

Washing the dog reduces dog allergen levels, but the dog needs to be washed twice a week

Tessa Hodson, BSc, Adnan Custovic, MD, Angela Simpson, MD, Martin Chapman, PhD, Ashley Woodcock, MD, and Rosalind Green, MD *Manchester, United Kingdom*

Background: Many asthmatic patients allergic to dogs refuse to part with their dog, and it is essential to develop techniques for lowering exposure with a dog in the home.

Objective: This study investigated the effect of dog washing on the subsequent recovery of Can f 1 from dog hair clippings and on the airborne allergen over a 7-day period.

Methods: Dogs, which had not been washed for at least the previous 3 weeks, were washed with a hand-held shower and proprietary shampoo. Hair clippings and dander samples from 25 dogs were collected before and immediately after washing. After these initial studies, 16 dogs had a small tuft of hair clipped from the collar or spinal area before washing and then daily for the next 7 days. Air sampling was performed in 5 homes, and the air samples were collected (airflow rate, 9 L/min) over an 8-hour period per day on 10 consecutive days (3 days of baseline sampling before washing and then 7 consecutive days after washing). Can f 1 level was measured by using 2-site ELISA.

Results: Washing significantly reduced recoverable Can f 1 from clippings (84% reduction: from 73 µg/g to 12 µg/g [geometric mean]; $P < .0001$) and from dander samples (86% reduction: from 347 µg/g to 50 µg/g [geometric mean]; $P < .0001$). There was a significant reduction in Can f 1 levels in dog hair over the observed 8-day period ($F = 18.4$, $P < .0001$). By using a multiple comparison test, this observed significance was found to be due to the difference between the baseline levels and those on days 1 and 2 after washing, with no difference in the baseline Can f 1 compared with days 3 to 7. Airborne Can f 1 levels showed a downward trend, which reached statistical significance when the data were grouped into 3 sampling periods as follows: baseline (ie, mean of 3 days before sampling) was compared with days 1 to 4 after washing (41% reduction, 95% CI 13%-60%) and days 5 to 7 after washing (61% reduction, 95% CI 2%-84%; $P = .014$).

Conclusions: Washing the dog reduces recoverable allergen from dog hair and dander. The dog needs to be washed at least twice a week to maintain the reduction in recoverable Can f 1 from its hair. Washing the dog achieves a modest reduction in the level of airborne Can f 1 in homes with a dog. (*J Allergy Clin Immunol* 1999;103:581-5.)

Key words: Dog allergen, airborne, dog washing, asthma

It is estimated that there are 7.3 million dogs in the United Kingdom (Royal Society for the Prevention of Cru-

Abbreviation used

GM: Geometric mean

elty to Animals, personal communication, 1997), a significant proportion of which are likely to live in the homes of individuals allergic to dogs. Sensitization to dog is an important cause of asthma, particularly in the areas where exposure to dust mite allergens is low.¹ In addition, animal materials are major allergenic sources in house dust in areas where house dust mite allergens are prevalent.²

Dander, pelt hair, and saliva are the most important sources of dog allergens, and urine does not exhibit significant allergenic activity.³⁻⁵ Up to 92% of dog-sensitized individuals react to a major allergen, Can f 1, which has been shown to account for at least half of the allergenic activity in the extracts of dog hair and dander.⁴ Varying degrees of Can f 1 were reported among different dog breeds and among individual dogs.⁵ In addition, the existence of breed-specific dog allergens has been suggested because about 15% of patients with hypersensitivity to dogs have different skin test responses to different dog breeds.⁶ This is, however, still a controversial issue because it was suggested that from a biologic point of view, it does not seem likely that true breed-specific allergens exist,⁷ and no breed-specific allergens have yet been purified.

We have previously shown that in a house with a dog, whatever the absolute quantity of the dog allergen in the air, a significant proportion (~20%) will be associated with small particles.⁸ Some particles will be inhaled and deposited in the tracheobronchial tree and alveoli. Furthermore, a proportion of the inhaled particles will inevitably deposit on the area between mucociliary and alveolar clearance, thus remaining in the lungs for a longer period of time. It is thus not surprising that dog-sensitized patients with asthma may have severe disease when they live in a home with a dog.

Removal of the dog from the home is the best method of reducing exposure for asthmatic patients allergic to dogs. However, patients often refuse to part with their pet, and effective environmental strategies that reduce dog allergen exposure with the dog in the home could help alleviate symptoms in these patients.

We have undertaken a study to investigate the effect of washing the dog on recoverable Can f 1 levels in dog hair and dander and on the airborne levels of Can f 1 in homes with dogs.

From the North West Lung Centre, Wythenshawe Hospital, Manchester. Received for publication May 22, 1998; revised Oct 1, 1998; accepted for publication Oct 1, 1998.

Reprint requests: Ashley Woodcock, MD, North West Lung Centre, Wythenshawe Hospital, Southmoor Rd, Manchester M23 9LT, UK.

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0091-6749/\$8.00 + 0 1/1/94895

METHODS

Dog washing and Can f 1 in dog hair

Recoverable Can f 1 levels from 25 domestic dogs that had not been washed for at least 3 weeks were measured before and once immediately after a thorough washing. The experiment was carried out at a dog-grooming parlor, and the dog owners gave their verbal consent. One side of each dog was vacuumed for 2 minutes with a Medical Dust Sampler (Medivac plc, Wilmslow, UK; airflow rate, 45 L/sec) with a brush attachment onto a 5- μ m vinyl filter (Plastok Associates Ltd, Wirral, UK; "dander"). A small tuft of hair was clipped from the other flank ("clipping"). The dog was then washed thoroughly for 5 minutes with shampoo in a bath under a hand-held shower head by using water at 40°C to 50°C before being rinsed clean. The excess water was blown from the dog with a jet of air before the dog was dried in a purpose-built drying cabinet at 30°C. Any damp patches were subsequently dried with a hair dryer. A second tuft of hair was clipped, and the side of the dog opposite to that from which the dander sample was collected before the washing was vacuumed for another 2 minutes.

To investigate whether vacuuming itself has any effect on allergen levels, 9 dogs were vacuumed for 5 minutes with a vacuum cleaner (airflow rate, 45 L/sec) with a special brush attachment. Dander samples were collected after 1 hour, and then 3 and 7 days after vacuuming as described above by sampling the whole dog for 4 minutes. These procedures were performed in dog owners' homes.

Further experiments were conducted to determine the duration of the effect of a single wash. Clippings of hair were taken from the neck or over the spine of 16 dogs (which had not been washed for at least the previous 3 weeks). The dogs were washed in their owners' family bath/shower unit by using a proprietary shampoo and then towel-dried. Clippings of hair were collected daily for the next 7 days. All samples were coded and stored at -40°C.

Dog washing and airborne Can f 1

The effect of washing the dog on airborne Can f 1 was investigated in 5 homes with a dog. The sampling was performed over a 10-day period in a living room in each of the houses. All of the rooms were carpeted and contained a sofa. The age of the houses ranged from 30 to 74 years. The windows were closed during the sampling period, and no air cleaning devices were present in any of the houses. The dogs were free to wander throughout the house. Three days of baseline sampling before washing the dog were followed by 7 subsequent follow-up days after the dog had been washed in the home as described above.

To minimize the variability in airborne allergen levels within each home, which we previously observed when the samples were obtained with high-volume air samplers over short periods (eg, 60 L/min for 1 hour),⁸ we decided to use a low-volume sampler (airflow rate, 9 L/min; Medic-Aid, West Sussex, UK) for a longer period (8 hours/day). The sampling head was loaded with a 3.7-cm glass microfiber filter (pore size, 0.3 μ m, Whatman) and positioned at a height of 1 m in the living room. The air samples were collected during normal domestic activities. After sampling, each filter was removed by forceps, placed into a petri dish, coded, and kept at 4°C until extraction.

Processing of samples and Can f 1 assay

Dog hair/dander extraction of Can f 1. One hundred milligrams of dog hair or dander sample obtained by vacuuming the dog was placed in a test tube. Three milliliters of 1% BSA in PBS with 0.1% Tween 20 (BSA PBS-T) were added. Samples were agitated by using a vortex mixer before being mixed end over end for 2 hours on an orbital rotator at room temperature. They were then centrifuged for 30 minutes at 3000 rpm at 4°C, and the supernatant was stored at -40°C for future analysis of allergen content.

Air sampler filter extraction. Each filter was cut into 4 pieces and placed in a syringe, and 1 ml of 1% BSA PBS-T was added. After overnight extraction at 4°C, the extraction liquid was aspirated backwards and forwards several times through a 3-way stop lock into a second syringe, then transferred into a test tube and centrifuged at 3000 rpm for 30 minutes at 4°C. The supernatant was stored at -40°C for future analysis of allergen content.

Can f 1 ELISA. Can f 1 was measured by a 2-site monoclonal/polyclonal antibody-based ELISA by using anti-Can f 1 mAb 6E9 for allergen capture and polyclonal rabbit anti-Can f 1 for detection as previously described.^{1,8} Dander and clipping extracts were initially assayed at 10-, 50-, and 250-fold dilutions. Air samples were assayed neat and at 2- and 4-fold dilutions. For concentrations lying off the linear portion of the standard curve, the assays were repeated at an appropriate dilution.

The assay was quantitated by using doubling dilutions of dog allergen standard (UVA 94/02) from 500 IU/mL to 1 IU/mL Can f 1. The UVA 94/02 (10,000 IU Can f 1/mL) was substandardized against the World Health Organization/International Union of Immunologic Societies International Reference Preparation of dog hair and dander (NIBSC 84/685), which contains 100,000 IU/mL Can f 1. One international unit is approximately 1 ng Can f 1 protein, and this value was used to calculate the results.

Statistical analysis

The data were found to follow a log-normal distribution, and the results are thus presented as geometric means (GMs). For all hair and dander samples the results are expressed as allergen concentration in micrograms per gram; for air samples, the airborne allergen levels in nanograms per cubic meter were calculated. The analysis was performed by using ANOVA with repeated measures. Statistical significance was set at the conventional 5% level.

RESULTS

Dog washing and Can f 1 in dog hair

Washing the dog significantly reduced recoverable Can f 1 by 84% from clippings (from a GM of 73.0 μ g/g [95% CI 48.9-109.0] to a GM of 11.8 μ g/g [95% CI 7.6-18.3]; $P < .0001$) and by 86% from vacuum samples (dander; from a GM of 347.2 μ g/g [95% CI 224.5-537.0] to a GM of 50.4 μ g/g [95% CI 35.0-72.5]; $P < .0001$). Can f 1 concentration in dander samples obtained by vacuuming the dogs was 4.7-fold higher than the corresponding levels in the clippings of dog hair.

There were no significant effects of the vacuuming on recovered dog allergen, both when the results were expressed in micrograms of Can f 1 per gram and as the total amount of allergen. GM concentrations of Can f 1 (95% CI) were 118.4 μ g/g (46.1-304), 42.4 μ g/g (9.4-189.1), 51.5 μ g/g (14.7-180.5), and 52.5 μ g/g (19.9-138.4) at the baseline, 1 hour, 3 days, and 7 days after vacuuming the dog, respectively ($F = 1.6$, $P = .2$). Total amount of Can f 1 (GM) recovered by sampling (95% CI) was 4.8 μ g (1.3-17.8) at baseline, and 1.2 μ g (0.2-10.2), 2.5 μ g (0.5-12.0), and 1.7 μ g (0.5-5.3) at 1 hour, 3 days, and 7 days after vacuuming, respectively ($F = 1.45$, $P = .25$).

Can f 1 concentrations in dog hair clippings from 16 dogs before washing and over the 7 consecutive days after washing are shown in Table I. Recovered Can f 1 was reduced by 98.7% on the first day after washing the dog, and the levels then gradually built up over the next

few days. The observed reduction in Can f 1 levels over the 7-day period was highly significant (ANOVA with repeated measures, $F = 18.4$; $P < .00001$). By using a multiple comparison test, it was found that the observed significance was due to the difference between the baseline levels and those on days 1 and 2 after washing. There was no difference in the recovered Can f 1 at baseline compared with days 3 to 7. Allergen levels from each individual dog are presented in Fig 1.

Dog washing and airborne Can f 1

Tables II and III show the effect of dog washing on the Can f 1 levels in the air in 5 homes with a dog. The mean values showed a downward trend, but the range of airborne levels obtained in different homes, as well as within the same homes on different sampling days, was substantial (Table II). The airborne Can f 1 measured in each of the homes during the baseline 3-day period and on 7 consecutive days after dog washing are shown in Fig 2. When the analysis was performed on this set of data, the observed downward trend failed to reach statistical significance ($F = 1.72$, $P = .14$).

In an attempt to minimize the day-to-day variability in airborne allergen levels within each home, the 7-day period subsequent to dog washing was divided into 2 periods that corresponded to the 3-day baseline period (period 1, days 1 to 4 after washing; period 2, days 5 to 7 after washing). When the GMs for airborne Can f 1 levels for days 1 to 4 and days 5 to 7 after washing were calculated and compared with the baseline value, the observed trend reached significance ($F = 7.53$, $P = .014$; Table III). As an alternative to a control group, all the air samples were assayed for major cat allergen Fel d 1 because it should not be affected by the dog washing. No cats were present in any of the homes, but detectable levels of airborne Fel d 1 were found in 3 of 5 homes. When the same analysis used for Can f 1 was repeated for Fel d 1, no difference between the 3 sampling periods was observed (Fel d 1 GMs: 0.83, 0.77, and 0.72 ng/m³ in baseline and periods 1 and 2, respectively; $F = 0.15$, $P = .9$).

DISCUSSION

Complete avoidance of dog allergens in developed countries with a high proportion of dog ownership is probably impossible. Sensitized patients can be exposed to dog allergen not only in homes with dogs, but also in those that have never housed an animal.^{2,8} In addition, exposure to airborne Can f 1 can occur inside a number of public buildings (including hospital outpatient departments).⁹⁻¹¹ Nonetheless, dog allergen levels are much higher (up to 250-fold) in homes with dogs,⁸ and a substantial reduction in exposure to these very high levels in sensitized patients with allergic disease (eg, asthma and allergic rhinitis) may improve the control of symptoms.¹²

The major dog allergen Can f 1 has very similar aerodynamic characteristics to the cat allergen Fel d 1.^{8,13,14} In addition, sensitized patients are similarly reluctant to get rid of their dogs as they are to get rid of their cats. Several studies have investigated the effect of cat washing on

Can f 1 (μg/g)

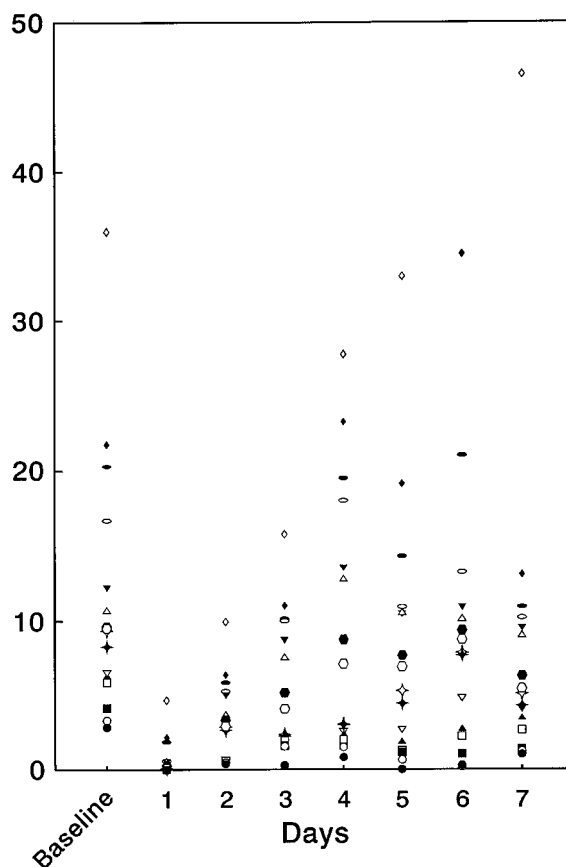


FIG 1. Major dog allergen Can f 1 recovered from samples of dog hair in 16 dogs before and on 7 days after washing.

Can f 1 (ng/m³)

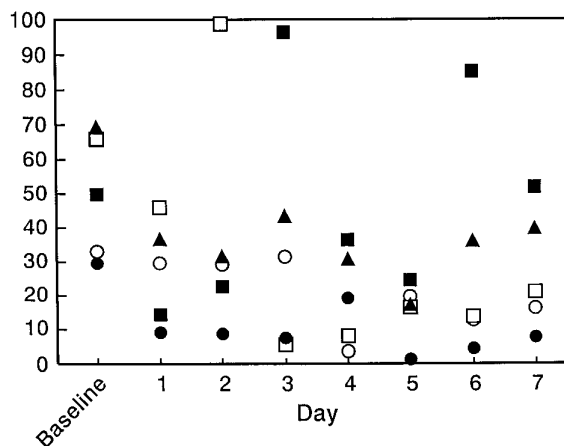


FIG 2. Airborne Can f 1 collected with an air sampler with a low airflow rate (9 L/min) over the 8-hour period/day before and on 7 subsequent days after dog washing. Baseline value is mean of 3 days before dogs had been washed.

levels of Fel d 1.¹⁵ Klucka et al¹⁶ compared the relative efficacy of washing with water, Allerpet-C spray, and acepromazine in decreasing cat allergen shedding in 24

TABLE I. Effect of dog washing on Can f 1 concentrations in dog hair clippings

	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
GM Can f 1 ($\mu\text{g/g}$ [95%CI])	9.11 (6.29-13.19)	0.12 (0.038-0.38)	1.98 (1.09-3.59)	3.62 (2.11-6.24)	5.4 (2.95-9.88)	3.71 (1.44-9.53)	4.82 (1.94-11.95)	5.18 (3.06-8.77)
Range ($\mu\text{g/g}$)	2.85-36.0	0.01-4.65	0.37-9.9	0.32-15.75	0.02-33	1.03-46.5	0.84-27.75	0.08-34.5
Percent reduction (95% CI)	—	98.7 (96.3-99.9)	78.3 (64.27-86.8)	60.2 (24.3-79.1)	40.7 (-21.3-71)	59.3 (-11.9-85.1)	47.1 (-45.5-80.8)	43.1 (-8.1-70.1)
Fold reduction (95% CI)	—	76 (26.9-213.8)	4.6 (2.8-7.6)	2.5 (1.3-4.8)	1.7 (0.8-3.4)	2.5 (0.9-6.7)	1.9 (0.69-5.2)	1.8 (0.9-3.3)

TABLE II. Effect of dog washing on airborne concentrations of Can f 1

	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
GM Can f 1 (ng/m^3 [95% CI])	46.7 (28.7-75.9)	23.02 (9.9-53.5)	28.2 (9.6-82.8)	22.2 (5-99.4)	14.42 (4.3-48.1)	11.03 (2.36-51.5)	18.78 (4.63-76.2)	22.14 (8.6-56.7)
Range (ng/m^3)	29.48-69.35	9.07-45.91	8.72-98.77	5.54-96.3	3.61-36.51	1.22-24.4	4.36-85.19	7.64-51.85
Percent reduction (95% CI)	—	50.7 (8.6-73.5)	39.5 (-34-72.7)	52.4 (-115.8-89.5)	69.1 (3.4-90.1)	76.4 (12.7-93.6)	59.8 (-30.6-87.6)	52.6 (6.9-75.8)
Fold reduction (95% CI)	—	2 (1.1-3.8)	1.7 (0.75-3.7)	2.1 (0.46-9.5)	3.2 (1.04-10.1)	4.2 (1.1-15.6)	2.5 (0.77-8.1)	2.1 (1.07-4.1)

TABLE III. Effect of dog washing on airborne concentrations of Can f 1

	Baseline (mean of 3 days before washing)	Period 2 (mean of days 1 to 4 after washing)	Period 3 (mean of days 5 to 7 after washing)
GM Can f 1 (ng/m^3 [95% CI])	46.7 (28.7-75.9)	27.4 (13.8-54.6)	18.2 (5.7-58.1)
Range (ng/m^3)	29.48-69.35	11.13-42.46	4.41-53.83
Percent reduction (95% CI)	—	41.3 (13.3-60.2)	60.95 (1.8-84.4)
Fold reduction (95% CI)	—	1.7 (1.15-2.5)	2.6 (1.02-6.43)

cats.¹⁶ Although no beneficial effect was observed with any of the treatments, it should be noted that only 2 L of water were used to wash the cats. In contrast, in the current study we washed the dogs in a bath with a hand-held shower unit and shampoo, and the dogs were rinsed thoroughly. In addition, Klucka et al¹⁶ collected the samples no earlier than 2 days after washing. From the results of our study, it is apparent that, at least in dogs, the allergen buildup is quite rapid, and recovered Can f 1 may reach the baseline level as soon as 3 days after washing. Interestingly, we found no effect of vacuuming on allergen levels. Vacuuming the dog for 5 minutes removed a relatively small amount of allergen (range, 0.5 to 32 μg in our sample of 9 dogs) and did not appear to affect the subsequent concentration of Can f 1 in dander samples.

In a recent study Avner et al¹⁷ examined the effect of different techniques of cat washing on airborne Fel d 1, with airborne levels being assessed immediately before and 3 hours after washing in the experimental room. Three cats were washed weekly with warm water and soap at a veterinarian's office, resulting in a 44% reduction in airborne Fel d 1. Immersion of 3 cats for 3 minutes in approximately 30 L of warm water resulted in an aver-

age decrease in allergen level of 79%. When this was accompanied in 2 cats by an additional rinsing in warm water, the airborne Fel d 1 was reduced to 16% of the baseline level. However, the allergen levels returned to baseline within a week. By shaving 6 cats, the authors measured the total quantity of Fel d 1 per cat and found it to range between 3 and 142 mg, with a mean of 67 mg. Washing removed between 1 and 35 mg of Fel d 1 per cat, with the largest quantities being removed from the cats with the highest concentrations of allergen. The same group of investigators studied the effect of Allerpet-C (a commercially available spray claimed by the manufacturer to reduce cat allergen shedding) on airborne cat allergen and reported a mean reduction of 62% after the first treatment.¹⁸ In addition, although wiping the cat with Allerpet-C removed allergen, the amount removed was 5-fold lower compared with that removed by washing the cat. All these experiments suggested that cat washing may reduce airborne allergen in the experimental room, but the effect in homes with cats remained largely unknown.

It is very difficult to estimate the size of the effect of dog washing on airborne Can f 1 in homes with dogs. This is not surprising because very high levels of Can f 1

can be found in the settled dust in carpets and/or soft furnishings from these homes. Thus the shedding of allergen from the animal, the reservoir levels, and the amount of artificial disturbance will influence airborne allergen levels. In a field trial it is impossible to standardize for some of these contributing factors. This study was designed to minimize the considerable variability in airborne dog allergen between different homes and within the same homes when the samples were taken on several occasions. Air samples were collected by using samplers with low airflow rates comparable to the average ventilation rate of an adult. The sampling was performed over a long period (8 hours/day), and mean values over the consecutive 3-day periods were used in the analysis. However, in spite of this, a considerable variability on different days within same houses was observed (Table II, Fig 2), which calls into question the validity of a single measurement of the airborne allergen as an index of exposure. The values, however, did show an overall downward trend, whereas at the same time the airborne level of major cat allergen (that would not have been affected by the dog washing) remained unchanged.

De Blay et al¹⁹ reported a reduction in airborne Fel d 1 after washing one cat weekly over a 4-week period. We showed a similar reduction in airborne Can f 1 in 5 homes with dogs. It is interesting that we have observed a very substantial reduction in allergen recovered from the dog hair immediately after washing, with a relatively rapid buildup over the following few days. The effect on airborne allergen, however, was much more modest but appeared to last longer (up to a week). It is tempting to speculate that a proportion of airborne allergen may be carried on dry flakes and that the newly produced allergen following the washing of the animal may not be associated with particles that can shed off the animal for some time. Nevertheless, one should be very cautious when interpreting the airborne allergen data. Not only was there a tremendous variability in these levels, but the sampling itself could have led to behavioral changes in the homes that might have contributed to lower allergen levels. Thus the main benefit of washing the dog regularly may be the reduction in the buildup of allergen in dust reservoirs (eg, carpets and upholstered furniture).

In conclusion, the results of the present study show that washing the dog resulted in reduction in Can f 1 concentration in dog hair and dander. However, the level of Can f 1 increased after washing and was not significantly lower compared with baseline by the third day afterwards. This would suggest that washing needs to be done a minimum of twice a week to be effective. Twice weekly washing could decrease the buildup of allergen in the dust reservoirs within the home. In addition, washing the dog regularly may reduce the level of airborne Can f 1 in homes with a dog. However, it is unlikely that the short-term and modest reduction in the airborne allergen achieved by dog washing would significantly improve asthma control in

sensitized individuals. Further research is required to establish the clinical effect of washing the dog in the management of patients with asthma who are sensitized and exposed to dogs. An integrated approach with the combination of environmental control measures resulting in a substantial reduction in airborne allergen may have a desired clinical effect, but it is essential that this be confirmed or refuted in a clinical trial.

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