

Cross reactivity of airborne allergens based on 1000 intradermal test results

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Objective The purpose of this study was to evaluate concurrent positive reactions of related versus nonrelated allergens based on 1000 intradermal tests of dogs with atopic dermatitis.

Procedure Pairs of closely related allergens (based on botanical relations and results of research in human allergic disease) and nonrelated allergens were evaluated. Significance of the difference between group means of log odds ratios was estimated using a Bootstrap percentile confidence interval.

Results There was a significant difference in the mean log odds ratio between related and nonrelated allergen pairs. However, there was also a significant difference between most groups consisting of nonrelated allergen pairs.

Conclusion These results provide conflicting evidence and emphasise the need for further studies. Numbers of concurrent reactions of related allergens indicate that immunotherapy should be formulated based on testing with single allergens to avoid exposure to allergens not involved in the dog's atopic disease.

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PNU Protein nitrogen units
w/v Weight per volume

Atopic dermatitis is a common disorder in small animal practice and one of the most common allergies in dogs and cats.¹ It is diagnosed by history, clinical examination and ruling out other pruritic skin diseases, such as food adverse reactions and scabies that present with similar clinical signs of facial, pedal, ventral or perianal pruritus or otitis externa.² Symptomatic treatment modalities for atopic dermatitis include glucocorticoids, antihistamines and fatty acid supplementation.^{1,2} Currently, the only specific treatment for the disease is allergen-specific immunotherapy, in which allergens involved in the patient's disease are injected subcutaneously.^{1,2} Intradermal testing has been used in human and veterinary medicine for decades to identify aeroallergens involved in atopic disease and to formulate an extract for allergen specific immunotherapy. Extracts of individual allergens are injected intradermally and the wheal and flare reaction is compared with a negative and a positive control.^{3,4} Recently, serum testing for allergen-specific IgE has become available. Serum testing is more convenient and more readily available to many veterinarians and may also be used as a basis for formulation of treatment extracts. False-positive reactions have been reportedly common,⁵⁻⁷ but evidence for a higher sensitivity of serum testing has been presented.⁸ Reproducibility and correlation with skin testing is variable.⁹⁻¹¹

In human medicine, significant cross reactivity of closely related allergens is observed based on intradermal tests,¹² Prausnitz-Kuestner extinction,¹³ Ouchterlony gel double immunodiffusion¹⁴ and RAST inhibition.^{15,16} Skin and serum testing utilise allergen-mixes of closely related allergens, such as grasses, due to this cross reactivity. In canine atopy, data on cross reactivity has been scarce.¹⁷ Thus, group testing for related allergens in veterinary medicine remains controversial.

The purpose of this study was to evaluate concurrent positive reactions of related versus nonrelated allergens in canine atopy based on 1000 skin tests.

Materials and methods

One thousand dogs were selected for skin testing on the basis of a history and clinical examination consistent with atopic dermatitis. Clinical signs included otitis externa, pedal, facial and ventral pruritus and dermatitis. Concurrent infections with bacteria or yeast organisms were identified cytologically and treated with antimicrobial agents. Differential diagnoses such as food adverse reaction or scabies were ruled out with appropriate tests or trials such as home-cooked elimination diets, skin scrapings and/or therapeutic trials with ivermectin at 300 µg/kg weekly for 4 weeks. Intradermal testing was performed as previously described.³ Individual allergens and a negative and a positive control were injected intradermally with 29 gauge needles. The negative control was the diluent of the allergen extracts, a solution of 0.04% potassium phosphate, 0.11% sodium phosphate, 0.50% sodium chloride and 0.40% phenol (Sterile diluent for allergenic extracts, Greer Laboratories, Lenoir, NC, USA). The positive control was histamine phosphate 0.275 mg/mL (Histatrol®, Center Laboratories, Port Washington, New York 11050, USA). Allergen extracts were obtained from Greer Laboratories (Lenoir, NC, USA). All pollens and mould extracts were used at 500 PNU/mL. House dust mite allergens were diluted to 1:7,500 and 1:75,000 w/v respectively. The skin reactions were graded subjectively from 0 to 4 in comparison to the negative control (graded as 0) and the positive control (graded as 4). Reactions graded as 2,3 or 4 (evaluation A) and 3 or 4 (evaluation B) were considered positive.¹⁻³ Chosen examples of closely related allergens with reported cross-reactivity in human allergic disease were perennial rye grass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*), red top (*Agrostis alba*) and Kentucky blue grass (*Poa pratensis*),^{15,16} short ragweed (*Ambrosia elatior*) and perennial ragweed (*Ambrosia psilostachia*), fat hen (*Chenopodium album*) and Mexican tea (*Chenopodium ambrosioides*),^{15,18} and the house dust mites *Dermatophagoides farinae* and *D pteronyssinus* (group 1). Pine (*Pinus* sp) and melaleuca (*Melaleuca quinquerivra*), pine and birch (*Betula* sp),¹⁵ sweet vernal (*Anthoxanthum odoratum*) and perennial rye grass, couch grass (*Cynodon dactylon*) and perennial rye grass and short ragweed and fat hen^{15,16} were selected as examples of pairs showing less or no cross reactivity in human allergic individuals (group 2). In addition, three groups of nonrelated allergens were evaluated. One group consisted of pairs

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chosen from the different plant groups grasses, weeds and trees. Pairs were Kentucky blue grass and melaleuca, pine and fat hen, paspalum grass and short ragweed, rye grass and Mexican tea and wattle tree and plantain (group 3). The second group was formed with allergens from plant pollens and dust mites, feathers and mould spores, respectively. Birch tree was paired with feathers, *Alternaria* with rye grass, birch with *D farinae*, elm with *D pteronyssinus* and *Cladosporium* with couch grass (group 4). The last group consisted of nonplant-derived, nonrelated allergen pairs with mixed feathers and penicillium, mixed feathers and *D farinae*, mixed feathers and *D pteronyssinus*, *Penicillium* and *D farinae*, and *Penicillium* and *D pteronyssinus* (group 5).

For each pair of allergens considered, the degree of association between the positive responses was estimated using the 'odds ratio'. The odds ratio is the odds of a positive response on allergen 1 among those having a positive response on allergen 2, divided by the odds of a positive response on allergen 1 among those having a negative response on allergen 2. An odds ratio of 1.0 indicates no association; an odds ratio greater than 1.0 indicates positive association; an odds ratio less than 1.0 indicates a negative association. To compare groups the logarithm (base *e*) of each odds ratio was computed and the average of the log odds computed for each group. Taking logarithms makes the responses more normally distributed and reduces the influence on the group average of unusually high values for individual comparisons. Any log odds greater than zero indicates a positive association. Since the logarithm of zero is not defined, pairs of allergens having no concurrent positive reactions were effectively removed from the calculations.¹⁹

Significance of the difference between group averages of log odds ratios was estimated using a 'Bootstrap percentile' confidence interval. Bootstrap replications of size *n* = 1000 were randomly selected, with replacement from the data. The difference between the group averages of log odds ratios was computed for each of the 2000 bootstrap replications. The (p/2) and 1-(p/2) quantiles of the 2000 differences were then used as the upper and lower limit of a two-sided 100% confidence interval. If the value zero was at the edge of a two-sided 100% confidence interval, then the P-value assigned was P. More sophisticated versions of the bootstrap are available, however, this method was judged to be adequate due to the large sample size and the approximate normality of the log odds ratios. A P value of < 0.05 was considered significant.

Results

The frequency of positive reactions to individual allergens is outlined elsewhere.³ Positive reactions and concurrent reactions for the selected allergen pairs are listed in Table 1 for evaluation A

Table 1. Single and concurrent positive reactions of selected allergen pairs with positive defined as a 2, 3 or 4+ reaction

Allergen	Number of positive reactions	Number of concurrent positive reactions	Number of positive reactions	Allergen
Group 1				
Perennial rye	116	51	132	Cocksfoot
Red top	103	59	115	Kentucky blue
Perennial ragweed	101	45	134	Short ragweed
Fat Hen	149	83	156	Mexican tea
<i>D pteronyssinus</i>	138	108	336	<i>D farinae</i>
Group 2				
Couch	113	66	116	Perennial rye
Perennial rye	116	65	163	Sweet vernal
Short Ragweed	134	64	149	Fat hen
Birch	100	27	102	Pine
Pine	102	38	192	Melaleuca
Group 3				
Kentucky blue	115	56	192	Melaleuca
Pine	102	33	149	Fat Hen
Paspalum	112	56	134	Short ragweed
Perennial Rye	116	61	156	Mexican Tea
Wattle	107	50	153	Plantain
Group 4				
Birch	100	16	108	Mixed feathers
<i>Alternaria</i>	40	10	116	Perennial Rye
Birch	100	39	336	<i>D farinae</i>
Elm	126	25	138	<i>D pteronyssinus</i>
<i>Cladosporium</i>	51	6	113	Couch
Group 5				
<i>Penicillium</i>	101	21	108	Mixed feathers
Mixed feathers	108	59	336	<i>D farinae</i>
<i>Penicillium</i>	101	54	336	<i>D farinae</i>
Mixed feathers	108	37	138	<i>D pteronyssinus</i>
<i>Penicillium</i>	101	15	138	<i>D pteronyssinus</i>

and Table 2 for evaluation B. According to both evaluations, the number of positive concurrent reactions between closely related allergen pairs was significantly higher than that of allergen pairs not closely related. There was also a significant increase of concurrent positive reactions of plant-derived allergen pairs not closely related compared to allergen-pairs not exclusively derived from plants. However, with both evaluations there was no significant difference in the number of positive concurrent reactions between plant-derived allergen pairs of nonrelated allergens within the same groups (grasses versus weeds versus trees) and allergen pairs from different plant groups. The P-values of the individual comparisons of the mean log odds ratios are listed in Table 3.

Discussion

Cross-reactivity of IgE antibodies against different allergens in human medicine was initially suspected based on frequent concurrent skin test reactions in allergic individuals to two related allergens.¹² Concurrent positive reactions to two allergens may indicate cross reactivity of allergen-specific IgE antibodies, but may also be due to concurrent true hypersensitivity to two allergens without any cross reactivity. However, a positive reaction to one allergen and a concurrent negative reaction to the other allergen is evidence against cross-reactivity of IgE to these two allergens. Skin test reactivity was used as evidence of IgE cross-

reactivity against conifer pollens in humans.²³ Immunization of rabbits with pollen extracts by these authors and subsequent immunodiffusion with the rabbit antiserum and various antigens showed cross-reacting antibodies to various conifer pollens. More evidence for true cross-reactivity in human medicine was presented using inhibition and absorption studies.^{15,16,18} These studies revealed considerable cross-reactivity between antibodies to perennial rye grass, Kentucky blue grass, cocksfoot and meadow fescue and poor cross-reactivity between that group and IgE against couch as well as sweet vernal grass.¹⁶ A similar report duplicated these results.¹⁵ This particular study also revealed a clear cross-reactivity of IgE antibodies against short, giant, false and western ragweed and a complete lack of allergenic association between a conifer (mountain cedar, *Juniperus sapinoides*) and trees belonging to the angiosperms such as oak (*Quercus alba*), beech (*Fagus grandifolia*) and elm (*Ulmus americana*).¹⁵ More recently a number of major antigens of various grass, weed and tree pollens and house dust mites have been isolated. A detailed review of the literature is beyond the scope of this article, however, what seems to emerge is a concept of some shared antigens between various pollens, particularly grass pollens and, in addition, highly individual antigens. A similar situation seems to be present with various mites - house dust mites as well as parasitic mites.

In veterinary medicine, antigens are not characterised very well and studies evaluating cross-reactivity of allergen-specific IgE indirectly by assessing concurrent intradermal test reactions,²⁴ or directly by inhibition studies, are rare. Noli et al²⁵ showed that dust mite antigens relevant in human allergic disease are not as important in canine atopy. A major allergenic fraction of *Dermatophagoides farinae* in dogs was isolated and characterised recently.²⁶ The two components of this fraction differ from the major characterised antigens of *D farinae* in humans. No such studies have been published in regard to pollen or mould antigens in veterinary medicine to the authors' knowledge.

Allergen-specific immunotherapy is based on results of intradermal testing or serum testing for allergen-specific IgE.^{1,2} If allergen mixes are part of a diagnostic test and a positive reaction is observed to such an allergen mix, all allergens involved are included in the extract used for immunotherapy. A positive reaction to an allergen mix without involvement of all allergens of the mix in the disease process is possible and then leads to administra-

Table 2: Single and concurrent positive reactions of selected allergen pairs with positive defined as a 3 or 4+ reaction

Allergen	Number of positive reactions	Number of concurrent positive reactions	Number of positive reactions	Allergen
Group 1				
Perennial rye	56	44	65	Cocksfoot
Red top	47	40	59	Kentucky blue
Short ragweed	43	25	53	Perennial ragweed
Mexican tea	63	50	85	Fat hen
D pteronyssinus	49	39	178	<i>D farinae</i>
Group 2				
Perennial rye	56	38	65	Couch
Perennial rye	56	45	89	Sweet vernal
Short ragweed	43	34	85	Fat hen
Pine	21	12	51	Birch
Pine	21	13	74	Melaleuca
Group 3				
Kentucky blue	59	30	74	Melaleuca
Pine	21	11	85	Fat Hen
Short ragweed	43	30	69	Paspalum
Perennial Rye	56	37	63	Mexican Tea
Wattle	41	27	59	Plantain
Group 4				
Birch	51	3	11	Mixed feathers
Alternaria	13	3	56	Perennial Rye
Birch	51	18	178	<i>D farinae</i>
Elm	34	6	49	<i>D pteronyssinus</i>
Cladosporium	5	0	45	Couch
Group 5				
Mixed feathers	11	0	29	Penicillium
Mixed feathers	11	4	178	<i>D farinae</i>
Penicillium	29	5	178	<i>D farinae</i>
Penicillium	29	0	49	<i>D pteronyssinus</i>
Mixed feathers	11	5	49	<i>D pteronyssinus</i>

Table 3: P-values for comparisons of the group mean log odds ratios for different groups of allergen pairs (considering 2, 3 and 4 as positive reactions/considering 3 and 4 as positive reactions)

	Group 2	Group 3	Group 4	Group 5
Group 1	< 0.0001*/0.041*	< 0.0001*/< 0.0001*	< 0.0001*/< 0.0001*	< 0.0001*/< 0.0001*
Group 2		0.697/0.129	< 0.0001*/< 0.0001*	< 0.0001*/< 0.0001*
Group 3			< 0.0001*/0.0001*	< 0.0001*/< 0.0001*
Group 4				0.531/0.448

*considered statistically significant

tion of irrelevant allergens to the patient. Administration of subcutaneous injections of allergen extract to normal dogs increased skin reactivity to these allergens,²⁰ however, another study failed to show induction of positive intradermal reactions by immunotherapy with irrelevant allergens.²¹ Similar studies performed in atopic patients are lacking. Dogs with a predisposition to develop atopy have been sensitised by injections of allergens and subsequently developed clinical atopic dermatitis.²² Thus, injections of allergens not involved in a patient's atopic dermatitis may have the potential to induce allergic reactions. The purpose of this study was to evaluate possible allergen cross reactivity in atopic dogs based on 1000 intradermal tests. If two closely related allergens are either both positive or both negative



in any given patient, the two allergens may be used in diagnostic procedures or therapeutic extracts interchangeably or as a mixture of both allergens. Sensitisation of a patient to a previously nonrelevant allergen would not be of concern. The mean log odds ratio of concurrent positive reactions in closely related allergens was significantly different from those of unrelated plant allergens in this study, which may be evidence for cross-reactivity of allergens in canine atopic dermatitis. However, in approximately one in two (evaluation A), or one in three (evaluation B) dogs with positive reactions to an allergen, a concurrent positive reaction to a closely related allergen was not present. Furthermore, there was also a significant difference in the mean log odds ratio of concurrent positive reactions of most other groups. Neither would be expected if the difference in the mean log odds ratio of the nonrelated allergens and related allergens were predominantly due to cross reactivity of allergen-specific IgE. The high number of evaluated skin tests in this study decreased the chance of misleading results.

Evaluation of intradermal skin test reactions with a grading of 0 to 4 in comparison with a positive and negative control is used by most veterinary dermatologists.^{1,2,5,20} It is however a subjective evaluation. Therefore, available data in this study was evaluated twice, once defining any reaction stronger than 1 as positive and then defining only reactions graded as 3 and 4 as positive. Significant differences were either present or absent in both evaluations for all comparisons. This is in contrast to a previously published study evaluating crossreactivity of canine allergen-specific IgE using a smaller number of intradermal tests in a different environment.²⁴ In that study, there was a significant difference between the mean log odds ratio of nonrelated plant allergen pairs from different plant groups (trees versus grasses versus weeds) and those from nonrelated grass, weed or tree pollen allergen pairs with evaluation A. The trend with all comparisons was for a stronger significance of results with lower P-values using evaluation B. One possible explanation given was a decreased ability of shared plant epitopes to cause mast cell degranulation in intradermal tests. Whether this explanation holds true for the allergens used in that study, but does not apply to all allergens in general or particularly for the allergens in the current study, is not clear. Possible further explanations include statistical chance and differences in the way intradermal tests were graded by different dermatologists.

There was a significant difference in the mean log odds ratio of concurrent reactions between plant-derived allergens (related or nonrelated) and mould-derived, dust-mite-derived and animal-derived allergens. This difference could be due to different life styles of the patients, with subsequent exposure to different allergens and development of hypersensitivities against the allergens predominantly exposed to. Unfortunately, information on life style of the 1000 dogs included in the study was not available. It could also indicate a true, although low-grade, cross-reactivity of all plant allergens.

Although these results indicated a lower incidence of cross-reactivity of IgE to environmental allergens in the dog than in humans, they certainly did not rule out such cross reactivity. Further studies using inhibition and absorption techniques are needed to evaluate the degree of cross-reactivity in the dog. Based on the results of this study, mixing of allergen extracts for identifi-

cation of allergens involved in a dog's atopic dermatitis is discouraged and the use of single allergens for diagnostic procedures or therapeutic extracts in canine atopic dermatitis is recommended.

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