

Cat shedding of *Fel d I* is not reduced by washings, Allerpet-C spray, or acepromazine

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Background: No published studies have compared the effectiveness of several treatments proposed to reduce cat allergenicity. Cat washing studies demonstrating efficacy involved very small sample sizes or infrequent washings. Allerpet-C (Allerpet, Inc., New York, N.Y.), a widely advertised topical spray, and acepromazine, a tranquilizer advocated as efficacious in subsedating doses, have never been scientifically studied.

Objective: We compared the effects of cat washing, Allerpet-C spray, and acepromazine with that of no treatment on the shedding of the primary cat allergen, *Felis domesticus I* by cats.

Methods: In a blinded, comparative, controlled study, we measured the amounts of *Fel d I* shed during an 8-week treatment period with a sample of 24 female mongrel cats randomly assigned to four groups; one group received weekly distilled water washings, one received weekly Allerpet-C spray applications, one received daily oral acepromazine, and one had no treatment (control). Thirty-minute, twice-weekly air samples were collected from each cat with a laminated plastic-acrylic chamber and air sampler.

Results: One-sample, two-sided *t* tests comparing baseline to final-week measurements revealed no significant change in *Fel d I* within each group (mean change $\pm$ SD: washing; 487.6 ± 1896.4 mU per 30 minutes, $p = 0.63$; Allerpet-C spray, 429.2 ± 871.6 mU per 30 minutes, $p = 0.46$ acepromazine; -620.6 ± 1031.2 , $p = 0.52$ per 30 minutes). Furthermore, analysis of covariance revealed no significant change in *Fel d I* levels between groups ($p = 0.72$).

Conclusions: Our data do not show significant reductions in *Fel d I* shedding as a result of any of these treatments. Therefore we cannot recommend them to patients allergic to cats. (*J ALLERGY CLIN IMMUNOL* 1995;95:1164-71.)

Key words: Acepromazine, allergen shedding, Allerpet-C spray, cat, cat washing, *Fel d I*

Cat allergy presenting as rhinoconjunctivitis and asthma is a widespread clinical problem, ranking first among pet allergies.¹ In the United States it is estimated that more than 2% of the population, or 6 million Americans, are allergic to cats, and of these, more than a third have a cat in their homes.^{2,3} Indeed, because 31% of homes in the United States have at least one cat, the prevalence of pet cats makes avoidance difficult for the sensitive person.⁴ Moreover, the problem is not unique to the United States; studies from other countries suggest that pets are kept in more than 50% of

Abbreviations used

BSA:	Bovine serum albumin
<i>Fel d I</i> :	<i>Felis domesticus I</i> , the primary cat allergen
PBS:	Phosphate-buffered saline solution

households.⁵ A study from The Netherlands reported that 51% of patients had positive skin test reactions to cat extract and that allergy to cats was more frequent than allergy to dogs.⁶

The most effective treatment of cat allergy is simply cat avoidance, yet removal of the cat from the home is viewed as an unacceptable option by many cat owners.⁶ Pharmacologic treatment with antihistamines, β_2 -agonists, antiinflammatory agents, and immunotherapy are currently used to help control symptoms resulting from cat allergy, but each form of treatment has significant expense, limitations, and/or risks. A preferable alternative

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would be to reduce or eliminate allergen shedding by cats. No published studies have compared the effectiveness of several treatments that have been thought to reduce the allergenicity of cats, including cat washings, topical sprays, and acepromazine.

A limited number of prior studies have suggested that cat washings are efficacious; consequently, many clinicians, including practicing allergists, recommend that their patient bathe their cat regularly. Allerpet-C spray (Allerpet, Inc., New York, N.Y.) is a commercially available topical preparation, widely marketed and used to help persons with allergy tolerate cats; however, scientific testing of the product's efficacy is lacking. Acepromazine, an animal tranquilizer, has been reported as efficacious for reducing cat allergenicity in an informal survey of pet owners and veterinarians in the Detroit area (P. Bloom, M. Christlieb, T. Ryan, R. Thoms, personal communications).⁷ It is generally given daily in subdosing doses.

The purpose of this study was to compare the relative efficacy of cat washings, Allerpet-C spray and acepromazine in decreasing the shedding of the major cat allergen, *Fel d I*. We conducted a blinded, controlled, 8-week study with a sample of 24 healthy, female mongrel cats. Randomized groups of equal numbers of cats received weekly distilled water washings, weekly Allerpet-C spray applications, daily oral acepromazine, or no treatment (control).

METHODS

Project approval was obtained through the Care of Experimental Animals Committee of Henry Ford Health System, Detroit, Mich.

Twenty-four adult, healthy, short- and medium-haired, female mongrel cats were obtained from a pet supplier. All were weighed, examined by a veterinarian, and given flea baths on admission to the animal research facility at Henry Ford Hospital. All were negative for feline leukemia virus and for feline immunodeficiency virus. The cats were housed in a single room in separate standard wire cages covered on all sides, except for the front, throughout the study period. The cat room floor was washed and the litter boxes were changed daily; cages were cleaned weekly.

Air sampling was conducted in a fashion similar to the method used by Wentz et al.⁸ We used a 61 × 46 × 30 cm laminated plastic-acrylic sampling chamber perforated with 1.0 cm holes at one end of the chamber to allow free air flow through the chamber. At the other end of the chamber we placed a polytetrafluoroethylene filter leading into a 3.5 cm diameter hose connected to a high-flow air sampler (Quan-Tec-Air Inc., Minneapolis, Minn.) with a 200 L/min flow rate, as used by Swanson et al.⁹ An acrylic divider within the box, perforated with 1.0

cm holes, prevented the cat from having direct contact with the filter. Throughout the study 30-minute air samples were collected from all cats on Tuesday mornings and Friday afternoons. The air sampling room and room where the cats were kept were on the same floor but had separate ventilation systems. The walls and floor of the sampling room were wiped down with a damp cloth and dried after each sampling session. The sampling chamber was also completely cleaned in similar fashion after each 30-minute collection. Separate cloths were used for each cleaning; none of them was used more than once. Control air samples were obtained periodically to ensure the adequacy of the cleaning procedures. The mean *Fel d I* level for a series of four control air samples was negligible, at 4.08 mU per 30-minute sample.

Weekly cat washings with 2000 ml of distilled water heated to 40° C were administered on Wednesday mornings. The pelt was heavily drenched and then towel-dried during a procedure lasting 15 minutes each. No anesthetic agents were used because the cats tolerated the washings well. No attempt was made to recover the wash water for measurement of *Fel d I*.

In accordance with the recommendations of a survey of three local veterinarians, 0.3 mg (0.15 ml of a 2 mg/ml solution) of acepromazine was administered daily by syringe into the mouth of each cat assigned to receive acepromazine treatment.

Allerpet-C spray was applied weekly by means of a cloth over the cat's pelt, with care to avoid the ears and perirectal area according to manufacturer's recommendations. Each cat was treated with a total of 60 sprays per week (15 sprays/cloth application, four applications per treatment) on Wednesday mornings.

The study was done in a blinded manner, with animal care technicians performing the weekly baths, Allerpet-C applications, and acepromazine administration. These technicians were not involved in the air sampling or in the laboratory *Fel d I* determinations. Personnel conducting air sampling and *Fel d I* determinations were blinded to treatment group.

The "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication No. 86-23, revised 1985) was used by the investigators and by the Department of Bioresources, Henry Ford Hospital, for the care of the treatment cats during the study. (The bioresources department is accredited by the American Association for the Accreditation of Laboratory Animal Care.)

Extraction of protein from polytetrafluoroethylene filters

Cat hairs were removed from the polytetrafluoroethylene filter, and each filter was cut to a circle 5.0 cm in diameter corresponding to the surface area exposed to air flow through the air sampling port. The membrane surface was separated from its mesh backing and placed into a test tube with 2 ml of 1% bovine serum albumin

TABLE I. Baseline *Fel d I* levels

Group	No. of cats	<i>Fel d I</i> level (mU/30 min)			
		Baseline*	SD	95% CI bounds	
				Lower	Upper
Acepromazine	6	1578.0	857.4	678.0	2478.0
Washing	6	803.0	658.4	111.8	1494.2
Allerpet-C	6	810.6	486.0	300.6	1320.8
Control	6	1927.0	3243.2	-1477.0	5331.0

CI, Confidence interval.

*The baseline *Fel d I* level did not differ significantly between groups (Kruskal-Wallis nonparametric test, $p = 0.42$).

and phosphate-buffered saline solution (BSA-PBS) with 0.002% polysorbate-20. Elution was completed overnight in an end-over-end tumbling mixer; the extracts were separated from the membranes and frozen at -20°C for later assay.

Fel d I determinations

The assay used in this study was a modification of the two-site monoclonal immunoassay developed by Chapman et al.¹⁰ Briefly, the monoclonal antibody to *Fel d I* (University of Virginia, Charlottesville; lot 6F9 A4) was diluted with coupling buffer (60 mmol/L sodium carbonate/bicarbonate; pH 9.8) and coupled to microtiter wells (1 μg /antibody per well) by overnight incubation. Non-specific binding was blocked with the addition of 2.5% BSA-PBS with 0.1% polysorbate 20. Fifty microliters of unknown samples, or dilutions of a *Fel d I* standard, were added to duplicate wells and incubated overnight at room temperature. The wells were washed with PBS and polysorbate 20, then incubated for 2 hours at room temperature with 50 μl /well of polyvalent rabbit anti-cat serum diluted 1:200 with 1% BSA-PBS. After further washing with PBS, 50 μl /well of alkaline phosphatase-labeled goat anti-rabbit serum diluted 1:1000 with 1% BSA-PBS was added, and the mixture was incubated for 1 hour at room temperature. After a final wash with PBS-polysorbate 20, 100 μl /well of dinitrophenol phosphate (1 mg/ml) was added. The resulting color change was read approximately 20 minutes later at wavelength 405 nm. The absorbance of unknown samples was converted to milliunits per milliliter of *Fel d I* by comparison with the standard curve, having a range of 0.05 to 13.3 mU/ml *Fel d I*. If the absorbance of an unknown was near or higher than that of the highest standard, the sample was reassayed at a greater dilution until a value in the midportion of the curve was obtained. The standard used for the assay was FDA Cat E3, the same as that used by Chapman et al.,¹⁰ containing 10.5 FDA units/ml *Fel d I* (1 FDA unit = 4 μg *Fel d I*).¹¹ Results were recorded as milliunits of *Fel d I* shed per 30-minute air sample per cat.

Statistical methods

Two measurements of *Fel d I* shedding were obtained weekly for each cat. The average weekly measurement

was used in the data analysis for individual cats and for group means. A test for overall differences in group means at baseline was performed with a Kruskal-Wallis nonparametric test. Change in weight was calculated as posttreatment weight minus pretreatment weight and tested within each group with one-sample, two-sided t tests. Change in *Fel d I* shedding was calculated as the average final-week value minus baseline value and was tested within each group with analysis of covariance, adjusted for pretreatment weight. A test for overall differences in group means for *Fel d I* was done with analysis of covariance, adjusted for pretreatment weight.

A separate analysis of Friday (2 days after washing or Allerpet-C spray application) air samplings was done to determine whether there was an effective, but short-lived treatment benefit, assuming that reaccumulation of *Fel d I* may have occurred by the posttreatment day 6 each week. Furthermore, an analysis of area under the curve was done as an estimate of total *Fel d I* allergen shed by each treatment group throughout the study period. This would determine whether there was any significant drop in *Fel d I* during the study period before the final week.

The work of Wentz et al.⁸ on the variability of cat allergen shedding revealed significant sixfold variation in rates of *Fel d I* production among 12 studied cats, with an even higher 100-fold difference in total allergen between cats. In designing this study we sought to minimize the large variability in *Fel d I* by using only female cats, taking all observations at the same time of day, and stratifying the randomization by baseline *Fel d I* production status (high or low). To be clinically useful, the reduction in *Fel d I* must be substantial. The sample size of six cats per group was chosen to detect a reduction in *Fel d I* of 90% or more, with 80% power and a 0.05 probability of type I error.

RESULTS

Twenty-four cats were used, divided into four groups of six cats each. All cats tolerated the treatments well, including the washed cats. One cat had rhinorrhea throughout the study; however, this did not appear to affect its *Fel d I* production. Baseline *Fel d I* levels were not significantly different between groups ($p = 0.42$) (Table I). Each

TABLE II. Weight changes during study

Group	Weight (kg)		<i>p</i> value*
	Mean	SD	
Acepromazine (<i>n</i> = 6)			
Pretreatment	3.25	0.83	0.99
Posttreatment	3.25	0.50	
Change	0.00	0.39	
Washing (<i>n</i> = 6)			
Pretreatment	3.62	0.84	0.62
Posttreatment	3.75	1.14	
Change	0.13	0.62	
Allerpet-C (<i>n</i> = 6)			
Pretreatment	3.53	0.98	0.63
Posttreatment	3.63	1.02	
Change	0.10	0.48	
Control (<i>n</i> = 6)			
Pretreatment	3.27	0.52	0.91
Posttreatment	3.28	0.41	
Change	0.01	0.34	

*No group of cats had a significant weight change during the study.

group showed a slight, but insignificant average weight gain during the study, likely because of inactivity ($p > 0.62$), (Table II).

Three internal standards were used in each assay. The average interassay coefficient of variation for these internal standards was 22.3% for all assays. As shown in Table III, after adjustment for pretreatment weight, comparison of baseline with final weekly measurements revealed no significant change in *Fel d I* within any group (mean change $\pm$ SD: wash, 487.6 ± 1896.4 mU per 30 minutes, $p = 0.63$; Allerpet-C spray, 429.2 ± 871.6 mU per 30 minutes, $p = 0.65$; acepromazine, -620.6 ± 1031.2 mU per 30 minutes, $p = 0.52$; control, -695.6 ± 3342.0 mU per 30 minutes, $p = .46$). Adjustment for each cat's weight was done because we assumed that larger cats might shed more allergen. This adjustment did not alter the results. Fig. 1 shows the average weekly *Fel d I* levels for each treatment group. There was no significant change in *Fel d I* levels between treatment groups ($p = 0.72$) after adjustment for pretreatment weight.

Furthermore, analysis of air samplings alone after 2-days washing and after-Allerpet-C spray revealed no significant decline within or among groups (Fig. 2; Table IV). Thus it is unlikely that these treatments were beneficial in reducing *Fel d I* levels in the short term. Furthermore, an analysis

TABLE III. *Fel d I* change during treatment

Group	<i>Fel d</i> level (mU/30 min)		<i>p</i> value*
	Mean	SD	
Acepromazine (<i>n</i> = 6)			
Baseline	1578.0	857.4	0.52
Final	957.6	436.6	
Change	-620.4	1031.2	
Washing (<i>n</i> = 6)			
Baseline	803.0	658.4	0.63
Final	1290.6	2192.2	
Change	487.6	1896.4	
Allerpet-C (<i>n</i> = 6)			
Baseline	810.6	486.0	0.65
Final	1239.8	1126.6	
Change	429.2	871.6	
Control (<i>n</i> = 6)			
Baseline	1927.0	3243.2	0.46
Final	1231.4	824.4	
Change	-695.6	3342.0	

The differences between baseline and final-week values (changes) are presented as unadjusted means.

*No significant change in *Fel d I* noted within groups. Furthermore, changes in *Fel d I* between groups were not significant ($p = 0.72$).

of area under the curve as an estimate of total *Fel d I* allergen shed by each treatment group revealed no significant difference between groups ($p = 0.58$) when adjusted for baseline values and pretreatment weight (Table V).

DISCUSSION

Fel d I is the major cat allergen previously demonstrated to cause symptoms in most patients allergic to cats,¹²⁻¹⁴ and in 20% to 30% of patients with asthma.¹⁵⁻¹⁷ *Fel d I* is an approximately 36,000 kd acidic protein dimer that is produced by both the salivary glands and the skin of the cat, that is, primarily by sebaceous gland cells and to a lesser extent by basal squamous epithelial cells.¹⁸ It is airborne on particles varying from less than 1 to more than 10 μ m in diameter, some of which remain airborne for prolonged periods even without disturbance, in contrast to dust mite allergens.^{19,20} Previous studies suggest that *Fel d I* is carried into homes and public places on the clothing of persons exposed to cats.²¹ Levels in homes without cats typically range from 1 to 8 μ g/gm of dust, and in homes with cats levels usually exceed

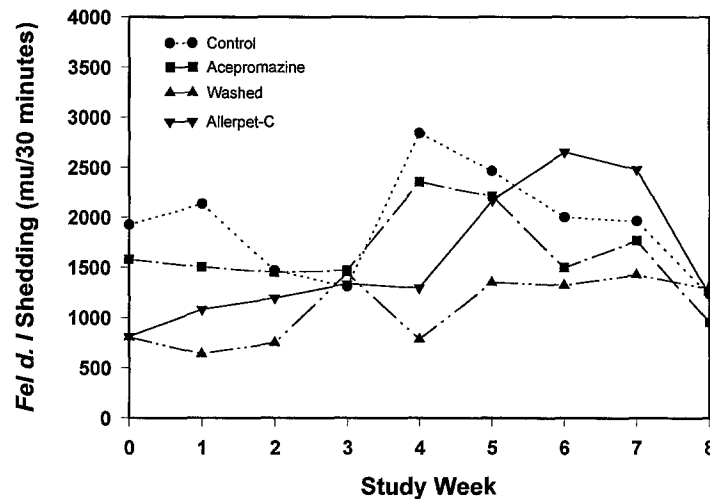


FIG. 1. Weekly shedding of *Fel d I* expressed as average milliunits of *Fel d I* per 30 minute air sample for each of four groups of cats. *Circles*, Control group; *squares*, acepromazine-treated group; *triangles*, washed group *inverted triangles*, Allerpet-C-treated group.

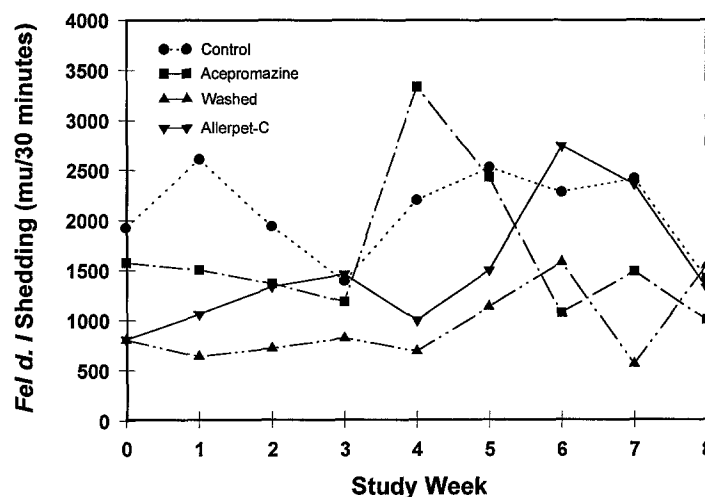


FIG. 2. Average shedding of *Fel d I* on Fridays (2 days after washing and Allerpet-C treatments) by cats in each of four groups. *Circles*, control group; *squares*, acepromazine-treated group; *triangles*, washed cats; *inverted triangles*, Allerpet-C-treated group.

8 $\mu\text{g/gm}$ dust. A *Fel d I* concentration of 8 $\mu\text{g/gm}$ is proposed as the threshold level for sensitization and asthma.²²

To our knowledge this is the first study to evaluate scientifically the effects of three different treatments on the shedding of *Fel d I* by cats. The study was performed in a prospective and blinded fashion. On the basis of prior reports, this study had adequate power to detect a major (>90%) reduction in allergen shedding. Although we may have failed to detect small changes in allergen shedding, we do not believe that small changes would be clinically significant. None of the treatment groups showed a consistent trend toward

lower allergen shedding during the study, suggesting that no effect would be seen even with a larger number of cats. It is also possible that one or more of the treatments tested may have reduced shedding of allergens other than *Fel d I*. This would have been missed by our test methods, because we measured only *Fel d I*. We do not think that this is a serious flaw in our study because many studies have shown that *Fel d I* is the allergen most consistently associated with patient symptoms.

We analyzed the data obtained by using the average of final-week treatment values and by reviewing only the 2-day posttreatment (after Allerpet-C or washing) values during the final

TABLE IV. *Fel d I* change during treatment

Group	<i>Fel d I</i> level (mU/30 min)		<i>p</i> value†
	Mean change*	SD	
Acepromazine (<i>n</i> = 6)	-569.0	1186.4	0.58
Washing (<i>n</i> = 6)	749.0	2487.1	0.46
Allerpet-C (<i>n</i> = 6)	521.0	813.1	0.60
Control (<i>n</i> = 6)	-507.0	3239.8	0.62

*The differences between baseline and final-week values, taken 2 days after treatment (Friday), are presented as unadjusted means.

†No significant change in *Fel d I* was noted within groups. Furthermore, changes in *Fel d I* between groups were not significant (*p* = 0.72).

week. The latter was done to anticipate whether there was a significant but short-lived treatment benefit, assuming that reaccumulation of *Fel d I* may occur by the end of each treatment week. Given the variability of shedding, it was also possible that levels may have declined overall during the majority of the study period, only to rise again during the final week. To study this we conducted an analysis of total *Fel d I* allergen shed by each group throughout the study period by measuring the total area under the curve and took into account all study measurements obtained. No significant benefit of treatment was found in viewing the data in any of these ways.

Trends in *Fel d I* shedding were noted for individual cats during the study. Certain cats began the study with low baseline levels and remained that way. Likewise, high shedders tended to remain high. This phenomenon is not new and was noted by both Wentz et al.⁸ and Glinert et al.²³ However, Wentz et al. also noted that high producers varied considerably in *Fel d I* production on a day-to-day, or even hourly, basis.⁸ Our attempt therefore was to conduct air sampling in all cats at the same time of day and on the same days of each week, to minimize the chances of diurnal variation affecting results. Variability, however, still existed in certain cats and was not associated with any particular treatment group. Male cats in the study of Wentz et al.⁸ did shed more allergen than female cats. We studied only female cats to remove this sex bias in *Fel d I* production. The sex bias was also confirmed by Zielonka et al.,²⁴ who were further able to demonstrate that castration of males significantly decreased *Fel d I* production. A hormonal relationship, then, could certainly play a role in *Fel d I* production. The cat's menstrual cycle may also

TABLE V. Estimated total *Fel d I* shedding area under the curve

Group	Area under curve (mU)	
	Adjusted mean	SD
Acepromazine (<i>n</i> = 6)	86,435.0	28,378.4
Washing (<i>n</i> = 6)	75,126.6	28,609.6
Allerpet-C (<i>n</i> = 6)	105,052.2	28,468.6
Control (<i>n</i> = 6)	94,483.2	28,767.6

After adjustment for baseline and pretreatment weight, there were no differences within groups (*p* = 0.89) or between groups (Kruskal-Wallis *p* = 0.58) for mean area.

play a role. Some cats went into heat during the study period; however, the days a cat is in heat are difficult to determine precisely. Thus the potential effect of menstrual cycles on *Fel d I* was not evaluated.

The season of the year may also affect *Fel d I* shedding. Our study was conducted in the spring and early summer months. However, the rooms had no windows, and lighting was controlled by timers to give cats the impression of a stable, nonchanging season. However, because our study was of a relatively short duration, we cannot totally rule out a seasonal effect.

Although the idea of washing one's cat has been promoted by the medical community for some time, only a limited number of cat washing studies have been reported. A study by deBlay et al.,²⁵ demonstrating a decline in *Fel d I* with washing, involved only one cat, washed weekly during only a 4-week period. Modest reaccumulation of *Fel d I* between washes was evident. An abstract published by Glinert et al.²³ used a slightly larger sample size of 10 cats, but washes were conducted only monthly. Recoverable *Fel d I* was obtained only by analyzing filtered bath water, not by air sampling, which seems to be far more important with regard to the probable clinical effects of airborne allergens. Glinert et al. acknowledged "significant variability" for individual cats for the first 3 to 7 months of washings, and it took 9 months for all cats to have a "significant decrease" in recoverable *Fel d I*. Further evaluation of their statement of significance was not possible, because their study was published only in abstract form. Critical review of an article by Ohman et al.²⁶ revealed data showing that three of six cats washed at weekly intervals had an increase in recoverable *Fel d I*, whereas the other three cats showed only a moderate decline. Again, *Fel d I* was analyzed only from recovered wash water, consisting of only 300

ml/wash. These three studies are the extent of the published data identified by an extensive literature search addressing the effect of cat washings on *Fel d* I shedding. It is possible that other washing methods, such as totally immersing the cat or adding detergents, may have been more effective at reducing *Fel d* I, but other methods were not evaluated because previous studies had reported that water alone was effective.

Topical pet sprays have been commercially available for some time; Allerpet spray is a product widely distributed in the Detroit area. Allerpet-C spray, specifically for cats, contains primarily deionized, ultraviolet-sterilized water, quaternium 22,26, hydrolyzed animal protein, allantoin, aloe vera gel, imidazolidinyl urea, and collagen. The product has never been scientifically tested. Manufacturer-sponsored field research involved 168 persons, only four of whom claimed to have asthma, who claimed they were allergic to their pets. Subjective reports were accepted at face value. Only 54 of the 168 persons had cats in their homes; the rest had dogs or other pets. Forty-six of 54 cat owners reported a "significant improvement in their ability to tolerate their pet."²⁷ The study was not blinded and did not involve objective measurements.

The use of acepromazine for cat allergy is promulgated as an urban legend, according to an informal survey of local Detroit veterinarians. We have been unable to find scientific studies in the medical or veterinary science literature to confirm any effect of acepromazine on cat allergy. However, the willingness of the mass media to convey information suggesting efficacy of treatments such as acepromazine for cat allergy ensures continued use of such measures.⁷

Anecdotal cases touting the effectiveness of acepromazine, Allerpet-C spray, and cat washings exist.^{7, 23, 26} (An isolated report of vitamin E given to the cat in a dosage of 100 IU/day has also been proposed as efficacious.²⁸) Our data, obtained under blinded, controlled conditions during an 8-week treatment period, however, do not show any of these three treatments to be effective in diminishing *Fel d* I shedding. Therefore we cannot recommend any of these treatments to patients allergic to cats.

The data presented here suggest that practicing allergists and others in the medical community reconsider their advice for avoidance measures to patients allergic to cats. Removal of the cat from the home, together with a regular household cleaning program, is still the most effective, undisputed

avoidance measure. Future studies with these treatments should be undertaken with the cat in a household environment, should note the effects on persons allergic to cats, and should correlate the response with *Fel d* I shedding.

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