Quantitation of house dust mites and house dust mite allergens in the microenvironment of dogs

Amy Randall, DVM; Andrew Hillier, BVSc; Lynette K. Cole, DVM, MS; Kenneth W. Kwochka, DVM; Glen Needham, PhD; Donald L. Wassom, PhD

Objective—To quantitate the density of *Dermatophagoides farinae* and *D pteronyssinus* and concentrations of house dust mite (HDM) allergens (Der f 1, Der p 1, and Group 2 allergens) in the indoor microenvironment of dogs.

Sample Population—50 homes in Columbus, Ohio.

Procedures—In each home, samples of dust were collected from 3 locations in which dogs spent most time. Whenever possible, the species of mites collected was identified. Mite density (mites/g of dust) was assessed, and allergen concentrations were assayed by standardized ELISAs. Relative humidity and temperature in each home were monitored during a 5-day period. Characteristics of homes and sample sources were evaluated.

Results—Dust samples from all 50 homes contained ≥ 1 HDM allergen; Der f 1 and Der p 1 were detected in 100 and 74% of homes, respectively. Fifteen homes had HDMs; compared with *D pteronyssinus*, *D farinae* was found more commonly (14/15 homes) and at a higher density. Basements, homes without central air-conditioning, and dog beds that were ≥ 1 year old had high HDM allergen concentrations. Homes with ≥ 2 µg of Der f 1 or Group 2 allergens/g of dust or ≥ 100 mites/g of dust were significantly more likely to have a maximum relative humidity ≥ 75%.

Conclusions and Clinical Relevance—Results indicate the presence of HDMs and HDM allergens in the specific microenvironment of dogs in homes. Factors associated with high levels of exposure were identified, which may be associated with increased risk for sensitization and development of atopic diseases. (*Am J Vet Res* 2003;64:1301–1309)

Atopic dermatitis (AD) in dogs is a genetically predisposed inflammatory and pruritic allergic skin disease that has characteristic clinical features and is associated most commonly with IgE antibodies against environmental allergens.[1] Atopic dermatitis is a common skin disease of dogs and is estimated to affect 3 to 30% of the dog population; it requires life-long symptomatic treatment, immunotherapy, and avoidance of the identifiable allergens (if possible).[2,3] **House dust mites (HDMs)**, in particular *Dermatophagoides farinae* and *D pteronyssinus*, are reported to be among the most common allergens that cause hypersensitivity in dogs with AD (affecting 30 to 100% of dogs).[4-7] In humans worldwide, HDM allergens are an important cause of IgE-mediated respiratory disease. In the United Kingdom, skin test reactivity to these mites is seen in 45 to 80% of patients with asthma, compared with 5 to 30% of the general population.[8,9] In humans, the relationship between exposure to mite allergens and the development of sensitization is well established.[10-13] Not only does there appear to be an association between HDM allergens and asthma but also between HDM allergens and other allergic diseases such as AD and allergic rhinitis.[14,15]

As allergen exposure is necessary for sensitization, numerous studies[10,16-20] have elucidated the distribution and abundance of HDMs and HDM allergens within homes. The highest numbers of HDMs and HDM allergens are found in bedding, carpets, fabric-upholstered furniture, and living room floors.[16-18,21] These studies have focused on areas that are commonly frequented by humans and most likely to provide an environment suitable for mite proliferation. The main criteria governing mite survival in a particular environment are relative humidity and the availability of food, primarily dander.[16] Additional studies[10,19,20,22] have been conducted to evaluate household and sample-site characteristics that may be specific risk factors for allergen exposure.

Despite the high incidence and significance of HDM-induced AD in dogs, the prevalence of HDMs and HDM allergens in the environment of dogs is unknown. House dust mites and HDM allergens in carpeted floors and upholstered furniture have been investigated in numerous studies,[17,18,23,24] some of which were performed in homes with pets[9,19,25]; however, whether the pets were allowed on the carpets and upholstered furniture these locations were common resting areas for the pets was not investigated in those studies. To the authors' knowledge, the role of locations such as dog beds, blankets, carpeted floors, and upholstered furniture as reservoirs for HDMs or HDM allergens has not been studied. Dogs generate adequate heat and moisture and provide food (in the form of

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From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine (Randall, Hillier, Cole), and the Acarology Laboratory, Department of Entomology, College of Biological Sciences (Needham), The Ohio State University, Columbus, OH 43210; the Animal Dermatology Specialty Clinic, Marina del Rey, CA 90292 (Kwochka); and Heska AG, 16 Grands Pl, CH-1700 Fribourg, Switzerland (Wassom).
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Address correspondence to Dr. Randall.

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remnants of the dogs' meals, skin scales, oils, and other lipids) to promote the survival and proliferation of mites.[16] Thus, it may not be appropriate to make assumptions about the potential exposure of dogs to HDMs and HDM allergens and their role in AD in dogs from only data pertaining to human-occupied areas of homes. The purpose of the study reported here was to quantitate the density of *D farinae* and *D pteronyssinus* mites and concentration of HDM allergens in the specific microenvironment of dogs. In addition, this study was intended to identify areas of highest exposure and risk factors for exposure to HDMs and HDM allergens for dogs.

Methods and Materials

Selection of households—Homes in metropolitan Columbus, Ohio, were selected for inclusion in the study. Inclusion criteria were the presence of at least 1 dog living indoors in the home and wall-to-wall carpeting in rooms where these dogs spent time. An adult member of each household completed a questionnaire concerning household characteristics thought to be of importance in the establishment and maintenance of HDM populations. Specific questions were asked to characterize the type of housing (free-standing dwelling, apartment, or townhouse); heating system (central heating [electric or gas], noncentral heating [wood or coal], or none); cooling system (central air-conditioning, air-conditioning with window units, or no air-conditioning system); level of housing (at ground level [with or without a basement], consisting of 2 stories [with or without a basement], consisting of 3 stories [with or without a basement], or floor level [if an apartment]); floor level of bedrooms and living rooms (basement, ground or first floor, second floor, or third floor); pile of carpeting (short, medium, or long); age of carpeting (≤ 1 year, > 1 year, or unknown); number of indoor dogs; use of carpet fresheners (yes or no); and use of flea or tick preventatives on pets (yes or no). Specific questions were asked to identify the 3 locations in the home where the dogs resided most frequently. Additionally, the type of bedding used by the dogs was categorized as dog bed (store purchased-specific dog bed), dog bedding (blankets or comforters used exclusively by dogs), or 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 type of dog bed filling (foam or other synthetic material); frequency of washing (never, ≤ 6 months, or > 6 months); age of dog bed (< 1 year or ≥ 1 year); location of the dog bed (bedroom, living room, or other location); and floor level in home where the dog bed was situated (basement, ground or first floor, second floor, or third floor).

Collection of dust samples—Samples of house dust were collected by the same investigator (AR) during the period from July to the first week in November 2002. This time period was chosen because results of studies[18,26] indicate that the number of HDMs in homes in temperate climates (eg, Ohio) are highest between June and October. The house dust samples were collected from the 3 locations in the household that were identified (from owners' responses on the questionnaire) as areas in which the dogs resided most frequently. The samples of house dust were collected in a standardized manner, as described.[8,10] Briefly, a small portable canister vacuum cleaner[a] with a plastic clear adapter and dust trap[b] was used to collect all of the samples. The clear adapter and dust trap unit enabled connection 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 unit was fitted with a new filter for the collection of each sample. Sampling time was standardized at 2 min/m^2/sample site.[19,22] Each selected sampling location was measured (1 m $\times$ 1 m) and demarcated with duct tape to ensure accuracy. The first sample obtained was used for HDM quantitation and species identification; this was designated as sample A. The collection of sample A required the use of the low-suction power setting of the vacuum cleaner to reduce destruction of the mites' bodies. From a site (1 m $\times$ 1 m) adjacent to that used to obtain sample A, a second sample (designated as sample B) was obtained for HDM allergen quantitation; this required the use of the high-suction power setting of the vacuum cleaner. Thus, 6 samples (samples A and B from each of the 3 locations) were collected from each home. To obtain samples from dog beds that had a surface area of < 1 m^2, an area of 0.5 m^2 was measured and vacuumed for 1 minute. In some locations, the surface area available was insufficient to collect sample B from an area adjacent to that used to collect sample A; in these circumstances, the same area was vacuumed twice (with low-power suction to collect sample A, followed by high-power suction to collect sample B). Before sampling each new location, the adapter and dust trap unit were wiped thoroughly with a disposable cloth, and the vacuum cleaner was switched on at 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 samplings at each of the 3 household locations. After collection of samples, the adapter and dust trap unit and nozzle attachments were rinsed thoroughly with tap water and allowed to air dry prior to use at another home.

At the completion of sample collection from each site, the filter paper was removed from the adapter and dust trap unit, folded at the midline twice (to confine mites), and placed into a sealable plastic bag.[c] Each bag was labeled to identify the home, location, and whether it was sample A or B. All samples were stored in the laboratory refrigerator at 4°C with 75% relative humidity for no longer than 2 days after collection before being processed.[18]

Processing of house dust samples to identify and quantitate HDMs—Standardized techniques for the quantitation of mites have been described.[18] Briefly, the weight of sample A was determined by measuring the weight of the sample and filter disk and subtracting the weight of the filter. The content of the filter was placed onto a 500-µm mesh[d] and sieved to remove large particulate matter. The sieved fine dust was apportioned into 3 subsamples of 10 mg each (weighed exactly by use of a plastic weighing dish[e]). The use of 2 or 3 subsamples of each dust sample has been reported to be accurate for the quantitation of mites.[18,27] Each subsample was suspended in 25 mL of saturated saline (0.9% NaCl) solution containing 3 drops of dish detergent[f] in a 50-mL polypropylene tube[g] and vortexed[h] for 20 seconds. The suspension was rinsed on a 235-µm mesh sieve[i]; crystal violet solution was passed through the sieve to stain the retained dust. After the excess stain was rinsed off with distilled water, the material retained on the sieve was washed with distilled water and placed into a 15- $\times$ 60-mm intergrid flint-glass Petri dish. Unlike the other components of dust samples, HDMs are not stained by crystal violet; this allowed easy identification of mites, which appeared white against a violet-stained background. Mite counts were performed via a stereomicroscope at low magnification (25$\times$ to 40$\times$). The stereomicroscope was focused through all planes of the fluid suspension in the Petri dish to identify and count HDMs. The number of HDMs in each of the 3 subsamples was assessed, and HDM density was calculated for each sample as the mean number of HDMs per gram of dust.[18,28] All mites viewed through the stereomicro-

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scope were removed and mounted on a microscope slide with Hoyer medium. Each microscope slide was labeled to identify house of origin and sample site. The identification and quantitation of the 2 common HDM species, *D farinae* and *D pteronyssinus*, were performed by the primary investigator (AR). Mites other than *D farinae* and *D pteronyssinus* were identified by an acarologist (HK) of the Arcology Laboratory, Department of Entomology, College of Biological Sciences, The Ohio State University. Dust samples that contained only damaged mites or parts of mites or those in which the species of the mites could not be identified were regarded as positive for mites but not recorded as having HDMs.

Processing of house dust samples to quantitate HDM allergens—Samples were weighed and sieved as described. One hundred milligrams of each sample B was extracted overnight at 4°C in 2.0 mL of PBS solution containing 0.05% Tween 20 at a pH of 7.4. For samples with < 100 mg of dust, extraction was performed in 0.2 mL of PBS-Tween 20 solution/10 mg of dust. The samples were centrifuged, and supernatants were removed and stored at –80°C. The samples were analyzed for HDM allergens (Der f 1, Der p 1, and Group 2 allergens) by use of a validated,[29] commercial 2-site monoclonal antibody ELISA.[j] Allergen concentrations were reported as microgram per gram of dust; in samples in which allergens were detected, the median allergen concentration (and range) of each of the 3 HDM allergens for each of the house and sample characteristics was calculated.

Household relative humidity and temperature—A computerized data logger[k] was placed in each home at the time of sampling. The logger was positioned on the floor adjacent to a location that was sampled and under a piece of furniture to prevent it from being damaged by the dogs. The logger was programmed to measure and record the relative humidity and temperature in the home every 30 minutes during a period of 5 days. Loggers were returned to the investigator after 5 days, and the data were downloaded onto a computer by use of a commercial software package.[l]

Statistical analyses—Comparisons between the types of HDMs and household and sample characteristics were made with the Fisher exact test. Because of the limited number of samples in which mite speciation was possible, further analyses were not performed. However, descriptive statistics for HDM density were calculated by use of household and sample categories. To compare the mean HDM density among rooms, an ANOVA was used. The type and concentration of each HDM allergen present were analyzed for each household and sample characteristic. The associations between types of HDM allergen detected (ie, Der f 1 only, Der p 1 only, or both Der f 1 and Der p 1) for identified species of HDM and each household and sample characteristic were assessed via the Fisher exact test. The relationship between the HDM allergen concentration and household and sample variables was evaluated for each HDM allergen type by the Kruskal-Wallis test. This nonparametric test was chosen because of the severe skewness of the HDM allergen concentration data. The Spearman rank correlation test was used for associations among the 3 individual groups of data: HDM allergens (Der f 1, Der p 1, and Group 2 allergens), HDM allergen concentrations, and the HDM density. For each household, the mean value for relative humidity was calculated; this and the minimum and maximum (range) values were used for analysis. For the mean, minimum, and maximum relative humidity data, threshold values of 50, 40, and 75% were selected, respectively. The associations of relative humidity values at or above and below these thresholds, HDM allergen concentrations at or > 2 µg/g, and homes with ≥ 100 HDM/g were assessed with the fisher exact test. All *P* values were 2-sided; values < 0.05 were considered significant.

Results

Fifty homes with at least 1 dog living indoors were sampled. There were 38 homes with 1 dog, 11 with 2 dogs, and 1 with 3 dogs. The homes were categorized as free-standing homes (n = 27), townhouses (10), and apartments (13). Of the 13 apartments, 6 were located on the first floor, 2 on the second floor, and 5 on the third floor of the building. Twenty-one of the homes had basements. All homes had central heating; 40 had central air-conditioning, and 10 had no air-conditioning or window air-conditioning units. Carpet fresheners were used at least monthly in 17 households; carpet fresheners were not used in 33 homes. Flea control products were used on indoor pets at least once a month in 45 households; flea control products were not used in 5 homes. The flea control agent used most frequently on pets was fipronil (39/45 homes); other topical flea control agents used on pets included imidacloprid (3/45) and selamectin (3/45). All products were used monthly. In some households, treatments were continued throughout the year; in other homes, treatments were given during the spring, summer, and fall months only.

Characteristics of sample sites—Samples A and B were obtained from 3 locations in 50 homes (300 samples in total). Samples from 4 locations (3 dog beds and 1 bedroom pillow) were sampled for only 1 minute because of surface area limitations. In 38 locations (33 dog beds, 4 chairs, and 1 bedroom pillow), a site adjacent to that used to obtain sample A was not available because of the size of the sampled item; therefore, sample B was obtained from the same site as sample A. The locations that were sampled were categorized as follows: room (living room [n = 81], bedroom [45], basement [7], and other [17]); type of item (chair or couch [29], carpet [76], dog bed [33], dog-exclusive bedding [8], and bedding shared by humans and dogs [4]); floor level (ground or first floor [89], second floor [36], third floor [15], and basement [10]); type of carpet pile (short [10], medium [64], and long [2]); and age of carpets (≤ 1 year [9], > 1 year [58], and unknown [9]).

Thirty-three dog beds were sampled and categorized as follows: location (living room [n = 14], bedroom [17], basement [0], and other [2]); floor level (ground or first [20], second [10], third [3], and basement [0]), age of dog bed (< 1 year [11] and ≥ 1 year [22]); frequency of washing (never [14], ≤ 6 months [18], and > 6 months [1]); and type of dog bed (foam-filled [20] and other synthetic filling [13]).

Mites—Mites were detected in 31 of 50 (62%) homes; 15 (30%) of these had populations of HDMs. *Dermatophagoides farinae* was detected in 14 homes, 4 of which also had *D pteronyssinus*. Only 1 home was found to be inhabited solely by *D pteronyssinus*. In the other 16 (32%) homes, either the mites could not be identified to the species level or other types of environmental mites were identified, such as oribitid mites (n = 5 homes), thrips (1), Tarsonemidae, *Xenotarsonemus* sp (3), and Tenuipalpidae, *Aegyptobia* sp (2).

The mean ± SD HDM density for the 15 homes with HDMs was 164 ± 167 HDMs/g of dust, whereas the mite density in the other 16 homes was 46 ± 19

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Table 1—Results of analyses of house dust mite (HDM) allergen concentrations (µg/g of dust) in 150 samples of house dust from 50 homes with ≥ 1 dog

Allergen	Concentration	No. of homes with detectable allergen	No. of samples with detectable allergen
Der f 1			
	None detected	0	12
	Allergen detected	50	138
	2 to < 10 µg/g of dust	10	17
	≥ 10 µg/g of dust	8	8
Der p 1			
	None detected	13	73
	Allergen detected	37	77
	2 to < 10 µg/g of dust	7	11
	≥ 10 µg/g of dust	2	3
Group 2			
	None detected	3	29
	Allergen detected	47	121
	2 to < 10 µg/g of dust	16	25
	≥ 10 µg/g of dust	2	3

mites/g of dust. For each HDM species, the mean HDM density was 284 ± 245 mites/g for *D farinae* and 91 ± 80 mites/g for *D pteronyssinus*. Nine of 15 homes with HDMs had a mean density of ≥ 100 HDMs/g of dust, of which 1 had a mean density of ≥ 500 HDMs/g of dust.

House dust mites were identified in samples from 21 locations and categorized as follows: room (living room [n = 10/21 locations; 48%], bedroom [7; 33%], and basements [4; 19%]); type of item (carpet [10; 48%], chair or couch [7; 33%], and dog beds [4; 19%]); floor level (basement [5; 24%], ground or first floor [12; 57%], second floor [3; 14%], and third floor [1; 5%]). Fourteen of the 21 (67%) locations with HDMs had a mean density of ≥ 100 HDMs/g, 7 (33%) had < 100 HDMs/g, and only 1 (5%) had ≥ 500 HDMs/g of dust.

With regard to HDM density and species, there were no significant differences between the household

Table 2—Quantitation (median and range) of HDM allergen concentrations (µg/g of dust) in allergen-positive samples with regard to house and sample characteristics

House and sample	HDM allergen								
	Der f 1			Der p 1			Group 2		
Characteristics	n	Median	Range	n	Median	Range	n	Median	Range
Item									
Carpet	70	0.32	0.02–25.20	39	0.14	0.02–8.44	60	0.28	0.02–10.90
Dog bed	28	0.34	0.02–9.50	13	0.06	0.02–33.20	26	0.15	0.02–4.76
Dog bedding	7	0.38	0.08–2.56	4	0.25	0.06–2.78	8	0.19	0.04–1.60
Furniture	29	0.98	0.04–31.36	20	0.15	0.02–10.30	25	0.68	0.02–9.72
Shared bedding	4	0.36	0.08–1.08	1	0.06	0.06–0.06	2	0.22	0.04–0.40
Room									
Bedroom	39	0.46	0.02–25.20	23	0.06	0.02–33.20	36	0.19	0.02–24.76
Living room	75	0.56	0.02–21.44	39	0.12	0.02–30.96	63	0.28	0.02–23.70
Basement	7	1.54*	0.10–31.36	7	0.24	0.04–8.44	7	0.88*	0.22–9.46
Other	17	0.24	0.04–1.78	8	0.07	0.02–0.88	15	0.10	0.02–1.92
Room level									
Basement	10	2.10	0.10–31.36	10	2.00†	0.04–10.30	10	4.74†	0.22–9.72
Ground or 1st floor	82	0.42	0.02–19.02	43	0.16	0.02–30.96	69	0.30	0.02–23.70
2nd floor	33	0.40	0.02–25.20	19	0.06	0.02–33.20	29	0.16	0.02–24.76
3rd floor	13	0.32	0.04–14.24	5	0.04	0.02–0.06	13	0.10	0.02–5.08
Type of carpet									
Medium-pile	58	0.30	0.02–25.20	30	0.16‡	0.02–8.44	50	0.23	0.02–10.90
Long- and short-pile	12	0.67	0.04–6.98	9	0.04	0.02–0.40	10	0.45	0.18–5.56
Age of medium-pile carpet									
> 1 year	46	0.35	0.02–25.20	26	0.02	0.02–8.44	71	0.36	0.02–10.90
≤ 1 year	6	0.19	0.10–0.22	2	0.05	0.02–0.08	4	0.10	0.02–0.18
Unknown	6	0.42	0.04–1.62	2	0.11	0.06–0.16	5	0.12	0.04–0.28
Housing									
With basement	61	0.38	0.02–31.36	44	0.13	0.02–10.30	56	0.31	0.02–9.72
No basement	77	0.46	0.02–25.20	33	0.10	0.02–33.20	65	0.22	0.02–24.76
Cooling system									
No central air-conditioning or window units	29	0.74	0.04–25.20	22	0.48§	0.02–33.20	27	0.36§	0.02–24.76
Central air	109	0.38	0.02–31.36	55	0.06	0.02–5.12	94	0.22	0.02–9.46
Carpet fresheners									
No	92	0.28	0.02–25.20	54	0.16	0.02–10.30	83	0.22	0.02–10.90
Yes	46	0.80¶	0.02–31.36	23	0.06	0.02–33.20	38	0.39	0.02–24.76
Flea prevention									
Treated	120	0.44	0.02–31.36	66	0.09	0.02–33.20	104	0.28	0.02–24.76
Not treated	18	0.41	0.04–8.40	11	0.24	0.02–10.30	17	0.12	0.02–9.72
Type of house									
Apartment	33	0.46	0.04–21.44	15	0.06	0.02–4.22	28	0.27	0.02–7.26
Free standing	77	0.42	0.02–31.36	46	0.15	0.02–10.30	68	0.25	0.02–9.72
Townhouse	28	0.44	0.02–25.20	16	0.16	0.02–33.20	25	0.30	0.02–24.76

*Significant ($P < 0.05$) difference between allergen concentrations of Der f 1 and Group 2 in the basement, compared with concentrations in the other types of rooms. †Significant ($P < 0.05$) difference between the concentrations of Der p 1 and Group 2 in the basement, compared with concentrations in the other types of rooms. ‡Significant ($P < 0.05$) difference between the concentration of Der p 1 allergen in medium-pile carpets, compared with concentrations in the other types of carpet. §Significant ($P < 0.05$) difference between the concentrations of Der p 1 and Group 2 allergens in the homes without central air-conditioning, compared with concentrations in homes with central air-conditioning. ¶Significant ($P < 0.05$) difference between the concentration of Der f 1 allergen in homes in which carpet fresheners were used, compared with the concentration in homes in which carpet fresheners were not used.

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and sample characteristics. However, HDMs were detected in 4 of 7 basements, compared with detection in 7 of 45 bedrooms and 10 of 81 living rooms. In addition, basement areas were found to have the highest mean HDM density (384 HDMs/g of dust), compared with that of bedrooms (104 HDMs/g of dust) or living rooms (88 HDMs/g of dust).

Allergens—House dust mite allergens were detected in samples from all 50 homes. The Der f 1 allergen was detected in all homes, whereas Der p 1 and Group 2 allergens were detected in 74% (37/50) and 94% (47/50) of homes, respectively **(Table 1)**.

House dust mite allergens were detected in 140 of 150 samples of house dust; 10 house dust samples did not contain Der f 1, Der p 1, and Group 2 allergens (Table 1). Of the 150 samples, Der f 1 allergen was detected in 92% (n = 138), whereas Der p 1 and Group 2 allergens were detected in 51% (77) and 81% (121), respectively. The mean ± SD allergen concentration of all allergen-positive samples was 2.25 ± 4.97 µg/g of dust for Der f 1, 1.80 ± 5.36 µg/g of dust for Der p 1, and 1.91 ± 3.89 µg/g of dust for Group 2 allergens.

In samples in which the HDM allergen was detected, the median allergen concentration (and range) of each of the 3 HDM allergens was calculated for each of the house and sample characteristics **(Table 2)**. For Der f 1, allergen concentration in basements was significantly ($P = 0.036$) higher than that detected in living rooms, bedrooms, and other locations (eg, hallways, kitchens, dining rooms, and dens). Furthermore, the concentration of Der f 1 was significantly ($P = 0.01$) higher in homes in which carpet fresheners were used than in homes in which they were not used.

For Der p 1, allergen concentration in basements was significantly ($P = 0.005$) higher than that detected on first, second, and third floor levels. The Der p 1 concentration was also found to be significantly ($P = 0.046$) higher in medium-pile carpets than that detected in short- or long-pile carpets and significantly (= 0.015) higher in homes without central air-conditioning than in homes with central air-conditioning.

For Group 2 allergen, the concentration was significantly ($P = 0.041$) higher in basements than that detected in living rooms, bedrooms, and other locations. The concentration of Group 2 allergen was also significantly ($P = 0.001$) higher in basements than that detected on the first, second, and third floor levels and significantly ($P = 0.\ 021$) higher in homes with no central air-conditioning, compared with homes with central air-conditioning.

For dog beds in which HDM allergens were detected, median allergen concentrations (and range) of each of the 3 HDM allergens were calculated **(Table 3)** and evaluated with regard to the characteristics of these dog beds. For Group 2 allergen, the concentration was significantly ($P = 0.008$) higher in dog beds that were ≥ 1 year old than the concentration detected in dog beds that were < 1 year old.

Significant ($P < 0.001$) correlations were detected between allergen concentrations of Group 2 and Der f 1 ($P = 0.79$) and between Group 2 and Der p 1 ($P = 0.69$), but not between concentrations of Der f 1 and Der p 1 ($P = 0.36$). There were high correlations between the concentration of HDM allergen and HDM density for Der f 1 ($P = 0.647$), Der p 1 ($P = 0.653$), and Group 2 allergens ($P = 0.778$).

Relative humidity—There were no associations between homes with a mean relative humidity ≥ 50% or a minimum relative humidity ≤ 40% and homes that were found to have concentrations of ≥ 2 µg of Der f 1, Der p 1, or Group 2 allergens/g of dust or ≥ 100 HDMs/g of dust. However, homes with a maximum relative humidity ≥ 75% were significantly associated with homes that contained ≥ 2 µg of Der f 1 ($P = 0.01$) or Group 2 ($P = 0.041$) allergens/g of dust or homes that contained ≥ 100 HDMs/g of dust ($P = 0.048$).

Table 3—Quantitation (median and range) of HDM allergen concentrations (µg/g of dust) in 28 allergen-positive dog beds with regard to dog bed characteristics

Dog bed	Der f 1			Der p 1			Group 2		
Charactistics	n	Median	Range	n	Median	Range	n	Median	Range
Location									
Bedroom	16	0.41	0.02–9.02	7	0.06	0.02–33.20	16	0.13	0.02–24.76
Living room	10	0.21	0.04–9.50	4	0.05	0.02–30.96	8	0.14	0.02–23.70
Other	2	0.95	0.12–1.78	2	0.13	0.02–0.24	2	0.38	0.22–0.54
Level									
1st floor	15	0.16	0.04–9.50	6	0.05	0.02–30.96	13	0.14	0.02–23.70
2nd floor	10	0.60	0.02–9.02	6	0.06	0.02–33.20	10	0.18	0.02–24.76
3rd floor	3	0.28	0.06–0.86	1	0.02	0.02–0.02	3	0.10	0.02–0.40
Age									
< 1 year	9	0.28	0.04–1.30	2	0.06	0.06–0.06	8	0.09	0.02–0.16
≥ = 1 year	19	0.42	0.02–9.50	11	0.04	0.02–33.20	18	0.26*	0.02–24.76
Interval since last washing									
≤ 6 months	15	0.26	0.02–9.50	8	0.04	0.02–33.20	13	0.30	0.02–24.76
> 6 months	13	0.40	0.06–6.54	5	0.06	0.02–1.18	13	0.10	0.02–4.90
Type									
Foam-filled	17	0.52	0.04–9.50	9	0.06	0.02–33.20	16	0.21	0.02–24.76
Other	11	0.26	0.02–1.02	4	0.04	0.02–0.06	10	0.11	0.02–0.58

*Significant ($P < 0.05$) difference in the concentration of Group 2 allergen in dog beds used for ≥ 1 year, compared to dog beds used for < 1 year.

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Discussion

House dust mite allergens were detected in all 50 (100%) homes that were included in the present study, of this report, whereas HDMs were detected in 15 (30%) homes. As HDMs are reported[4,30] to be important allergens for dogs with AD, the quantitation and evaluation of HDMs and HDM allergens in the microenvironment of dogs may help to define the link between allergen exposure and disease.

In studies[10,23,31] in humans, strong correlations between HDM allergens and numbers of HDMs have been revealed. In the present study of this report, significant correlations between all 3 HDM allergens and HDM density have been identified. However, HDMs were collected in only 15 (30%) of the homes where HDM allergen was detected. Although mites were detected in 31 of 50 (62%) homes, only 15 of these homes were confirmed to have *D farinae*, *D pteronyssinus*, or both species of mite. The other 16 homes from which mites were collected were not considered to have HDMs, because it was not possible to identify the species of those mites or the mites were definitively not *D farinae* or *D pteronyssinus*. The number of homes with detectable *D farinae* or *D pteronyssinus* in the present study was less than expected and may be the result of several factors. First, each home was sampled at a single time point rather than at multiple time points during a period of months or years. It has been reported that there is a wide variation in HDM densities between and within homes at any 1 time point[17,18,24]; therefore, multiple samplings are preferred for detection of HDMs. Second, seasonal fluctuations in HDM density have been reported, particularly in temperate climates.[17,32-34] In this study, most of the sample collections were performed during the months that were expected to have peak numbers of HDMs (ie, July, August, September, and October); however, some homes were sampled in the middle of October and during the first week of November when HDM numbers were likely to be decreasing.[18] House dust mite allergen concentrations in the environment are less affected by seasonal fluctuations.[18,34] Finally, homes and dust samples were only considered to have HDMs if intact mites were collected and the species could be identified definitively. Dust samples were recorded as containing mites, but not HDMs, when only damaged mites or mite parts were observed or the species of mites obtained could not be identified. This accounted for 16 of 50 (32%) homes. The inability to collect or identify the species of mites in these 16 homes was attributable to the difficulty in extraction and mounting of mites from the samples obtained and to the much lower mean mite density, compared with that in the 15 homes with HDMs (46 and 164 mites/g of dust, respectively). Thus, detection of HDM allergens was considered to be the more reliable indicator of potential allergen exposure. This is similar to the approach that has been adopted in human studies[10,20,29] in which the availability of validated assays for HDM allergens in dust samples has superceded the identification and quantitation of mites.

The distribution of HDM allergens and HDMs in the homes in our study is consistent with data regarding HDM distribution reported from human medical studies[17,18,35] conducted in Ohio. In the study reported here, all homes had Der f 1 allergen, whereas 74% of homes had detectable Der p 1 allergen. Furthermore, among 15 homes with HDMs, 10 had *D farinae* only, 4 had *D farinae* and *D pteronyssinus*, and 1 had *D pteronyssinus* only. In studies[17,18,35] of the microenvironment of humans, 95.8 to 100% of homes had *D farinae* and 39 to 81.3% had *D pteronyssinus*. In addition, in homes that contained *D farinae* and *D pteronyssinus*, 87% of the HDM population was identified as *D farinae*.[18] In our study, the mean *D farinae* density (284 mites/g of dust) was higher than the mean *D pteronyssinus* density (91 mites/g of dust). Thus, *D farinae* is the dominant and most common HDM in the household microenvironment of dogs.

For dogs with HDM hypersensitivity, Der f 1, Der p 1, and Group 2 allergens are reported to be minor allergens, whereas the high molecular weight allergen Der f 15 is considered to be a major allergen.[36] In the study reported here, it would have been ideal to quantitate the concentration of Der f 15 allergen. However, there is no commercially available assay to detect that allergen; therefore, the Der f 1, Der p 1, and Group 2 assays were used as markers for exposure to HDM allergens.

Although HDM allergens were detected on all floor levels of homes that were sampled, basements were found to be an important reservoir of these allergens. Significantly higher concentrations of Der p 1 and Group 2 allergens were detected in the samples from basements than in samples from other floor levels; analysis of data with regards to room type revealed significantly higher concentrations of Der f 1 and Group 2 allergens in samples from basements, compared with other room types. In support of these findings, 4 of 7 basements that contained HDMs had a higher mean HDM density than that of other floor levels. All basements had concrete floors, which have been reported to contain more Der p 1 allergen (7.4 µg/g of dust) than that contained in nonconcrete floors (3.5 µg/g of dust).[22] In addition, basements generally have a higher relative humidity than other floor levels, which further supports the HDM life cycle. Although previous studies have not obtained samples from basements, the low floor levels of houses (ie, ground or first floors) have been shown to have significantly higher concentrations of Der f 1 and Der p 1 allergens than the higher floor levels.[19,22,37] Thus, it appears that basements are notable reservoirs of HDM allergens for dogs and are also unrecognized reservoirs of HDM allergens for humans.

Homes without central air-conditioning were found to have significantly higher concentrations of Der p 1 and Group 2 allergens than were homes with central air-conditioning. This finding is compatible with reports[19,38] in the human medical literature. The greater Der p 1 concentration in homes without central air-conditioning supports data regarding the requirements for mite survival in terms of temperate climates and relative humidity.[32,39,40] Homes without central air-conditioning have a high ambient relative humidity[23]; this condition is favorable for comple-

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tion of the life cycle of *D pteronyssinus*, because this mite is prone to desiccation at low relative humidity. In comparison, *D farinae* is a more hardy species, and these mites are likely to survive in less than ideal environments (ie, homes with central air-conditioning); in our study, Der f 1 allergen was detected in all of the homes. This is probably the reason for the absence of a significant difference in the median level of Der f 1 concentration between homes with and without air-conditioning. Nevertheless, it should be noted that the median level of Der f 1 concentration in homes without air-conditioning (0.74 µg/g of dust) was approximately 100% greater than that in homes with air-conditioning (0.36 µg/g of dust).

Significantly higher concentrations of Der p 1 allergen were detected in medium-pile carpets, compared with concentrations in short- or long-pile carpets. Long-pile carpets have been reported to harbor more mites than do short-pile carpets.[18] The association between long-pile carpeting and high HDM density can be explained in terms of the life cycle requirements of the mite. Long-pile carpeting provides an excellent microenvironment for accumulation of food material and has higher moisture content; food sources and the mites themselves are ineffectively removed by vacuuming. Furthermore, HDMs are able to burrow deep into long-pile carpets and less likely to be removed by cleaning processes. Our data did not indicate a significant difference in HDM allergen concentration between samples from long- and short-pile carpets, which is probably because of the small sample size (2 and 10/76 carpet samples, respectively). Additionally, the Der f 1 allergen concentration was found to be significantly higher in homes in which carpet fresheners were used, compared with that in homes in which carpet fresheners were not used. This difference could be the result of interference with the Der f 1 assay caused by some of the additives in carpet fresheners. It has been reported that carpet fresheners that contain sodium bicarbonate (baking soda) increase the rate response in Der f 1 assays, thus potentially falsely elevating the reported concentration of Der f 1.[41] That study also evaluated another common additive in carpet fresheners (sodium sulphate), which did not interfere with the assay results. Although these findings are interesting and worthy of consideration, that study was performed under simulated conditions in a laboratory; the effects on results of the Der f 1 assay obtained from dust samples collected from homes in which carpet fresheners have been applied in a routine manner have not been evaluated. Furthermore, not all carpet fresheners contain baking soda, and the effects of the concentration of carpet freshener used and its frequency of application on Der f 1 allergen quantitation are unknown. In our study, carpet fresheners were≥ used in 46 homes, but the types of agent and their active ingredients were not recorded or evaluated.

Interestingly, data from homes in which flea control products were administered to the dogs were similar to those from homes in which flea control treatments were not used. However, this was not surprising, because the flea control products used were applied topically onto the skin of the dorsal portion of the dogs' necks and not directly into the environment of the homes. Environmental flea control was not used in the homes included in our study. Furthermore, the mechanisms of action of the topically applied flea control products are different from those of acaricidal agents, which have been studied for the control of HDM and HDM allergens. Even if a significant difference between findings from the households in which flea control products were or were not used had been found, interpretation of the importance of that difference would have been difficult, because there are conflicting results reported from a study[10] in which the use of acaricidal chemicals to control dust mites and their allergens was evaluated. Factors include the penetration rates of commercially available acaricidal sprays, foams, and powders into furniture and fabrics that harbor the mites, moisture, and heat. Additionally, most dog beds usually have a filling of synthetic materials (ie, foam, cotton, or polyester) that is rarely cleaned properly, thus providing a reservoir of food and moisture for HDMs. In the study of this report, foam-filled dog beds had higher median concentrations of all 3 HDM allergens, compared with those of dog beds that contained other types of filling materials. Compared with other filling materials, foam may provide a more favorable biologic microenvironment for mite survival and proliferation by trapping humidity and food and maintaining a more constant temperature. In addition, foam may retain HDM allergens more effectively than other fabrics. Because dogs spend a considerable proportion of time in dog beds, the detection of HDM allergens in dog beds suggested a potentially important allergen reservoir and exposure point. Furthermore, dog beds have not been previously evaluated as a source of HDM allergens and may represent a previously unrecognized source for HDM allergen exposure for humans with atopic diseases.

Most dog beds that were sampled had HDM allergens; of the 33 dog bed samples, 28 (85%) contained Der f 1, 13 (39%) contained Der p 1, and 26 (79%) contained Group 2 allergens. A significantly higher concentration of Group 2 allergens was found in dog beds that were ≥ 1 year old, compared with the concentration in dog beds that were < 1 year old. These results parallel findings in human mattresses in which the concentration of HDM allergens increases significantly with the age of the mattress.[19,22,38,42] Dog beds, like human mattresses, provide an ideal environment for HDM survival and proliferation; as the dog rests on the bed, it provides a continuous supply of skin scales, moisture, and heat.

Dermatophagoides farinae and *D pteronyssinus* gradually dehydrate and die when exposed to prolonged periods of relative humidity of < 50%.[21,43-46] However, HDMs will survive and proliferate if conditions of higher relative humidity (eg, 75 to 85%) occur for a few hours between periods of relative humidity of < 50%.[33,42,47] In our study, homes with ≥ 2 µg of Der f 1 or Group 2 allergens/g of dust or ≥ 100 HDMs/g of dust were more likely to have a maximum relative humidity of ≥ 75%. Furthermore, no association was found between HDM density or HDM allergen concentration and homes with a minimum relative humidity of ≤ 40%

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or a mean relative humidity of ≥ 50%. These findings supported other data which indicate that attaining an environmental relative humidity of ≥ 75%, albeit intermittently, is an important determinant of mite survival and proliferation.

In humans, HDM allergen concentrations of ≥ 2 µg/g of dust and HDM density of ≥ 100 mites/g of dust have been implicated as risk factors for sensitization to mite allergens.[10,11,18,24,48-50] Exposure to HDM allergen concentrations of ≥ 10 µg/g of dust or ≥ 500 HDMs/g increases the risk for development of overt clinical signs of atopic asthma.[10,11] Although concentrations of HDM allergens and HDM density that represent risk factors for sensitization or development of clinical signs of disease have not been established for dogs, our data indicated that many homes had allergen concentrations and mite densities greater than those regarded as important for humans. In our study, 22 of 50 (44%) homes had at least 1 sample that contained ≥ 2 µg of HDM allergen/g of dust. For both Der f 1 and Group 2 allergens, 18 of 50 (36%) homes had allergen concentrations of ≥ 2 µg/g of dust, whereas 9 (18%) homes had a similar concentration of Der p 1. In addition, 9 of 15 (60%) homes that had HDMs had ≥ 100 HDMs/g of dust. The association of HDM allergen concentration and HDM density with the potential for sensitization of dogs requires further elucidation.

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[a]Portable Filter Queen Majestic Triple Crown canister vacuum, HMI Industries Inc, Cleveland, Ohio.
[b]Adapter/dust trap, Fussnecker Sweeper, Springfield, Ohio.
[c]Ziploc sandwich bag, SC Johnson & Son Inc, Racine, Wis.
[d]500-µm mesh sieve, Small Parts Inc, Miami Lakes, Fla.
[e]Weighting dishes, Life Science Products Inc, Denver, Colo.
[f]Joy dish detergent, Proctor & Gamble, Cincinnati, Ohio.
[g]Falcon polypropylene tube, Fisher Scientific, Long Island, NY.
[h]Maxi-Mix, Barnstead International, Dubuque, Iowa.
[i]235-µm mesh sieve, Small Parts Inc, Miami Lakes, Fla.
[j]Der f 1, Der p 1, and Group 2 allergen ELISA kits, Indoor Biotechnologies Inc, Charlottesville, Va.
[k]HOBO data logger, Onset Computer Corp, Poccasett, Mass.
[l]Basic Box car software, Onset Computer Corp, Poccasett, Mass.

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