

Ubiquitous presence of cat allergen in cat-free buildings: probable dispersal from human clothing

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Fel d I has been found in almost all houses investigated, including those in which cats have never been present. These observations have not been explained. We measured Fel d I in samples of carpet dust from ten newly built model homes, 14 occupied homes, six allergists' offices, five hospital corridors, and three shopping mall stores. We also measured Fel d I on T-shirts of persons with and without cats. Measurable amounts of Fel d I were found in all the dust samples. Fel d I found in older model homes exceeded that found in newer model homes ($P < .05$). The amounts of Fel d I found in allergists' offices, hospital corridors, and retail stores were similar to the amounts found in occupied homes without cats. Fel d I was found in all T-shirts sampled and increased with increasing exposure to cats. Our findings confirm and extend previous reports of the ubiquitous presence of cat allergen and are consistent with the hypothesis that Fel d I is carried into cat-free buildings on the clothing of people exposed to cats.

INTRODUCTION

Allergens from domestic cats frequently elicit symptoms of asthma and allergic rhinitis in sensitive individuals.¹⁻⁴ The most important of the cat-derived allergens, *Fel d I*, accounts for about half of the allergen load produced by cats.⁵⁻⁸ Measurable amounts of *Fel d I* have been found in almost all homes investigated, including those without cats in residence.⁹⁻¹²

It has been postulated that cats belonging to former occupants are the source of cat allergens in homes without cats.¹⁰ Alternatively, persons exposed to cats or cat allergens outside the home might bring such allergens into that home via their cat-contaminated clothing. In this study, we measured *Fel d I* in dust that was collected in homes and a variety of public places where cats are not allowed. We also measured *Fel d I* on T-shirts of cat and non-cat owners before and after the shirts

were worn. Our findings suggest that cat allergens are carried into homes and public places on the clothing of persons exposed to cats.

MATERIALS AND METHODS

Sources of Material

Dust samples were collected with a Sample-Vac vacuum cleaner equipped with a 10 x 10-cm polytetrafluoroethylene filter (Quan-Tec Air, Inc., Rochester, MN). Monoclonal antibody to *Fel d I* (6F9A4) was provided by Dr. Martin Chapman, University of Virginia, Charlottesville. Reference cat allergen 1 (E3-Ct OBRR) was obtained from the US Food and Drug Administration, Bethesda, Maryland. Bovine serum albumin and Tween 20 were purchased from Sigma Chemical, St. Louis, MO; Immulon 4 plates from Dynateck Laboratories, Inc, Chantilly, VA; the microplate reader (Multiscan 340) from ICN/FLOW, Costa Mesa, CA; and the alkaline phosphatase-labeled goat anti-rabbit serum from K & P Laboratories, Gaithersburg, MD. Serum filters (LabcraftTM) were obtained from Curtin Matheson Scientific, Inc, Houston, TX.

Rabbit antiserum to cat was prepared in our laboratory using stand-

ard immunization techniques.¹³ Cat extract (Hollister-Stier, Spokane, WA) combined with Freund's adjuvant (Difco Laboratories, Detroit, MI) was periodically injected intramuscularly into New Zealand white rabbits until high titer antiserum was produced.

Collection of Dust Samples

Initially, ten newly constructed, unoccupied, carpeted model homes were studied. These ranged in age from 1 to 18 months. Each of these houses was for sale and available for public viewing; thus, the total number of persons passing through the house increased with increasing age. Cats had not been permitted in any of these houses. Vacuum samples were obtained from the carpets and subdivided as coming from houses less than or greater than 6 months old. Fourteen carpeted homes that were occupied were also studied. These homes were subdivided into homes with and without cats in residence. Subsequently, carpet dust was collected from different types of public places where cats were not normally permitted. Vacuum samples were obtained from the waiting rooms of allergists' offices, from hospital corridors, and from retail stores located within a large shop-

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ping mall. All samples were collected from one square meter areas of carpet by vacuuming for five minutes with the Sample-Vac. Each sampling site was thought to be a high traffic area.

In order to determine whether *Fel d I* found in cat-free buildings could be carried on clothing, we studied T-shirts in a variety of conditions. T-shirts were vacuumed for three minutes with the Sample Vac and the resultant dust samples were assayed for *Fel d I*. We vacuumed new T-shirts immediately after removal from their packaging, clean washed T-shirts, the same T-shirts after being worn at home for four hours, and T-shirts immediately after being worn by a person holding a cat for five minutes. Vacuuming of T-shirts before and after wear was done in the homes of persons with and without cats.

The nozzle of the Sample-Vac was carefully cleaned with dish-washing detergent between samples. In order to demonstrate that cleaning the nozzle prevented antigen carryover from one sample to another, we alternately sampled dust from a sofa on which a cat frequently slept and outdoor air, a source from which we expected no cat allergen. Two samples from each source were collected with appropriate cleaning of the Sample-Vac nozzle between each collection. Sofa dust samples contained 14,400 and 10,900 ng of *Fel d I*, air samples following each of sofa collections contained 0.2 ng and an undetectable (<0.2 ng) amounts respectively. This indicates that antigen carry over from sample to sample, following cleaning of the sampler, is less than 0.002%.

Extraction of Dust Samples

Particles more than 3 mm in diameter were removed with forceps from the dust samples. Usually identifiable, the removed particles were often stones, carpet fibers, or foodstuffs such as apple and orange seeds. The remaining dust was weighed and placed in 13 x 100-mm

test tubes. Samples of carpet dust weighed from 0.05 to 0.4 g. Samples from T-shirts were not weighed, and these results were expressed as micrograms of *Fel d I* per sample. One percent bovine serum albumen in phosphate buffered saline (PBS) with 0.002% Tween 20 was added to the fine dust in a 1:20 wt/vol ratio. Each test tube was turned end over end for 16 hours and was then centrifuged for 15 minutes at 1500 rpm (300 g). The resulting dust extract, ie, supernate, was collected with a serum filter and stored at -20 °C until assayed.

Fel d I Assay

The assay used in this study was a modification of the 2-site monoclonal immunoassay developed by Chapman et al.¹⁴ Briefly, the monoclonal antibody to *Fel d I* was coupled to microtiter wells by allowing the antibody diluted with coupling buffer (10 µg antibody per 100 mL of 60 mM Na carbonate/bicarbonate, pH 9.8, buffer) to sit in the wells overnight (1 µg/100 µL/well). Non-specific binding sites were blocked with the addition of 2.5% BSA-PBS. One hundred microliters of dust sample extracts (or reference *Fel d I* containing from 0.1 to 10.7 ng) were added to triplicate wells and incubated overnight at room temperature. The wells were washed 10 times with PBS and then incubated for two hours at room temperature with 100 µL/well of polyvalent rabbit anti-cat serum diluted 1:200 with 1% BSA-PBS. After ten washes with PBS, 100 µL per well of alkaline phosphatase-labeled anti-rabbit serum diluted 1:1000 with 1% BSA-PBS was added, and the mixture was incubated for one hour at room temperature. After ten washes with PBS, 100 µL/well of dinitrophenol phosphate 1 mg/mL) was added. The resulting color change was read approximately 60 minutes later at wavelength 405 nm. The absorbance of unknown samples was converted to ng of *Fel d I* by comparison to the standard curve. If the absorbance of the unknown was

near or higher than that of the highest standard, the sample was re-assayed at a greater dilution until a value in the linear portion of the curve was obtained.

Sensitivity and Specificity of the Fel d I Assay

To demonstrate the specificity of the assay for *Fel d I*, we assayed four dust samples containing high levels of *Fel d I* using both wells coupled with human serum albumin instead of monoclonal anti-*Fel d I* and wells to which no protein had been coupled. Both sets of wells were blocked as above and the remaining steps of the assay were unchanged. In both cases, no *Fel d I* was detectable, demonstrating that the monoclonal anti-*Fel d I* was absolutely necessary for the assay. Hence the assay was as specific for *Fel d I* as the monoclonal antibody.

Figure 1 illustrates the standard curve of the assay with a range of 0.2 to 10.7 ng of *Fel d I*. The net absorbance of the lowest assay point (0.2 ng) was consistently greater than the mean plus three standard deviations above the values of reagent controls. Also illustrated are the values obtained when a dust sample was assayed in serial 2-fold dilutions. The dilution results are parallel to the standard curve within the variation of the assay. The third 2-fold dilution became undetectable. The interassay coefficient of variation for the assays in this study was 14.1%.

Statistical Methods

Nonparametric tests were used in statistical calculations. The Wilcoxon signed rank test was used in comparing *Fel d I* on T-shirts before and after wear. The Mann-Whitney test was used for all other comparisons. Because cat allergen was postulated to increase with increasing carpet age, a single-tailed test was used in analyzing data from model homes.

RESULTS

Concentrations of *Fel d I* found in each of the carpet dust samples are

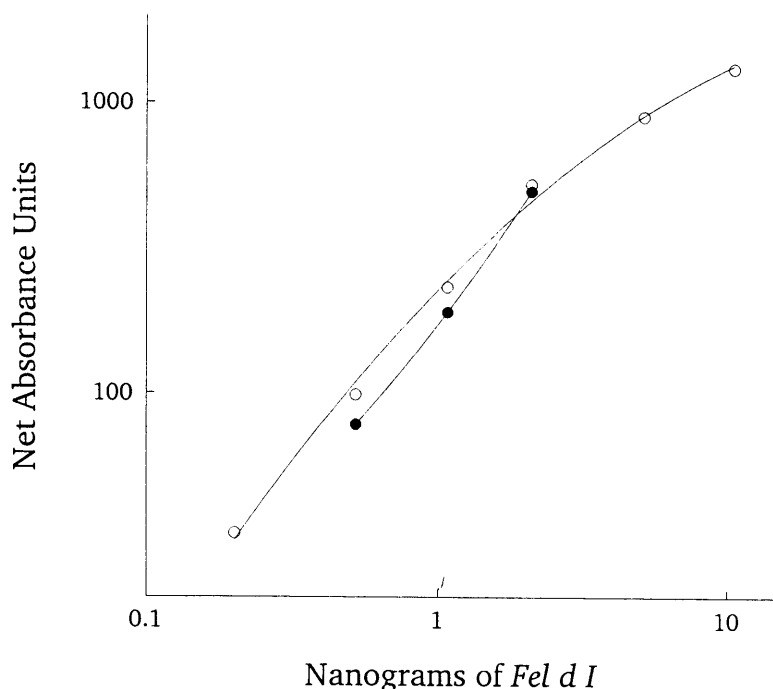


Figure 1. Open circles represent standard curve of *Fel d I* assay. Closed circles indicate 2-fold dilutions of a single dust sample. See text for details.

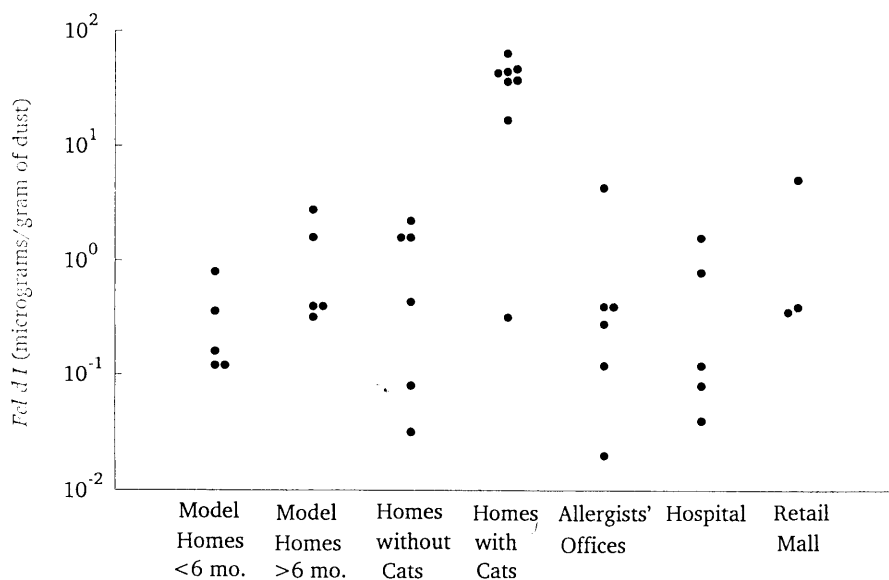


Figure 2. Concentration of *Fel d I* in carpet dust of various buildings.

shown in Figure 2. Model homes less than 6-months-old had less *Fel d I* than the older homes ($P < .05$). As expected, occupied homes with cats had more *Fel d I* than their counterparts without cats ($P = 0.008$). Concentrations of *Fel d I* found in allergists' offices, hospital

corridors, and shopping mall stores were similar to those found in the occupied homes without cats.

As shown in Figure 3, *Fel d I* was found on the new T-shirts or on clean, washed T-shirts of persons who did not own cats. Clean, washed T-shirts belonging to cat

owners contained significantly more *Fel d I*, however, than shirts belonging to persons without cats ($P = .007$). Furthermore, there was a significant increase of *Fel d I* on shirts of cat owners after four hours of wear in their homes ($P = .03$). During the interval when the shirts were being worn in homes with cats, cats jumped onto the laps of three individuals and may have touched their shirts momentarily. When shirts were worn in homes without cats, there was a small and statistically insignificant increase in measurable *Fel d I*.

DISCUSSION

Fel d I is almost universally found in homes. Wood and coworkers found measurable amounts of *Fel d I* in each of 90 homes of allergic patients in Baltimore.¹⁰ Similar results have been reported in Sweden.¹¹ In addition, the presence of *Fel d I* has been demonstrated in each of a group of Norwegian schools.¹⁵ Our data confirm and extend these findings. We found *Fel d I* in each of the 24 homes, 11 medical facilities, and three retail stores sampled. We suspect that *Fel d I* is ubiquitous in the inhabited buildings of any society where cats are frequently kept as pets.

The clinical significance of *Fel d I* levels found in buildings without cats is uncertain. Pollart and coworkers have suggested that more than 10 μg of *Fel d I*/g of dust is a risk factor for acute asthma attacks and that more than 2 μg of *Fel d I*/g of dust is a risk factor for sensitization.¹⁶ Of the cat-free buildings we studied, none had >10 μg *Fel d I*/g of dust. Four cat-free buildings, however, including a model home, an occupied home, an allergy office and a retail store contained >2 μg of *Fel d I*/g of dust.

In some instances, the presence of *Fel d I* is likely due to the former presence of cats in the building; however, cats had never been permitted in the model homes, medical facilities, or stores we sampled. We

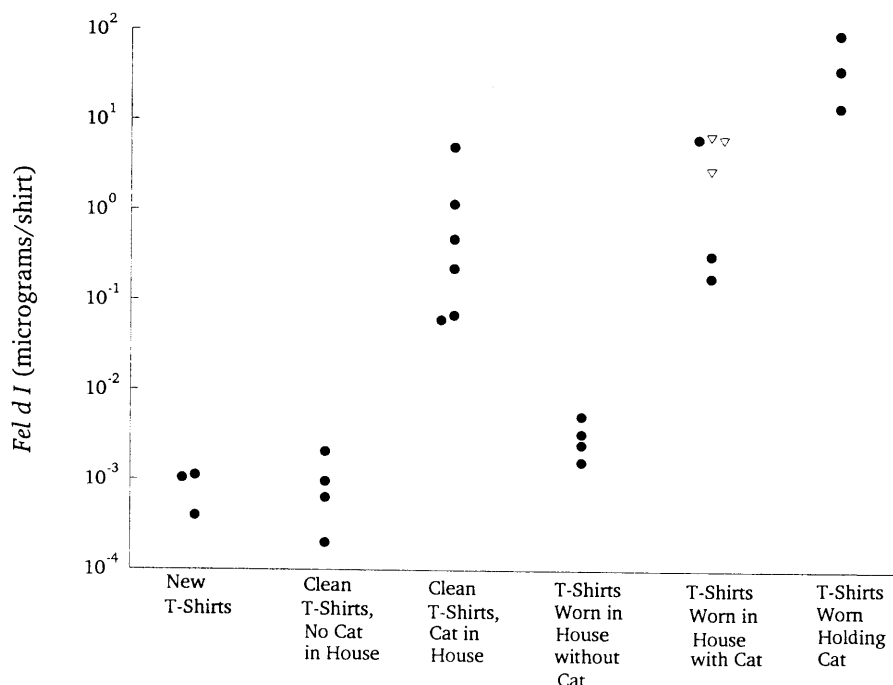


Figure 3. Quantity of *Fel d I* recovered from T-shirts. Cats may have briefly come into contact with shirts indicated by open triangles.

believe that the presence of *Fel d I* in these cat-free buildings resulted from the dispersal of *Fel d I* from the clothing of people who have regular contact with cats. This hypothesis is consistent with increased levels of cat allergen in older model homes and with the ubiquitous presence of cat allergen in public places. It is also consistent with the finding of increased allergen on the clothing of those exposed to cats and the increased amount of *Fel d I* when clothing is worn in homes occupied by cats.

We were surprised to find detectable amounts of cat antigen on new T-shirts. Having reviewed our procedures and shown that antigen contamination from previous samples is minimal (<0.002%), we doubt, but cannot totally exclude the possibility, that the *Fel d I* found in new T-shirts represents contamination related to sample collection. An alternative possibility is that cat owning workers contaminated the T-shirts during their manufacturing or packaging.

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