies, which focused on allergen levels in settled dust, by both measuring airborne cat allergen and attempting to assess the

clinical relevance of this low-level cat allergen exposure.

Similar to previous studies, we detected cat allergen in the settled dust of 38 of 40 homes without cats. More importantly, we were also able to measure Fel d 1 in baseline air samples in 10 of the 40 homes and in at least one sample from all homes in which repeat samples were obtained. These levels were overall about 10 times lower than those found in homes with cats, in which airborne Fel d 1 was detectable in all 37 homes. Other groups have been unsuccessful in finding measurable Fel d 1 in airborne samples from homes without cats.^{11, 14} This may be due to the sample size, because Sakaguchi et al.¹¹ only studied seven homes without cats, and the number of non-cat-containing homes studied by Luczynska et al.¹⁴ was not specified.

Settled dust samples were also obtained from the homes without cats to determine whether these samples, which are much easier to collect and analyze, might help to identify homes with higher airborne allergen levels. Fel d 1 was present in the dust of all 40 homes, and although airborne Fel d 1 was more likely to be detected in the homes with higher settled dust levels, this relationship was far from consistent. In fact, two homes with settled dust levels below 100 ng/gm had detectable airborne allergen. When repeat samples were analyzed, we again found that settled dust levels were only roughly predictive of airborne levels. It is

therefore possible to have significant airborne Fel d 1 levels in the absence of a readily identifiable reservoir, suggesting that settled dust samples must be interpreted with caution in the assessment of actual cat exposure.

The lack of correlation between air and dust Fel d 1 levels was also found by Swanson et al.¹³ This poor correlation again emphasizes the poor predictive value of dust samples. This variability could be due to differences in ventilation in the homes or variability in the activities of the human beings or animals, leading to differences in antigen disturbance.

We used our cat challenge model to assess the potential clinical significance of these home airborne allergen levels. Although this may not be a perfect model of natural exposure, it has been shown to closely mimic home environments in most respects, including Fel d 1 particle size distribution.¹⁷ We found that both upper and lower respiratory reactions were more likely to occur at higher allergen levels, although these differences were less dramatic than one might have expected. In fact, with regard to nasal symptoms, we were unable to demonstrate a significant difference between high-level and low-level challenges. Although a significant difference was found for FEV₁ changes, several subjects in the low-level challenge group experienced substantial decreases in FEV₁, even at very low airborne antigen concentrations. These results indicate that even the airborne Fel d 1 found in many homes without cats might induce symptoms in certain highly sensitive individuals.

Platts-Mills¹⁸ has suggested that Fel d 1 levels of 8 µg/gm of dust or greater in settled dust should be considered a threshold level for inducing both sensitization and asthma and that settled dust levels less than 1 µg/gm are not clinically significant. In our baseline samples only three of the 40 homes without cats had a Fel d 1 level greater than 1 µg/gm, and none had a level above 8 µg/gm, and yet 10 of these homes had detectable airborne Fel d 1. In the 12 homes without cats, in which weekly samples were collected, six of the 48 settled dust samples had a Fel d 1 level greater than 1 µg/gm, and only two of 48 had a level above 8 µg/gm. Of these, 29 of 48 air samples contained detectable Fel d 1. Given the fact that many homes had airborne Fel d 1 levels capable of causing symptoms, despite settled dust levels of less than 1 µg/gm, these proposed threshold levels need to be reconsidered.

These findings are consistent with the fact that it is common to find patients sensitive to cats who

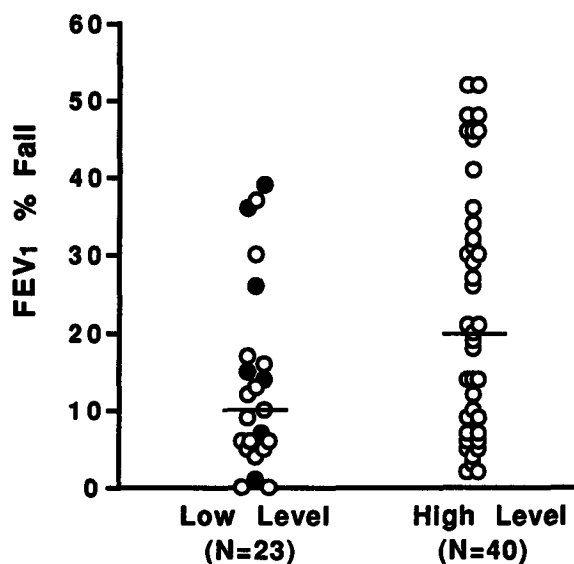


FIG. 6. Percent fall in FEV₁ for challenges performed at low levels of Fel d 1 (<500 ng/m³) and high levels of Fel d 1 (>500 ng/m³). Solid circles represent challenges performed at Fel d 1 levels less than 100 ng/m³.

have never lived with a cat. In fact, one study found that skin test reactivity to cat allergen occurred with equal frequency in children whether or not they had ever lived with a cat.¹² It is clear that the assessment of cat exposure, with regard to both sensitization and disease, should not be based solely on the presence or absence of a cat in the home.

Week-to-week variability of airborne Fel d 1 was substantial in homes both with and without cats. These results are similar to those of Sakaguchi et al.,¹¹ who found coefficients of variation of 15.8% to 55.8% in cat-containing homes, even though they used much longer sampling times. It is likely that these fluctuations are the result of varying degrees of disturbance on the part of both the human beings and the cats in the home. Such data are certainly consistent with anecdotal reports from patients, who often note marked variability in symptoms at different points in time.

Week-to-week variability was even more profound in the homes without cats. It was common for Fel d 1 to be undetectable during one week and easily detectable during another. This suggests that more active disturbance may be required for cat allergen to become airborne when the reservoir is more limited or that activity of the cats themselves is important to the creation of airborne allergen. Cat activity was clearly shown to be a cause of higher airborne allergen levels in our cat challenge room.¹⁷

In conclusion, we have shown that Fel d 1 is measurable in air samples not only in homes with cats, but also in homes without cats. In cat challenges acute upper and lower respiratory reactions were induced in sensitized individuals with a brief exposure to concentrations of airborne antigen that were comparable to those found in homes, including homes that do not contain cats. Any measurable airborne Fel d 1 might therefore be clinically significant, and the potential for cat exposure should be considered in all homes. Thus although cat avoidance should still be recommended as the first line of treatment for the patient allergic to cats, complete avoidance is clearly not possible, and the need for medications or immunotherapy must be carefully considered.

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