Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: Relation to sensitization and asthma among children living in Los Alamos, New Mexico

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Background: *Our objective was to identify the allergens associated with asthma among schoolchildren in an area of the United States where dust mite growth is expected to be poor. Los Alamos, N.M., was chosen because it has low rainfall and is at high altitude (7200 feet), making it very dry. One hundred eleven children (12 to 14 years old) from the middle school who had been previously classified according to bronchial hyperreactivity to histamine (BHR) were studied.*

Methods: *Sera were assayed for IgE antibodies to mite, cat, dog, cockroach, Russian thistle, and grass pollen, with both CAP system fluoroimmunoassay (Kabi Pharmacia, Uppsala, Sweden) and conventional RAST. Allergens were measured in dust samples from 109 homes with two-site assays for mite (Der p 1 and Der f 1), cat (Fel d 1), dog (Can f 1), and cockroach (Bla g 2).*

Results: *Concentrations of dog and cat allergens were elevated in almost all houses with pets but were also high in a significant proportion of the houses without pets. Levels of mite allergen were less than 2 μg/gm in 95% of the houses, and cockroach was undetectable in all but two of the houses. Among the 21 with BHR who had symptoms, 67% had IgE antibody to dog and 62% had IgE antibody to cat. For these allergens IgE antibody was strongly associated with asthma ($p < 0.001$). By contrast, the presence of IgE antibody to mite, cockroach, or grass pollen was not significantly associated with asthma.*

Conclusion: *The high prevalence of IgE antibody to cat and dog allergens among these children is in keeping with the presence of cat and/or dog allergen in most of the houses. Furthermore, sensitization (as judged by IgE antibodies) to cat and dog allergens was strongly associated with asthma. On the other hand, no clear relationship was found between sensitization or symptoms and the current level of allergen in individual houses. The results show that in this mite-and cockroach-free environment sensitization to domestic animals was the most significant association with asthma. (J ALLERGY CLIN IMMUNOL 1995;96:449-56.)*

Key words: *Asthma, dog, cat, dust mite, allergen, altitude*

Immediate hypersensitivity to indoor allergens is known to be associated with many cases of asthma among children and young adults. In temperate, humid climates, allergens from dust mites of the genus *Dermatophagoides* are an important cause of sensitization.[1-3] Indeed, several reports have suggested that increased dust mite allergen exposure, particularly in childhood, could be causally related to the marked increases in prevalence and morbidity of asthma during the last 35 years.[4, 5] In keeping

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Abbreviations used

ABTS:	2, 2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
BHR:	Bronchial hyperreactivity
BSA:	Bovine serum albumin
mAb:	Monoclonal antibody
PBS:	Phosphate-buffered saline solution
RU:	RAST unit

with this, decreased asthma prevalence or severity has been reported from areas that have low levels of mite allergen.[5, 6] However, in arid, high-altitude areas of the United States, asthma morbidity continues to be a problem among children and young adults. We chose to study risk factors for asthma among schoolchildren in Los Alamos, N.M., because the town is located at an elevation of 7200 feet in a dry area and would be expected to be free of dust mites.[7] This community was established in the 1940s around the U.S. government's nuclear research facilities. The stability of the population was evident in that almost half the children in the middle school were born in Los Alamos.

Many different sources can contribute foreign proteins to house dust and are known to be important as allergens.[8-12] These include cats, dogs, cockroaches, and fungi; however, cockroaches require warm temperatures, and the growth of fungi is inhibited by dry conditions. In keeping with other areas of the United States, numerous families in this region had dogs and/or cats. In Los Alamos most of these animals are kept indoors because of the presence of numerous wild coyotes and a strict leash law for dogs. Therefore exposure to animal dander allergens would be expected to be elevated in this setting. We have previously reported survey results, lung function results, and skin test results on middle-school children in Los Alamos, which demonstrated an association between skin test sensitization to cats and asthma.[7] However, the importance of dogs in this area was not appreciated before the survey and the dog skin tests were not included. In this report we present results of serum assays for IgE antibody to dog dander and other inhaled allergens, together with allergen assays on dust from the children's houses and the middle school. Dust was also assayed for the major dog allergen (Can f 1) with a newly developed monoclonal antibody (mAb)–based ELISA. The results suggest that in this climate, proteins from dogs and cats are the major allergens giving rise to sensitization and are an important risk factor for asthma.

METHODS

Subjects

Approval for the study was obtained from the school board and administration of the Los Alamos, N.M., school system, the University of Virginia Human Investigation Committee, and the Los Alamos Medical Center. Phase I of the study consisted of enrollment of 567 children (12 to 14 years old) in the seventh and eighth grades at Los Alamos Middle School during 1 week of March 1992. An in-school respiratory questionnaire was administered to all consenting children, and a similar questionnaire was mailed to the parents of each child. On the basis of the questionnaires, the children were divided into two groups: "wheezers" and control subjects. Sixty-four children with a history of wheezing and 57 control children (total, 121 subjects) were invited to participate in phase II of this study; of these, 111 children took part. Each child underwent (1) skin prick testing (n = 111) with lancets (Miles Inc., Hollister-Stier Div., Spokane, Wash.), (2) venipuncture for the measurement of allergen-specific IgE levels (n = 108), (3) bronchial provocation testing with histamine (n = 111) to measure BHR,[7, 13]; and (4) home visits for questionnaires and collection of house dust (n = 109). The children were examined at the Los Alamos Allergy Clinic during the spring and summer of 1992. Serum was obtained from 108 children and was stored at −20° C until tested. The children were classified in one of three groups: (1) children with bronchial hyperreactivity (BHR) and symptoms (asthma), (2) children without BHR with symptoms, and (3) control subjects. A follow-up telephone questionnaire was submitted to 36 students to qualify the presence or absence of pets in the home.

Home visits and dust assays

Home visits were completed during a 2-week period in September 1992, chosen as the moistest time of the year in Los Alamos. House dust was collected from 109 homes in Los Alamos and White Rock. Dust samples were also obtained from the classrooms in the Los Alamos Middle School. Dust was collected with a hand-held vacuum cleaner (Hoover Sprint 100, model S1211, The Hoover Co., N. Canton, Ohio) at up to five sites within each home, including the child's bedding, bedroom floor, family room floor, family room furniture, and kitchen. Dust samples were stored at 4° C until tested. Each sample was sieved and weighed, and 100 mg was extracted in 2 ml of borate-buffered saline solution overnight at 4° C. The samples were then centrifuged for 20 minutes at 2500 rpm and the supernatant was stored at −20° C for analysis of allergen content. Fel d 1, the major allergen for cat (*Felis domesticus*); Der f 1 and Der p 1, the group 1 allergens for dust mites; and Bla g 2, a major allergen from the German cockroach (*Blattella*

germanica) were each measured with a two-site mAb-based ELISA as described previously.[14-16] Can f 1, the major allergen for dog (*Canus familiaris*) was measured with a newly developed two-site monoclonal/polyclonal ELISA.[17, 18] Immulon 2 flat-bottomed ELISA plates (Dynatech, Alexandria, Va.) were coated with a 1:1000 dilution of 6E9 anti–Can f 1 mAb (kindly provided by Dr. R. Aalberse, Amsterdam, The Netherlands) in 0.1 mol/L carbonate-bicarbonate buffer, pH 9.6, overnight at 4° C. The plates were washed twice with phosphate-buffered saline solution (PBS)–polysorbate and blocked for 30 minutes with 1% bovine serum albumin (BSA)–PBS–polysorbate (assay diluent) at room temperature. After a further two washes, 100 μl aliquots of either dog allergen standard or dust eluates were added to the wells and incubated for 45 minutes at room temperature. The plates were washed five times with PBS-polysorbate, and 100 μl aliquots of a 1:16,000 dilution of polyclonal rabbit anti–Can f 1 antibody (kindly provided by Dr. C. Schou, ALK Laboratories, Copenhagen, Denmark) were incubated in each well for 45 minutes at room temperature. After five washes with PBS-polysorbate, 100 μl peroxidase conjugated goat anti-rabbit IgG (1:1000 solution; TAGO Inc., Burlingame, Calif.) was added and incubated for 45 minutes at room temperature. The plates were washed a final five times with PBS-polysorbate and developed with 100 μl 0.01 mol/L 2, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, Mo.) in hydrogen peroxide. The standard used for the assay was the World Health Organization/International Union of Immunological Societies International Reference for dog allergen (National Institute of Biological Standards and Controls code 84/685), which contained 100,000 IU/ml Can f 1. It has been estimated (by Dr. R. Aalberse) that 1 unit = 1 ng Can f 1, and this value was used to calculate results in the present report.

Immunoassays for specific IgE antibodies

Specific IgE antibodies to dog and cat allergens were measured by quantitative fluoroimmunoassays with the CAP System FEIA (Kabi Pharmacia Diagnostics, Uppsala, Sweden). This system is a complete modular system for in vitro testing of specific IgE response to allergens. ImmunoCAPs are specially designed cellulose sponges with covalently bound allergen. The allergens used were dog dander and cat dander. In each assay a standard curve was constructed with known IgE antibody standard solutions ranging from 0.35 to 50 kU/L IgE antibody calibrated against the WHO Second International Reference Preparation 75/502 human IgE. After incubation of the specific ImmunoCAP with the serum sample for 30 minutes, the ImmunoCAP sponge was washed and a rabbit β-galactosidase–anti-IgE complex was added; the bound complex on the ImmunoCAP was then incubated for 10 minutes with a developing agent (4-methylumbelliferyl-β-D-galactoside). After final elution of the ImmunoCAPs, the fluorescence of the eluate was measured. Both negative sera and serial dilutions of known positive sera were run in parallel with each assay. Conventional RASTs for mite, cat, cockroach, and pollens were carried out in parallel with RAST disks as previously described.[19] Samples analyzed by CAP system RAST were considered positive if values were at least 0.7 IU/ml (class 2), with the international unit being equal to 2.4 ng IgE (WHO IgE standard 75/502). Samples analyzed by conventional RAST were positive if values were at least 40 RAST units (RU)/ml. We have previously calculated the RU to be equivalent to 0.1 ng IgE antibody,[19] but our results suggest that 1 IU of IgE antibody used in the CAP system is approximately 12 RU. Therefore our RU may be closer to 0.2 ng IgE antibody.[20]

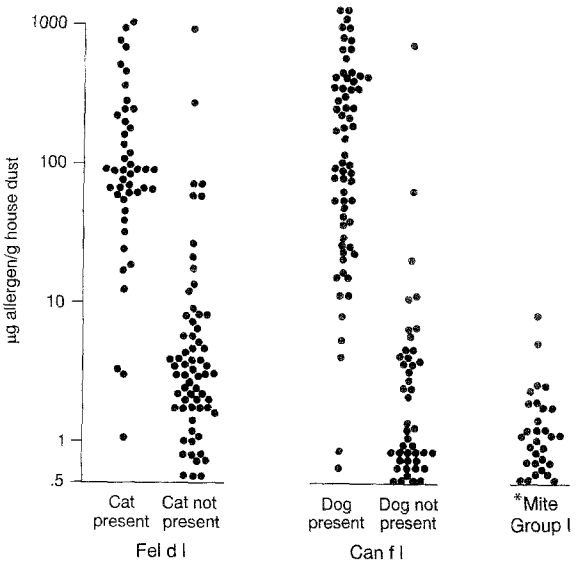

FIG. 1. Concentration of mite, cat, and dog allergen in each house; values (in micrograms per gram of dust) are the highest levels recorded of four or five samples obtained. The house shown with 900 μg/gm Fel d 1, but no cat had had a cat 3 months before study. Der p 1 and Der f 1 are added together to give group 1 mite levels. Sensitivity of assays: Fel d 1, 0.5 μg/g; Can f 1, 0.5 μg/gm; group 1 mite, 0.5 μg/gm.

Statistical analysis

Prevalence of IgE-specific sensitization in the three groups of children was compared by the chi-square test for trends. Spearman rank correlation (r_s) was used to compare the results of IgE antibody measurements of both the CAP and conventional RAST assays. Odds ratios were calculated as the odds that the disease will occur in exposed persons relative to the odds that disease will occur in unexposed persons, as described by Schlesselman.[21]

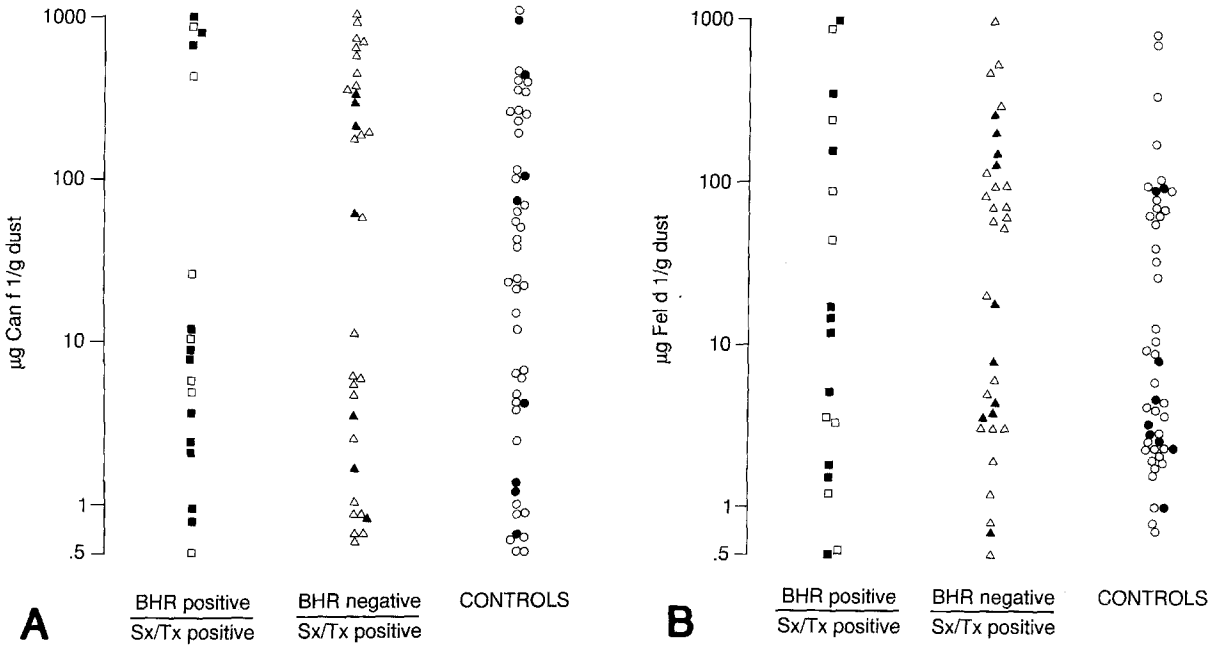

FIG. 2. Highest concentration of Can f 1 **(A)** and Fel d 1 **(B)** found in homes of children in each symptom group. *Sx,* Symptomatic; *Tx,* receiving therapy for asthma. Children who were sensitized to relevant allergens are indicated by *closed symbols* and nonsensitized children by *open symbols.*

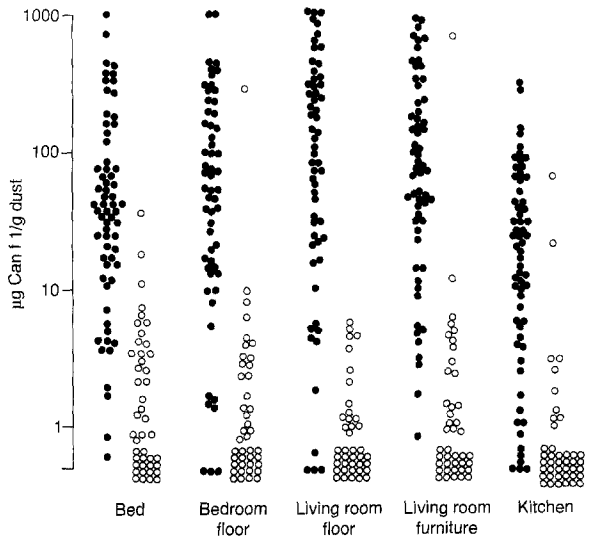

FIG. 3. Concentrations of dog allergen (Can f 1) in five locations of 109 homes in Los Alamos, N.M. Homes with a dog are indicated by *closed circles,* and those without a dog are indicated by *open circles.*

RESULTS

Exposure to indoor allergens

The homes of 109 children were visited and dust samples were assayed for dog, cat, dust mite, and cockroach allergens. The levels of dog and cat allergens were very high, in keeping with the fact that dogs were present in 65 homes and cats were present in 44 homes (Fig. 1). At least four samples of dust were obtained from each house, and in keeping with previous reports the values shown are the highest level found. At least 10 µg Can f 1 per gram of dust was found in 65% of the homes, and at least 8 µg Fel d 1 per gram of dust was found in 50% of the homes. Only two homes had significant levels of cockroach allergen (i.e., >2 U Bla g 2 per gram; data not shown) whereas only 4% were found to have mite allergen levels greater than 2 µg/gm. In general, concentrations of either dog or cat allergen greater than 10 µg/per gram of dust were found in homes where pets were present (see Fig. 1). The levels of dog allergen in houses without dogs were generally low; however, high levels of Can f 1 or Fel d 1 were found in some homes where cats and dogs were not present. Levels of dog allergen between 1 and 10 µg were present in 19 houses, whereas cat allergen between 1 and 8 µg were present in 39 houses. The percentage of homes with high levels of either allergen was very similar for children with symptoms and those without symptoms (Fig. 2). In addition, as is clear from Fig. 2, no difference was observed

TABLE I. Prevalence of IgE antibody to indoor allergens among middle-school children

Allergen	No. (%) of subjects with IgE antibody*			
	Subjects with symptoms			
	BHR$^+$ (n = 21)	BHR$^-$ (n = 36)	Control subjects (n = 54)	p Value†
Dust mite	1 (5)	6 (17)	2 (4)	0.38
Dog	14 (67)	7 (19)	8 (15)	<0.0001
Cat	13 (62)	10 (28)	9 (17)	<0.001
Cockroach	0 (0)	0 (0)	1 (2)	0.35
Russian thistle	10 (48)	10 (28)	12 (22)	0.045
Ryegrass	6 (29)	9 (25)	22 (41)	0.19

*Prevalence of at least 40 RU or CAP of at least grade II.
†Significance assessed by chi-square test for trends.

TABLE II. Subjects with both sensitivity to indoor allergens and significant home exposure to relevant indoor allergens

	Dog	Cat	Cockroach	Mite	Any indoor allergen
Group with BHR (n = 21)	8	7	0	0	10
Symptomatic group without BHR (n = 36)	5	6	0	2	9
Control group (n = 54)	4	2	0	0	5
Significance	*	†	NS	NS	†

NS, Not significant.
Significance of sensitization and exposure was judged for each allergen with chi-square test for trend in the three groups:
*$p < 0.01$.
†$p < 0.001$.

between the current exposure of dog- or cat-sensitized persons compared with nonsensitized persons.

The highest levels of Can f 1 were found in family room floors and furniture, whereas the kitchen contained the lowest levels (Fig. 3). A similar distribution was found for cat allergen in these homes (data not shown). Previous studies of homes in humid areas have shown that dust mite allergen was highest in beds and sofas, whereas for cockroach allergen the highest levels have been found in kitchens.[12, 15] The dust samples obtained from three classrooms in the middle school contained from less than 1.5 to 3.3 μg Can f 1 and from less than 0.5 to 1.1 μg Fel d 1 per gram of dust.

Specific IgE antibody measurement

The results from the fluoroimmunoassays correlated well with results from conventional RASTs for specific IgE antibody to cat and mite allergens (cat $r_s = 0.93, p < 10^{-5}$; mite $r_s = 0.89, p < 10^{-4}$). Sixty-seven percent and 62% of the 21 patients with BHR (asthma) had IgE antibodies to dog and cat, respectively. This compared with 19% and 28% of the 36 children with symptoms without BHR, and with 15% and 17% of the 54 control subjects (Table I). The prevalence of sensitization to dust mites and to cockroach, measured by conventional RAST, was much lower among all three groups of students. Overall, only 12% of the children who reported wheezing had positive RAST responses to mites, a very low prevalence of mite sensitization for patients with symptoms. The prevalence of sensitization to cockroach was 1%. In keeping with previous skin test results, the RAST data showed that many of the children had IgE antibody to both Russian thistle and grass pollen. Sensitization to Russian thistle was weakly correlated with asthma, whereas sensitization to

grass showed no significant correlation with asthma.

Sensitization and exposure

The combination of sensitization and significant exposure to at least one of the four indoor allergens was present in 10 of 21 children with BHR compared with 9 of 36 children with symptoms without BHR and only 5 of 54 control children. The combination of sensitization and increased exposure levels for either dog or cat showed a strong correlation with asthma (Table II). However, comparison with Table I shows that the correlations between asthma and sensitization to these allergens were as strong as the associations seen in Table II. This suggests that the correlation between asthma and the combination of sensitization and exposure is in large part attributable to the correlation between sensitization and asthma.

DISCUSSION

Our results showed that of the homes sampled in Los Alamos, 50% had more than 8 μg of cat allergen per gram of dust, whereas 65% had more than 10 μg dog allergen per gram. By contrast, only 4% and 2% had significant levels of dust mite or cockroach allergen, respectively. A threshold level for cat allergen has been proposed previously.[12] Threshold levels for dog allergen have not been proposed. From our results it is clear that at least 95% of the houses with a dog have more than 10 μg/gm of Can f 1 whereas at least 95% of the houses with a cat were found to have at least 8 μg Fel d 1/per gram of dust. According to these values, high levels of allergen were found in five houses without a dog and 11 homes without a cat. Several of these homes had had pets present in the past, whereas the allergen levels in the other houses probably resulted from passive transfer on clothing. These assays are highly specific, and the results cannot be explained by cross-reactivity of the assays.[17, 18] Previous studies have reported passive transfer of dog or cat allergen into schools and cat allergen into houses, and have suggested that passive exposure could contribute to continuing symptoms.[14, 18, 22-24] Our results confirm that significant levels of dog allergen can be present without a dog and that levels of at least 1 μg/gm are common in houses.[22] These levels are higher than the levels reported from homes without a dog and from schools in Scandinavia, or those found by us in the middle school in Los Alamos. Thus it is clear that children living in a house without an animal could be exposed to animal dander allergens in several ways: at school, in other children's houses, or in their own house from passive transfer.

The current results support previous data showing that sensitization can be assessed by in vitro assays. Our results show an excellent correlation between results for IgE antibody with the CAP system assay and conventional RAST with cellulose disks. Both systems have been quantified previously, and our calculations suggest that the RAST unit is approximately 0.1 to 0.2 ng of IgE antibody. This study illustrates two advantages of using in vitro assays for IgE antibodies in studies of this kind: (1) antibodies to additional allergens can be evaluated after the initial survey and (2) results with different techniques or sera from other studies can be directly compared in a central laboratory. The availability of quantitative in vitro assays for IgE antibody and two-site mAb-based assays for the major indoor allergens means that observations of exposure and sensitization can be standardized.

The low prevalence of sensitization to dust mite and cockroach in these children is not surprising, because exposure to both of these allergens was low. Indeed, the concentration of mite allergen in these houses was very low compared with results from the United States and from other countries.[5, 12, 23, 25, 26] Only two children had the combination of increased exposure levels to dust mites and sensitization. Both these children (and their furniture) had moved to Los Alamos only recently (one from Virginia and the other from Washington state). Our results strongly support the view that when children are reared in an area where the houses contain high levels of dog and cat allergen, sensitization to these allergens is associated with asthma. However, current exposure levels in Los Alamos were no higher among sensitized subjects than among unsensitized subjects. A previous prospective study has shown no correlation between current mite exposure levels and the presence of asthma at 11 years of age.[4] The present results show that sensitization to animal dander is strongly associated with asthma. However, because there was very little association with current exposure, the results do not answer the following questions: whether exposure in early childhood was more relevant, whether the children are being exposed in other homes and/or in school, or whether levels of dog or cat allergen less than these threshold levels are sufficient to maintain symptoms. Sensitization to outdoor pollens such as Russian thistle was prevalent among all groups of students and was correlated only weakly with asthma.

In countries where the prevalence of asthma has increased markedly (e.g., New Zealand, Australia, and the United Kingdom), some studies have shown such a high prevalence of mite sensitization that it seemed likely that increased levels of dust mite allergens were the single most important cause of the increase in asthma.[4, 5, 27, 28] In Los Alamos we have estimated the prevalence of symptomatic BHR (i.e., asthma) among schoolchildren as between 5.3% and 6.3%, depending on the level of BHR taken as diagnostic; this is lower than in some studies from humid areas but is still high.[7] However, there is no clear evidence that the prevalence of asthma or the number of indoor pets in this area has increased. It is possible that either tighter housing construction or increased indoor temperatures could have altered the response to inhaled allergens. However, the striking feature of the present results is that, as in most other studies, the strongest association with asthma is sensitization to foreign proteins found indoors rather than with outdoor allergen.

A logical conclusion from the present results is that measures to reduce exposure to allergens are relevant both to the treatment of symptomatic asthma and to efforts to reduce the prevalence of asthma. Our results show that dog and cat allergens were not only distributed throughout the houses where pets were present but also in many houses where pets were not present. Therefore controlling exposure may not be simply a matter of controlling the levels in a patient's house. Many questions are unanswered, including whether passively transferred allergens can give rise to airborne allergen and whether regular washing of dogs can reduce airborne allergen levels. It is clear that more studies are needed on methods for controlling animal dander, especially in environments such as Los Alamos, where indoor pets are so prevalent. However, the results for mite and cockroach allergens strongly suggest that if the concentration of these allergens were kept as low as they are in Los Alamos, then they would not contribute to sensitization or to symptomatic asthma.

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