To cite this article, please use DOI: 10.1111/j.1365-3164.2009.00840.x

1.6

Levels of house dust mite-specific serum immunoglobulin E (IgE) in different cat populations using a monoclonal based anti-IgE enzyme-linked immunosorbent assay

Jennifer Bexley*, Janice E. Hogg*, Bruce Hammerberg† and Richard E.W. Halliwell‡

*YorkTest Veterinary Services, York Science Park, York, UK
†College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
‡Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK
Correspondence: Jennifer Bexley, Avacta Animal Health, Biocentre, York Science Park, Heslington, York, YO10 5NY, UK.
Email: jennifer.bexley@avacta.com

Source of Funding
YorkTest Veterinary Services, York, UK.
Conflict of Interest
J. Bexley and J.E. Hogg are employees of YorkTest Veterinary Services, York, UK. R.E.W. Halliwell is a consultant for YorkTest Veterinary Services. B. Hammerberg and North Carolina State University own the rights to the antibody used in this assay.

Abstract

Levels of serum immunoglobulin E (IgE) specific for the house dust mites (HDMs) *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP) in 58 cats with clinical signs suggestive of atopic dermatitis (allergic dermatitis cats), 52 cats with no history of allergic or immunological disease (nonallergic cats) and 26 specific pathogen-free (SPF) cats were measured using a monoclonal anti-IgE enzyme-linked immunosorbent assay. Reactivity to both native and reduced HDM allergens was compared. SPF cats had significantly lower levels of HDM-specific serum IgE than cats with allergic dermatitis and nonallergic cats. The difference in levels of HDM-specific IgE in the serum of cats with allergic dermatitis and nonallergic cats was significant for native DF allergen, but not for native DP allergen or reduced HDM allergens. The results suggest that DF in its native form may be a significant allergen in cats with allergic dermatitis. The clinical relevance of these reactions, however, remains to be proven.

Accepted 27 July 2009

Introduction

Allergic skin diseases are reportedly common in cats. Feline atopic dermatitis (AD) is thought to exist, although the exact incidence is unknown,[1,2] and the constellation of clinical signs that constitute 'feline AD' is a subject of debate. Whilst canine AD has been recently redefined as 'a genetically predisposed inflammatory and pruritic skin disease with characteristic clinical features associated with immunoglobulin E (IgE) antibodies, most commonly directed against environmental allergens',[3] no such precise definition exists for feline AD. Indeed, apart from one report of disease in three members of a family of cats,[4] a genetic predisposition has not been documented. The finding of Langerhans cell hyperplasia,[5] increased numbers of CD4+ and CD8+ cells[6] and IL-4 production in the lesional skin of affected cats[7] suggests that the canine and feline diseases may have the same underlying immunopathogenesis. Furthermore, atopy patch tests undertaken in affected cats reveal a high degree of specificity for the implicated allergen, and an inflammatory infiltrate that is immunophenotypically similar to that seen in canine AD.[8,9] However, similar to findings in canine AD, the cytokine mRNA expression is not necessarily characteristic of a Th2 immune response.[10,11]

The spectrum of clinical signs that have been associated with the disease includes miliary dermatitis, self-induced ('barbered') alopecia and all components of the eosinophilic granuloma complex.[12] A significant proportion of cats referred for specialist evaluation with a presumptive diagnosis of psychogenic alopecia, furthermore, have been found to suffer from an adverse food reaction, AD or both.[13] In addition, there is circumstantial evidence that some cases of feline asthma have an allergic aetiology, which may therefore also be a manifestation of atopic disease.[12]

The majority of cases diagnosed as feline AD appear to be associated with IgE antibodies to environmental allergens,[14,15] and, although no placebo-controlled studies have been undertaken, the condition appears to respond to allergen-specific immunotherapy with a success rate similar to that recorded for canine AD.[12,16,17] Few studies on the prevalence of allergen-specific IgE have been undertaken in different cat populations, possibly because reagents specific for feline IgE have only

recently become available. Intradermal tests (IDTs) are useful in some animal species, but the very thin skin and poor wheal development limit their use in cats, although the use of fluorescein may aid identification of positive reactions.[14] Polyclonal antisera specific for feline IgE have been produced,[18] and a test utilizing the cloned α-chain of the human high-affinity IgE receptor (FcεRIα) is commercially available for the detection of feline allergen-specific IgE.[19]

The house dust mites (HDMs), *Dermatophagoides farinae* (DF) and *Dermatophagoides pteronyssinus* (DP) are thought to be major allergens in the pathogenesis of both canine and feline AD,[8,20,21] but published studies utilizing polyclonal anti-feline IgE and human (FcεRIα) have not demonstrated significant differences in allergen-specific IgE levels in sera from supposed atopic and healthy cats.[19,22] A role for HDMs in human AD has been more directly demonstrated using a double-blind controlled trial to investigate the effect of dust mite allergen avoidance on disease severity.[23] Similar studies have not been reported in cats.

The cloning of the feline IgE epsilon heavy chain revealed many similarities to its canine homologue.[24] The recent production of a monoclonal antibody (mAb) specific for a heat-stable, papain-degradable IgE epsilon chain epitope of canine IgE,[25] which was found to be highly conserved at similar areas on feline IgE, enabled the development of an enzyme-linked immunosorbent assay (ELISA) for the measurement of allergen-specific IgE in feline serum.

To further investigate the role of HDMs in feline allergic dermatitis, the above assay was used to measure levels of HDM-specific IgE in sera from a large population of cats with allergic dermatitis, from nonallergic cats, and from specific pathogen-free (SPF) healthy cats who were unlikely to have been exposed to HDMs. In addition, as it is known that dust mite allergens are liable to degradation, and that reduction of allergens may reveal epitopes that alter the frequency of positive reactions,[26,27] the antibody response both to native and reduced HDM allergens was compared.

Materials and methods

Animals

Allergic dermatitis cats

This group comprised 58 cats aged between 5 and 168 months (median 60 months), whose sera had been submitted to YorkTest Veterinary Services, York, UK for serological testing for allergen-specific IgE. Cats in this group were selected by one of the investigators (REWH) following scrutiny of the accompanying history form and were suffering from dermatological signs compatible with feline AD including miliary dermatitis, self-induced ('barbered') alopecia, head and neck pruritus and components of the eosinophilic granuloma complex. Cases that had any present or past history of flea infestation, or that had shown any response to ectoparasiticidal therapy were excluded, although the possibility of flea allergy dermatitis was not always ruled-out by repeated and exhaustive parasiticidal therapy. Similarly, the possibility of an adverse food reaction had not always been ruled-out by repeated hypoallergenic dietary trials. Twenty-three cats in this group were neutered females, six were entire females, 26 were neutered males, and three were entire males.

Nonallergic cats

Surplus serum was used from 52 cats aged between 6 and 204 months (median 66 months) that had been presented to the Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK with clinical signs that were not suggestive of allergic disease of any type. Cases were excluded that had any indication of neoplasia, or that had a history of gastrointestinal or respiratory disease. The ages of this group were not significantly different from those of the allergic dermatitis group ($P = 0.272$; Mann–Whitney *U*-test). Seventeen cats were neutered females, two were entire females, 29 were neutered males, and four were entire males. Reported symptomatology or diagnoses included hyperthyroidism, diabetes mellitus, cystitis, hypertension, anorexia and pancreatitis.

SPF cats

Serum was obtained from 26 healthy, laboratory-bred cats that were reared under SPF conditions at Charles River Laboratories, Co Mayo, Ireland. The cats were aged between 6 and 93 months (median 52.5 months). The SPF cats were found to be significantly younger than the nonallergic group but not significantly different from the allergic dermatitis group ($P = 0.01$ and 0.07 respectively; Mann–Whitney *U*-test). Eleven cats were entire females, and 15 were entire males.

Allergen extracts

Dermatophagoides farinae and DP allergen extracts (both 20 000 protein nitrogen units [PNU]/mL in phenol-saline preservative) were purchased from Greer Laboratories, Lenoir, NC, USA. The allergens were reduced by the addition of 2-mercaptoethanol at 5% (v/v) and heating at 100 °C for 5 min. The reduced extracts were then dialysed against 0.15 M phosphate buffered saline (PBS) pH 7.4.

ELISA

Unless otherwise indicated, all reagents were obtained from Sigma-Aldrich Co, Gillingham, Dorset, UK. Microtitre plates (Greiner Labortechnik, Frickenhausen, Germany) were coated with 100 µL/well each of native or reduced DF and native or reduced DP extracts diluted 1/50 in 0.05 M carbonate/bicarbonate buffer pH 9.6 at 4 °C overnight. Plates were then washed three times with 300 µL/well PBS containing 0.05% (v/v) Tween-20 (PBST) using an automatic plate washer (Bio-Tek Instruments, Winooski, VT, USA). Nonspecific binding sites were blocked with 300 µL/well of PBS containing 0.5% (w/v) polyvinylpyrrolidone (PVP10) and 0.5% (w/v) sucrose (blocking buffer) at room temperature for 2 h. Excess blocking buffer was removed and plates were dried at 37 °C. Plates were sealed in foil pouches with desiccant, and stored at 4 °C for 1 week prior to use. Cat serum samples were diluted 1/10 in PBST and 100 µL was added to duplicate wells. Each plate contained a six-point standard curve prepared by assaying serial twofold dilutions of a cat serum sample with high levels of DF- and DP-specific IgE, as determined by previous ELISA analysis. Undiluted, this serum was assigned a value of 2000 arbitrary units (AU). A negative control comprising serum from a cat with no detectable DF- or DP-specific IgE was included on each plate. Plates were then sealed and incubated at 4 °C overnight. After washing as before, 100 µL/well of biotinylated mAb (clone 5.91) diluted 1/2000 in PBST (0.5 µg/mL) was added and plates incubated at room temperature for 2 h. Plates were washed as before, and 100 µL/well of peroxidase-labelled streptavidin [Kirkegaard and Perry Laboratories (KPL), Gaithersburg, MD, USA] diluted 1/20 000 in PBST added. Plates were then incubated at room temperature for a further 1 h, followed by three washes as before. Plates were developed with 100 µL/well of 2,2'-azino-di (3-ethyl-benzthiazoline-6-suphonate) (ABTS) peroxidase substrate (KPL) at 4 °C overnight. Absorbances were determined at 405 nm using an ELISA plate reader (Molecular Devices Co, Sunnyvale, CA, USA). After subtraction of the mean blank (un-coated) well optical density (OD) from each value, mean standard OD values were fitted to a four-parameter standard curve using SOFTmax Pro ver.3 software (Molecular Devices Co). Levels of HDM-specific IgE in feline sera were determined from their mean duplicate OD values by extrapolation from the resulting standard curve.

Reproducibility

The mean inter-assay coefficient of variation (CV) was calculated from eight replicates of a positive sample on six separate days. The mean intra-assay CV was determined from eight replicates of a positive sample run on four plates on the same day.

Statistical analysis

As the data were not normally distributed, nonparametric Kruskal–Wallis analysis was used to compare levels of HDM-specific serum IgE in the three groups of cats. Mann–Whitney *U*-tests were used to compare levels of HDM-specific serum IgE between each group (SPSS 15.0 for Windows; SPSS Inc., Chicago, IL, USA). Results were considered significant when the *P*-value was <0.05.

Results

Specificity of mAb 5.91

Enzyme-linked immunosorbent assay assays were undertaken prior to this study to assess the specificity of the mAb for IgE. Wells were coated with 125 μg each of canine IgE, feline IgM, feline IgA and feline IgG. Before subtracting background, ODs were 0.470 for canine IgE, 0.029 for feline IgM, 0.015 for feline IgA, and 0.009 for feline IgG. IgE specificity was therefore confirmed by both strong signal towards canine IgE (against which the mAb was raised) and a lack of binding to other feline antibody isotypes.

Reproducibility

The mean intra-assay CV was 5.5%; the mean inter-assay CV was 9.4%.

Standardization

Standard curves were produced on each ELISA plate by fitting the standard OD data to a four-parameter model using SOFTmax Pro ver.3; the correlation coefficient (R^2) was always between 1 and 0.999.

DF- and DP-specific IgE levels

There were significant differences ($P < 0.01$; Kruskal–Wallis) in levels of native and reduced DF- and DP-specific serum IgE between the three groups of cats. Median levels of HDM-specific serum IgE antibodies in each of the three groups of cats are shown in Table 1.

Using native antigen

Overall, the SPF group had the lowest number of cats with measurable IgE antibody to HDM allergens. DF-specific serum IgE was detected in only two out of 26 SPF cats (7.7%), whilst three sera (11.5%) from this group had measurable DP-specific IgE. Of the 58 cats with allergic dermatitis, DF-specific serum IgE was detected in 36 (62.1%), and DP-specific serum IgE was detected in 30 (51.7%). In the nonallergic group, DF-specific serum IgE was detected in 22 of the 52 cats (42.3%), and DP-specific serum IgE was detected in 29 (55.8%).

Specific pathogen-free cats had significantly lower levels of DF- and DP-specific serum IgE ($P < 0.01$; Mann–Whitney *U*-test) than both the allergic dermatitis group and the nonallergic group. Levels of DP-specific IgE in the sera of the allergic cats were not found to be significantly different from the nonallergic cats ($P = 0.658$; Mann–Whitney *U*-test) (Figure 1).

Table 1. Median and range of HDM-specific serum IgE in SPF cats, cats with allergic dermatitis, and nonallergic cats (data given as AUs); and percentage (%) of cats in each group with measureable anti-HDM serum IgE

	SPF (n = 26)	Allergic dermatitis (n = 58)	Nonallergic (n = 52)
Native DF-specific IgE			
Median	0	10.3	0
Range	0–15	0–576	0–49
%	7.7	61.2	42.3
Native DP-specific IgE			
Median	0	2.2	1.5
Range	0–13	0–187	0–30
%	11.5	51.7	55.8
Reduced DF-specific IgE			
Median	0	5.1	4.2
Range	0–9	0–519	0–101
%	11.5	63.8	65.4
Reduced DP-specific IgE			
Median	0	4.2	8
Range	0–8	0–182	0–91
%	15.4	63.8	61.5

HDM, house dust mite; DF, *Dermatophagoides farinae*; DP, *Dermatophagoides pteronyssinus*; SPF, specific pathogen-free.

Using reduced (denatured) antigen

Three sera from the 26 SPF cats (11.5%) had demonstrable IgE against reduced DF, and four sera (15.4%) had IgE antibodies against reduced DP. Sera from 37 of 58 in the allergic dermatitis group (63.8%) showed positive reactions to both reduced DF and DP allergens. Of the 52 nonallergic cats, 34 (65.4%) had measurable serum IgE antibodies to reduced DF, whereas 32 (61.5%) had serum IgE antibodies to reduced DP.

Levels of reduced DF- and DP-specific IgE were significantly lower ($P < 0.01$; Mann–Whitney *U*-test) in the sera of SPF cats than both the allergic dermatitis group and the nonallergic cats. Levels of reduced DF- and DP-specific IgE in allergic dermatitis group were, however, not found to be significantly different from the nonallergic cats ($P = 0.804$ for reduced DF, and $P = 0.807$ for reduced DP; Mann–Whitney *U*-test).

Discussion

This study measured levels of HDM-specific IgE in the serum of cats with allergic dermatitis, nonallergic cats and SPF cats using a monoclonal anti-IgE ELISA. Whilst previous studies failed to demonstrate any significant relationship between levels of HDM-specific serum IgE and feline allergic skin disease,[19,22] the results of this study showed that cats with allergic dermatitis had significantly greater levels of native DF-specific serum IgE antibody than nonallergic cats living in a household environment and laboratory-reared SPF cats.

The groups were matched with respect to age, although there was some difference in the sex ratios, with greater numbers of females in the allergic dermatitis group. In addition, the SPF cats were all intact whereas the great majority in the other two groups were neutered. However, it is highly unlikely that this would have affected the results, as no influence of neutering on IgE production has been found in any species.

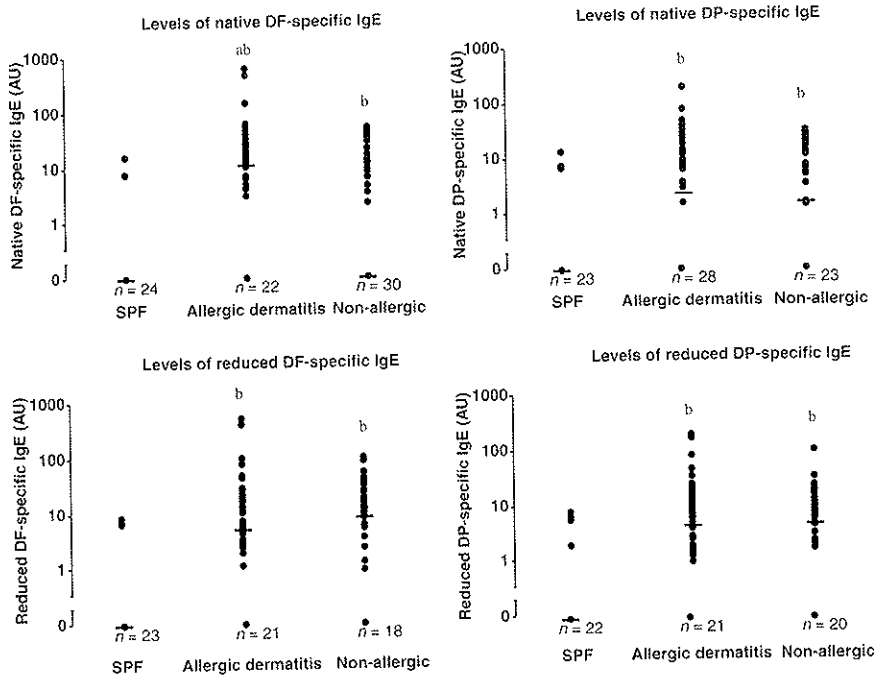

Figure 1. Levels of serum IgE against native and reduced house dust mite (HDM) in specific pathogen-free (SPF) (n = 26), cats with allergic dermatitis (n = 58) and nonallergic cats (n = 52). The median value of each group is represented by a horizontal bar. Cats with no HDM-specific serum IgE (negatives) are represented by a single dot at zero with the actual number of negatives in each group (n) indicated below. a, Significantly greater than the levels in the nonallergic group ($P < 0.03$; Mann–Whitney U-test). b, Significantly greater than the levels in the SPF group ($P < 0.01$; Mann–Whitney U-test).

The allergic dermatitis group were generally found to react more strongly to native DF than native DP, although this difference was not significant ($P = 0.697$; Mann–Whitney U-test) and differences were not evident in median responses to reduced allergens and in the other groups. The same finding has been reported in atopic dogs,[28–30] but this apparent bias toward DF is notably different from human AD, where DP reactivities predominate.[31] DP is the most prevalent species of HDM in the UK,[32,33] and why the response in human AD appears to mirror exposure, whilst that in dogs and cats does not is unclear, but may relate to recognition of cross-reacting epitopes between HDM and storage mites or the relative abundance of reactive allergens in commercial HDM extracts.[34] A greater percentage of the allergic dermatitis group reacted to reduced DF and DP than their native allergen counterparts, although reactions were generally weaker towards the reduced DF allergen than native DF. Moreover, as measurement of serum IgE to reduced DF allergen did not differentiate cats with allergic dermatitis from nonallergic cats, the use of reduced HDM allergens, in this instance, seemed less discriminatory. Such findings suggest that whilst denaturation of the DF allergen may have made some epitopes more accessible, it also appears to have reduced binding to other, possibly more significant epitopes, resulting in an overall reduction in the detection of anti-DF serum IgE in the allergic dermatitis group. A similar situation has been reported in mite-allergic humans, where disruption of the disulphide bonds in one of the major HDM antigens, *Der p 2*, resulted in up to a 100-fold reduction in skin test reactivity.[35]

Over one-third of the cats with allergic dermatitis had no detectable serum IgE antibody towards native DF, and nearly half had no measurable native DP-specific serum IgE. The diagnosis of AD is difficult and, as in dogs, is primarily based upon exclusion of other possible causes of the presenting signs. Although careful analysis of the history forms was made to exclude other causes – particularly FAD – it is possible that some cats were not suffering from AD or indeed from allergic disease. It is also possible that the assay was not sufficiently sensitive to detect low levels of HDM-specific IgE that might be clinically relevant, or that the disease resulted from reactivity against dietary or other environmental allergens. Levels of blocking IgG may also have been higher in these samples. IgG auto anti-IgE antibodies have been demonstrated in dogs, where they form a complex with IgE.[25] Circulating IgE immune complexes can interfere with IgE analysis, particularly in monoclonal-based assays, where they may mask the epitope recognised by the mAb.[36] A more accurate determination of HDM-specific IgE could, therefore, have been obtained by using a mixture of mAbs with different IgE epitope specificities. Unfortunately, the lack of such antibodies makes this difficult to investigate. Cats may also suffer from non-IgE mediated AD. In human AD there are two, clinically indistinguishable forms of AD: intrinsic and extrinsic.[37] Cases with intrinsic AD (approximately 30% of cases) have normal IgE levels, no allergen-specific IgE, are IDT-negative and have low IL-4 levels. Extrinsic AD, in contrast, is associated with high levels of total IgE and allergen-specific IgE, is IDT-positive and has high IL-4 levels.[38] A similar situation appears to exist in canine AD, where the term 'atopic-like dermatitis' has been introduced to describe dogs with clinical signs typical of AD but no demonstrable allergen-specific IgE.[3]

The incidence of positive reactions to DF and DP in sera from nonallergic cats and some SPF cats, as well as from the allergic dermatitis group is consistent with previous studies in both cats[19,22] and dogs,[20] although the reasons for this are unclear. Synthesis of IgE is normally tightly controlled, with production regulated via feedback mechanisms involving the low-affinity IgE Fc receptor, FcεRII (also known as CD23). However, there is increasing evidence to suggest that cleavage of CD23 from the surface of IgE-secreting B cells by dust mite proteases disables the negative feedback system, resulting in excessive

production of IgE.[39] Moreover, following proteolytic cleavage, soluble CD23 (sCD23) fragments released into the extracellular environment are believed to up-regulate IgE synthesis in an autocrine manner.[40] Thus, measurable levels of HDM-specific serum IgE in nonallergic subjects may be the result of biochemical activity by the dust mites themselves. It has also been shown that allergen-specific IgE can be experimentally induced in normal cats.[22] However, despite having demonstrable serum IgE, not all of the sensitized cats developed positive skin test reactivity. Furthermore, only sera from the IDT-positive cats resulted in positive Prausnitz–Küstner (PK) tests.[41] Such findings could be taken as evidence of IgE heterogeneity in cats.

Subclasses of IgE antibodies could differ in terms of their ability to respond to cytokines known as histamine releasing factors (HRFs). HRFs only induce basophil histamine release in a specific, IgE-dependent fashion when bound to a suitable type of IgE.[42] Other subclasses of IgE may bind to basophils, but are unable to induce degranulation as readily. Moreover, it has been found that basophils from atopic dogs have a greater tendency to release histamine than those of normal and artificially sensitized dogs, irrespective of the concentration of total serum IgE or antigen-specific IgE.[43] Thus, one of the key differences that separate atopic and nonallergic populations may not be the production of IgE antibodies *per se*, but the ability of IgE antibodies in atopic individuals to interact with HRF to induce activation of basophils. Whether such findings hold true in atopic cats has yet to be determined. An alternative explanation for the presence of HDM-specific IgE antibodies in the serum of some nonallergic cats could be the existence of a subclinical form of AD. In this case, anti-HDM serum IgE may serve as an important marker of the disease in cats that otherwise appear clinically normal or present with clinical signs not normally associated with AD.

The presence of anti-HDM IgE antibody in the sera of some of the SPF cats was unexpected, as these cats have, presumably, never had any exposure to HDMs. Levels of both DF- and DP-specific serum IgE in this group were, however, significantly lower than in both the cats with allergic dermatitis and nonallergic cats. One explanation for the presence of HDM-specific serum IgE in these cats may be their diet; the SPF cats were fed a pelleted dry diet. Dried food can harbour the storage mites *Acarus siro* and *Tyrophagus putrescentiae*,[44] and cross-inhibition studies have shown that DF strongly cross-reacts with DP, *A. siro* and *T. putrescentiae*.[34] The food fed to the SPF cats was, however, pasteurized so contamination would have been unlikely (although no analysis of the food was conducted in this study, and storage mites could have been present in the food prior to pasteurisation). It is therefore possible that these represent false-positive reactions, as suggested in a recent study using an Fcε receptor-based assay.[45]

In conclusion, this study has shown that cats with allergic dermatitis have significantly higher DF-specific IgE titres than nonallergic or SPF cats. However, the clinical importance of this finding is limited by the presence of HDM-specific serum IgE in nonallergic cats and the absence of HDM-specific serum IgE in some cats with allergic dermatitis. It would, therefore, not be appropriate to make a diagnosis of AD on the results of serological measurement alone. Nonetheless, such tests may help to identify allergens for avoidance and immunotherapy. The clinical relevance of these allergens and therapies in feline skin disease remains to be proven.

Acknowledgements

Grateful thanks are due to Elspeth Milne and her colleagues in the Clinical Pathology Laboratory, Royal (Dick) School of Veterinary Studies, The University of Edinburgh for provision of the sera from the nonallergic cats; and to Geoffrey Hardman for the statistical analysis.

References

1. O'Dair H, Markwell PJ, Maskell IE. An open investigation into the etiology in a group of cats with suspected allergic skin disease. Veterinary Dermatology 1996; 7: 193–202.
2. Prost C. Diagnosis of feline allergic diseases: a study of 90 cats. In: Kwochka KW, Willemse T, von Tscharner C, eds. Advances in Veterinary Dermatology III, Oxford, Butterworth Heinemann, 1998: 516–7.
3. Halliwell R. Revised nomenclature for veterinary allergy. Veterinary Immunology and Immunopathology 2006; 114: 207–8.
4. Moriello KA. Feline atopy in three littermates. Veterinary Dermatology 2001; 12: 177–81.
5. Roosje PJ, Whitaker-Menezes D, Goldschmidt MH *et al.* Feline atopic dermatitis. A model for Langerhans cell participation in disease pathogenesis. American Journal of Pathology 1997; 151: 927–32.
6. Roosje PJ, van Kooten PJ, Thepen T *et al.* Increased numbers of CD4+ and CD8+ T cells in lesional skin of cats with allergic dermatitis. Veterinary Pathology 1998; 35: 268–73.
7. Roosje PJ, Dean GA, Willemse T *et al.* Interleukin 4-producing cells in the skin of cats with allergic dermatitis. Veterinary Pathology 2002; 29: 228–33.
8. Roosje PJ, Thepen T, Rutten VP *et al.* Immunophenotyping of the cutaneous cellular infiltrate after atopy patch testing in cats with atopic dermatitis. Veterinary Immunology and Immunopathology 2004; 101: 143–51.
9. Sinke JD, Thepen T, Bihari IC *et al.* Immunophenotyping of skin-infiltrating T-cell subsets in dogs with atopic dermatitis. Veterinary Immunology and Immunopathology 1997; 57: 13–23.
10. Taglinger K, Van Nguyen N, Helps CR *et al.* Quantitative real-time RT-PCR measurement of cytokine mRNA expression in the skin of normal cats and cats with allergic skin disease. Veterinary Immunology and Immunopathology 2008; 122: 216–30.
11. Nuttall TJ, Knight PA, McAleese SM *et al.* Expression of Th1, Th2 and immunosuppressive cytokine gene transcripts in canine atopic dermatitis. Clinical and Experimental Allergy 2002; 32: 789–95.
12. Halliwell REW. Efficacy of hyposensitization in feline allergic diseases based upon results of *in vitro* testing for allergen-specific immunoglobulin E. Journal of the American Animal Hospital Association 1997; 33: 282–8.
13. Waisglass SE, Landsberg GM, Yager JA *et al.* Underlying medical conditions in cats with presumptive psychogenic alopecia. Journal of the American Veterinary Medical Association 2006; 228: 1705–9.
14. Schleifer SG, Willemse T. Evaluation of skin test reactivity to environmental allergens in healthy cats and cats with atopic dermatitis. American Journal of Veterinary Research 2003; 64: 773–8.
15. Foster AP, O'Dair H. Allergy testing for skin disease in the cat *in vivo* vs. *in vitro* tests. Veterinary Dermatology 1993; 4: 111–5.

16. Willemse A, Van den Brom WE, Rijnberk A. Effect of hyposensitization on atopic dermatitis in dogs. Journal of the American Veterinary Medical Association 1984; 184: 1277–80.
17. Trimmer AM, Griffin CE, Boord MJ *et al.* Rush allergen immunotherapy protocol in feline atopic dermatitis: a pilot study of 4 cats. Veterinary Dermatology 2005; 16: 324–9.
18. Gilbert S, Halliwell REW. Production and characterisation of antisera against feline IgE. Veterinary Immunology and Immunopathology 1998; 63: 223–33.
19. Taglinger K, Helps CR, Day MJ *et al.* Measurement of serum immunoglobulin E (IgE) specific for house dust mite antigens in normal cats and cats with allergic skin disease. Veterinary Immunology and Immunopathology 2005; 105: 85–93.
20. Lian TM, Halliwell RE. Allergen-specific IgE and IgGd antibodies in atopic and normal dogs. Veterinary Immunology and Immunopathology 1998; 66: 203–23.
21. Marsella R, Olivry T, Nicklin C *et al.* Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs. Veterinary Dermatology 2006; 17: 24–35.
22. Gilbert S, Halliwell REW. Feline immunoglobulin E: induction of antigen-specific antibody in normal cats and levels of spontaneously allergic cats. Veterinary Immunology and Immunopathology 1998; 63: 235–52.
23. Tan BB, Weald D, Strickland I *et al.* Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. Lancet 1996; 347: 15–8.
24. Weber ER, Helps CR, Foster AP *et al.* Molecular cloning and phylogenetic analysis of a cDNA encoding the cat (*Felis domesticus*) Ig epsilon constant region. Veterinary Immunology and Immunopathology 2000; 76: 299–308.
25. Hammerberg B, Bevier D, DeBoer DJ *et al.* Auto IgG anti-IgE and IgG x IgE immune complex presence and effects on ELISA-based quantitation of IgE in canine atopic dermatitis, demodectic acariasis and helminthiasis. Veterinary Immunology and Immunopathology 1997; 60: 33–46.
26. Lombardero M, Heymann PW, Platts-Mills TA *et al.* Conformational stability of B-cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. Journal of Immunology 1990; 144: 1353–60.
27. Le Mao J, Mayer CE, Peltre G *et al.* Mapping of *Dermatophagoides farinae* mite allergens by two-dimensional immunoblotting. Journal of Allergy and Clinical Immunology 1998; 102: 631–6.
28. Noli C, Bernadina WE, Willemse T. The significance of reactions to purified fractions of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in canine atopic dermatitis. Veterinary Immunology and Immunopathology 1996; 52: 147–57.
29. Masuda K, Tsujimoto H, Fujiwara S *et al.* IgE sensitivity and cross-reactivity to crude and purified mite allergens (Der f 1, Der f 2, Der p 1, Der p 2) in atopic dogs sensitive to *Dermatophagoides* mite allergens. Veterinary Immunology and Immunopathology 1999; 72: 303–13.
30. Bensignor E, Carlotti DN. Sensitivity patterns to house dust mites and forage mites in atopic dogs: 150 cases. Veterinary Dermatology 2002; 13: 37–42.
31. Nuttall TJ, Hill PB, Bensignor E *et al.* House dust and forage mite allergens and their role in human and canine atopic dermatitis. Veterinary Dermatology 2006; 17: 223–35.
32. Jackson AP, Foster AP, Hart BJ *et al.* Prevalence of house dust mites and *Dermatophagoides* group 1 antigens collected from bedding, skin and hair coat of dogs in south-west England. Veterinary Dermatology 2005; 16: 32–8.
33. Raffan E, Lawrence H, Henderson T *et al.* Prevalence of the group 1 *Dermatophagoides* allergens Der p 1 and Der f 1 in homes with no dogs, healthy dogs and *Dermatophagoides*-sensitized atopic dogs in Liverpool. Veterinary Dermatology 2005; 16: 253–60.
34. Saridomichelakis MN, Marsella R, Lee KW *et al.* Assessment of cross-reactivity among five species of house dust and storage mites. Veterinary Dermatology 2008; 19: 67–76.
35. Mueller GA, Smith AM, Chapman MD *et al.* Hydrogen exchange nuclear magnetic resonance spectroscopy mapping of antibody epitopes on the house dust mite allergen Der p 2. Journal of Biological Chemistry 2001; 276: 9359–65.
36. Peng Z, Simons FER, Becker AB. Measurement of ragweed-specific IgE in canine serum by use of enzyme-linked immunosorbent assays containing polyclonal and monoclonal antibodies. American Journal of Veterinary Research 1993; 54: 239–43.
37. Novak N, Kruse S, Kraft S *et al.* Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: The dichotomy of extrinsic and intrinsic atopic dermatitis. Journal of Investigative Dermatology 2002; 119: 870–5.
38. Bardana EJ Jr. Immunoglobulin E- (IgE) and non-IgE-mediated reactions in the pathogenesis of atopic eczema/dermatitis syndrome (AEDS). Allergy 2004; 59: 25–9.
39. Hewitt CRA, Brown AP, Hart BJ *et al.* A major house dust mite allergen disrupts the immunoglobulin IgE network by selectively cleaving CD23: Innate protection by antiproteases. Journal of Experimental Medicine 1995; 182: 1537–44.
40. Gordon J, Flores-Romo L, Cairns JA *et al.* CD23: a multifunctional receptor/lymphokine? Immunology Today 1989; 10: 153–7.
41. Halliwell REW, Gilbert SM, Lian TM. Induced and spontaneous IgE antibodies to *Dermatophagoides farinae* in dogs and cats: evidence of functional heterogeneity of IgE. Veterinary Dermatology 1998; 9: 179–84.
42. MacDonald SM, Lichtenstein LM, Proud D. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. Journal of Immunology 1987; 139: 506–12.
43. Jackson HA, Miller HRP, Halliwell REW. Canine leukocyte histamine release: response to antigen and to anti-IgE. Veterinary Immunology and Immunopathology 1996; 53: 195–206.
44. Brazis P, Serra M, Sellés A *et al.* Evaluation of storage mite contamination of commercial dry dog food. Veterinary Dermatology 2008; 19: 209–14.
45. Loft KE, Pedersen K. The presence of pollen and house dust mite allergen-specific IgE in serum samples of 15 SPF cats and prevalence of house dust mite allergens (*Dermatophagoides pteronyssinus 1, Dermatophagoides farinae 1*, and mite group 2) in their microenvironment. Veterinary Dermatology 2007; 18: 184.

Résumé Les taux sériques d'immunoglobulines E (IgE) spécifiques d'acariens de la poussière de maison (HDMs) *Dermatophagoides farinae* (DF) et *Dermatophagoides pteronyssinus* (DP) de 58 chats présentant des signes cliniques de dermatite atopique (dermatite allergique féline), 52 chats sans anamnèse de maladie allergique ou immunitaire (chats non-allergiques) et 26 chats « specific pathogen-free » (SPF) ont été mesurés à l'aide d'un test ELISA (enzyme-linked immunosorbent assay) monoclonal anti-IgE. La réactivité aux allergènes normaux et diminués des acariens de la poussière de maison ont été comparés. Les chats att- SPF avaient significativement des taux sériques d'IgE spécifiques d'HDM plus faibles que les chats atteints de dermatite allergique ou les chats non-allergiques. La différence entre les résultats des chats allergiques et non-allergiques était significative pour les allergènes DF initiaux mais pas pour les allergènes DP initiaux ou les HDM diminués. Ces résultats suggèrent que DF sous sa forme initiale pourrait être un aller-