

High allergen-specific serum immunoglobulin E levels in nonatopic West Highland white terriers

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dogs. The clinical significance of this and whether it characterizes a protective phenotype is unclear.

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Introduction

Human atopic dermatitis (AD) or eczema is an inflammatory, chronically relapsing, pruritic skin disease, with an increasing prevalence in industrialized countries,¹ which is associated with a systemic tendency to develop IgE-mediated responses to environmental allergens.² Many similarities exist between canine and human AD, at the clinical, immunopathological and therapeutic levels. The use of canine models to study homologous diseases in humans can be a valuable resource, and a model of canine AD has recently been proposed for AD and other atopic disorders in humans.³

Canine AD is described in many breeds, although West Highland white terriers (WHWTs) are highly predisposed, with a reported prevalence of up to 25%.⁴ Atopic dermatitis was originally described as a type I anaphylactic, immediate hypersensitivity reaction, although the pathophysiology is now known to be more complex, involving a variable range of immune cells, cytokines and inflammatory mediators.⁵ It is most commonly, but not always, associated with IgE antibodies to environmental allergens.⁶ Other factors that play a role in development of canine AD include defects in epidermal barrier function and altered innate skin immunity.^{7,8}

Common allergens associated with canine AD throughout the world include house dust and storage mites, epidermal antigens, moulds, and pollens of grass, trees and weeds.⁹ A study of 1000 intradermal tests (IDT) in atopic dogs in eastern Australia determined that house dust mites, *Dermatophagoides farinae* (34%) and *Dermatophagoides pteronyssinus* (14%), were the most common reactive allergens in this region.¹⁰ In this population of dogs, reactivity to outdoor allergens (grass, tree and weed pollens) was generally below 20%, while positive reactions to moulds varied between 4% and 10%.

The diagnosis of AD in dogs is based largely on history and clinical signs, along with exclusion of other possible causes of pruritic skin disease.¹¹ Serological and IDT can provide supportive evidence for a diagnosis of AD, but are preferentially used to identify causative allergens for avoidance and/or immunotherapy in animals with a clinical diagnosis of AD.¹¹

In dogs, IDT is generally reported to be more accurate than serological testing and is considered the gold-standard test for allergen identification in AD.¹² A positive

Abstract

Human and canine atopic dermatitis (AD) share an association with IgE specific to environmental allergens, but few studies have evaluated serum allergen-specific IgE in nonatopic dogs. This study compared serum allergen-specific IgE levels in 30 atopic and 18 nonatopic West Highland white terriers. Atopic dermatitis was confirmed using standard criteria. Nonatopic dogs were over 5 years of age and had no clinical signs or history of AD. Serum allergen-specific IgE levels were measured with Allercept® IgE ELISAs using a 48-allergen Australian panel. Positive reactions were defined as ≥ 150 ELISA absorbance units. Intradermal tests were performed in 16 atopic dogs, either at the time of or at various times prior to serum collection. In atopic dogs, the most common positive ELISA and intradermal test results were to *Dermatophagoides farinae* (11 of 30 dogs), but there were no statistically significant correlations between results from the two methods for any allergen. In nonatopic dogs, multiple high-positive ELISA reactions were reported to 45 of 48 allergens, most commonly *D. farinae* and *Tyrophagus putrescentiae* (17 of 18 dogs each). Positive ELISA results in nonatopic dogs were statistically significantly higher than those in atopic dogs for 44 of 48 allergens, including two allergens (*D. farinae* and *Dermatophagoides pteronyssinus*) commonly regarded as significant in canine AD. In conclusion, positive allergen-specific IgE ELISAs were not specific for canine AD, and high allergen-specific IgE levels were seen in nonatopic

skin test indicates the presence of allergen-specific IgE in skin previously sensitized to that allergen; however, its clinical significance is determined from the patient's history and other regional factors, including seasonal availability of the allergen, timing of the dog's pruritus, previous therapy, and age of the dog.¹²

Alternatively, relative levels of allergen-specific IgE may be measured in the serum. However, several studies have questioned the reliability and reproducibility of some commercial serological allergy tests,^{13–15} specifically the high incidence of false-positive reactions in healthy dogs. In a study with 24 normal dogs and 65 atopic dogs, positive allergen-specific IgE reactions were frequently reported in the normal dogs, including house dust (100%), *D. farinae* (95.8%), flea (91.7%) and *D. pteronyssinus* (83.3%).¹⁶ In that study, the median range of relative antibody units (RAU) to *D. farinae* and *D. pteronyssinus* did not significantly differ between the atopic dogs (171.5 RAU for *D. farinae* and 139.7 RAU for *D. pteronyssinus*) and the normal dogs (126.0 RAU for *D. farinae* and 96.3 RAU for *D. pteronyssinus*). Additionally, specific IgE levels are not always detectable in the sera of some atopic dogs, which could indicate that some cases of canine AD are not IgE mediated (atopic-like dermatitis),⁶ that the allergens are not always clinically relevant¹⁷ and/or that IDTs are more sensitive. The results of serological allergy tests may correlate poorly or only moderately with those of IDTs.¹⁸ However, no significant differences seem to exist in the response of atopic dogs to immunotherapy using either of these two methods.¹⁹ Thus, the significance of circulating allergen-specific IgE in the pathogenesis of AD remains unclear.

An IgE test using the extracellular part of the α -chain of the high-affinity binding mast cell receptor for IgE (Fc ϵ RI α) to detect allergen-specific IgE in serum has been introduced recently in Australia. This test is reported to have improved specificity and sensitivity due to a strong affinity of the receptor to canine IgE and lack of reactivity with

other serum antibodies, such as IgG.²⁰ Additionally, the ability of this assay to detect allergen-specific IgE is reported to be unaffected by total serum IgE levels.²¹ A good level of agreement between IDT results and the Fc ϵ RI-based test has been reported, especially for house dust mites and fleas.²²

A few studies have evaluated the use of the Fc ϵ RI-based anti-IgE enzyme-linked immunosorbent assay (ELISA) test for detection of specific IgE in the serum of atopic dogs.^{20,22,23} However, similar independent studies including a large number of nonatopic dogs have not been reported. Hence, the aim of this study was to evaluate the levels of allergen-specific IgE in atopic and nonatopic WHWT dogs using a commercially available Fc ϵ RI-based anti-IgE ELISA test. A secondary aim was to compare intradermal and allergen-specific IgE serum results, where both tests were performed.

Materials and methods

Selection criteria

The University of Queensland Animal and Human Ethics Committees and University of Sydney Animal Ethics Committee approved this study. The atopic WHWTs participating in this study originated from two sources. Atopic dogs from source A ($n = 17$) were diagnosed with AD by two veterinary dermatologists. These dogs fulfilled at least four criteria for canine AD, adapted from Willemse^{24,25} and Prélard *et al.*²⁶ and at least six of Favrot's diagnostic criteria for canine AD²⁷ (Table 1). Other causes of pruritic skin disease were excluded using appropriate diagnostic tests and/or therapy.

Atopic dogs from source B ($n = 13$) were selected prospectively, based on clinical history and clinical examination by dermatologists. These dogs fulfilled at least three major criteria for canine AD, adapted from Willemse^{24,25} and Prélard *et al.*²⁶ and at least five of Favrot's diagnostic criteria for canine AD²⁷ (Table 1). Primary bacterial, fungal and parasitic skin diseases were excluded in these dogs using appropriate diagnostic tests.

Details of a diet trial were available for 14 of 30 atopic dogs, all from source A; eight of 30 dogs did not respond to the elimination diet and six of 30 had associated cutaneous adverse food reaction or food-induced AD, diagnosed by partial clinical improvement on

Table 1. Diagnostic criteria for selection of the dogs used in the present study

Group of dogs	Diagnostic criteria
Atopic A ($n = 17$)	≥4 diagnostic criteria for canine AD, adapted from Willemse and Prélard*, fulfilled. ≥6 Favrot[†] diagnostic criteria for canine AD fulfilled. Other causes of pruritic skin disease were excluded at the time of presentation.
Atopic B ($n = 13$)	≥3 diagnostic criteria for canine AD, adapted from Willemse and Prélard*, fulfilled. ≥5 Favrot[†] diagnostic criteria for canine AD fulfilled. Other causes of pruritic skin disease had not been excluded at the time of presentation but were subsequently.
Nonatopic ($n = 18$)	Line of WHWTs minimally affected by AD and at least 5 years old. No history or clinical symptoms of AD, pyoderma, otitis, pruritus, foot chewing or face rubbing.
Intermediate ($n = 7$)	WHWTs that did not meet the criteria for inclusion in any of the other three groups. No skin changes consistent with AD, but had: • either one episode of otitis externa and/or pyoderma • no more than occasional mild pruritus on any part of the body.

WHWT, West Highland white terrier.

*Willemse^{24,25} and Prélard *et al.*²⁶ diagnostic criteria for canine atopic dermatitis (AD) include the following six possibilities: individual or family history of AD; onset of symptoms before 3 years of age; history of chronic or chronically relapsing dermatitis; pruritus; distribution of the skin lesions including the feet and metatarsal/metacarpal surfaces, muzzle, periocular area, ventrum, ears, ventral neck and axillae; types of lesions including erythema, papules, pustules, excoriations, epidermal collarettes, complete or partial alopecia, lichenification, hyperpigmentation, seborrhoea; and positive response to specific AD therapy including immunotherapy, systemic and topical medication or allergen-avoidance therapy.

[†]Favrot diagnostic criteria for canine AD²⁷ include the following seven possibilities: onset of signs under 3 years of age; pruritus without lesions at onset; affected front feet; affected ear pinnae; dog living mostly indoors; nonaffected ear margins; nonaffected dorsolumbar area and glucocorticoid-responsive pruritus.

elimination diet and relapse on rechallenge. In these six atopic dogs, hypersensitivity to environmental allergens was demonstrable on their IDTs. Sixteen dogs never had a diet trial. Exclusion of cutaneous adverse food reaction or food-induced AD in these dogs was made by dermatologists based only on exclusion criteria, including seasonality of clinical signs, distribution of lesions, age at onset of clinical signs, response to corticosteroids and absence of GI signs. In the absence of a food trial, the diagnosis of AD in these dogs was done based on current diagnostic criteria.

Fifteen dogs in the nonatopic group ($n = 18$) were selected from one family line of WHWTs with very few individuals affected by AD (Table 1). The onset of clinical signs in atopic dogs is generally before 3 years of age,¹¹ thus all dogs in the nonatopic group were at least 5 years of age. These dogs had no history of signs consistent with AD, including pruritus, foot chewing or face rubbing, pyoderma or aural disease. All dogs in this group underwent thorough clinical examinations by the veterinary dermatologists, and owners completed a questionnaire to carefully screen for previous and/or current skin disease.

A fourth group of dogs did not meet the criteria for inclusion in any of the other groups (i.e. atopic groups A and B, and the nonatopic group) and were classified as the intermediate group ($n = 7$; Table 1). These dogs were over 5 years of age and had no skin changes consistent with AD at clinical examination, but their owners reported either one episode of otitis externa and/or pyoderma, and/or occasional mild pruritus on any part of the body. Five of the dogs in this group were from the same minimally atopic line as the animals in the nonatopic group, and the two other dogs were unrelated to this minimally atopic line for at least five generations.

Twenty-three dogs, including 18 dogs from the nonatopic group and five dogs from the intermediate group, were routinely treated against helminthic and heartworm infections, and had long-term preventative treatment for ectoparasites, including fleas; however, the status of the other 32 WHWTs (30 from the atopic groups A and B, and two from the intermediate group) was uncertain in this regard.

Fifteen of 18 nonatopic dogs and nine of the 30 atopic dogs lived in New South Wales and the remaining dogs lived in Queensland. Thirteen nonatopic dogs lived in a semi-rural area and spent most of the time outdoors or were kept in kennels. Five nonatopic dogs lived inside houses in an urban environment. All dogs in the atopic groups lived inside houses in an urban environment, except for one dog that came from a semi-rural area.

Sample collection and IgE measurement

Two millilitres of blood were collected by cephalic or jugular vein venipuncture from all dogs. Serum samples were obtained from 15 nonatopic dogs in winter and three in spring, while they were obtained from atopic dogs throughout the year.

Serum IgE was measured using an ELISA test that uses the high-affinity mast cell Fc ϵ receptor α -chain protein (Fc ϵ RI α) to detect allergen-specific canine IgE.²⁰ This commercially available IgE Test Panel (ALLERCEPT[®]; Heska Corp., Loveland, CO, USA) was performed by Gribbles Veterinary Pathology (Clayton, Vic., Australia) according to the manufacturer's instructions. The allergen extracts were obtained from Greer Laboratories, Inc. (Lenoir, NC, USA) and included a comprehensive Australian panel of 48 individual allergens (Table 2). The 55 samples were randomly allocated using Excel[®] (Microsoft, Washington, DC, USA) into six batches of five to ten samples. A high-positive control, a low-positive control, a negative control and a blank for each allergen were included in each batch. Adjustments were made for interbatch variation, according to the laboratory's internal quality control procedures.

This semi-quantitative assay was considered positive when the optical density (OD) measured at 405 nm was >150 units. A normalization scheme was used to standardize the results of the assay. The observed optical density values were compared with expected optical density values obtained from a curve that was established from average results of repeated experiments with a pooled positive reference serum standard. The scale was based on sera from dogs known to be highly reactive to allergens, with high levels of allergen-specific

IgE (positive canine serum pool) compared with a pool of sera from young dogs having no history of allergy and negative IDT (negative canine serum pool). The normalized results were expressed in ELISA absorbance units (EA units).

According to the manufacturer, the level of canine IgE antibodies in the serum detected by the Fc ϵ RI α -receptor is directly correlated with the OD measured at 450 nm. In addition, this detection is dose dependent, as shown by measuring the OD at 450 nm of two dilutions (1:10 and 1:100) of positive and negative canine serum.²⁸ Thus, we assumed that EA units reported in the IgE Test Panel were directly correlated with allergen-specific IgE levels in the serum of the participating dogs.

Intradermal testing

Sixteen dogs in the atopic group A underwent both intradermal testing and determination of serum allergen-specific IgE via the Allercept[®] assay (11 dogs in Queensland and five dogs in New South Wales). In two of 16 dogs, IDT and serum testing were performed at the same time. In the remaining 14 dogs, IDT testing was performed 2 months (two dogs), 1–2 years (four dogs), or >2 years (eight dogs) prior to serum testing. Fifteen of 16 dogs from group A that had an IDT received allergen-specific immunotherapy. Three received immunotherapy for <1 year prior to sampling. Twelve dogs completed courses 1–3 years (five dogs), 3–5 years (four dogs) or >5 years (three dogs) prior to the study. The duration of immunotherapy was 12–18 months.

Eleven atopic dogs, nine from group A ($n = 17$) and two from group B ($n = 13$), had received immunosuppressive medications, either at the time of the study (seven of 11), 3–4 months (two of 11) or 1–2 months (two of 11) prior to the serum collection. In four dogs, these included a combination of prednisolone (5 mg) and chlorphenamine maleate (2 mg; Niralone[®]; Mavlab, Logan City, Qld, Australia). Three atopic dogs received prednisolone (0.5 mg/kg daily or every other day), and the remaining four were receiving ciclosporin (1–5 mg/kg daily) at the time of the study.

The panel of allergens used for IDT was similar in Queensland and New South Wales (Table 2). Aqueous solutions of allergen extracts from a commercial company (Greer Laboratories) were prepared and used according to the manufacturer's instructions. Diluent control (Greer Laboratories) was used as a negative control, and 0.275 mg/mL (active histamine base, 0.1 mg/mL) histamine phosphate (Greer Laboratories) was used as a positive control. Prior to the IDT, the dogs were sedated with either xylazine (Rompun[®]; Bayer Australia Ltd, Bentley, WA, Australia) for dogs tested in Queensland, or medetomidine (Domitor[®]; Novartis Animal Health Australasia North Ryde, NSW, Australia), for dogs tested in New South Wales. Approximately 0.05 mL of each allergen and control solutions were injected intradermally, with allergen concentrations recorded in Table 2. If medetomidine was used, atipamezole (Antisedan[®]; Novartis Animal Health Australasia) was injected intramuscularly or intravenously to reverse sedation at the end of the skin testing. Skin test reactions were evaluated after 15–20 min and graded from 0 to 4, with 4 having a wheal the same size/turgor/degree of erythema as that of the positive control. Only reactions graded 2+ and stronger were considered positive.¹⁰

Analysis

Data were entered in an Excel[®] spreadsheet. All statistical analyses were carried out within the R statistical computing environment.²⁹ ELISA absorbance units were log transformed for statistical analysis and tested for effects of key covariates, such as clinical diagnosis, age and sex of the animal. Statistical analysis included comparison of the specific IgE levels (measured in EA units) between different clinical groups, using linear (ANOVA) and nonparametric tests (Mann–Whitney *U*-test). Additionally, the effect of previous or concurrent treatment, prior food trial, age and time from onset of clinical signs on the specific IgE levels was investigated using ANOVA and linear regression methods. Multiple-comparison adjustment was applied using the Bonferroni or the Benjamini and Hochberg correction for a level of statistical significance of $P < 0.05$.

Table 2. Allergens (Greer Laboratories, Inc., Lenoir, NC, USA) used in this study for intradermal testing and serum allergen-specific IgE testing

Allergen	ALLERCEPT®	Intradermal test (allergen concentration)
Grass pollens		
Paspalum, <i>Paspalum notatum</i>	Yes	Yes (1000 PNU/mL **†)
Couch (Bermuda grass) <i>Cynodon dactylon</i>	Yes	Yes (1000 PNU/mL **†)
Brome grass, <i>Bromus inermis</i>	Yes	Yes (1000 PNU/mL **†)
Canary grass, <i>Phalaris arundinacea</i>	Yes	Yes (1000 PNU/mL **†)
Sweet vernal, <i>Anthoxanthum odoratum</i>	Yes	Yes (1000 PNU/mL **†)
Kentucky blue grass, <i>Poa pratensis</i>	Yes	Yes (1000 PNU/mL **†)
Cocksfoot grass, <i>Dactylis glomerata</i>	Yes	Yes (1000 PNU/mL **†)
Bent grass (red top), <i>Agrostis alba</i>	Yes	Yes (1000 PNU/mL **†)
Timothy grass, <i>Phleum pratense</i>	Yes	Yes (1000 PNU/mL **†)
Perennial rye grass, <i>Lolium perenne</i>	Yes	No
Yorkshire fog (velvet grass), <i>Holcus lanatus</i>	Yes	No
Johnson grass, <i>Sorghum halepense</i>	Yes	Yes (1000 PNU/mL **†)
Oat, <i>Avena sativa</i>	No	Yes (1000 PNU/mL **†)
Wheat, <i>Triticum aestivum</i>	No	Yes (1000 PNU/mL **†)
Perennial rye grass, <i>Lolium perenne</i>	No	Yes (1000 PNU/mL **†)
Red clover, <i>Trifolium pratense</i>	No	Yes (1000 PNU/mL *)
English couch, <i>Elytrigia repens</i>	No	Yes (1000 PNU/mL **†)
Corn, <i>Zea mays</i>	No	Yes (1000 PNU/mL *)
Yorkshire fog (velvet grass), <i>Holcus lanatus</i>	No	Yes (1000 PNU/mL **†)
Tree pollens		
Silver poplar, <i>Populus alba</i>	Yes	Yes (1000 PNU/mL †)
Liquidambar, <i>Liquidambar styraciflua</i>	Yes	Yes (1000 PNU/mL †)
Plane tree (sycamore), <i>Platanus recemosa</i>	Yes	Yes (1000 PNU/mL **†)
Privet, <i>Ligustrum vulgare</i>	Yes	Yes (1000 PNU/mL **†)
Peppercorn, <i>Schinus</i> spp.	Yes	Yes (1000 PNU/mL **†)
Cypress, <i>Cupressus</i> spp.	Yes	Yes (1000 PNU/mL †)
Black willow, <i>Salix nigra</i>	Yes	Yes (1000 PNU/mL **†)
Pine mix, <i>Pinus</i> spp.	Yes	Yes (1000 PNU/mL **†)
Melaleuca, <i>Malaleuca quinquenervia</i>	Yes	Yes (1000 PNU/mL **†)
Grey birch, <i>Betula populifolia</i>	Yes	Yes (1000 PNU/mL <i>Betula</i> spp. **†)
Red oak, <i>Quercus rubra</i>	Yes	Yes (1000 PNU/mL **†)
Eucalyptus, <i>Eucalyptus globulus</i>	Yes	Yes (1000 PNU/mL **†)
Casuarina, <i>Casuarina equisetifolia</i>	No	Yes (1000 PNU/mL **†)
Maple, <i>Acer</i> spp.	No	Yes (1000 PNU/mL †)
Willow arroyo, <i>Salix lasiolepis</i>	No	Yes (1000 PNU/mL *)
Elm, <i>Ulmus</i> spp.	No	Yes (1000 PNU/mL **†)
Wattle, <i>Acacia</i> spp.	No	Yes (1000 PNU/mL **†)
Palm, <i>Arecastrum romansoffianum</i>	No	Yes (1000 PNU/mL **†)
Western juniper, <i>Juniperus occidentalis</i>	No	Yes (1000 PNU/mL **†)
Casuarina, <i>Casuarina equisetifolia</i>	Yes	No
Wattle, <i>Acacia</i> spp.	Yes	No
Weed/cultivated pollens		
Curled dock, <i>Rumex crispus</i>	Yes	Yes (1000 PNU/mL **†)
Sheep sorrel, <i>Rumex acetosella</i>	Yes	Yes (1000 PNU/mL **†)
Rough pigweed, <i>Amaranthus retroflexus</i>	Yes	Yes (1000 PNU/mL **†)
Lamb's Quarter (Fat Hen), <i>Chenopodium album</i>	Yes	Yes (1000 PNU/mL **†)
English plantain, <i>Plantago lanceolata</i>	Yes	Yes (1000 PNU/mL **†)
Perennial ragweed, <i>Ambrosia psilostachya</i>	Yes	Yes (1000 PNU/mL **†)
Dandelion, <i>Taraxacum officinale</i>	Yes	Yes (1000 PNU/mL **†)
Daisy, <i>Chrysanthemum leucanthemum</i>	Yes	Yes (1000 PNU/mL **†)
Elm, <i>Ulmus</i> spp.	Yes	No
Maple, <i>Acer</i> spp.	Yes	No
Mugwort, <i>Artemisia vulgaris</i>	Yes	No
Mustard weed, <i>Brassica rapa</i>	Yes	Yes (1000 PNU/mL †)
Mexican tea, <i>Chenopodium ambrosioides</i>	No	Yes (1000 PNU/mL †)
Dog fennel, <i>Eupatorium capillifolium</i>	No	Yes (1000 PNU/mL **†)
Moulds		
Alternaria, <i>Alternaria tenuis</i>	Yes	Yes (1000 PNU/mL **†)
Aspergillus, <i>Aspergillus niger</i>	Yes	Yes (1000 PNU/mL **†)
Cladosporium, <i>Cladosporium herbarum</i>	Yes	Yes (1000 PNU/mL **†)
Penicillium, <i>Penicillium notatum</i>	Yes	Yes (1000 PNU/mL **†)
Insects		
Flea, <i>Ctenocephalides</i> spp.	Yes	Yes (1000 PNU/mL **†)
Ant, <i>Camponotus pennsylvanicus</i>	Yes	Yes (1000 PNU/mL **†)
Cockroach, <i>Periplaneta americana</i>	Yes	Yes (1000 PNU/mL **†)
Mosquito, <i>Culicidae</i> spp.	No	Yes (1000 PNU/mL **†)
Moth, <i>Heterocera</i> spp.	No	Yes (1000 PNU/mL *)

Table 2. Continued

Allergen	ALLERCEPT®	Intradermal test (allergen concentration)
Horse fly, <i>Tabanus</i> spp.	No	Yes (1000 PNU/mL**†)
House fly, <i>Musca domestica</i>	No	Yes (1000 PNU/mL*)
House dust mites		
<i>Dermatophagoides pteronyssinus</i>	Yes	Yes [1:4000, 1:10 000 and 1:25 000 (w/v)**†]
<i>Dermatophagoides farinae</i>	Yes	Yes [1:4000, 1:10 000 and 1:25 000 (w/v)**†]
Storage mites		
<i>Tyrophagus putrescentiae</i>	Yes	Yes [1:4000, 1:10 000 and 1:25 000 (w/v)**†]
<i>Acarus siro</i>	No	Yes (1000 PNU/mL**†)
<i>Lepidoglyphus destructor</i>	No	Yes (1000 PNU/mL**†)
<i>Blomia tropicalis</i>	No	Yes (1000 PNU/mL**†)

PNU, protein nitrogen units; w/v, weight/volume.

*Eleven West Highland white terriers (WHWTs) with atopic dermatitis (AD) diagnosed by veterinary dermatologist in Queensland.

†Five WHWTs with AD diagnosed by veterinary dermatologist in New South Wales.

Results

Dogs in the study

A total of 55 WHWTs entered this study. Thirty dogs were considered atopic: 17 from group A (nine females, mean age 5.7 years) and 13 from group B (seven females, mean age 6.5 years). Eighteen dogs were considered nonatopic (15 females, mean age 7.6 years). Seven dogs were in the intermediate group (six females, mean age 8.2 years). The nonatopic dogs ($n = 18$) were more likely to be female (83%) and were slightly older (mean age 7.6 years) than the atopic dogs ($n = 30$; 53% females, mean age 6.1 years), although these differences were not statistically significant.

Allergen-specific IgE serology in all four groups of dogs enrolled in the study

From the total of 55 dogs that entered this study, 43 of 55 (78%) dogs showed positive serology (EA > 150) to at least one tested allergen, as follows: 18 of 18 (100%) dogs in the nonatopic group ($n = 18$), three of seven (43%) dogs in the intermediate group ($n = 7$), ten of 17 (59%) dogs in the atopic group A ($n = 17$) and 12 of 13 (92%) in the atopic group B ($n = 13$). Overall, the most prevalent positive reactions were reported against *D. farinae* (31 of 55 or 56% dogs with positive serology), followed by *Tyrophagus putrescentiae* (26 of 55 or 47% dogs). Within each clinical group, *D. farinae* remained as the most prevalent allergen, with 17 of 18 (94%) dogs in the nonatopic group, three of seven (43%) dogs in the intermediate group, four of 17 (24%) dogs in the atopic group A and three of 13 (23%) dogs in the atopic group B reacting positively to this allergen. The median value for *D. farinae*-specific IgE levels in nonatopic dogs (774 EA units; range 145–3458 EA units) was nine times higher than in atopic dogs (82 EA units; range 0–3650 EA units), and this difference was statistically significant (Mann–Whitney U -test, $P = 1.6 \times 10^{-4}$).

Allergen-specific IgE serology in two combined groups of dogs enrolled in the study

When comparing the specific log IgE levels between the different clinical groups, we found that these were significantly higher in the nonatopic ($n = 18$) than in the other three groups for 45 of 48 allergens, and this effect remained significant for 44 of 48 allergens after adjusting for age and sex (best ANOVA adjusted for sex and age

$P = 3.3 \times 10^{-6}$; highest ANOVA adjusted $P = 0.9$). However, no significant differences were detected in the log IgE levels between the two atopic groups (A and B). Furthermore, log IgE levels in the intermediate group seemed to lie between the nonatopic and the atopic groups. Testing the differences in the means between the different clinical groups showed that the nonatopic group ($n = 18$) was significantly different from the atopic group A ($n = 17$, Tukey's HSD, $P = 2.9 \times 10^{-6}$), the atopic group B ($n = 13$, $P = 0.024$) and the intermediate group ($n = 7$, $P = 0.001$). Thus, to simplify the analysis and increase the power of the study, atopic dogs from groups A and B were combined for subsequent statistical tests into a single atopic group ($n = 30$). The seven dogs from the intermediate group were removed from the following comparisons because they presented characteristics intermediate between the other groups.

Statistical results for Fat Hen log-specific IgE were the most significantly increased in nonatopic dogs ($n = 18$) compared with atopic dogs ($n = 30$), with ANOVA unadjusted for covariates $P = 4.4 \times 10^{-7}$ and adjusted for age and sex $P = 1.4 \times 10^{-6}$. These results were followed by *T. putrescentiae* IgE levels (ANOVA adjusted $P = 1.5 \times 10^{-6}$). The difference between these two groups was also significant for the house dust mites, *D. farinae* ($P = 1.5 \times 10^{-4}$) and *D. pteronyssinus* ($P = 0.001$). The same pattern but with relatively higher P -values was observed for 40 remaining allergens. Figure 1 plots results for relevant allergens (*D. farinae*, *D. pteronyssinus* and Fat Hen), while Figure 2 shows detailed results for *D. farinae* allergen testing.

We evaluated the effect of previous or concurrent therapy on the IgE testing of atopic dogs. We found that specific IgE levels were not significantly different between the atopic dogs from group A that were on previous or current treatment (including immunotherapy, corticosteroids and/or ciclosporin, $n = 9$) and the atopic dogs from group B, which did not have any therapy prior to IgE testing ($n = 11$), for any of the 48 allergens (best ANOVA corrected $P = 0.2$).

We also compared the specific IgE levels in the atopic dogs with a negative response to an elimination diet ($n = 8$), the atopic dogs with a partial response ($n = 6$) and the atopic dogs with an unknown response to a diet trial ($n = 16$). We found that there were no differences between these groups for any of the tested allergens (best ANOVA corrected $P = 0.1$).

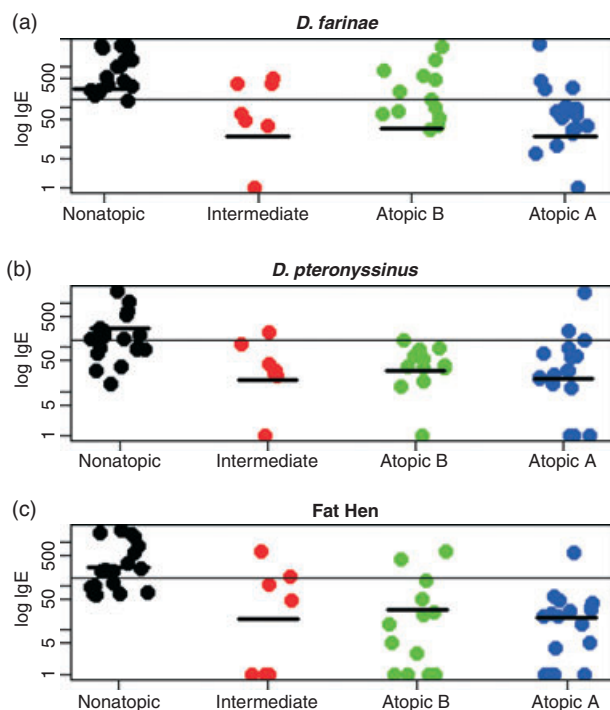


Figure 1. Plots of the ELISA absorbance log-transformed levels for *Dermatophagoides farinae* (a), *Dermatophagoides pteronyssinus* (b) and Fat Hen (c), adjusted for age and sex, versus clinical diagnosis for atopic dermatitis. The bold bars indicate median values.

Additionally, age and time from onset of clinical signs did not significantly influence log IgE specific levels in atopic dogs ($n = 30$). These two predictors accounted for minimal variance in the IgE levels ($r^2 = -0.098$), which was not significant ($F_{2,15} = 0.244$) at $P = 0.786$. Under this model, age ($\beta = 0.058$, $P = 0.699$) was a more influential, albeit nonsignificant, predictor than time from onset of clinical signs ($\beta = -0.009$, $P = 0.964$).

Intradermal testing

When comparing positive reactions on IDT and serum allergen-specific IgE testing, none of the correlations between the results from the two methods for any allergen was statistically significantly different from zero (maximal Kendall τ for any allergen $n = 0.32$). Similarly, considering only the presence of at least one positive reaction to any allergen in both panels (i.e. whether a dog was sensitized to at least one allergen in the panel), there was no significant evidence of agreement (Cohen's $\kappa = -0.31$, $P = 0.18$), with six concordant diagnoses and ten discordant diagnoses.

Discussion

In this study, serum allergen-specific IgE levels were significantly higher for 44 of 48 allergens in the nonatopic group ($n = 18$) than in both groups of atopic WHWTs (group A, $n = 17$; and group B, $n = 13$), and this effect remained significant after adjusting for age, sex and previous or concurrent therapy. This was in contrast to the findings in atopic WHWTs, which had comparatively low allergen-specific IgE levels in the serum, except for

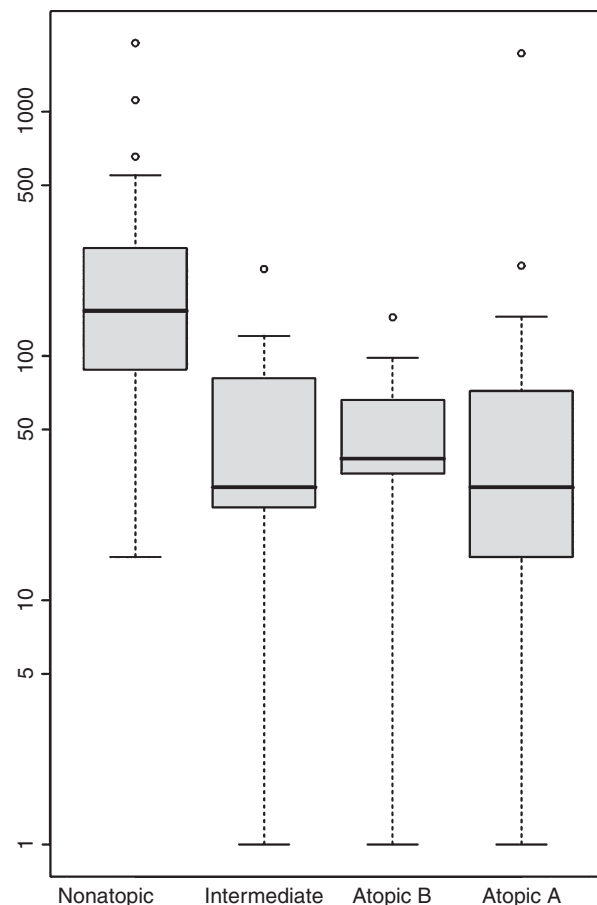


Figure 2. Boxplot of *Dermatophagoides farinae* ELISA absorbance log-transformed levels versus clinical diagnosis for atopic dermatitis. The bold bars indicate median values, and the upper and lower limits of the boxes are the 75th and 25th percentiles, respectively, with the bars extending up to 1.5 times the interquartile range (the hinges).

increased reactivity to common allergens, especially house dust mites.

Other studies have reported significant levels of allergen-specific IgE in clinically normal dogs against different allergens.^{13,16,30,31} However, in previous studies, specific IgE levels reported in normal dogs against a relevant allergen (*D. farinae*) were not statistically significantly different from those reported in atopic dogs (median 126.0 RAU in normal dogs versus 171.5 RAU in atopic dogs, Mann-Whitney U -test $P \geq 0.05$).¹⁶ In the present study, the median for *D. farinae*-specific IgE levels (represented by EA units) in nonatopic dogs (774 EA units) was nine times that reported in atopic dogs (82 EA units), and this difference was statistically significant.

The levels of allergen-specific IgE detected by serological tests may be affected by several factors, including total IgE levels in the serum, the season in which the test was performed, age of the patient, geographic and husbandry factors and previous medication, specifically immunotherapy and corticosteroid administration.¹⁸ The IgE test used in this study is reported to be unaffected by the total serum IgE in the sample,²¹ as well as lacking reactivity with other serum antibodies, such as IgG.²⁰ Furthermore, a study with nonatopic dogs of different breeds found no correlation between total IgE serum

concentrations and the number of allergen-positive results obtained in a specific ELISA IgE test.³¹ In dogs, unlike humans, total IgE levels cannot be used for the diagnosis of atopic disease, because no significant differences exist in total IgE levels between healthy and affected dogs, possibly due to the high frequency of parasitic infections in dogs.³²

In the present study, serum samples were collected from the majority of nonatopic dogs in winter, whereas they were collected from atopic dogs throughout the year. In humans, season is unlikely to cause false-positive reactions in allergen-specific IgE tests;³³ however, mixed results have been reported in dogs.^{34,35} Exposure to indoor allergens, such as house dust mites, which are commonly associated with canine AD, is usually perennial. Pollination periods for some plants in Australia, especially weeds and grasses, can occur all year round,¹⁰ although levels are generally lower in winter. Thus, seasonal variations are unlikely to explain the differences in IgE levels in atopic and nonatopic WHWTs in this study.

Age and sex were also unlikely to explain the differences in allergen-specific IgE between atopic and nonatopic WHWTs in this study, because results were adjusted for these variables. Total serum IgE may be affected by sex and age in dogs. An inverse relationship between total IgE and age is reported in atopic dogs³⁶ and mixed results in nonatopic dogs,^{31,37} and higher total IgE is reported in female nonatopic dogs than males.³⁷ The effect, if any, of age or sex on allergen-specific IgE levels in dogs is unknown. In humans, however, specific IgE levels are typically higher in children and young adults compared with infants,³³ and total and specific IgE levels against house dust mites may be higher in males than females.³⁸

As production of allergen-specific IgE in dogs appears to be dependent on the allergen exposure, especially in the first few years of life,³⁹ differences in geography and husbandry between groups could have resulted in varying allergen exposure. The majority of nonatopic dogs lived in New South Wales, while the majority of atopic dogs lived in Queensland. More than half of the nonatopic dogs lived as a group in a semi-rural area, and spent most of the time outdoors or were kept in kennels, while most of the atopic dogs lived inside houses in an urban environment. While this could explain the high exposure to pollens in the nonatopic group and higher titres of IgE antibodies for these allergens, it does not explain the dogs' lack of clinical signs. The nonatopic dogs also had very high levels of specific IgE to dust house mites and other indoor allergens, although these may have been prevalent in the kennel environment.

In humans, several studies suggest that long-term helminth infection (associated with persistent immunological challenge) could modulate the susceptibility to allergy.⁴⁰ Total IgE levels are relatively higher in children from developing countries compared with industrialized regions; however, the prevalence of atopic disease is relatively lower in the former.⁴⁰ A possible explanation for this difference could be a variation in the exposure to infectious and parasitic agents in different geographic areas. In this study, however, parasitism does not explain the lack of clinical signs of AD in the nonatopic WHWTs, because the majority of these dogs had excellent preven-

tative treatment for internal and external parasites. Additionally, no association is reported between the incidence of false-positive reactions in allergy tests and parasitism in dogs,¹³ and a recent epidemiological study using WHWTs failed to show an association between routine deworming treatment and development of AD.⁴¹ Vaccination is also unlikely to have contributed to the findings of this study because, while it may increase the total IgE levels detected in the serum, it should not significantly affect the specific IgE values other than for the target antigen.⁴²

A possible explanation for the higher allergen-specific IgE levels observed in the nonatopic WHWTs in this study is the possibility that treatment of the atopic WHWTs resulted in reduced allergen-specific IgE. Mixed results have been reported in specific IgE levels in response to the influence of corticosteroids and other immunosuppressive drug administration.¹⁸ Allergen-specific immunotherapy can significantly decrease serum IgE levels for specific allergens in atopic dogs by increasing the production of immunosuppressive cytokines and IgG antibodies that restrict the production of both total and allergen-specific IgE, although WHWTs may be less sensitive to this effect than other breeds.⁴³ This is also reported in human allergic patients up to 3–5 years postimmunotherapy.³³ There were, however, no significant differences in IgE levels between the treated and untreated atopic dogs.

In this study, the presence of cutaneous adverse food reactions was excluded in eight of 30 atopic dogs. In six of the remaining 22 atopic dogs, results from an elimination diet showed that, in these dogs, the clinical signs of AD were also associated with food allergens, suggesting that they had food-induced AD.⁴⁴ However, in 16 dogs with a clinical diagnosis of AD, details of an elimination diet were not available. Thus, failure to detect IgE-mediated reactivity to environmental allergens in these dogs could be explained by the presence of cutaneous adverse food reactions, a clinically indistinguishable entity that may be associated with AD.⁴⁴ In contrast to this, however, six of fourteen atopic dogs tested had only a partial response to an elimination diet. This suggests it is unlikely that all 16 atopic dogs which did not have an elimination diet trial would have had solely diet-related skin disease. Furthermore, differences in the IgE levels between the atopic dogs with partly food-induced AD and the dogs in which cutaneous food reactions were excluded as a cause of their AD were not statistically significant for any of the tested allergens.

The low prevalence of positive reactions to environmental allergens in the atopic group may also be explained by low levels of allergen-specific IgE antibodies in the serum of dogs with atopic-like dermatitis. This form of disease, where IgE-mediated hypersensitivity cannot be documented, may be present in dogs with a definite clinical diagnosis of AD that failed to show positive results in the IgE test.⁶ Alternatively, IgG molecules rather than IgE may predominate in the serum of some atopic dogs with chronic skin lesions and concurrent secondary *Malassezia* infection.⁴⁵ These IgG antibodies, while not detected by the specific FcεRI receptor of the IgE test, could play a role in the pathogenesis of AD in these dogs.

Serum samples for the FcεRI-based anti-IgE ELISA test were not run in duplicate, so intrasample variability could not be assessed in the analysis. The use of EA units as an indirect measurement of allergen-specific IgE levels, while supported by the existence of a dose-dependent binding of the FcεRI receptor to canine IgE antibodies, was only used as a reference, and it does not represent quantitative allergen-specific IgE values. Furthermore, positive reactions in the IgE test recorded in nonatopic dogs may have been caused by reactivity to irrelevant epitopes (antigenic determinants) of some allergens, although the manufacturer states that the incidence is under 10%. A few independent studies have concluded that the FcεRI-based anti-IgE ELISA test is reliable.^{22,23} However, the need for independent quality assurance programmes for validation of commercial serological tests was also emphasized in these studies.

The finding of significantly higher levels of allergen-specific IgE in nonatopic WHWTs compared with atopic WHWTs in this study was unexpected. The ability to produce high IgE levels in response to different allergens has been suggested to be inherited in a dominant manner in atopic dogs.⁴⁶ However, this predisposition is not always associated with clinical disease, and it seems to be dependent on environmental triggers, such as early and repetitive allergen challenge, skin barrier defects and other genetic factors.³⁹ In addition, there may be functional heterogeneity of the IgE antibodies.³⁰ Alternatively, any component of a molecular pathway directly or indirectly involved with IgE expression and/or regulation, including antigen processing and presentation, T cell or B cell receptor signalling, and FcεRI signalling, may lead to changes in the cellular response to the IgE molecule or variations in mediator releasability.⁴⁷ Thus, these nonatopic WHWTs may have important variations in immunological pathways involved in the atopic phenotype. It is unclear whether the high IgE levels represent a protective phenotype or whether the absence of clinical AD is associated with other factors, e.g. an intact skin barrier or tolerance to environmental allergens.

There was poor agreement between the allergen-specific serology and the IDTs. It is difficult to interpret the significance of this because the tests were performed at different times. The differences could therefore be due to the effect of allergen-specific immunotherapy and/or other medications, or simply the time between the two tests, which was >1 year for 75% of the tested dogs. For example, IDT results can differ with season and the age of the dog, especially for very young animals.¹² Seasonal variability appears to affect specific serum IgE levels more significantly than skin reactivity in dogs, as artificially sensitized dogs maintain their skin hypersensitivity for many months even in the absence of allergen exposure.⁴⁸ However, concurrent IDTs and serology on the same patient have also been reported to differ.¹⁸ Similar discrepancies have been reported using the same FcεRI-based assay for 72% of the tested allergens.²² This may be due to the tests assessing different pools of antibodies in different tissues and/or differences in sensitivity.²¹ Furthermore, different antigens were used in both tests in this study; a flea saliva extract in the ELISA test (to which 23% of the atopic dogs reacted) and a whole flea

extract used in the IDT (to which none of these dogs reacted). Finally, although regarded as the 'gold standard' for allergy testing in dogs, false-positive and -negative reactions can also occur in IDT.¹²

In conclusion, this study found that related nonatopic WHWTs had significantly higher levels of serum allergen-specific IgE compared with atopic WHWTs, although the clinical significance of this and whether it represents a protective phenotype is unclear.

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Résumé La dermatite atopique canine et humaine (AD) est associée avec des IgE spécifiques d'allergènes environnementaux mais peu d'études ont porté sur les IgE sériques spécifiques d'allergènes chez les chiens non-atopiques. Cette étude compare les taux d'IgE sériques spécifiques d'allergènes chez 30 atopiques et 18 non-atopiques West Highland white terriers (WHWTs). L'AD a été confirmée par des critères standard. Les chiens non-atopiques avaient plus de cinq ans et ne présentaient aucun signe clinique ou anamnestique de AD. Les taux d'IgE sériques spécifiques d'allergènes ont été mesurés par une gamme australienne de 48 allergènes (Allercept® IgE ELISAs). Une réaction ELISA ≥150 unités d'absorbance (EA) était considérée comme positive. Des tests intradermiques ont été réalisés chez 16 chiens atopiques, antérieurement ou au moment du prélèvement de sérum. Chez les chiens atopiques les tests ELISA et intradermiques les plus souvent positifs étaient ceux à *Dermatophagoides farinae* (11 sur 30 chiens) mais il n'y avait aucune différence statistique significative entre les résultats des deux méthodes pour aucun des allergènes. Chez les chiens non-atopiques, de multiples réactions ELISA positives élevées ont été rapportées pour 45 des 48 allergènes; le plus souvent pour *D. farinae* et *Tyrophagus putrescentiae* (17 sur 18 chiens chacun). Les résultats ELISA positifs chez les chiens non-atopiques étaient statistiquement

significativement plus élevés que ceux des chiens atopiques pour 44 des 48 allergènes, incluant *D. farinae* et *Dermatophagoides pteronyssinus*, les deux allergènes les plus fréquemment associés à la dermatite atopique canine. En conclusion, les tests ELISA positifs n'étaient pas spécifiques pour la dermatite atopique canine et des taux élevés d'IgE spécifiques d'allergènes étaient obtenus chez les chiens non-atopiques. La signification clinique de ces résultats et si ils caractérisent un phénotype particulier n'est pas clair.

Resumen La dermatitis atópica humana y canina (AD) comparten una asociación con IgE específica a alérgenos ambientales, pero hay pocos estudios que hayan evaluado la presencia de IgE específicas de alérgeno en perros no atópicos. Este estudio compara los niveles de IgE específicas de alérgeno en 30 perros atópicos y 18 perros no atópicos de raza West Highland White Terrier (WHWT). AD se confirmó usando los criterios estándar. Los perros no atópicos eran mayores de cinco años y no presentaban signos clínicos o historia de AD. Los niveles de IgE específicas de alérgeno se midieron con pruebas de ELISA Allercept® utilizando 48 alérgenos del panel Australiano. Las reacciones positivas se definieron como ≥ 150 unidades de absorción de ELISA (EA). Las pruebas intradermales se hicieron en 16 perros atópicos, al tiempo de colección de suero o en varios momentos antes de la toma de suero. En perros atópicos los resultados positivos mas frecuentes por ELISA y por pruebas intradermales fueron a *Dermatophagoides farinae* (11 de 30 perros) pero no hubo correlación estadística entre los resultados de los dos métodos para ninguno de los alérgenos. En perros no atópicos se obtuvieron muchas reacciones positivas con alto título de ELISA para 45 de los 48 alérgenos; principalmente *D. farinae* y *Tyrophagus putrescentiae* (17 de 18 perros cada uno). Los resultados positivos de ELISA en perros no atópicos fueron significativamente mas altos que los de perros atópicos para 44 de los 48 alérgenos, incluyendo dos alérgenos (*D. farinae* y *D. pteronyssinus*) que generalmente se consideran importantes en AD. En conclusión, las pruebas de ELISA positivas para IgE específicas de alérgenos no fueron exclusivas de AD y niveles altos de IgE se detectaron en perros no atópicos. La significación clínica de este resultado o si caracteriza un fenotipo protectivo no esta clara.

Zusammenfassung Die atopische Dermatitis (AD) beim Menschen und beim Hund teilen die Assoziierung mit IgE, welches spezifisch auf Umweltallergene gebildet wird, aber wenige Studien haben bisher Allergen-spezifisches IgE im Serum bei nicht-atopischen Hunden beurteilt. In dieser Studie wurden Allergen-spezifische IgE-Werte im Serum von 30 atopischen und von 18 nicht-atopischen West Highland White Terriern (WHWTs) verglichen. Die AD wurde mittels Standardkriterien bestätigt. Die nicht-atopischen Hunde waren über 5 Jahre alt und hatten keine klinischen Anzeichen oder eine Anamnese von AD. Die Allergen-spezifischen IgE-Werte im Serum wurden mit Hilfe von Allercept® IgE ELISAs, die ein Panel von 48 australischen Allergenen verwendeten, gemessen. Positive Reaktionen wurden definiert als ≥ 150 ELISA Absorptionseinheiten (EA). Bei 16 atopischen Hunden wurden Intradermaltests entweder zum Zeitpunkt der Serumentnahme oder zu verschiedenen Zeitpunkten vor der Serumentnahme durchgeführt. Bei atopischen Hunden waren die häufigsten positiven ELISA und Intradermaltestergebnisse zu *Dermatophagoides farinae* (11 von 30 Hunden), aber es bestand für kein Allergen eine statistisch signifikante Korrelation zwischen den Ergebnissen beider Untersuchungsmethoden. Bei nicht atopischen Hunden wurden bei 45 von 48 Allergenen multiple hoch positive ELISA Reaktionen beschrieben; am häufigsten *D. farinae* und *Tyrophagus putrescentiae* (bei jeweils 17 von 18 Hunden). Positive ELISA Ergebnisse waren bei nicht atopischen Hunden bei 44 von 48 Allergenen statistisch signifikant höher als jene bei atopischen Hunden. Diese Allergene beinhalteten zwei Allergene (*D. farinae* und *Dermatophagoides pteronyssinus*), die häufig als bedeuend bei caniner AD betrachtet werden. Zusammenfassend waren positive Allergen-spezifische IgE ELISAs nicht spezifisch für canine AD und es wurden auch bei nicht atopischen Hunden hohe Allergen-spezifische IgE Werte gefunden. Die klinische Signifikanz dieser Befunde und ob es sich dabei um die Charakteristika eines schützenden Phänotyps handelt sind noch unklar.

要約 ヒトと犬のアトピー性皮膚炎 (AD) は環境アレルゲンに対する特異的 IgE が病態に関連するという点で共通しているが、しかし血清アレルゲン特異的 IgE を非アトピー犬で評価している研究はほとんどない。この研究では血清アレルゲン特異的 IgE レベルを 30 頭のアトピーと 18 頭の非アトピーであるウェストハイランドホワイトテリア (WHWTs) で比較した。AD は一般的な基準を用いて裏付けた。非アトピー犬は 5 歳以上で AD の病歴や臨床症状を示さない犬とした。血清アレルゲン特異的 IgE レベルは Allercept® IgE ELISA にてオーストラリアパネルの 48 アレルゲンを測定した。陽性は ≥ 150 ELISA 吸光度 (EA) 単位と定義した。16 頭のアトピー犬で血清採取前または複数回、皮内反応試験を実施した。アトピー犬では *Dermatophagoides farinae* (30 頭中 11 頭) が最も ELISA と皮内反応で最も多く陽性がみられたが、いずれのアレルゲンに対しても 2 つの統計学的手法を用いても有意な相関は認められなかった。非アトピー犬では 48 アレルゲン中 45 で複数の高い ELISA 陽性反応が見られ；最も多かったのは *D. farinae* と *Tyrophagus putrescentiae* (それぞれ 18 頭中 17 頭) であった。非アトピー犬における ELISA 陽性の結果は、一般的に犬 AD で重要と評価される 2 つのアレルゲンを含む 48 アレルゲン中 44 アレルゲンに対して統計学的にアトピー犬より高かった。結論として、アレルゲン特異的 IgE ELISA 反応が陽性であるということは犬 AD に特異的ではなく、高 IgE レベルは非アトピー犬で認められた。臨床的な重要性あるいはこの結果が新しい表現型を示すかどうかは不明である。