Reactivity to intradermal injection of extracts of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, house dust mite mix, and house dust in dogs suspected to have atopic dermatitis: 115 cases (1996–1998)

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Objective—To compare reactivities to intradermal injection of extracts of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, house dust mite mix, and house dust in dogs suspected to have atopic dermatitis.

Design—Retrospective study.

Animals—115 dogs.

Procedures—Records of all dogs suspected to have atopic dermatitis that underwent intradermal testing between October 1996 and July 1998 were reviewed. Reactivities to intradermal injection of crude mixed house dust mite (1:25,000 wt/vol) and crude house dust (25 PNU/ml) extracts were compared with reactivities to intradermal injection of individual extracts of *D farinae* and *D pteronyssinus* (1:50,000 wt/vol).

Results—Ninety dogs were confirmed to have atopic dermatitis including 61 of the 69 dogs with positive reactions to either or both of the individual house dust mite extracts. Intradermal testing with the mixed house dust mite extract had sensitivity of 75%, specificity of 96%, and accuracy of 83%. Intradermal testing with the house dust extract had sensitivity of 30%, specificity of 93%, and accuracy of 56%.

Conclusions and Clinical Relevance—Results suggest that use of crude mixed house dust mite and crude house dust extracts for intradermal testing in dogs is not as accurate a method of determining house dust mite hypersensitivity as is the use of individual *D farinae* and *D pteronyssinus* extracts mainly because of the high percentage of false-negative results. Extracts of individual house dust mites are recommended for intradermal testing of dogs suspected to have atopic dermatitis. (*J Am Vet Med Assoc* 2000;217:536–540)

In dogs, **atopic dermatitis (AD)** is a chronic relapsing pruritic dermatitis associated with IgE-mediated hypersensitivity to aeroallergens.[1] House dust mites, in particular *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, are reported to be among the most common allergens causing hypersensitivity in dogs with AD.[2-5]

Intradermal testing (IDT) is a well-recognized and common method of determining allergen-specific hypersensitivity in vivo.[6,7] The results allow clinicians to advocate allergen avoidance measures that are appropriate for individual patients and to formulate allergen-specific vaccines for immunotherapy.

In veterinary medicine, crude extracts of individual house dust mites (*D farinae* and *D pteronyssinus*), mixed house dust mite extracts containing both *D farinae* and *D pteronyssinus*, and house dust extracts have been used for IDT.[8-11] Two recent studies[8,9] recommend use of a mixed house dust mite extract for IDT of dogs suspected to have atopic dermatitis. However, use of mixtures of allergens for IDT is controversial, and it is believed that testing with individual allergens should give more accurate results.[1,7] To the authors' knowledge, there are no reports on the correlation between reactivity to individual house dust mite extracts and mixed house dust mite or house dust extracts in dogs undergoing IDT. Therefore, the purpose of the study reported here was to compare reactivities to intradermal injection of extracts of *D farinae*, *D pteronyssinus*, house dust mite mix, and house dust in dogs suspected to have atopic dermatitis.

Criteria for Selection of Cases

Medical records of all dogs that underwent IDT through the dermatology service at The Ohio State University's College of Veterinary Medicine between October 1996 and July 1998 were reviewed. Dogs that underwent IDT were suspected to have atopic dermatitis on the basis of suggestive historical features, typical clinical signs, and elimination of other possible causes of the clinical abnormalities.[6] The diagnosis of atopic dermatitis was confirmed when positive IDT reactions were consistent with the known seasonality of the dog's disease and the likelihood of exposure to allergens that resulted in positive reactions. Food-related dermatitis was ruled out on the basis of a lack of response to feeding a commercial or home-cooked hypoallergenic elimination diet for a minimum of 4 but usually 8 weeks. If a dog improved while fed a hypoallergenic elimination diet, the diagnosis of food-related dermatitis was confirmed if clinical signs worsened within 1 week after the dog was returned to its previous diet.

Procedures

In all dogs, IDT consisted of intradermal administration of a panel of 70 aeroallergens specifically formulated for the central Ohio area. Reactivity to *D fari-*

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nae[a] and *D pteronyssinus*[b] was tested individually, using a 1:50,000 wt/vol concentration of the crude extracts. The mixed house dust mite extract[c] was a 1:1 mixture of crude *D farinae* and *D pteronyssinus* extracts; a 1:25,000 wt/vol concentration was used. House dust extract[d] was used at a concentration of 25 PNU/ml. All extracts were prepared and diluted in sterile diluent[e] according to standard protocols.[6]

In all dogs, administration of anti-inflammatory drugs was discontinued prior to IDT; routine recommendations for withdrawal times were followed.[6] All dogs were sedated with xylazine hydrochloride and atropine sulfate, and IDT was performed, using a standard protocol, on the left lateral aspect of the thorax.[6] A volume of 0.05 ml of each allergen and each control solution was injected intradermally at separate sites. Reactivity was assessed subjectively 15 minutes after injection of allergens and was graded on a scale from 0 to 4 on the basis of size, intensity of erythema, and turgidity. A score of 4 was assigned if the reaction was equivalent to that induced by the histamine control solution[f]; a score of 0 was assigned if the reaction was equivalent to that induced by the negative control solution (ie, sterile diluent[e]). Reactions scored as 2 (half the size and turgidity of the reaction induced by the histamine control solution) or greater were considered to be positive and indicative of hypersensitivity.[6]

Data analyses—Sensitivity ([number of true-positive results]/[number of true-positive results + number of false-negative results]), specificity ([number of true-negative results]/[number of true-negative results + number of false-positive results]), predictive value of a positive result ([number of true-positive results]/[number of true-positive results + number of false-positive results]), predictive value of a negative result ([number of true-negative results]/[number of true-negative results + number of false-negative results]), and accuracy ([number of true-positive results + number of true-negative results]/[number of true-positive results + number of true-negative results + number of false-positive results + number of false-negative results]) of using the mixed house dust mite extract and of using the house dust extract for IDT were assessed, with reactivity to the individual mite extracts regarded as the criterion standard.

Results

One hundred fifteen dogs met the criteria for inclusion in the study. Dogs ranged from 9 months to 10 years old. Sixty-eight (59%) were male, and 47 (41%) were female. There were 26 (23%) Labrador Retrievers, 24 (21%) mixed-breed dogs, and 9 (8%) Golden Retrievers; the remaining 56 dogs represented 32 other breeds with ≤ 4 dogs each.

In 90 (78%) dogs, the diagnosis of AD was confirmed on the basis of results of IDT; 81 of the 90 (90%) dogs with AD had year-round disease. For the remaining 25 dogs, results of IDT did not correlate with the known history and clinical signs. Final diagnoses for these dogs included AD (diagnosed on the basis of combined intradermal and serum allergy test results), food-related dermatitis, insect hypersensitivity, and idiopathic pruritic dermatitis.

Hypoallergenic elimination diets were fed to 79 of the 81 dogs with year-round AD, and 4 (5%) were determined to have concurrent food-related dermatitis. The remaining 2 dogs were not fed a hypoallergenic elimination diet for a minimum of 4 weeks because of lack of owner compliance in 1 instance and refusal on the part of the dog to eat the prescribed diet in the other.

Four dogs had a history of *Sarcoptes scabeii* or *Otodectes cyanotis* infestation, although none were known to be infested at the time of IDT. Two of these dogs had positive reactions to *D farinae*, mixed house dust mite, and house dust extracts; 1 had been treated for scabies 4 months, and the other 1 year, prior to IDT. Two dogs had negative reactions to both individual house dust mite extracts, mixed house dust mite extract, and house dust extract; 1 was treated for ear mite infestation > 1 year prior to IDT, and the other was treated for scabies 6 months prior to IDT.

Of the 115 dogs, 69 (60%) had positive reactions to *D farinae* alone (n = 31), *D pteronyssinus* alone (2), or both *D farinae* and *D pteronyssinus* (36); 54 (47%) had positive reactions to the mixed house dust mite extract; and 24 (21%) had positive reactions to the house dust extract. Sixty-one of the 69 (88%) dogs that had positive reactions to *D farinae*, *D pteronyssinus*, or both were confirmed to have AD.

Using responses to the individual house dust mite extracts as the criterion standard, IDT with the mixed house dust mite extract had sensitivity of 75%, specificity of 96%, positive predictive value of 96%, negative predictive value of 72%, and accuracy of 83% (**Table 1**). Six of the 36 (17%) dogs that had positive reactions to both individual mite extracts, 10 of the 31 (32%) dogs that had a positive reaction to *D farinae* alone, and 1 of the 2 (50%) dogs that had a positive reaction to *D pteronyssinus* alone had a negative reaction to mixed house dust mite extract.

Using responses to the individual house dust mite extracts as the criterion standard, IDT with the house dust extract had sensitivity of 30%, specificity of 93%, positive predictive value of 88%, negative predictive value of 47%, and accuracy of 56% (**Table 2**). Eighteen of the 36 (50%) dogs that had positive reactions to both individual mite extracts, 29 of the 31 (94%) dogs that had a positive reaction to *D farinae* alone, and 1 of the 2 (50%) dogs that had a positive reaction to *D pteronyssinus* alone had a negative reaction to house

Table 1—Cross-tabulation of results of intradermal testing, using extracts of individual house dust mites and a mixed house dust mite extract in 115 dogs suspected to have atopic dermatitis

	Reaction to extracts of individual house dust mites		
Reaction to mixed house dust mite extract*	**Positive†**	**Negative**	**Total**
Positive	52	2	54
Negative	17	44	61
Total	**69**	**46**	**115**

*Concentration of 1:25,000 wt/vol. †Positive reaction to a *Dermatophagoides farinae* extract (1:50,000 wt/vol), a *Dermatophagoides pteronyssinus* extract (1:50,000 wt/vol), or both.

Table 2—Cross-tabulation of results of intradermal testing, using extracts of individual house dust mites and a house dust extract in 115 dogs suspected to have atopic dermatitis

	Reaction to extracts of individual house dust mites		
Reaction to house dust extract*	Positive†	Negative	Total
Positive	21	3	24
Negative	48	43	91
Total	**69**	**46**	**115**

*Concentration of 25 PNU/ml.
See Table 1 for remainder of key.

dust extract.

Discussion

It has recently been recommended that a mixed house dust mite extract with equal parts of *D farinae* and *D pteronyssinus* be used for IDT of dogs suspected to have atopic dermatitis.[8,9] Results of the present study, however, indicated that use of a mixed house dust mite extract had an accuracy of only 83% when reactions to individual house dust mite extracts were used as the criterion standard. Specificity (96%) and predictive value of a positive result (96%) were high, with only 2 of 54 (4%) dogs having false-positive results. However, sensitivity (75%) and predictive value of a negative result (72%) were lower, and 17 of 69 (25%) dogs had false-negative results when the mixed house dust mite extract was used for IDT. Closer examination of the data indicated that sensitivity of using mixed house dust mite extract was better in dogs that had positive reactions to both individual mite extracts (30/36; 83%) than in dogs that had positive reactions to *D farinae* alone (21/31; 68%) or to *D pteronyssinus* alone (1/2; 50%). Thus, in dogs living in environments in which only 1 of the dust mite species is present, the accuracy of using the mixed house dust mite extract for IDT is likely to be less than the 83% found in the present study.

Reasons for the high number of false-negative reactions to the mixed house dust mite extract are not obvious. According to the manufacturer's label,[c] the mix contains equal parts of crude *D farinae* and *D pteronyssinus* extracts. The concentration of the mixed house dust mite extract used for IDT in the present study was 1:25,000 wt/vol, which means that there was a concentration of 1:50,000 wt/vol of each of the individual mite species. This is the same concentration of the individual house dust mite extracts used for IDT. It is known that some allergenic extracts, including house dust mite extracts, contain proteases,[12] and it is possible that proteases in the mixed house dust mite extract degraded some of the allergenic proteins in the extract, altering their allergenicity. However, further study is needed to determine whether this hypothesis is true.

A premise of the use of allergen extracts containing > 1 allergen is that only allergens known to have a high degree of immunologic cross-reactivity in the species tested should be mixed in a single extract.[7] Although cross-reactivity between *D farinae* and *D pteronyssinus* is well established in human patients with atopy,[13,14] similar cross-reactivity has yet to be demonstrated in dogs. In fact, examination of the results of 1 study suggests that there is poor cross-reactivity between these 2 house dust mite species in dogs.[15] Recently, a high molecular weight protein (between 90 and 98 kd) has been reported by 3 groups of investigators as the major allergen of *D farinae* for dogs.[15-17] It has yet to be determined whether the same or a closely related protein is present in *D pteronyssinus*.

In this regard, it is interesting to examine the distribution of reactivity to *D farinae* and *D pteronyssinus* among dogs in the present study. Of the 69 dogs that had positive reactions to the individual house dust mite extracts, 36 had positive reactions to *D farinae* and *D pteronyssinus*, 31 had a positive reaction to *D farinae* alone, and 2 had a positive reaction to *D pteronyssinus* alone. Thus, 67 of the 69 (97%) had positive reactions to *D farinae,* and 38 (55%) had positive reactions to *D pteronyssinus*. This pattern of reactivity may be reflective of exposure to these mites in the environment of the dogs in this study. In a study of mite populations in 19 homes in Cincinnati and Dayton, Ohio,[18] it was found that all 19 homes were infested with *D farinae*, but only 7 were infested with *D pteronyssinus,* and none were infested with *D pteronyssinus* alone. In a study of the prevalence of *Dermatophagoides* spp in 48 homes in Cincinnati,[19] 96% of homes were infested with *D farinae,* and 81% of homes were infested with *D pteronyssinus*, but only 4% were infested with *D pteronyssinus* alone. On the other hand, the pattern of reactivity to these 2 house dust mites among dogs in the present study may be indicative of an increased allergenicity of *D farinae* in dogs so that dogs develop hypersensitivity to this mite more readily than they do to *D pteronyssinus*. Evaluation of house dust mite numbers and concentrations of house dust mite allergens in the microenvironment of dogs included in studies of house dust mite hypersensitivity may be helpful in clarifying the predominance of *D farinae* reactivity. However, we suggest that the reactivity pattern is more likely a reflection of relative exposure to the individual mite species rather than an increased allergenicity of *D farinae* in dogs.

The fact that 36 dogs had positive reactions to both individual house dust mite extracts suggests that there may be a subpopulation of dogs with house dust mite hypersensitivity in which there is immunologic cross-reactivity between these 2 species of house dust mites. However, these 36 dogs represented only 52% of the 69 dogs that had positive reactions to 1 or both of the individual mite extracts, and we do not believe this demonstrates sufficient cross-reactivity to warrant the use of a single extract containing both house dust mite species for IDT.

In the present study, use of the crude house dust extract had an accuracy of only 56% in identifying dogs with hypersensitivity to the individual house dust mites. In particular, there were a large number of false-negative reactions (48 of the 69 dogs that had positive reactions to the individual house dust mites had a negative reaction to the house dust extract), resulting in low sensitivity (30%) and negative predictive value (47%). Closer examination of these results indicated that sensitivity of using the house dust extract was 50%

(18/36) in dogs that had positive reactions to both individual mite extracts, 6% (2/31) in dogs that had positive reactions to *D farinae* alone, and 50% (1/2) in dogs that had positive reactions to *D pteronyssinus* alone. Thus, as for mixed house dust mite extract, the house dust extract had lower sensitivity and negative predictive value in dogs that had positive reactions to *D farinae* alone.

Crude house dust extract is a mixture of many allergens and includes not only house dust mites but also danders, mold spores, other insect allergens, pollens, and nonallergenic proteins.[20,21] This extract is standardized only in regard to the total amount of protein per unit volume, and the relative amount of each individual allergen may vary tremendously. Reactivity to house dust is consequently not specific for any particular allergen, and the amounts of house dust mite allergen in house dust extracts may vary by as much as 4,000%.[20] For this reason, and because of the low accuracy associated with using house dust extract in the present study, we believe that reactivity to crude house dust extracts is of little diagnostic value.

In the study by Codner and Tinker,[8] it was determined that mixed house dust mite extract should be used at a concentration of 1:50,000 wt/vol (equivalent to a concentration of 1:100,000 wt/vol of each of the 2 individual house dust mite species). In the present study, concentrations of *D farinae, D pteronyssinus,* and mixed house dust mite extracts used were double these concentrations. It has been suggested that the threshold concentration of an allergenic extract for use in IDT should be the maximum concentration for which a minimum number of healthy dogs have a positive reaction.[11] However, this suggestion is based on the notion that IDT can be used to distinguish dogs with atopy from dogs without. We believe, on the other hand, that with currently available allergen extracts and knowledge, the true use of any allergy test, including IDT, is to distinguish between animals that have hypersensitivity to a specific allergen and animals that do not have hypersensitivity to that allergen. The clinical importance of IDT reactivity can be assessed only in conjunction with the history and clinical signs and only after other potential causes of the clinical signs have been ruled out.[6,22,23] Studies in humans have shown that up to 40% of the population may have house dust mite hypersensitivity, but that 50% of those with house dust mite hypersensitivity do not have allergic disease.[24-27] If the situation in dogs is similar, then in the study by Codner and Tinker,[8] positive reactions to mixed house dust mite extract at concentrations of 1:10,000 wt/vol and 1:25,000 wt/vol in 5 of 24 (21%) and 4 of 24 (17%), respectively, healthy dogs may have been a reflection of true, albeit subclinical, hypersensitivity to house dust mites.

House dust mite and house dust extracts are potential irritants, as is any allergen extract. There is concern that the use of these extracts at high concentrations will cause nonspecific irritant reactions leading to false-positive IDT results. In a recent report, 5 of 5 laboratory-raised Beagles were found to have negative reactions to intradermal injection of a *D farinae* extract at a concentration of 1:10,000 wt/vol,[28] suggesting that at concentrations even higher than those used in the present study, an irritant effect was unlikely.[28] Threshold concentrations will need to be further evaluated by means of passive cutaneous anaphylaxis testing and determination of the minimal concentration of house dust mite extract that causes irritant reactions in dogs specifically reared in an environment free of house dust mites.

Before an optimal concentration of house dust mite extracts for use in IDT in dogs can be determined, a method of standardizing house dust mite extracts quantitatively and qualitatively, according to the major allergenic peptides of house dust mites for dogs, must be developed.[29] During the present study, more than 1 batch of concentrated allergen extract for each of the mixes and the individual mites was purchased to prepare the dilutions used for IDT. This may have been the cause of some of the differences in skin test reactivity over the course of the study. However, as none of these extracts are standardized for biological potency in dogs, even extracts purchased at the same time and the use of the same concentrated extracts throughout the study would not necessarily have provided a better basis for comparison of IDT reactivity.

Cross-reactivity between *D farinae* and *Sarcoptes scabeii* in dogs and between *Dermatophagoides* spp and *Otodectes cynotis* in cats has been reported.[30,31] Thus, infestation with acarine mites other than *Dermatophagoides* spp at the time of IDT may lead to difficulties in interpretation of positive reactions to *Dermatophagoides* spp. The duration of such cross-reactivity following resolution of parasitic mite infestations and the clinical relevance of this in dogs is still unclear. In the present study, only 4 (3%) dogs were known to have previously been infested with parasitic mites, and with so few dogs, we are unable to draw any conclusions regarding cross-reactivity to other mites.

The success of immunotherapy is dependent on the correct identification of allergens to which the patient is hypersensitive.[6,21,32] Use of mixtures of allergens for IDT that are not highly cross-reactive may create difficulties in interpretation of IDT results and in formulation of vaccines for immunotherapy. If mixed allergenic extracts are used for IDT, false-negative results may be obtained. In the present study, false-negative results were obtained for 25% of the dogs when mixed house dust mite extract was used and for 70% of the dogs when house dust extract was used. Reliance on these results would have led to exclusion of clinically relevant allergens from an immunotherapeutic vaccine. Additionally, false-positive reactions were identified. For instance, 21 of 31 dogs that had positive reactions to *D farinae* alone had positive reactions to the mixed house dust mite extract and would have been interpreted to have been hypersensitive to *D pteronyssinus* as well, leading to inclusion of an irrelevant allergen in an immunotherapeutic vaccine. Consequently, dogs in which IDT is performed with mixed extracts may have a poor response to immunotherapy as a result of exclusion of relevant allergens and may theoretically become sensitized to an irrelevant allergen that has been included in a vaccine.

In conclusion, this study indicates that use of a

mixed house dust mite extract for IDT in dogs is not as accurate a method of determining house dust mite hypersensitivity as is use of individual *D farinae* and *D pteronyssinus* extracts. This is particularly true in dogs that are hypersensitive to only 1 of these 2 house dust mite species. Intradermal test reactions to crude house dust extracts are nonspecific and not clinically useful in detecting hypersensitivity to individual house dust mites in dogs.

[a]B51 *D farinae*, Greer Laboratories, Lenoir, NC.
[b]B58 *D pteronyssinus*, Greer Laboratories, Lenoir, NC.
[c]B060 GS mite mix, Greer Laboratories, Lenoir, NC.
[d]D9 House dust, Greer Laboratories, Lenoir, NC.
[e]07030 Sterile diluent for allergenic extracts, Greer Laboratories, Lenoir, NC.
[f]Histatrol, Center Laboratories, Port Washington, NY.

References

1. Reedy LM, Miller WH, Willemse T. Urticaria, angioedema and atopy. In: Reedy LM, Miller WH, Willemse T, eds. *Allergic skin diseases of dogs and cats*. 2nd ed. Philadelphia: WB Saunders Co, 1997;24–49.

2. Schick RO, Fadok VA. Response of atopic dogs to regional allergens: 268 cases (1981–1984). *J Am Vet Med Assoc* 1986;189: 1493–1496.

3. Bevier DE, Mondesire RL, Rose BJ, et al. FceRIa-based ELISA technology for in vitro determination of allergen-specific IgE in a population of intradermal skin-tested normal and atopic dogs. *Compend Contin Educ Pract Vet* 1997;19:10–16.

4. Carlotti DN, Costargent F. Analysis of positive skin tests in 449 dogs with allergic dermatitis. *Eur J Comp Anim Pract* 1994;4:42–59.

5. Sture GH, Halliwell REW, Thoday KL, et al. Canine atopic disease: the prevalence of positive intradermal skin tests at two sites in the north and south of Great Britain. *Vet Immunol Immunopathol* 1995;44:293–308.

6. Scott DW, Miller WH, Griffin CE. Immunologic skin diseases. In: Scott DW, Miller WH, Griffin CE, eds. *Muller and Kirk's small animal dermatology*. 5th ed. Philadelphia: WB Saunders Co, 1995;484–626.

7. Demoly P, Michel F-B, Bousquet J. In vivo methods for study of allergy: skin tests, techniques and interpretation. In: Middleton E, Reed CE, Ellis EF, et al, eds. *Allergy principles and practice*. 5th ed. St Louis: Mosby Year Book Inc, 1998;430–439.

8. Codner EC, Tinker MK. Reactivity to intradermal injections of extracts of house dust and housedust mite in healthy dogs and dogs suspected of being atopic. *J Am Vet Med Assoc* 1995;206: 812–816.

9. Wellington J, Miller WH, Scarlett JM. Determination of skin threshold concentration of an aqueous house dust mite allergen in normal dogs. *Cornell Vet* 1991;81:37–42.

10. August JR. The reaction of canine skin to the intradermal injection of allergenic extracts. *J Am Anim Hosp Assoc* 1982;18: 157–163.

11. Willemse A, Van Den Brom WE. Evaluation of intradermal allergy test in normal dogs. *Res Vet Sci* 1982;32:57–61.

12. Nelson HS, Ikle D, Buchmeier A. Studies of allergen extract stability: the effects of dilution and mixing. *J Allergy Clin Immunol* 1996;98:382–388.

13. Heymann PW, Chapman MD, Platts-Mills TAE, et al. Antigen Der f 1 from the dust mite *Dermatophagoides farinae*: structural comparison with Der p 1 from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 1986;137:2841–2847.

14. Heymann PW, Chapman MD, Aalberse RC, et al. Antigenic and structural analysis of group II allergens (Der f 2 and Der p 2) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83:1055–1067.

15. Noli C, Bernadina WE, Willemse T. The significance of reactions to purified fractions of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in canine atopic dermatitis. *Vet Immunol Immunopathol* 1996;52:147–157.

16. McCall C, Hunter S, Stedman K, et al. Characterization and cloning of the major house dust mite (*Dermatophagoides farinae*) allergen for dogs, in *Proceedings*. 15th Annu Meet AAVD/ACVD 1999;111–112.

17. Esch RE, Plaster J, Girone C, et al. Isolation and characterization of a major dust mite (*Dermatophagoides farinae*) allergenic fraction of dogs, in *Proceedings*. 13th Annu Meet AAVD/ACVD 1997;87–88.

18. Arlian LG, Bernstein IL, Gallagher JS. The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. *J Allergy Clin Immunol* 1982;69:527–532.

19. Arlian LG, Bernstein D, Bernstein IL, et al. Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. *J Allergy Clin Immunol* 1992;90:292–300.

20. Platts-Mills TAE. Indoor allergens. In: Middleton JR, Reed CE, Elliot EF, et al, eds. *Allergy principles and practice*. 5th ed. St Louis: Mosby Year Book Inc, 1998;393–403.

21. Reedy LM, Miller WH, Willemse T. Immunotherapy. In: Reedy LM, Miller WH, Willemse T, eds. *Allergic skin diseases of dogs and cats*. 2nd ed. Philadelphia: WB Saunders Co, 1997;116–149.

22. Reedy LM, Miller WH, Willemse T. Allergy testing. In: Reedy LM, Miller WH, Willemse T, eds. *Allergic skin diseases of dogs and cats*. 2nd ed. Philadelphia: WB Saunders Co, 1997;83–115.

23. Ownby DR. Clinical significance of immunoglobulin E. In: Middleton JR, Reed CE, Elliot EF, et al, eds. *Allergy principles and practice*. 5th ed. St Louis: Mosby Year Book Inc, 1998:770–782.

24. Peat JK, Tovey E, Toelle BG, et al. House dust mite allergens: A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;153:141–146.

25. Shibasaki M, Tajima K, Morikawa A, et al. Relation between frequency of asthma and IgE antibody levels against *Dermatophagoides farinae* and total serum IgE levels in schoolchildren. *J Allergy Clin Immunol* 1988;82:86–94.

26. Horak F. Manifestation of allergic rhinitis in latent-sensitized patients. *Arch Otorhinolaryngol* 1985;242:239–245.

27. Brown WG, Halonen MJ, Kaltenborn WT, et al. The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 1979;63:328–335.

28. Halliwell REW, Gilbert SM, Lian TM. Induced and spontaneous IgE antibodies to *Dermatophagoides farinae* in dogs and cats: evidence of functional heterogeneity of IgE. *Vet Dermatol* 1998;9:179–184.

29. Dreborg S. Standardization of allergenic preparations by in vitro and in vivo methods. *Allergy* 1993;48(suppl 14):63–70.

30. Prelaud P, Guagere E. Sensitization to the house dust mite, *Dermatophagoides farinae,* in dogs with sarcoptic mange. *Vet Dermatol* 1995;6:205–209.

31. Saridomichelakis MN, Koutinas AF, Gioulekas D, et al. Sensitization to house dust mites in cats with *Otodectes cynotis* infestation. *Vet Dermatol* 1999;10:89–94.

32. Nelson HS. Immunotherapy for inhalant allergens. In: Middleton E, Reed CE, Ellis EF, et al, eds. *Allergy principles and practice*. 5th ed. St Louis: Mosby Year Book Inc, 1998;1050–1062.