

Evaluation of different techniques for washing cats: Quantitation of allergen removed from the cat and the effect on airborne Fel d 1

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Background and Objective: The purpose of this study was to examine the quantity and distribution of the major cat allergen, Fel d 1, on cats and to evaluate the efficacy of washing, both in removing allergen from the cat and reducing airborne allergen levels.

Methods: Airborne samples were collected on four glass fiber filters in a 30 m³ room, before and 3 hours after serial washing of eight cats (45-minute sampling at 18 L/min for each filter). Aliquots of hair and bath water were also collected and assayed for Fel d 1 content.

Results: Extracting cat hair with tap water or pet shampoo for 3 minutes removed mean levels of 191 and 245 µg of Fel d 1 per gram of hair, respectively; the quantity of allergen on samples of cat hair ranged from 1 µg/gm to more than 1770 µg/gm. The highest concentration of allergen was found on hair from the neck. Estimates of the total Fel d 1 on the cat, based on shaving the whole cat, ranged from 3 to 142 mg (mean = 67 mg). Washing cats reduced airborne allergen 3 hours later. Washing three cats at weekly intervals for 5 weeks in a veterinarian's office produced a mean decrease of 44% in airborne Fel d 1 ($n = 15$, $p < 0.02$). Washing three cats by immersion for 3 minutes at weekly intervals for a 1-month period produced a mean decrease in airborne allergen of 79% ($n = 12$, $p < 0.001$). However, after repeated washing, the airborne levels before the next wash were not consistently decreased. The quantity of Fel d 1 removed by immersion varied from 1 to 35 mg.

Conclusion: Cats carry large quantities of Fel d 1, only a small proportion of which (~0.002%/hr) becomes airborne. Washing cats by immersion will remove significant allergen from the cat and can reduce the quantity of Fel d 1 becoming airborne. However, the decrease is not maintained at 1 week. (*J Allergy Clin Immunol* 1997;100:307-12.)

Key words: Fel d 1, cat washing, airborne allergen, allergen avoidance

Approximately six million Americans are allergic to cats.¹⁻³ Persons who are sensitive to cats often report a rapid onset of symptoms, including allergic rhinitis and

Abbreviation used

mAb: Monoclonal antibody

asthma, when they come into contact with a cat.^{1,4} Symptoms caused by cat allergen can also occur when the cat is not present, has been removed for days or weeks, and even on exposure to passively transferred allergen.⁵ This is in part due to the ability of the major cat allergen, Fel d 1, to become and remain airborne on small particles (1 to 10 µm in diameter) even in undisturbed conditions.^{3,6-8} Despite the obvious health risks, it has been estimated that one third of the persons in the United States who are allergic to cats live with at least one cat in the house.^{1,2,9} Traditionally, clinicians have recommended complete avoidance for patients who are cat-sensitive. However, many pet owners become too attached to their animals for this to be a viable option. Other alternatives include immunotherapy or modifying the furniture and carpets in a house. These approaches also have significant limitations. A preferable alternative would be to reduce or eliminate the allergen coming off the cat, thereby reducing airborne Fel d 1 levels. Several studies in the past have suggested that repeated washing of a cat yields progressively lower amounts of allergen and also decreases the amount of allergen becoming airborne directly from the animal.^{10,11} However, a subsequent study reported little effect on airborne allergen after washing the cat or wiping it with Allerpet/c.¹²

The purpose of this study was to examine the effects of different techniques of washing cats on airborne allergen. In addition, these studies have calculated the total amount of allergen on the cat, described the distribution of allergen over the cat's body, and estimated the quantity that can be removed by washing.

METHODS

Extraction of Fel d 1 from cat hair

Cats were volunteered by their owners for this study. All cats were healthy, neutered males. Between samplings the cats were returned home. Cat hair was obtained from a local pet groomer to determine the solutions that were most efficient at removing Fel d 1. Samples of 0.05 gm were extracted overnight at 4° C in 3 ml of tap water; 1 mmol/L HCl, pH 3; borate-buffered saline, pH 8; or a commercially marketed pet shampoo diluted 1:16 in water, pH 7 (HyLy; DVM Pharmaceuticals Inc., Miami, Fla.).

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After extraction, the samples in 1 mmol/L HCl were titrated to pH 7.5 with sodium hydroxide solution. All samples were assayed for Fel d 1 content.

Measurement of total Fel d 1 present on cats

The total hair removed by shaving was collected from six different cats and was used to estimate the amount of Fel d 1 on a cat. Four of the cats were shaved because of matting, and two others were shaved before euthanasia. The hair from each cat was weighed and assayed for Fel d 1 content. The distribution of Fel d 1 on cats was studied by collecting samples of hair from the chest, neck, and back regions and from the rear haunches of an additional six cats; animals were brushed for 1 minute at different sites with a grooming glove to obtain hair samples weighing at least 0.1 gm. Samples were assayed for Fel d 1 content.

Air sampling

Air sampling was conducted in a 30 m³ clean room by using methods described previously.^{3,6} The vents and windows were sealed, and the floor was covered with plastic sheeting. Four filter holders (Cassella Lovidon Ltd., Bedford, U.K.) were loaded with glass fiber filters (type AP20; Millipore, Bedford, Mass.) and assembled in parallel in the center of the room at a height of 18 inches from the floor. Air was sampled for 45 minutes at a flow rate of 18 L/min through each filter. Airflow was monitored by two flowmeters (Wright Respirometer; Ferraris Development and Engineering Co. Ltd., Edmonton, London, U.K.). Samples were collected immediately before washing and 3 hours after washing. Between measurements the floor in the clean room was wiped down, and the air was filtered for at least 3 hours with a high efficiency particulate air filter (Envirocaire Corp., Hagerstown, Md.). Samples were taken between measurements to ensure that the room had returned to a baseline level of approximately 4.3 ng of Fel d 1/m³. On completion of sampling, glass fiber filters were eluted separately in 1.0 ml of phosphate-buffered saline-Tween-20 containing 1% bovine serum albumin in a 3 ml syringe. After rotation overnight at 4° C, the plunger was used to compress the filter and express the eluate, which was then assayed for Fel d 1 content. Results were expressed as the mean of four values obtained from individual filters. The variability in values obtained from individual filters was $\pm 20\%$ of the mean.

Cat washing

Cats were divided into three groups. The first group consisted of three cats that were washed weekly for 5 weeks at a local veterinarian's clinic. Before each washing, 0.05 gm of hair was obtained from each cat by using a grooming glove (Purebred; Project Strategies Corporation, Deerfield Beach, Fla.). The glove was washed before each use. The cat was then placed in the clean room, and air was sampled for 45 minutes. After air sampling, the cat was taken to a veterinarian and washed with soap and warm water from a hose, rinsed, and allowed to air dry. Total washing time was approximately 60 seconds. After 3 hours, the cat was returned to the clean room, airborne measurements were repeated, and an additional hair sample was collected.

The second group of three cats was washed weekly for 4 weeks by immersion. A wash basin was filled with 30 L of tap water at 38° C, a comfortable temperature for the cats. Each cat was immersed for 3 minutes in water and secured by a chest harness attached to the basin floor while its head was kept above water. The cat's head was thoroughly soaked with water,

and its body was massaged to ensure adequate penetration of the pelt. The cat was removed from the water, wiped with a clean towel, and allowed to dry for 30 minutes. Airborne measurements and hair samples were collected before and 3 hours after each immersion. In addition, samples of bath water were collected and assayed for Fel d 1 content.

The third washing method consisted of weekly washes of two cats by immersion in tap water at 38° C by using the methods described above. Cats were rinsed for an additional 3 minutes in 30 L of fresh tap water heated to the same temperature. Airborne measurements, bath water, and hair samples were collected as described above and assayed for Fel d 1 content.

Two-site monoclonal antibody (mAb) ELISA for quantification of Fel d 1

A two-site mAb binding assay was used to determine the concentration of Fel d 1 in collected samples.¹³ In brief, a 96-well microtiter plate was coated overnight at 4° C with mAb 6F9 at 1 μ g/well in carbonate-bicarbonate buffer, pH 9.6. All subsequent incubations were at room temperature, and plates were washed with phosphate-buffered saline containing 0.05% Tween-20 between stages. After blocking with 1% bovine serum albumin-phosphate-buffered saline-Tween-20 for 1 hour, samples were added to each well in 100 μ l aliquots; cat allergen standard (UVA 94/01, 1.6 U/ml) was diluted in the range of 84 ng/ml to 0.04 ng/ml (1 mU = 4 ng of Fel d 1) to establish a control curve. After 1-hour incubation and washing, biotinylated anti-Fel d 1 mAb 3E4 (100 μ l/well) was added, and plates were incubated for an additional hour. Streptavidin peroxidase (0.25 μ g/ml) (Sigma) was added to the plate at 100 μ l/well, and the plate was incubated for 30 minutes. Assays were developed with 0.01 mol/L 2,2'-azino-bis 13-ethylbenzthiazoline-6-sulfonic acid (Sigma A1888) in 0.07 mol/L citrate-phosphate buffer, pH 4.2, containing 0.03% H₂O₂, and the optical density was read at 405 nm. Values for samples were interpolated from the linear part of the control curve.

Statistical analysis

Student's paired *t* test was used to compare changes in Fel d 1 concentrations in both airborne samples and cat hair extracts before and after washing.

RESULTS

Estimation of total Fel d 1 on cats and variation with anatomic site

Preliminary experiments established that elution of Fel d 1 from cat hair can be achieved with water, pet shampoo, or buffered saline solution. The quantity of Fel d 1 eluted with 1 mmol/L HCl was lower, but this may reflect denaturation during elution. None of the eluants interfered with the ELISA. Hair was extracted for 6 minutes; aliquots removed at 1-minute intervals were assayed for Fel d 1 content. Greater than 95% of the elutable Fel d 1 was removed within 3 minutes (Fig. 1). The quantity of Fel d 1 varied widely from 1 μ g/gm to more than 1770 μ g/gm of hair. The mean levels of Fel d 1 extracted by using shampoo, water, or buffered saline solution were not significantly different (245, 191, and 102 μ g/gm, respectively, *n* = 34).

Hair from six shaved cats was collected and weighed to determine the total amount of allergen present on a cat. The mean weight of hair was 121 gm. Ten samples of

TABLE I. Estimate of total Fel d 1 on cats*

Cat	Approx. weight of hair on cat (gm)	Concentration of Fel d 1 ($\mu\text{g/gm}$)†	Total amount of allergen on cat (mg)
1	188	754	142
2	74	852	63
3	124	410	51
4	75	36	3
5	133	970	129
6	129	94	12
Mean	121	519	67

*Allergen on cat = (Approx. weight of hair) $\times$ (Concentration of Fel d 1). Hair was obtained from cats treated for matting by a veterinarian. Most of the hair was removed from the cats.

†Values represent the mean of 10 0.05 gm hair samples from each cat extracted for 6 minutes in 3 ml of water.

hair from each cat were extracted in tap water, and the Fel d 1 content was assayed. The estimated total quantity of Fel d 1 on each cat studied ranged from 3 to 142 mg, with a mean of 67 mg (Table I). The allergen distribution on cats was also examined. Hair extracts were prepared from samples obtained from the chest, stomach, neck, back, and rear haunches of six cats; and the Fel d 1 concentration was measured. The highest concentrations of Fel d 1 were located in the neck region; mean levels were significantly higher than concentrations at other anatomic sites (Table II).

Effect of washing cats on allergen removal and airborne Fel d 1 levels

Three cats were washed at a veterinarian's clinic over a period of 5 weeks (Table III). The mean reduction in airborne Fel d 1 after these washes was 44% ($n = 15$, $p < 0.02$). The cat with the highest airborne Fel d 1 levels before washing showed the greatest reductions in airborne allergen concentrations. However, the reductions were not consistent, and lower levels did not persist from week to week; in the case of one cat, a 290% increase in airborne Fel d 1 levels was measured before washing compared with the prewashing level of the previous week (cat 3, Table III). Given that the washing procedure took approximately 60 seconds, it seemed possible that insufficient allergen was being removed. However, in the veterinarian's office it was not possible to measure the quantity of allergen removed from the cats.

A second group of cats was washed by immersion for 3 minutes. Hair and airborne samples were collected before and after bathing. In addition, aliquots of bath water were assayed for Fel d 1 content. The airborne Fel d 1 level measured 3 hours after washing was reduced by 79% ($p < 0.001$), and the mean reduction each week ranged from 91% in the second week to 59% in the fourth week (Table IV, Fig. 2). The mean percentage of Fel d 1 removed by washing, based on changes in Fel d 1 concentrations on cat hair, was 66% ($p < 0.002$) (Table IV). Estimates of the percentage change in allergen, based on the quantity of Fel d 1 in

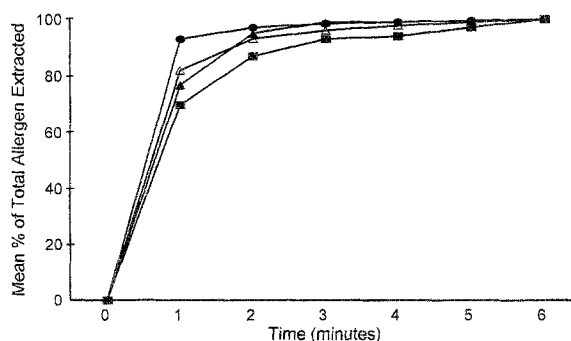


FIG. 1. Time course of Fel d 1 extraction from 0.05 gm aliquots of cat hair with borate-buffered saline, pH 8 (filled squares); water (filled triangles); shampoo, pH 7 (open circles); and 1 mmol/L HCl, pH 3 (open triangles). Values represent the mean of 10 samples.

the bath water, were lower; in addition, the quantity of allergen removed decreased with sequential washes. Mean percentage reductions in allergen on the cat ranged from a maximum of 53% for week 1 to 9.1% for week 4 (data not shown). After repeated washing, the airborne levels before the next wash were not consistently decreased (Table IV, Fig. 2).

Two cats were washed for 3 minutes and then rinsed for an additional 3 minutes in fresh tap water. Washing was repeated weekly for 4 weeks. The results for the two cats were different; in one case a progressive decrease in airborne allergen before washing was seen (Fig. 3, A). However, in the second case the airborne allergen did not consistently decrease, but the quantity removed at each wash was progressively reduced (Fig. 3, B). Washing and rinsing were carried out eight times with a mean reduction in airborne Fel d 1 of 84% 3 hours after rinsing (Fig. 3).

DISCUSSION

Previous studies have shown that Fel d 1 is the major cat allergen responsible for causing symptoms in patients allergic to cats.¹⁴⁻¹⁶ Fel d 1 is a 36 kd acidic protein dimer secreted primarily by the sebaceous glands.¹⁶⁻¹⁹ The antigen can accumulate on cats' hair, and when airborne, is associated with small particles ranging in size from 1 to 10 μm in diameter.^{3,20-21} Cat allergen can remain airborne for prolonged periods, even in undisturbed conditions.^{3,7,8,22-24}

This study examined the quantity and distribution of Fel d 1 on cats and evaluated the effects of several washing methods on Fel d 1 shedding. Previous studies have shown that the quantity of allergen shed varies between cats.^{11,25} Furthermore, it has been shown that the amount of allergen shed by individual cats may vary from time to time.^{11,25} Wentz et al.²⁵ reported that male cats secreted more Fel d 1 than females, whereas other studies demonstrated reductions in Fel d 1 production after castration.^{26,27} Neutered males were used for our study to avoid variations in Fel d 1 secretion induced by hormonal fluctuations. Our calculations suggested that

TABLE II. Distribution of allergen on cats

Cat	Chest*	Stomach†	Neck	Back†	Back haunches*
1	1042	703	1720	275	540
2	653	1480	1450	356	355
3	487	281	1530	1680	376
4	82	40	426	83	114
5	82	136	931	118	152
6	298	347	1770	288	366
Mean	441	498	1300	464	318

Hair samples were obtained by using a grooming glove and assayed for Fel d 1 content by ELISA. Values are expressed as micrograms of Fel d 1 per gram of hair. Values from the neck region were compared with results for other anatomic sites by using Student's paired *t* test.

**p* < 0.002.

†*p* < 0.05.

TABLE III. Airborne allergen levels before and after washing at a veterinarian's clinic

	Airborne Fel d 1 (ng/m ³)*								
	Cat 1			Cat 2			Cat 3		
	Before	After	Reduction† (%)	Before	After	Reduction† (%)	Before	After	Reduction (%)
Week 1	30.7	5.8	81.1	5.5	3.6	34.6	6.5	6.0	7.7
Week 2	48.3	6.0	87.6	7.8	3.5	55.1	7.5	2.6	65.3
Week 3	17.0	8.4	50.6	9.7	12.9	+33	21.8	10.7	50.9
Week 4	40.9	5.0	87.8	12.7	11.9	6	7.5	10.5	+40
Week 5	30.7	3.5	88.6	7.0	3.9	44.3	4.3	3.4	21
Mean	33.5	5.7	79.1	8.5	7.2	32.1	9.5	6.6	20.1

Samples were obtained before and 3 hours after washing.

*Values represent the mean of samples collected from four parallel filters at a flow rate of 18 to 20 L/min for 45 minutes.

†Percentage reduction in airborne allergen. Values after washing were significantly lower (*p* < 0.02).

TABLE IV. Repeated washing by immersion of three cats: Quantity of allergen removed and effects on airborne Fel d 1

	Week 1	Week 2	Week 3	Week 4
Fel d 1 on hair (μg/gm)				
Before wash	385	420	278	575
After wash	54	99	223	103
Reduction of Fel d 1 on hair after wash (%)	84	63	39	77
Estimated total Fel d 1 on cat (mg)*	46	51	45	70
Total Fel d 1 in bath water (mg)†	25	8.3	4.1	8.0
Airborne Fel d 1 levels (ng/m ³)‡				
Before wash	23	12	20	17
After wash	2.0	1	3.7	4.6
Reduction of airborne Fel d 1 after wash (%)	85	91	82	59

Cats were washed by immersion in 30 L of tap water for 3 minutes. All values represent the mean for three cats.

*Determined by [Concentration of Fel d 1 on hair before wash (μg/gm)] × [Mean total hair weight (gm)] (see Table I).

†Calculated by [Volume of water (ml)] × [Concentration of Fel d 1 in bath water (μg/ml)].

‡Values for each cat were the mean of samples collected from four parallel filters. Samples were collected before and 3 hours after washing. Airborne Fel d 1 values after washing were significantly reduced (*p* < 0.001).

the average amount of Fel d 1 on a cat was 67 mg with a range of 3 to 142 mg. The amount of hair and the concentration of Fel d 1 varied between cats. It is important to note that this estimate of the total quantity of allergen on a cat is 10- to 100-fold greater than previously reported values.^{6, 10} Levels of airborne allergen measured before washing ranged from 4.3 to 48.3

ng/m³ (Table III), representing between 0.17 and 1.5 μg of Fel d 1 coming off the cat in a 45-minute period. These levels were consistent with airborne levels measured in previous studies.^{3, 6, 7, 23} Assuming a total quantity of allergen on the cat of 67 mg, the quantity shed is only 0.002% of the total allergen present on the cat. Thus other factors that influence the ease with which

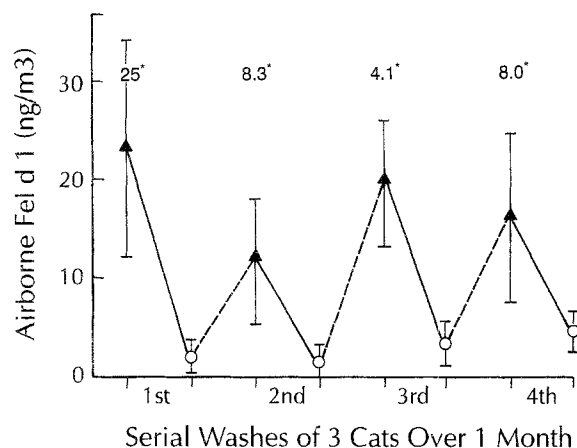
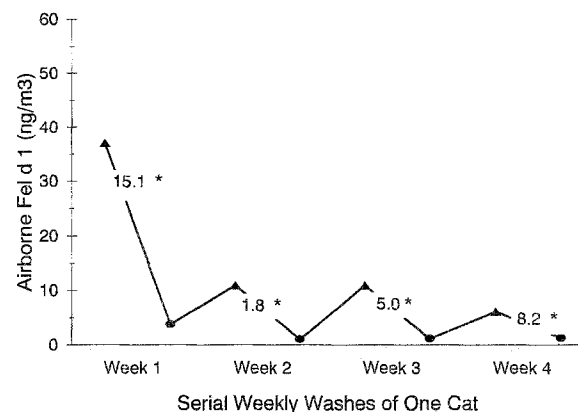


FIG. 2. Airborne Fel d 1 (nanograms per cubic meter) derived from cats in a 30 m³ room before (filled triangles) and 3 hours after (open circles) washing by immersion in 30 L of water. Values represent the mean for three cats. *Total allergen removed by washing (in milligrams). Bars represent 1 standard deviation from the mean. Airborne levels were significantly reduced 3 hours after washing ($p < 0.001$).

allergen particles become airborne may be as important as or more important than the total quantity on the cat. Our study showed that concentrations of Fel d 1 varied significantly between different anatomic sites on the cat; concentrations in the neck region were at least threefold higher than those at other sites. These findings are in keeping with the high concentration of sebaceous glands in the neck region.²⁵ We reported progressive decreases in airborne allergen levels and a reduction in the amount of allergen collected after weekly washings; however, that study used only one cat and a relatively small amount of water (1 L).⁶ Glinert et al.¹¹ washed cats on a monthly basis and measured the allergen removed in bath water, but no attempts were made to measure airborne levels. Significant variations for individual cats occurred during the first 3 to 7 months of washing; nine months was required for all cats to show significant decreases in recoverable Fel d 1. Ohman et al.¹⁰ reported a progressive decrease in the amount of recoverable Fel d 1 in three of six cats with weekly washing, whereas in two cats allergen re-accumulated. Ohman et al.¹⁰ also stated that "while washing at short intervals appears to reduce the yield of Fel d 1, it reappears in its original concentration within a month." More recently, Klucka et al.¹² reported no significant reduction in Fel d 1 shedding after weekly washings. However, those experiments did not assess the quantity of allergen removed by the washing procedure (2 L of water) and evaluated allergen becoming airborne in artificial airflow conditions (200 L/min). Those experiments are also difficult to interpret because of a major difference in the baseline measurements (the washed group had a mean level of only 40% of the control group).

Our initial results suggested that cats should be bathed in tap water or pet shampoo for at least 3

A.



B.

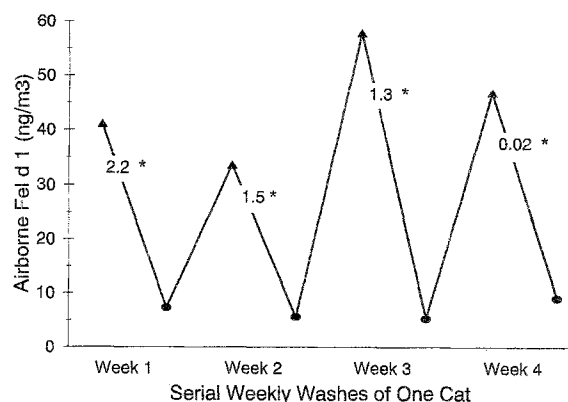


FIG. 3. Airborne Fel d 1 (nanograms per cubic meter) derived from two cats studied separately in a 30 m³ room before (filled triangles) and 3 hours after (filled circles) washing, followed by rinsing. In one case a progressive reduction in airborne allergen levels was detected (A), and in the other the decrease after washing was not maintained 1 week later (B). *Total allergen removed by washing (in milligrams).

minutes to solubilize Fel d 1. Washings carried out by a veterinarian took approximately 60 seconds and decreased airborne allergen levels by an average of 44%. Immersion of cats in water for 3 minutes produced a highly significant reduction in airborne allergen 3 hours later, but the quantity of allergen becoming airborne from the cat did not remain low 1 week later. Our calculations suggested that the amounts of Fel d 1 removed by immersion represented a substantial proportion of the total estimated allergen on the cat (19% to 92%). Regular removal of large quantities of allergen from the cats' fur might reduce the amount of Fel d 1 accumulating on furnishings and carpets in the home; however, this has not been studied. Two cats were subjected to washing and rinsing repeatedly over 4 weeks. This procedure produced significant reductions in airborne allergen 3 hours later (i.e., up to 84% reduction). However, even with washing and rinsing, one

of the cats did not demonstrate a progressive decrease in the quantity of airborne allergen 1 week later.

Our results have clarified the effects of washing cats and have established that this procedure can decrease allergen shed from the cat. It is clear that cats carry large quantities of Fel d 1, of which a small quantity becomes airborne while a larger amount accumulates in the carpet and furniture.⁶ Washing cats by immersion can remove up to 35 mg of Fel d 1 and can decrease the amount becoming airborne from the cat. The rate of re-accumulation of allergen is not known; however, our results show that the airborne allergen coming off cats was not reduced 1 week later. Although it is possible that cats pick up allergen from the rest of the house, it is likely that cats secrete sufficient Fel d 1 to replace protein removed by washing. The original experiments on reducing cat allergen in homes with a cat recommended that the combination of removal of reservoirs (i.e., carpets and sofas), room air filtration, and regular washing of cats should be tested.^{6, 11} It is clear that these measures on their own are not effective.²⁸ However, two recent studies have suggested that the combination of measures is effective in decreasing exposure.^{29, 30} What remains to be answered is (1) whether the regimen of washing cats combined with reduction of allergen reservoirs and the use of air filtration devices can reduce symptoms in a controlled trial, (2) whether repeated washing over a prolonged period will lead to a progressive reduction in allergen becoming airborne from the cat, and (3) the effect of regular washing on the accumulation of Fel d 1 in reservoirs such as carpets and sofas.

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