Serum immunoglobulin E against storage mite allergens in dogs with atopic dermatitis

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Objective—To determine the prevalence of serum IgE against the storage mites *Acarus siro, Blomia tropicalis,* and *Tyrophagus putrescentiae* in a population of dogs with atopic dermatitis.

Sample Population—Sera from 84 dogs with atopic dermatitis residing in various regions of the United States and Europe.

Procedure—Immunoblotting of sera from atopic dogs was used to identify proteins in mite extracts that bound IgE.

Results—94% of the dogs had serum IgE against proteins in extracts of 1 or more of the storage mite species. Ninety-five, 92, and 89% of the storage mite-sensitive dogs had serum IgE against proteins in extracts of *A siro, B tropicalis,* and *T putrescentiae,* respectively. Eighty-two percent had serum IgE against at least 1 protein in all 3 species. Most of the major allergens had molecular weights > 80 kd. A greater percentage of the dog sera had IgE against storage mite proteins, compared with proteins of the house dust mites *Dermatophagoides farinae* and *D pteronyssinus.*

Conclusion and Clinical Relevance—Many dogs with atopic dermatitis have serum IgE against many allergens of storage mites. Most of these allergens, like allergens of dust mites, had molecular weights > 80 kd. Storage mite sensitivity in dogs may be as important, if not more important, than dust mite sensitivity. (*Am J Vet Res* 2003;64:32–36)

Atopic dermatitis is common in dogs worldwide.[1-6] A survey of 31,484 dogs that were admitted to 52 veterinary practices in the United States revealed that 8.7% had atopic-allergic dermatitis, allergy, or atopy.[4] Many dogs with atopic dermatitis have circulating serum IgE, positive intradermal test results, or both to the astigmatid house dust mites, *Dermatophagoides farinae*, *D pteronyssinus*, and *Euroglyphus maynei*.[5,7-15] It is likely that exposure of dogs to house dust mites occurs in the indoor environment mainly by contact with the skin and by inhalation of airborne mite allergens, just as it does for humans. These dust mites also are occasionally found in packaged processed food (eg, pancake mix)[16,17] and have been grown in the laboratory on dog food.[18] Anaphylactic reactions have occurred in sensitized humans after ingestion of whole-wheat bread and baked goods contaminated with dust mites.[16,17] Therefore, sensitization and allergic reactions in humans and dogs may result following ingestion of dust mites and their allergens in contaminated food. Many astigmatid storage mite species such as *Glycyphagus domesticus*, *Acarus siro*, *Tyrophagus putrescentiae*, *Blomia tropicalis*, and *Lepidoglyphus destructor*, which are phylogenetically closely related to house dust mites, are also known to sensitize and induce allergic reactions in humans. These storage mites are common in stored hay, straw, and grains; in the facilities used to store and transfer these materials; in livestock feeding facilities; and also in processed foods made from these grains. Although some storage mite species may be found in home furnishings (eg, carpets, bedding, and fabric-covered furniture), they are usually not prevalent and are rarely present in US homes.[19,20] Human exposure to these mites usually occurs in an occupational setting (eg, farm workers).[21-23] Dogs living in an agricultural setting may be exposed to them in a similar way. In addition, these mites and their immunogenic products may contaminate dry dog and livestock food. The mites may also invade and grow on some dry dog food once the package is opened. In either case, ingested mites and mite products may sensitize and induce allergic reactions in dogs, just as they do in humans.

Despite the many avenues for exposure, sensitivity and allergic reactions to stored product mites in dogs have not been extensively investigated. A few studies have used intradermal tests to assess sensitivity to storage mites in atopic dogs. A large percentage of these dogs have sensitivity with 18 to 50% reacting positively to *A siro*, 30 to 50% to *T putrescentiae*, 27% to *G domesticus*, and 23% to *L destructor*.[5,8,15,24,25] An even higher percentage of positive test results (57 to 86%) was recorded in healthy dogs with previously documented cutaneous reactivity to *Ascaris suum*.[26]

Some immunogens of storage mites that sensitize and induce allergic reactions in humans have been characterized. Using serum samples from occupationally exposed farmers, *T putrescentiae* was shown to be the source of at least 14 allergens, with individual human patients recognizing from 5 to 11 of these.[23] *Blomia tropicalis*, a prevalent storage mite in homes in some tropical climates, is the source of at least 21 allergens.[27] Allergens Blo t 5, 12, and 13 have been characterized and have molecular weights of 14 to 17 kd. A major allergen of *L destructor* (Lep d 2) has a molecular weight of 14 kd.[17]

To our knowledge, storage mite allergens that sensitize and induce allergic reactions in dogs have not been characterized. The purpose of the study presented here was to determine whether dogs with atopic dermatitis had circulating IgE against the storage mites *A siro*, *B tropicalis*, or *T putrescentiae*.

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Materials and Methods

Serum samples—Immunoglobulin E-specific proteins in extracts of *A siro*, *B tropicalis*, and *T putrescentiae* were characterized by probing serum samples from 84 atopic dogs. Surplus sera from the 84 dogs were donated by Biomedical Services (Austin, Tex). Seventy-six of the serum samples were sent by veterinarians from various regions of the United States including California, Great Plains, Midwest, Northeast, Northwest, Rocky Mountains, Southeast, South Florida, Southwest, and Texas. The remaining 8 serum samples came from 4 European countries (Denmark, Finland, Spain, and Sweden).

Forty-two of the 84 serum samples had been probed for circulating IgE by immunoblot analysis in a prior study, and 21 of these had circulating IgE against proteins of at least 1 of the 2 species of house dust mites (*D farinae* or *D pteronyssinus*).[14] One of these 42 serum samples came from Denmark, and the rest of the dogs were from various regions of the United States.

Serum IgE specific for proteins of *D farinae* and *D pteronyssinus* were determined for 38 of the remaining 42 dogs by use of the method described in the previous study.[14] Thirty-two of the dogs were from the United States and 6 were from Europe. Four of the serum samples were not probed for IgE against the proteins of the 2 species of house dust mites because of a lack of sufficient sera.

Antigen extracts—*Acarus siro* and *T putrescentiae* were grown on a whole-wheat flour-baker's yeast medium (1:35, wt/wt) and were collected as they wandered out of thriving laboratory cultures as described by Arlian et al.[28] *Blomia tropicalis* was donated. Extracts from the 3 species of storage mites were prepared similarly as reported for other mite species.[14,29] Briefly, the mites were suspended in glass-distilled water at 1:10 (wt/vol) for 24 hours at 4°C and then homogenized with 10 strokes of a TenBroeck homogenizer on ice. The homogenate was allowed to extract for an additional 24 hours at 4°C. Extracts were centrifuged and the supernatants collected. Supernatants were sterile-filtered and stored at 4°C until used. The Bradford method was used to determine the protein concentration of each extract by use of bovine serum albumin as the standard.[30] *Dermatophagoides farinae* and *D pteronyssinus* extracts were prepared as previously described.[14] Because storage mite extracts could contain culture medium, a control extract of this material was also prepared as described.

SDS-PAGE and immunoblotting—Sodium dodecylsulfate-polyacrylamide gel electrophoresis was used under nonreducing conditions to separate the proteins in the antigen extracts. An aliquot containing 50 µg of mite protein was loaded into the sample well of a 12% resolving gel with a 4% stacking gel.[a] Molecular weight markers[a] were loaded in parallel in the reference well. Sodium dodecylsulfate-polyacrylamide gel electrophoresis-separated proteins were transferred to a polyvinylidene difluoride membrane[b] and blocked as reported.[14,31] The membrane was then placed in a multiscreen apparatus[a] and incubated overnight with 17 individual dog sera each diluted 1:5 (vol/vol) in buffer. The immunoblot was placed in peroxidase labeled goat anti-dog IgE detecting antibody,[c] and IgE binding was viewed by developing with the chromogenic substrate of Young.[32]

Data analysis—Digital images of the blots were prepared and molecular weights of the IgE binding proteins were determined by use of computer software[d] to compare the mobilities of the proteins of interest with the mobilities of the reference proteins of known molecular weight.

Results

Sensitization to multiple storage mite species—Serum from 79 (94%) of the 84 dogs had circulating IgE that bound to proteins in extracts of 1 or more of the 3 storage mite species (*A siro, B tropicalis*, or *T putrescentiae*; **Table 1**). Seventy-five, 73, and 70 of the 79 storage mite-sensitive dogs had serum IgE that recognized proteins in extracts of *A siro, B tropicalis*, and *T putrescentiae*, respectively. Sixty-five (82%) of the 79 dogs had serum IgE that recognized at least 1 protein in extracts of all 3 species. Nine dogs had serum IgE that recognized the proteins from 2 of the 3 mite extracts, and 5 dogs had serum IgE that recognized only 1 mite species. The highest number of proteins recognized by the serum IgE of an individual dog for any species of mite was 22, with most dogs having IgE that bound to ≥ 5 proteins in at least 1 extract.

Table 1—Presence (+) or absence (–) of serum IgE against storage mite and house dust mite allergens among the 84 serum samples from atopic dogs

Storage Mites			Dust Mites		No. of dogs
AS	BT	TP	DF	DP	
+	+	+	+	+	23
+	+	+	+	–	23
+	+	+	–	–	14
+	+	+	0	0	4*
+	+	–	+	+	3
+	–	+	–	–	3
+	–	–	–	–	2
–	+	–	+	–	2
+	+	+	–	+	1
+	+	–	+	–	1
+	+	–	–	–	1
–	+	+	–	–	1
–	–	+	+	–	1
–	–	–	+	–	1
–	–	–	–	–	4
75	73	70	54	27	84

*Four serum samples were not probed for serum IgE against house dust mites (0) because of a lack of sufficient serum.
AS = *Acarus siro*. BT = *Blomia tropicalis*. TP = *Tyrophagus putrescentiae*. DF = *Dermatophagoides farinae*. DP = *D pteronyssinus*.

Table 2—Distribution of serum IgE binding of 63 atopic dogs to 27 allergens of various molecular weights in an extract of *A siro*

Molecular Weight (kd)	Dogs with IgE (No.)	Dogs with IgE (%)
179.8	6	9
160.4	6	9
133.3	10	16
112.6	20	31
100.8	56	89
92.1	45	71
86.6	5	8
82.2	2	3
76.3	18	29
72.7	47	75
65.6	9	14
61.3	34	53
57.6	39	62
51.3	9	14
47.7	14	22
42.6	2	3
41.0	1	2
39.6	10	16
38.7	7	11
36.9	4	6
35.1	3	5
32.8	1	2
31.2	1	2
30.6	1	2
28.2	1	2
25.7	1	2
19.9	1	2

Sixty-five of the 79 dogs also had IgE directed at proteins in the whole wheat-yeast growth medium used to culture the *A siro* and *T putrescentiae*. The number of culture medium proteins recognized by serum IgE from any single dog ranged from 1 to 11 with the mean being 3. Most dogs had serum IgE that recognized more allergenic proteins in the mite extracts, compared with the number recognized in the culture medium extract. Molecular weights of these culture medium proteins that bound IgE were mostly < 80 kd.

***A siro* allergens**—Seventy-five (95%) of the 79 dogs had circulating IgE directed at 1 or more of the proteins in an extract of *A siro*. Immunoblots of 12 serum samples resulted in diffuse binding, and the molecular weights of the proteins bound could not be determined. The remaining 63 serum samples had specific IgE binding to 27 different molecular weight proteins (**Table 2**). Mean number of proteins recognized by the circulating IgE of an individual dog was 6 (range, 1 to 22). One dog had serum IgE against 22 *A siro* proteins, 2 dogs had IgE against 10 to 14 proteins, 37 had IgE against 5 to 9 proteins, and 23 dogs had IgE that detected < 5 proteins. Molecular weights of the 27 IgE-specific proteins that were detected ranged between 179.8 and 19.9 kd. Eighty-nine, 75, 71, 62, and 53% of the 63 dogs had serum IgE that recognized proteins with molecular weights of 100.8, 72.7, 92.1, 57.6, and 61.3 kd, respectively.

***B tropicalis* allergens**—Seventy-three (93%) of the 79 mite-sensitive dogs had circulating IgE against the proteins in an extract of *B tropicalis*. Immunoblots of 16 serum samples resulted in diffuse binding, and the molecular weights of the proteins bound could not be determined. Fifty-seven dogs, however, had circulating IgE that specifically bound to 15 *B tropicalis* proteins, with molecular weights between 231.2 and 35.8 kd (**Table 3**). Mean number of IgE-specific proteins recognized by the serum IgE of individual dog serum was 6 (range, 2 to 11). Specifically, 1 dog had serum IgE that bound to 11 *B tropicalis* proteins, 49 dogs had IgE directed at 5 to 10 proteins, and 7 of the dogs had serum IgE that bound < 5 proteins. Molecular weights of the proteins with the most frequent IgE binding were 231.2, 215.0, 197.7, 119.8, 113.7, and 79.2 kd; ≥ 77% of the 57 dogs had serum IgE against these proteins.

***T putrescentiae* allergens**—Seventy (89%) of the 79 mite-sensitive dogs had circulating IgE specific for proteins in the *T putrescentiae* extract. Immunoblots of serum samples from 12 of the 70 dogs revealed diffuse binding, and specific molecular weights of proteins could not be identified. Immunoblots of the remaining 58 serum samples revealed IgE binding to 16 proteins, with molecular weights ranging from 186.6 to 38.1 kd (**Table 4**). On average, individual dog sera had IgE that bound to 6 (range, 1 to 10) *T putrescentiae* proteins. Three dogs had serum IgE that bound to 10 proteins, and 47 dogs had serum IgE that recognized from 4 to 9 proteins. The remaining 20 dogs had serum IgE that recognized < 4 proteins. The *T putrescentiae* proteins most frequently bound by circulating IgE had molecular weights of 125.7, 120.3, 111.8, 81.4, 79.8, and 70.7 kd.

Table 3—Distribution of serum IgE binding of 57 atopic dogs to 15 allergens of varying molecular weights in an extract of *B tropicalis*

	Dogs with IgE	
Molecular Weight (kd)	(No.)	(%)
231.2	56	98
215.0	56	98
197.7	57	100
154.9	21	37
132.9	15	26
119.8	50	88
113.7	50	88
94.1	7	12
84.0	3	5
79.2	44	77
66.5	8	14
58.6	2	3
46.5	1	2
40.6	1	2
35.8	1	2

Table 4—Distribution of serum IgE binding of 58 atopic dogs to 16 allergens of varying molecular weights in an extract of *T putrescentiae*

	Dogs with IgE	
Molecular Weight (kd)	(No.)	(%)
186.6	8	14
147.6	23	39
125.7	38	66
120.3	55	95
111.8	48	83
105.4	9	15
87.6	9	15
81.4	48	83
79.8	42	72
76.6	7	12
73.7	6	10
70.7	42	72
61.6	3	5
48.8	2	3
41.8	1	2
38.1	2	3

***D farinae* and *D pteronyssinus* allergens**—Serum samples were also evaluated for the presence of IgE directed at *D farinae* and *D pteronyssinus* proteins. The IgE binding patterns to *Dermatophagoides* allergens were previously reported for 42 of these serum samples.[14] Twenty-one of the serum samples had IgE against the storage mites but not the dust mites (Table 1).

Discussion

Results of our study indicate that dogs with atopic dermatitis may be sensitized to several species of storage mites. Ninety-four percent of the dog sera had IgE directed at 1 or more proteins in the extracts of *A siro*, *B tropicalis,* or *T putrescentiae*. The majority (82%) of dog sera had IgE directed at proteins in all 3 species. Serum samples of individual dogs often had IgE directed at a different number of allergens for each mite species, although the mean number of allergens of each mite species recognized by serum IgE of an individual dog was 6.

It is possible that some of the recognition of allergens of multiple species by individual dogs was the

result of the presence of cross-reactive epitopes on molecules in the extracts of various species. If this were the case, serum IgE built to an epitope on 1 species would bind to a cross-reactive epitope on a different allergen molecule of another species. However, results of research on human sera and molecular studies[17,22,33-37] indicate that there is limited cross-reactivity between these species of storage mites. The results of these human studies, taken together with our finding that serum IgE of an individual dog recognized varied numbers of allergens of each species and that some individual dogs had IgE against only 1 or 2 species, suggest that most of these dogs had been sensitized to multiple storage mite species.

It is not known where or how dogs are exposed and sensitized to storage mites. Storage and dust mites have been reported to occur in dog and cat beds.[38] Therefore, dogs may become sensitized by inhalation of mite allergen or by skin contact with the allergen when occupying mite-infested sleeping areas. Dog food or components of dog food may contain mites or their allergens. Also, mites may invade and grow on dog food after the package is opened. Occasionally, storage mites have been found in a wide variety of processed human foods,[16,17,39] and this is likely to occur with animal foods as well. Broadly based longitudinal studies to investigate the occurrence of storage mites and storage mite allergens in dog food or ingredients used in dog food are needed to determine whether this is an important source of sensitization for dogs.

It was possible in our study that some of the allergens in the mite extracts were from the culture medium on which the mites were grown. Culture medium may have remained in the gastrointestinal tract or adhered to the surfaces of the bodies when mites were harvested and the extracts were made. Some of these culture medium proteins may be allergens. To investigate this possibility, proteins in extracts made from the culture medium used to grow *A siro* and *T putrescentiae* were also probed for IgE binding by immunoblotting in parallel with these mite extracts. The results of our study revealed that most of these dogs had serum IgE against proteins in the culture medium (mostly whole-wheat flour) in addition to IgE specific for mites. This high frequency of serum IgE against proteins in the culture medium suggests that whole-wheat flour (or baker's yeast) is an important source of allergens for dogs. This is not surprising because other studies[11-13,40] have reported sensitivity by intradermal test to wheat (*Triticum aestiuum*). It is not clear how dogs are exposed and sensitized to whole-wheat flour or baker's yeast. However, it is likely a result of ingestion or inhalation of allergens in the diet. Conversely, because flour is often contaminated with storage mites, we cannot rule out the possibility that the allergens present in the culture medium extract are actually of mite origin.

Our immunoblots revealed that the molecular weights of the IgE binding proteins in the culture medium extract were generally < 80 kd. In comparison, the IgE binding proteins in the mite extracts were mostly > 80 kd. Thus, most of the serum IgE was specific for either mite proteins or culture medium proteins, because there were few IgE binding proteins in the mite and culture medium extracts that had comparable molecular weights. In addition, 11 dogs had IgE against mite proteins but not to culture medium proteins. Taken together, these data suggest that most dogs were cosensitized to storage mites and culture medium.

Results of our study indicate that for dogs, most of the allergenic proteins of storage mites were > 80 kd. The exceptions were proteins of 70.7 and 76.6 kd in extracts of *T putrescentiae*, a 79.2-kd protein from *B tropicalis*, and a 72.7-kd protein from *A siro*. These results are consistent with the fact that the major allergens from house dust mites for dogs are > 90 kd,[14,41] whereas those for humans are typically < 60 kd.[42] Most of the storage mite proteins to which humans react have not been characterized.

An important finding in our study was that 94% of the dogs had serum IgE against *A siro*, *B tropicalis,* or *T putrescentiae*. In contrast, only 68 and 34% had IgE against the house dust mites *D farinae* and *D pteronyssinus* allergens, respectively. Twenty-seven percent (n = 21) of the dogs that had serum IgE against storage mites (79) did not have serum IgE against *D farinae* or *D pteronyssinus*. All but 8 of the serum samples came from dogs residing in homes located throughout the various geographic regions of the United States These sensitization data suggest that storage mites may be a more important source of allergens for dogs in the United States than are house dust mites. The higher frequency of serum IgE against storage mites we found is consistent with the high frequency of positive intradermal test results to several species of storage mites reported for dogs in the United States by Hogen et al.[26] Our results revealed a higher frequency of sensitization to these storage mites, compared with the frequency of positive intradermal test results to selected storage mites reported in dogs from London, Edinburgh, France, Greece, and Tokyo.[5,8,12,15] In these later studies, the highest frequency of a positive skin reaction was to the house dust mites *D farinae* and *D pteronyssinus*. The varied geographic frequency of sensitivity to the various species of mites may reflect varying amounts of exposure to the different species in these countries.

[a]Mini PROTEAN II system with Tris HCl 2D/Prep well Ready Gels, Bio Rad, Hercules, Calif.
[b]ProBlott, Applied Biosystems, Foster City, Calif.
[c]Bethyl Labs, Montgomery, Tex.
[d]Kodak 1D software, Kodak Research and Development, Rochester, NY.

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