

Quantitation of house dust mite allergens (Der f 1 and group 2) on the skin and hair of dogs

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Objective—To determine the concentration of house dust mite (HDM) allergens, Der f 1 and group 2, on the skin and hair of dogs and whether associations exist between the presence of Der f 1 and group 2 allergens on the skin and hair of dogs and household and dog characteristics.

Animals—63 pet dogs from 50 homes.

Procedure—Dogs were weighed and body surface area in square meters was determined. Skin and hair samples were obtained by vacuuming dogs. Collected dust was analyzed by use of standard ELISA techniques.

Results—HDM allergen was detected in 21 of 59 skin and hair samples. Presence of group 2 allergen on skin and hair of dogs was significantly associated with long hair, compared with short or medium length hair. Median house dust sample concentrations of Der f 1 and group 2 allergens were high in homes with dogs that had skin and hair samples that were positive for Der f 1 and group 2 allergens. Dogs with skin and hair samples that were positive for Der f 1 and group 2 allergens resided in homes with a high number of house dust samples that were positive for Der f 1, group 2, or both allergens and in homes with a mean house dust sample allergen concentration of ≥ 2 $\mu\text{g/g}$ of dust.

Conclusions and Clinical Relevance—Associations exist between environmental HDM allergen concentrations and HDM allergens on the skin and hair samples of dogs. Environmental allergen load is a major factor in accumulation of allergens on the skin and hair of dogs. (*Am J Vet Res* 2005;66:143–149)

Atopic dermatitis in dogs is a genetically-predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features and associated most commonly with IgE antibodies to environmental allergens.¹

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House dust mites (HDMs), in particular *Dermaphagoides farinae* and *Dermatophagoides pteronyssinus*, are reported to be among the most common allergens causing hypersensitivity in dogs with atopic dermatitis, affecting between 30% to 100% of the dogs.^{2,5}

In humans, HDM allergens are a major cause of IgE-mediated atopic diseases worldwide.^{6,7} Additionally, in humans, the relationship between exposure to HDM allergens and the development of sensitization is well established.^{8,9} Because allergen exposure is necessary for sensitization, numerous studies^{10–14} have quantitated and determined the distribution of HDMs and HDM allergens within homes, thereby elucidating household and sample site characteristics that may be specific risk factors for sensitization in humans. Despite the high incidence and importance of HDM-induced atopic dermatitis in dogs, the presence of HDMs and HDM allergens in the specific microenvironment of dogs has only recently been reported.¹⁵ In that study, significantly higher allergen concentrations were reported in basements, homes without central air-conditioning, and dog beds > 1 year old, thus identifying specific factors that may be associated with the increased risk for sensitization and development of atopic dermatitis in dogs.

House dust mites and HDM allergens have not only been found in the household microenvironment of humans but also on their clothes, skin, and hair, all of which were previously unrecognized reservoirs.^{16–19} Both HDMs and an HDM allergen, Der p 1, have been detected in hair dust and on the scalp of humans.^{17,20} Authors of another study documented Der p 1 allergen from human skin dust of the trunk and limbs, excluding the neck and head.¹⁸ Recently, the presence of the Der f 1 allergen was reported in dust samples collected from the skin and hair of dogs.²¹ The purposes of the study reported here were to investigate the presence and concentration of HDM allergens, Der f 1 and group 2, on the skin and hair of dogs and determine whether associations exist between the presence of Der f 1 and group 2 allergens on the skin and hair of dogs and various household and dog characteristics.

Materials and Methods

Animals—Dust samples from the skin and hair were collected from pet dogs belonging to staff and students at the College of Veterinary Medicine at The Ohio State University. Dogs were resident in 50 homes in metropolitan Columbus, Ohio, that were included in a recently reported study.¹⁵ Inclusion criteria for homes were the presence of at least 1 dog living indoors in the home and wall-to-wall carpeting in the rooms where the dog(s) spends time.

The dog owners completed questionnaires to detail dog characteristics, management practices, and whether dogs had any history of skin disease, ear disease, or both. Specific questions were asked to characterize the following: breed of dog (pure breed [type] or mixed), hair type (short, medium length, or long), age, sex, and weight. Additional questions were asked to obtain data on the following: date of last bath (< 1 month ago or ≥ 1 month ago), use of insecticidal shampoo (yes or no), last application of a flea or tick preventive (< 1 month ago, ≥ 1 month ago, or none used), chronic allergic skin disease (yes or no), and chronic otitis externa (yes or no). For all dogs with chronic allergic skin disease, otitis externa, or both, specific questions were asked to characterize the allergic disease as follows: type of allergic skin disease or allergic otitis externa (flea allergy dermatitis, atopic dermatitis, cutaneous adverse food reaction, prior skin infection [recurrent bacterial dermatitis, recurrent *Malassezia* dermatitis, or none], pruritus [yes or no], location of pruritus [feet, face, ears, distal portion of limbs, axillary or inguinal regions, base of the tail, caudal thighs, or unknown], and seasonality of clinical signs [seasonal, nonseasonal, nonseasonal with seasonal exacerbation, or unknown]). A diagnosis of atopic dermatitis was made according to accepted criteria,²² which included compatible history, typical clinical features, and diagnostic testing to rule out differential diagnoses such as flea allergy dermatitis, sarcoptic acariasis, cutaneous adverse food reactions, bacterial dermatitis, and *Malassezia* dermatitis. Dogs with atopic dermatitis had positive reactions on intradermal testing performed according to standard procedure²³ or had positive allergen-specific IgE serologic test^a results to allergens that were suspected or known to be in their environment and that were consistent with the nature of the disease in the individual dog.

Household characteristics—Specific questions were asked to characterize the following: type of housing (free standing, apartment, or townhouse), heating system (central with electric or gas, noncentral with wood or coal, or none), cooling system (central air-conditioning, air-conditioning with window units, or no air-conditioning system), level of housing (ground level with or without basement, 2 stories with or without basement, 3 stories with or without basement, or in the case of an apartment, floor level or not), floor level of bedrooms and living rooms (basement, ground or first floor, second floor, or third floor), pile of carpeting (short, medium length, or long), age of carpeting (≤ 1 year, > 1 year, or unknown), number of indoor pets (dogs and cats), use of carpet fresheners (yes or no), and use of flea or tick preventatives on pets (yes or no). Specific questions regarding the dog(s) were asked of the dog owner to identify the 3 locations in the home where the dog(s) most frequently resided. Additionally, the type of bedding used by the dog(s) was characterized into 3 categories as follows: dog bed (store purchased, specific dog bed), exclusive dog bedding (blankets or comforters used exclusively by the dog[s]), and shared bedding (blankets or comforters on beds used by humans and dogs). If samples were obtained from a dog bed, specific questions were asked to characterize the following: type of dog bed (foam filled or other synthetic filling material with cotton or other type of covering), frequency of washing (never, ≤ 6 months, or > 6 months), age of dog bed (≤ 1 year or > 1 year), location of dog bed (bedroom, living room, or other), and floor level where dog bed was situated (basement, ground or first floor, second floor, or third floor).

Dust sample collection from skin and hair of dogs—Samples were collected from each dog within 2 weeks from the time samples were collected from the respective home. Each dog was weighed, and body surface area in

square meters was determined by use of the following standard equation²⁴:

$$m^2 = (10.1 \times \text{body weight in grams}^{2/3}) \times 10^{-4}$$

The standard protocol for collection of dust samples for allergen identification and quantification from environmental areas by vacuuming 1 m² for 2 minutes^{6,25} was used for collecting dust samples from each dog. The time of vacuuming of the hair and body surface was calculated according to the calculated body surface area. A dog with 1 m² of body surface area was vacuumed for 2 minutes, and dogs with < 1 or > 1 m² of body surface area were vacuumed for 12 seconds for each 0.1 m² of body surface area that differed from 1 m². The entire body was vacuumed.

Briefly, a small, portable, canister vacuum cleaner^b with a plastic clear adapter and dust trap^c was used to collect all samples. The clear adapter and dust trap connects the tubing of the vacuum cleaner hose to the vacuum cleaner canister. A standard plastic removable nozzle was attached to the distal end of the vacuum cleaner hose. The adapter and dust trap was fitted with a new filter for the collection of each sample. The adapter and dust trap and nozzle attachments were rinsed with tap water thoroughly and allowed to air dry in between sample collections.

At the completion of sample collection from each dog, the filter paper was removed from the adapter and dust trap, folded at the midline twice, and placed into a sealable sandwich bag.^d Each sandwich bag was labeled to identify the dog and corresponding home. All samples were stored in the laboratory refrigerator at 4°C for ≤ 2 days after collection before processing.

Dust sample collection from homes—The house dust samples were collected by the same investigator (AJR) between July 2002 and the first week in November 2002. The house dust samples were collected from the 3 locations in the home where the dog(s) most frequently resided. These locations were identified from the responses of the owners on the questionnaire. House dust samples were collected in a standardized manner, as previously described.^{6,25} The same portable canister vacuum cleaner with a dust trap was used for the collection of skin and hair samples from dogs. Sample collection time was standardized at 2 min/m²/sample site.^{13,14} Each selected sample collection location was measured (1 × 1 m) and demarcated with duct tape to ensure accuracy. A total of 3 samples for HDM allergen quantitation were collected from each home. Before sample collection at each new location, the adapter and dust trap was wiped out thoroughly with a disposable cloth and the vacuum cleaner was turned on high power for 30 seconds to remove any residual dust in the hose. The corrugated design of the hose prevented washing or cleaning. The plastic removable nozzle was replaced with a new nozzle between each of the 3 locations where samples were obtained in the home. The adapter and dust trap and nozzle attachments were rinsed with tap water thoroughly and allowed to air dry in between sample collection from homes.

At the completion of sample collection from each site, the filter paper was removed from the adapter and dust trap, folded at the midline twice, and placed into a sealable sandwich bag.^d Each sealable bag was labeled to identify the home and location. All samples were refrigerated in the laboratory at 4°C and 75% humidity for ≤ 2 days after collection before processing.¹²

Processing of skin and hair dust samples to quantitate HDM allergens—Briefly, the skin and hair dust sample weight was determined by first weighing the sample and filter disk and then subtracting the weight of the filter. One

hundred milligrams of each sample was weighed and extracted in 2 mL of PBS solution containing 0.05% Tween 20 in PBS solution (pH, 7.4) overnight at 4°C. When < 100 mg of dust was obtained, samples were extracted with 0.2 mL of solution/10 mg of dust. Samples were centrifuged, and supernatants were removed and stored frozen at -80°C. Samples were analyzed for Der f 1 and group 2 allergen content by use of a validated²⁶ commercial 2-site monoclonal antibody ELISA.^c Allergen concentrations were reported as microgram per gram of dust.

Processing of house dust samples to quantitate HDM allergens—Samples were weighed and sieved as previously described.¹⁵ One hundred milligrams of each sample was weighed and extracted in 2 mL of PBS-Tween 20 solution overnight at 4°C. When < 100 mg of dust was obtained, samples were extracted with 0.2 mL of solution/10 mg of dust. Samples were centrifuged, and supernatants were removed and stored frozen at -80°C. Samples were analyzed for Der f 1, Der p 1, and group 2 allergen content by use of a validated²⁶ commercial 2-site monoclonal antibody ELISA.^c Allergen concentrations were reported as microgram per gram of dust.

Statistical analyses—Of the 63 skin and hair samples, only 59 samples were of sufficient quantity for allergen analysis. Descriptive statistics were provided for skin and hair samples that were positive for Der f 1 and group 2 allergens. The associations between types of HDM allergen (Der f 1 only, group 2 only, or Der f 1 and group 2) on skin and hair samples and dog characteristics were assessed via the Fisher exact test. The relationship between skin and hair samples that were negative or positive for Der f 1 and group 2 allergens and mean concentrations of Der f 1 and group 2 allergens in house dust samples was evaluated by the Wilcoxon rank sum test. Because the concentrations of allergens in house dust samples were not normally distributed, all com-

parisons between concentrations of allergens in house dust samples were made by use of the Mann-Whitney rank sum test for 2-group comparisons or the Kruskal-Wallis test for multiple-group comparisons. Comparisons between skin and hair samples that were positive or negative for Der f 1 and group 2 allergens and mean concentrations of allergens in house dust samples (Der f 1 and group 2) on the basis of dog characteristics and household characteristics (limited to household characteristics determined to be significant previously)¹⁵ were assessed by the Wilcoxon rank sum test. Associations were found between the number of house dust sample sites that were positive for Der f 1 and group 2 allergens in each house and the number of skin and hair samples that were positive or negative for Der f 1 and group 2 allergens. All *P* values were 2-sided and considered significant if < 0.05.

Results

Skin and hair samples were collected from a total of 63 dogs residing in 50 homes. Samples from 4 dogs had < 10 mg of recoverable dust and were excluded from our study, resulting in a total of 59 skin and hair samples available for analysis. The mean weight for samples collected from the 59 dogs was 67.5 mg (range, 20 to 105 mg). Of the 59 dogs, 39 were purebred and 20 were mixed breed. Of the 39 purebred dogs, 17 were categorized as others because only 1 dog represented each breed and the remaining 22 purebred dogs included Golden Retrievers (*n* = 8), Labrador Retriever (5), Greyhound (4), Cocker Spaniel (2), and Jack Russell Terrier (3).

Characteristics of dogs—Dogs were categorized as follows: type of hair (long [*n* = 17], medium length

Table 1—Characteristics of dogs with Der f 1 and group 2 allergens in skin and hair samples.

Dog Characteristics	No. of dogs	Skin and hair samples		
		Der f 1 allergen Median and range (µg/g of dust)	No. of dogs	Group 2 allergen Median and range (µg/g of dust)
Breed				
Golden Retriever	4	0.12 (0.06–0.14)	3	0.12 (0.06–0.14)
Labrador Retriever	1	0.10	0	NA
Greyhound	1	0.14	1	0.26
Cocker Spaniel	1	0.10	0	NA
Mixed	4	0.11 (0.08–0.22)	0	NA
Others	4	0.09 (0.08–0.10)	5	0.12 (0.06–0.84)
Type of coat				
Long	5	0.12 (0.06–0.14)	7	0.30 (0.06–0.84)*
Medium length	5	0.10 (0.08–0.12)	1	0.12
Short	5	0.10 (0.08–0.22)	1	0.26
Date of last bath				
< 1 month ago	4	0.08 (0.06–0.10)	3	0.14 (0.12–0.84)
≥ 1 month ago	11	0.12 (0.08–0.22)	6	0.10 (0.06–0.26)
Insecticidal shampoo				
No	14	0.10 (0.06–0.22)	9	0.12 (0.06–0.84)
Yes	1	0.10	0	NA
Flea prevention				
< 1 month ago	8	0.10 (0.06–0.14)	6	0.13 (0.06–0.84)
≥ 1 month ago	5	0.12 (0.08–0.22)	2	0.16 (0.06–0.26)
Not used	2	0.10 (0.10–0.10)	1	0.08
Otitis externa				
No	11	0.10 (0.06–0.22)	6	0.11 (0.06–0.84)
Yes	4	0.09 (0.08–0.14)	3	0.12 (0.12–0.26)
Chronic skin disease				
No	8	0.10 (0.06–0.22)	3	0.14 (0.06–0.84)
Yes	7	0.10 (0.08–0.14)	6	0.12 (0.06–0.26)

*Significant (*P* < 0.05) difference among median values for a given dog characteristic.
NA = Not applicable.

[18], or short [24]), date of last bath (< 1 month [24] or ≥ 1 month [35]), bathed with insecticidal shampoo (yes [5] or no [54]), date of last application of a flea or tick preventive (none used [7], < 1 month [27], or ≥ 1 month [25]), prior or current otitis externa (yes [11] or no [48]), history of chronic allergic skin disease (yes [29] or no [30]), type of allergic skin disease if present (atopic dermatitis [7]; flea allergy dermatitis [1]; possible flea allergy dermatitis, cutaneous adverse food reaction, or atopic dermatitis [10]; unknown [12]; or recurrent bacterial dermatitis [2]), and seasonality of allergic skin disease if present (seasonal [12], nonseasonal [10], nonseasonal with seasonal exacerbation [3], or unknown [4]).

Allergens on skin and hair—House dust mite allergens were detected in skin and hair samples from 21 of 59 (35%) dogs. Der f 1 allergen was detected in samples from 15 of 59 (25%) dogs, whereas group 2 allergen was detected in 9 of 59 (15%) samples. Only 3 dogs had skin and hair samples that were positive for Der f 1 and group 2 allergens. Mean (± SD) allergen concentration of all samples that were positive for Der f 1 and group 2 allergens was 0.11 ± 0.04 µg/g and 0.22 ± 0.25 µg/g of dust, respectively.

Median (and range) concentrations of Der f 1 and group 2 allergens in skin and hair samples were evaluated on the basis of each dog's characteristics (Table 1). Concentration of group 2 allergen in skin and hair samples was significantly ($P = 0.002$) higher on dogs

with long hair than dogs with medium length or short hair. No other significant differences were found between dog characteristics and allergen concentrations in skin and hair samples for either Der f 1 or group 2 allergens.

Median concentrations of Der f 1 and group 2 allergens in house dust samples from homes with dogs that had skin and hair samples that were positive for Der f 1 and group 2 allergens and house dust samples from homes with dogs that had skin and hair samples that were negative for Der f 1 and group 2 allergens were compared (Table 2). Median house dust sample concentrations of Der f 1 and group 2 allergens were higher in homes with dogs that had skin and hair samples that were positive for Der f 1 and group 2 allergens, compared with median concentrations of Der f 1 and group 2 allergens in homes with dogs that had skin and hair samples that were negative for Der f 1 or group 2 allergens. Differences in median concentrations of group 2 allergen in house dust samples were significantly ($P < 0.001$) different between homes with dogs that had skin and hair samples that were positive for group 2 allergen and homes with dogs that had skin and hair samples that were negative for group 2 allergen.

Comparisons were made between skin and hair samples that were positive or negative for Der f 1 or group 2 allergens and mean concentrations of Der f 1 and group 2 allergens in house dust samples on the basis of selected household characteristics (Table 3). In homes without air-conditioning ($P = 0.032$) and

Table 2—Association between Der f 1 or group 2 allergens in skin and hair samples of dogs and concentration of these house dust mite (HDM) allergens in house dust samples.

Skin and hair sample results	House dust samples			
	Der f 1 allergen		Group 2 allergen	
	No. of dogs	Median and range (µg/g)	No. of dogs	Median and range (µg/g of dust)
Der f 1 allergen				
Positive	15	1.38 (0.05–8.95)	NA	NA
Negative	44	0.62 (0.01–10.27)	NA	NA
Group 2 allergen				
Positive	NA	NA	9	2.95 (1.41–18.91)*
Negative	NA	NA	50	0.20 (0.01–5.41)

*Significant ($P < 0.05$) difference in allergen concentration in house dust samples between skin and hair samples positive and negative for allergen. NA = Not applicable.

Table 3—Association between HDM allergens in skin and hair samples of dogs and concentrations of HDM allergens in house dust samples on the basis of selected household characteristics.

Household characteristics	Skin and hair sample results for Der f 1 or group 2 allergens	Der f 1 and group 2 allergens in house dust samples	
		No. of dogs	Median and range (µg/g)
No central air-conditioning	Positive	8	5.21 (0.18–12.65)*
	Negative	7	0.41 (0.40–0.54)
Basement positive for allergen	Positive	4	1.55 (1.39–2.38)
	Negative	5	0.41 (0.19–2.02)
Maximum relative humidity ≥ 75%	Positive	9	4.19 (0.38–5.96)*
	Negative	7	0.54 (0.41–1.38)

*Significant ($P < 0.05$) difference in allergen concentration in house dust samples between skin and hair samples positive and negative for allergen for a given household characteristic.

Table 4—Association between detection of HDM allergens in skin and hair samples of dogs and concentration of HDM allergen in house dust samples.

House dust samples (mean concentration of allergens)	Hair and skin samples			
	Positive for group 2 allergen		Positive for Der f 1, group 2, or both allergens	
	No. of dogs	Median and range ($\mu\text{g/g}$ of dust)	No. of dogs	Median and range ($\mu\text{g/g}$ of dust)
Group 2 allergen				
$\geq 2 \mu\text{g/g}$	8	0.13 (0.06–0.84)	NA	NA
$< 2 \mu\text{g/g}$	1	0.12	NA	NA
Der f 1, group 2, or both allergens				
$\geq 2 \mu\text{g/g}$	NA	NA	12	0.11 (0.06–0.84)*
$< 2 \mu\text{g/g}$	NA	NA	9	0.10 (0.06–0.22)

*Significant ($P < 0.05$) difference in allergen concentration in hair and skin samples between homes with high and low allergen concentrations in house dust samples. NA = Not applicable.

Table 5—Association between detection of HDM allergens in skin and hair samples of dogs and number of house dust samples that were positive for HDM allergens.

No. of house dust samples positive for allergen	Hair and skin samples			
	Positive for group 2 allergen		Positive for Der f 1, group 2, or both allergens	
	No. of dogs	Median and range ($\mu\text{g/g}$ of dust)	No. of dogs	Median and range ($\mu\text{g/g}$ of dust)
Group 2 allergen				
> 2 samples	9	0.12 (0.06–0.84)*	NA	NA
≤ 2 samples	0	0	NA	NA
Der f 1, group 2, or both allergens				
> 4 samples	NA	NA	20	0.10 (0.06–0.84)*
≤ 4 samples	NA	NA	1	0.14

*Significant ($P < 0.05$) difference in allergen concentration in hair and skin samples between homes with high and low numbers of house dust samples that are positive for allergens.

with a maximum relative humidity of $\geq 75\%$ ($P = 0.022$), median HDM allergen concentrations in house dust samples were significantly higher for homes with dogs that had skin and hair samples that were positive for HDM allergens, compared with homes with dogs that had skin and hair samples that were negative for HDM allergens.

Associations between homes with mean allergen concentrations of ≥ 2 or $< 2 \mu\text{g/g}$ of dust were evaluated for homes with dogs that had skin and hair coat samples that were positive or negative for allergens (Table 4). No significant association was found between concentrations of Der f 1 allergen in house dust samples and whether dogs had skin and hair coat samples that were positive or negative for Der f 1 allergen. However, dogs with skin and hair samples that were positive for group 2 allergen were significantly ($P < 0.001$) associated with homes with a mean group 2 allergen concentration of $\geq 2 \mu\text{g/g}$ of dust. Dogs with skin and hair samples that were positive for either allergen were significantly ($P = 0.002$) associated with homes with a mean allergen concentration of $\geq 2 \mu\text{g/g}$ of dust.

The number of house dust samples that were positive for each HDM allergen (Der f 1 and group 2) in homes with dogs that had skin and hair samples that were positive for Der f 1 and group 2 allergens was evaluated and compared with the number of house

dust samples that were positive for each HDM allergen in homes of dogs with skin and hair samples that were negative for Der f 1 and group 2 allergens (Table 5). No significant difference in the number of house dust samples that were positive for Der f 1 allergen was found between dogs with skin and hair samples that were positive for Der f 1 allergen and dogs with skin and hair samples that were negative for Der f 1 allergen. However, dogs with skin and hair samples that were positive for group 2 allergen were significantly ($P = 0.048$) associated with homes with > 2 house dust samples that were positive for group 2 allergen. Dogs that had skin and hair samples that were positive for either allergen were significantly ($P = 0.041$) associated with homes with > 4 house dust samples that were positive for Der f 1 or group 2 allergens.

Discussion

In our study, HDM allergens were detected in 21 (35%) of the skin and hair samples. Der f 1 allergen was detected in the skin and hair samples from more dogs (15/21 [71%]) than group 2 allergen (9/21 [43%]). However, mean concentration of group 2 allergen in skin and hair samples was double that of the mean concentration of Der f 1 allergen in skin and hair samples.

Our results support the findings of a recent study²¹ where Der f 1 allergen was detected in skin and hair

samples from 6 of 29 (21%) dogs by use of the same standardized ELISA technique. In that study, an additional 14 of 29 (48%) skin and hair samples were positive for Der f 1 allergen when an amplified ELISA technique that lowers the detection limit for allergen 100-fold to 0.01 ng/mL was used. Because this amplified ELISA technique was not available to us at the time of our study, we used the standardized ELISA technique with its higher detection limit for allergen of 1 ng/mL. In our study, we further documented the presence of group 2 allergen in skin and hair samples from dogs and we have evaluated dog characteristics and the household microenvironment for factors that may be associated with the presence of HDM allergens on the skin and hair.

The importance of identifying allergens on the skin and hair of dogs is 2-fold. Firstly, it confirms the presence of another reservoir for allergen exposure to dogs and humans and a possible mode of allergen transmission and dispersal within the household microenvironment. Secondly, in atopic dermatitis in dogs, evidence exists for an epidermal route of allergen entry, which suggests that environmental antigens directly transfer through the stratum corneum and contact antigen-presenting cells in the epidermis, thereby initiating the cutaneous inflammatory cascade.²⁷ If further evidence continues to support this theory, it becomes imperative that the allergen sources and magnitude of the allergen concentrations on the skin and hair of dogs be evaluated.

Results of our study indicate that allergen in the microenvironment of dogs is an important contributor to the presence of allergen on the skin and hair of dogs. Median concentrations of Der f 1 and group 2 allergens in house dust samples were higher (significantly for group 2 allergen) and the number of house dust samples that were positive for Der f 1 and group 2 allergens was significantly higher in homes of dogs with skin and hair samples that were positive for allergens, compared with homes of dogs with skin and hair samples that were negative for allergen. These results reveal the correlation and interplay between the allergens in the microenvironment of dogs and the detection of allergens in the skin and hair samples. Thus, allergen concentration in house dust samples as well as number of sample sites that were positive for Der f 1 and group 2 allergens in the home are important determinants for the transmission and presence of allergen on the skin and hair of dogs.

Findings in our study support the importance of the role of household allergen load. We determined that in homes without air conditioning and with a maximum relative humidity of $\geq 75\%$, median HDM allergen concentrations in house dust samples were significantly higher for homes with dogs that had skin and hair samples that were positive for HDM allergens, compared with homes with dogs that had skin and hair samples that were negative for HDM allergens. In our previous study,¹⁵ these 2 household characteristics were significantly associated with higher concentrations of HDM allergens. Thus, the concentration of allergen in the microenvironment and 2 of the most important determinants of HDM survival in the environment sig-

nificantly influence the amount of allergen transmission onto the skin and hair of dogs.

Although median concentration of Der f 1 allergen in house dust samples from homes of dogs with skin and hair samples that were positive for Der f 1 allergen was double that of median concentration of Der f 1 allergen in house dust samples from homes of dogs with skin and hair samples that were negative for Der f 1, this difference was not significant. We speculate that if a greater sample size of dogs with skin and hair samples that were positive for Der f 1 allergen was evaluated (only 15 of 59 samples were positive for Der f 1 allergen in our study), a significant difference may have been revealed. Further, use of the highly sensitive amplified ELISA technique would likely have increased our number of samples that were positive for Der f 1 allergen.

In our previous study¹⁵, dog beds that were ≥ 1 year old had significantly higher allergen concentrations than newer dog beds.¹⁵ In our study reported here, none of the dog bed characteristics were significantly associated with skin and hair samples that were positive or negative for Der f 1 or group 2 allergens. However, median HDM allergen concentrations in dog beds ≥ 1 year old from homes of dogs with skin and hair samples that were positive for allergens was higher than those from homes with skin and hair samples that were negative for allergens (3.82 vs 0.40 $\mu\text{g/g}$ of dust, respectively). As indicated in our previous study, older dog beds are a risk for exposure to higher allergen concentrations.¹⁵ Thus, as expected, it appears that allergen accumulation in dog beds over time leads to increased exposure to allergen for dogs with dog beds > 1 year of age. This further supports the close association between HDM allergen concentrations in the environment and HDM allergens on the skin and hair of dogs.

The only dog characteristic significantly associated with the presence of HDM allergens was the type of hair. Dogs with long hair are more likely to have hair samples that are positive for group 2 allergen than dogs with medium length or short hair, with 78% of positive samples for group 2 allergen coming from dogs with long hair. We speculate that the positive association of group 2 allergen with long hair could be the result of the length. Typically with increased length of hair in dogs an added component of thickness and fullness exists, which may allow for enhanced allergen trapping without easy removal. However, Der f 1 allergen would also be expected to be significantly associated with long hair. A further possibility involves the interaction between the hair and the molecular structure of each allergen. Der f 1 allergen has a molecular weight of about 25 kd and has been characterized as a cysteine protease, whereas group 2 allergen has a molecular weight of about 14 to 15 kd and is thought to be an epididymal protein (biological function unknown).^{28,29} Because group 2 allergen has a lower molecular weight than Der f 1 allergen, group 2 allergen may be more readily translocated from the environment onto the hair of dogs. Additionally, the greater stability of group 2 allergen²⁹ in the environment, compared with Der f 1 allergen, may also account for the high number of hair samples that were positive for group 2 allergen from

dogs with long hair. In this regard, it was interesting to note that the mean concentration of group 2 allergen in hair samples of all dogs (regardless of coat type) was higher than the mean concentration of Der f 1 allergen in hair samples.

In humans, HDM allergen concentrations $\geq 2 \mu\text{g/g}$ of dust have been implicated as significant risk factors for sensitization to mite allergens.^{6,12,30,31} Further, exposure to allergen concentrations of $\geq 10 \mu\text{g/g}$ of dust increases the risk for development of overt clinical signs of atopic asthma.^{6,30} Although concentrations of HDM allergens that represent risk factors for sensitization or clinical signs of atopic dermatitis in dogs have not been established, we found significant differences between mean concentrations of allergen in house dust samples from homes of dogs with skin and hair samples that were positive or negative for HDM allergens. Dogs with skin and hair samples that were positive for HDM allergens were significantly associated with homes with a mean allergen concentration of $\geq 2 \mu\text{g/g}$ of dust. In addition, dogs that had skin and hair samples that were positive for group 2 allergen were significantly associated with homes with a mean group 2 allergen concentration of $\geq 2 \mu\text{g/g}$ of dust.

Throughout our study, we identified significant associations between the HDM allergen concentrations in house dust samples from specific homes and characteristics of the microenvironment and the presence of HDM allergens on the skin and hair of dogs. This establishes the association between high allergen concentrations in the environment and the risk of these allergens translocating onto the skin and hair of dogs. Unlike humans, who bathe regularly, most dogs are not bathed even on a weekly to monthly basis. This further adds to the risk of dogs for continual exposure and accumulation of allergens on the skin and hair, thus potentially predisposing to the development of sensitization.

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- a. Allerecept ELISA, Heska Corp, Ft Collins, Colo.
 - b. Portable filter queen canister vacuum, HMI Industries Inc, Cleveland, Ohio.
 - c. Adapter/dust trap, Fussnecker Sweeper, Springfield, Ohio.
 - d. Ziploc sandwich bag, SC Johnson & Son Inc, Racine, Wis.
 - e. Der f 1, Der p 1, Group 2 allergen ELISA kits, Indoor Biotechnologies Inc, Charlottesville, Va.
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