

Serum IgE and IgG responses to food antigens in normal and atopic dogs, and dogs with gastrointestinal disease

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Abstract

In human food allergy, with or without concurrent atopy, there may be significant increases in serum allergen-specific IgE. Serological methods have been tried but are not currently recommended for diagnosis of suspected food allergy in dogs. The aim of this study was to investigate humoral immune responses to food antigens in dogs. Serum IgG and IgE antibodies specific for food antigens were measured by enzyme linked immunosorbent assay (ELISA) using polyclonal anti-dog IgG and IgE reagents. Antigens tested were beef, chicken, pork, lamb, chicken, turkey, white fish, whole egg, wheat, soybean, barley, rice, maize corn, potato, yeast and cow's milk. Three groups were examined: normal dogs, dogs with atopic dermatitis (AD); and dogs with one of four types of gastrointestinal (GI) disease: small intestinal bacterial overgrowth (SIBO), inflammatory bowel disease (IBD), food-responsive disease, and infectious diarrhoea. Statistically significant differences in food-specific antibodies were not detected between the GI subgroups. There were statistically significant differences in the IgE concentration between the normal dogs, and dogs with atopic or GI disease, for all of the antigens tested. There were statistically significant differences in the average IgG concentrations between the normal dogs, and dogs with atopic or GI disease, for all of the antigens tested, except egg and yeast. The relationship of antigen responses for pooled data was analysed using principle component analysis and cluster plots. Some clustering of variables was apparent for both IgE and IgG. For example, all dogs (normal and diseased) made a similar IgG antibody response to chicken and turkey. Compared with other groups, atopic dogs had more food allergen-specific IgE and this would be consistent with a Th₂ humoral response to food antigens. Dogs with GI disease had more food allergen-specific IgG compared with the other groups. This may reflect increased antigen exposure due to increased mucosal permeability which is a recognised feature of canine intestinal disease.

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Abbreviations: PBST, 0.1% phosphate buffered saline–Tween; HRP, horse radish peroxidase; ELISA, enzyme linked immunosorbent assay; SIBO, small intestinal bacterial overgrowth; CFU, colony forming units; TMB, 3,3',5,5'-tetramethylbenzidine; AD, atopic dermatitis; GI, gastrointestinal

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1. Introduction

In humans, a range of different adverse reactions to food are recognised. These include food allergy which may be defined as an immunologically-mediated, excessive reaction to food substances. Food allergy

must be distinguished from food intolerance where non-immunological mechanisms are implicated.

Food allergic reactions are often considered to be IgE-mediated, where cross-linking of food allergen-specific IgE molecules on the surface of mast cells and basophils occurs following ingestion of the food allergen to which the individual is sensitive, causing immediate and late-phase reactions. The clinical manifestations of such IgE-mediated adverse food reactions may include signs of cutaneous and gastrointestinal (GI) disease. Some patients show signs of vomiting, diarrhoea and abdominal pain. In others, there may be urticaria and angioedema, asthma and rhinitis and, in severe cases, anaphylaxis. The late-phase IgE-mediated reactions may include food-induced eczema and urticaria. Alternatively, other immunopathological mechanisms have been implicated in the causation of food allergy. These non-IgE-mediated mechanisms may involve immune complex or delayed hypersensitivity based reactions (Metcalf, 1991; Sampson, 1999a).

In human medicine, the method of choice for the demonstration of food allergy is an elimination diet followed by the introduction of selected foods in a sequential fashion; the double-blind placebo-controlled food challenge method is considered the “gold standard” (Sampson, 1999b). A number of *in vitro* methods have been used to evaluate human patients with suspected food allergy. The detection of serum allergen-specific IgG (notably IgG₄) antibodies is assumed to reflect previous exposure to food antigens and is not considered indicative of a specific food-related pathogenesis (Sampson, 1999b). The measurement of food-specific serum IgE can be a useful negative predictive factor—a negative result is considered reliable for ruling out an IgE-mediated reaction to a food. However, the specificity of a positive result is low, although careful measurement of food-specific IgE concentrations has been used to calculate positive and negative predictive values in patients with confirmed food allergy (Sicherer and Sampson, 1999; Yunginger et al., 2000; Sampson, 2001).

In veterinary medicine, food allergy is considered to cause dermatitis more commonly than gastrointestinal disease, and the prevalence of food allergy varies from rare to common according to different authors (Chesney, 2001; Hillier and Griffin, 2001; Scott et al., 2001). Dermatological signs are variable; the most important

sign is pruritus, which may start at any age and can be generalised or localised. The pathophysiology of food allergy in animals is not completely understood. Consequently, it has been proposed that the term ‘cutaneous adverse food reactions’ should be used rather than food allergy or food intolerance because the immunopathogenic mechanism is unknown (Hillier and Griffin, 2001).

Attempts to demonstrate an IgE-mediated basis in dogs in naturally occurring cases have previously been unsuccessful. This has shed doubt on the role of IgE and a type 1 hypersensitivity reaction in cutaneous adverse food reactions (Jeffers et al., 1991; Mueller and Tsohalis, 1998). The role of food allergy in causing gastrointestinal disease is even less well characterised. It is presumed that food allergy may arise through abnormal responses by the mucosal immune system to ingested food. The gut is the largest immunological organ in the body and normally generates a local immune response, but systemic tolerance to, an ingested antigen. The current understanding of the normal mucosal immune system is limited and proposed mechanisms of allergy are generally speculative (Hall and Simpson, 2000).

The aims of the present study were to measure the serum concentrations of IgG and IgE antibodies specific for a variety of common food antigens in three groups of dogs. Antibody concentrations were compared between dogs with atopic dermatitis (AD), gastrointestinal diseases and dogs with no clinical disease. Measuring serological responses in large populations of dogs may provide some insight into the type of immune mechanisms that may be present in such conditions.

2. Materials and methods

The study used serum samples collected from two groups of diseased dogs referred to the University of Bristol Small Animal Hospital, Langford as part of their clinical investigation. Group 1 comprised of dogs with suspected atopic dermatitis ($n = 91$). Group 2 comprised of dogs with suspected gastrointestinal disease ($n = 72$).

Dogs with pruritic skin disease were diagnosed with atopic dermatitis on the basis of a detailed history, the presence of appropriate clinical signs, and considera-

tion of the role of microbial infections and parasite burdens using standard diagnostic techniques. The role of food antigens as a cause of the skin condition was assessed using an elimination diet of 6–8 weeks duration and this was usually based on a single diet trial with a commercial diet chosen on the basis of the previous dietary antigens (where known), some cases had two diet trials if compliance with the first was considered poor. All of the dogs were intradermally tested using Greer antigens (Greer, Lenoir, USA). Cutaneous adverse food reactions were not diagnosed as a cause of the pruritic skin condition. Serum samples from 91 dogs were analysed by enzyme linked immunosorbent assay (ELISA) for food antigen-specific IgG and antigen-specific IgE.

All dogs with gastrointestinal signs were treated with oral fenbendazole before investigation to reduce the possibility of *Giardia lamblia* infection and metazoan parasites. They were then classified into four groups on the basis of the results of preliminary investigations, endoscopic biopsy and response to treatment as described previously (German et al., 2000). Routine clinical investigations included a detailed history, physical examination, complete blood count, serum biochemical analysis, urinalysis, faecal analysis (direct smear and zinc sulphate centrifugation), selective faecal bacterial culture (for *Campylobacter*, *Salmonella* and *Clostridium difficile*), assay of serum trypsin-like immunoreactivity, folate and cobalamin, and abdominal radiography. Duodenal mucosal biopsies and duodenal juice for quantitative culture were obtained by flexible endoscopy. Biopsies were examined by light microscopy after haematoxylin and eosin staining. A standardised, sequential treatment regime of oral antibiotics (oxytetracycline), exclusion diet and immunosuppression (prednisolone) was used. Exocrine pancreatic insufficiency and endoparasitism were ruled-out and all remaining cases were categorised on the following criteria:

1. Cases of infectious diarrhoea, by isolation of a known pathogen and a positive response to antibiotics.
2. Idiopathic small intestinal bacterial overgrowth (SIBO) was diagnosed when there were $>10^5$ colony forming units (CFU) total, or $>10^4$ anaerobic, bacteria per millilitre of duodenal juice in the absence of known pathogens; characteristic

serum folate and cobalamin concentrations; minimal histological changes; a positive response to antibiotics and signs of relapse and remission on withdrawal and re-institution of antibiotic treatment, respectively.

3. Food sensitive dogs were defined as those responding completely to an exclusion diet with evidence of relapse and remission on withdrawal and re-institution of the diet, respectively.
4. Inflammatory bowel disease (IBD) was defined by histological evidence of inflammation; a failure to respond to antibiotics or diet; and a positive response to immunosuppressive therapy with prednisolone.

Serum samples from 72 dogs with GI disease (Group 2) were analysed by ELISA for food antigen-specific IgG and 64 of the 72 dog serum samples for food antigen-specific IgE.

A third group comprised serum samples from dogs with no history of skin or gastrointestinal disease. These samples were obtained by TLC from local veterinary practices (Group 3). Serum samples from 91 dogs were analysed by ELISA for food antigen-specific IgG and a further 40 dog serum samples for food antigen-specific IgE.

2.1. IgG and IgE ELISA

All reagents were obtained from Sigma, UK unless otherwise specified. Plates were washed six times in phosphate buffered saline (pH 7.4, 0.05 M) containing Tween-20 (0.1% PBST) between steps, and were incubated at room temperature for each stage of the assay.

2.2. IgE ELISA

Plates were coated with food antigens (Antigen Laboratories, Inc., Missouri, USA) diluted in carbonate/bicarbonate buffer (pH 9.6, 0.05 M), see Table 1. The quantity of each food allergen extract used to coat the ELISA plate for both IgG and IgE ELISA were derived experimentally and represent the amount required to obtain maximum antibody binding. This quantity varied considerably from IgG to IgE assays for the majority of the allergens investigated. This was presumed to reflect differences in the nature and quantity of allergens and allergen epitopes present

Table 1
Optimised concentrations of food antigens used in IgG and IgE ELISAs

	IgG ELISA ($\mu\text{g/ml}$)	IgE ELISA ($\mu\text{g/ml}$)
Beef	25	5
Pork	5	10
Lamb	25	5
Chicken	10	15
Turkey	10	10
Whole egg	10	1
White fish	5	1
Wheat	5	5
Soybean	5	10
Barley	5	15
Rice	20	5
Corn	2	20
Potato	1	1
Yeast	5	1
Cow's milk	20	2

in the allergen extracts and the extent to which these were recognised by the sera selected to optimise the assay.

Plates were incubated at 4 °C overnight with 100 μl of extract per well. Wells were blocked with 400 μl of PBST/0.5% sucrose/1% fish gelatin, at room temperature for 1 h, the blocking buffer was then decanted and the plates were left at 37 °C overnight. Serum samples were diluted 1/10 (GI and atopic samples) and 1/15 for normal dogs in PBST/0.1% human serum albumin and 100 μl was added to each well in duplicate for 1 h. Following washing of the plate, goat anti-dog IgE ϵ chain specific antibody, conjugated to horse radish peroxidase (HRP) (Bethyl, USA) diluted 1/6000 in PBST/0.5% polyvinyl pyrrolidone (PVP)/0.2% fish gelatin was added to each well. Plates were incubated for 1 h.

The use of the anti-dog IgE ϵ has been described by Griot-Wenk et al. (1999). The specificity of the anti-dog IgE ϵ reagent was evaluated by immunoblotting of anti-dog IgE ϵ against purified canine IgG (Sigma) and canine IgE (Bethyl hybridoma cell line). There was no reaction with IgG (heavy or light chain) and there was reaction versus IgE (heavy or light chain) data not shown.

3,3',5,5'-Tetramethylbenzidine substrate (TMB) (Kirkegaard and Perry Laboratories, UK) was applied for 10 min. The reaction was stopped with 0.5 M sulphuric acid and plates were read using a Dynex

ELISA plate reader at 450 nm. The results were converted into arbitrary units (AU) using a calibration curve included on each plate. A calibration curve was prepared from a pool canine serum with a high titred IgE anti-wheat antibody assigned a value of 10 arbitrary units. The concentration of food-specific IgE antibody in each test specimen was derived from the calibration curve, corrected for the dilution factor.

2.3. IgG ELISA

Plates were coated with food antigens (Antigen Laboratories, Inc., Missouri, USA) diluted in carbonate/bicarbonate buffer, see Table 1.

Plates were incubated at 4 °C overnight with 100 μl of extract per well. Plates were subsequently blocked with 400 μl per well of PBST/0.5% sucrose/1% fish gelatin, at room temperature for 1 h. The blocking buffer was then decanted and the plates were left at 37 °C overnight. Serum samples were diluted in PBST/3% PVP 10 kD to 1/400 and 100 μl was added to each in duplicate wells for 30 min. After washing, rabbit anti-dog IgG (whole molecule) HRP conjugated, was diluted 1/2000 in PBST/3% PVP 10 kD and 100 μl was applied to each well for 10 min with shaking on a Titramax 100 plate shaker (Heidolph, Germany). TMB substrate was applied to each well for 10 min. The reaction was stopped using 50 μl per well of 0.5 M sulphuric acid and the plates were read using a Dynex ELISA plate reader at 450 nm. The mean absorbance of each test specimen was compared to the absorbance of a positive control serum for each food antigen applied to each ELISA plate at a 1/400 dilution. The results were expressed as a percentage of the absorbance obtained with the positive serum. Positive food allergen ELISA control sera were identified from extensive preliminary screens and were defined as having a minimum absorbance greater than three times that of a control negative pool of canine serum.

The specificity of the anti-dog IgG reagent was evaluated by immunoblotting against purified canine IgG (Sigma) and canine IgE (Bethyl hybridoma cell line). There was no reaction with IgE (heavy chain); there were reactions to IgG (heavy and light chain) and IgE light chain (data not shown). It was assumed that the light chain reactivity of the anti-IgG reagent was insignificant in view of the presumed greater concentrations of serum IgG compared with serum IgE.

However, standardised assays for reporting the calibrated measurement of canine serum IgE have not been widely reported and the exact relationship of the concentrations of serum IgE to IgG antibodies is unknown (DeBoer and Hillier, 2001; Stedman et al., 2001).

2.4. Statistical analysis

For the statistical analysis, the natural log of the IgG response was used to transform the data to a normal distribution. The IgE response was treated as a binary variable, as for each antigen there were a considerable number of zero responses. A one-way analysis of variance (ANOVA) was carried out for each antigen to test for differences in levels of IgG between the groups. Tamhane's T2 test was used to test for pairwise differences between the groups. This test is robust to non-homogeneity of variances. An exact Chi-square test was used to test for an association between IgE response and group for each antigen. Pairwise comparisons of dog groups were made using further Chi-square tests based on exact statistics. Following these tests, the four sub-groups within the group of dogs with GI disease were tested for differences using the approaches outlined above. For all IgG data, and also for all IgE data, a hierarchical cluster analysis, using the Ward

method and the squared Euclidean distances, generated dendrograms to identify antigens which produced similar responses across dogs. For readers unfamiliar with cluster analysis, an accessible introduction is given in the chapter Cluster analysis (StatSoft, Inc., 2001). Lastly, multinomial regression was used to fit models predicting group membership using either IgG response or IgE response. The statistics package SPSS v11.01 running on Windows XP Professional was used for the analyses.

3. Results

There were significant differences between the three groups of dogs in their IgG and IgE responses to the different antigens. There were no detectable differences between the four sub-groups of Group 2 with GI disease in terms of either IgG or IgE response to any of the antigens, and no individual results from these analyses are presented.

3.1. IgG ELISA

The mean natural log ($\log_e$) IgG responses of each of the three groups of dogs to each antigen are shown

Table 2

Summary of the IgG responses to food antigens showing the mean $\ln(\text{IgG})$ serum concentration within each group of dogs, the standard error of the mean and the F -value and the P -value from the analysis of variance

	Atopic dermatitis ($n = 91$)		Gastrointestinal ($n = 72$)		Normal ($n = 91$)		F -value	P -value
	Mean	Standard error of mean	Mean	Standard error of mean	Mean	Standard error of mean		
Barley	3.5788 ^a	0.0552	3.4167 ^{a,b}	0.1578	3.1103 ^b	0.0684	6.721	0.001
Beef	2.6470 ^a	0.0446	3.5223 ^b	0.0693	3.3001 ^c	0.0608	61.360	0.000
Chicken	3.0022 ^a	0.0474	3.1590 ^a	0.0796	4.1235 ^b	0.0441	123.253	0.000
Corn	3.4965 ^a	0.0594	4.0946 ^b	0.0977	3.2081 ^a	0.1129	22.195	0.000
Egg	3.7960 ^a	0.0549	3.7120 ^a	0.0769	3.9138 ^a	0.0883	1.780	0.171
Fish	3.2761 ^a	0.0759	3.9370 ^b	0.0777	3.2403 ^a	0.0537	29.559	0.000
Lamb	2.9455 ^a	0.0532	3.3262 ^b	0.0752	2.6324 ^c	0.0852	21.787	0.000
Milk	3.0090 ^a	0.0573	3.6535 ^b	0.1597	3.5404 ^b	0.0570	13.685	0.000
Pork	3.1741 ^a	0.0377	3.5397 ^b	0.0583	2.9778 ^c	0.0486	33.372	0.000
Potato	3.5768 ^a	0.0698	3.6636 ^a	0.1242	1.9342 ^b	0.1088	96.071	0.000
Rice	2.3958 ^a	0.0846	3.1267 ^b	0.0683	2.0098 ^c	0.0926	41.942	0.000
Soy	3.0457 ^a	0.0685	3.6091 ^b	0.0897	3.2637 ^{a,c}	0.0773	12.491	0.000
Turkey	3.0655 ^a	0.0587	3.7522 ^b	0.0756	3.8590 ^b	0.0642	45.157	0.000
Wheat	4.0160 ^a	0.0515	3.4387 ^b	0.1055	3.6592 ^b	0.0657	15.320	0.000
Yeast	2.5191 ^a	0.1097	2.6165 ^a	0.1544	2.3885 ^a	0.0893	0.925	0.398

Means with different superscripts across rows were significantly different ($P < 0.05$).

in Table 2 together with the results of the analyses of variance. All antigens except egg and yeast produced an IgG response that differed between groups. The normal dogs (Group 3) on average had more, but not statistically significant, IgG antibodies than the dogs in Groups 1 and 2 to chicken, turkey and egg antigens. The dogs with skin disease (Group 1) had significantly more antibodies than dogs of the other two groups to wheat antigen. The dogs with GI disease (Group 2) had significantly more antibodies than dogs of the other two groups for beef, pork, lamb, soy, corn, rice and fish. There were greater concentrations of IgG antibodies in the dogs with GI disease than the other two groups for milk, yeast, and potato antigens, although this was not statistically significant.

3.2. IgE ELISA

The percentage of dogs within each of the three groups showing an IgE response is shown in Table 3, together with the results of the Chi-square tests. There was a significant association in response/non-response between the normal, atopic and GI groups of dogs, for all of the antigens tested.

In terms of IgE antibodies, the normal dogs (Group 3) on average had significantly more than dogs in the other two groups for chicken and lamb antigens. In normal dogs the average antibody concentrations were higher, but not statistically significant, for barley and milk antigens.

The dogs with skin disease (Group 1) on average had significantly more IgE antibodies than dogs of the other two groups to egg, fish, pork, turkey, rice, soy, and yeast antigens. The Group 1 dogs had more antibodies to beef, corn, potato and wheat antigens, but this was not statistically significant.

The cluster analysis and the dendrograms (Figs. 1 and 2), suggest possible relationships in the serological responses when data from all of the dogs are considered together for certain antigens. For example, in terms of IgG responses there appears to be an association between dogs that respond to beef and pork; between chicken, turkey and egg; between barley and wheat, and between corn and milk. In terms of IgE responses there were apparent shared responses to corn and wheat, between beef and milk, between chicken and lamb, and between potato, turkey, soy and rice.

Table 3
Summary of the IgE responses to food antigens

	Percentage positive ELISA result			Pearson Chi-square value	Exact two-sided significance
	Atopic dermatitis (n = 91)	Gastro-intestinal (n = 72)	Normal (n = 40)		
Barley	80.2 ^a	98.4 ^b	100 ^b	19.613	0.000
Beef	76.9 ^a	46.9 ^b	67.5 ^a	15.062	0.000
Chicken	60.4 ^a	50.0 ^a	87.5 ^b	15.108	0.000
Corn	78 ^a	71.9 ^a	55 ^b	7.211	0.025
Egg	79.1 ^a	39.1 ^b	57.5 ^b	25.821	0.000
Fish	92.3 ^a	31.3 ^b	42.5 ^b	67.656	0.000
Lamb	71.4 ^a	37.5 ^b	92.5 ^c	36.031	0.000
Milk	69.2 ^a	31.3 ^b	75 ^a	28.247	0.000
Pork	81.3 ^a	21.9 ^b	55 ^c	54.036	0.000
Potato	83.5 ^a	62.5 ^b	65 ^{a,b}	9.940	0.007
Rice	91.2 ^a	39.1 ^b	52.2 ^b	49.822	0.000
Soy	80.2 ^a	46.9 ^b	55 ^b	19.967	0.000
Turkey	91.2 ^a	40.6 ^b	62.5 ^c	45.630	0.000
Wheat	78 ^a	68.8 ^{a,b}	52.5 ^b	8.622	0.014
Yeast	82.4 ^a	34.4 ^b	47.5 ^b	38.986	0.000

The percentage of dogs positive for IgE within each group are shown together with the Chi-square statistic and an exact *P*-value for the test of association between response/non-response and the groups of dog. Different superscripts across rows indicate which groups differed when tested in pairs.

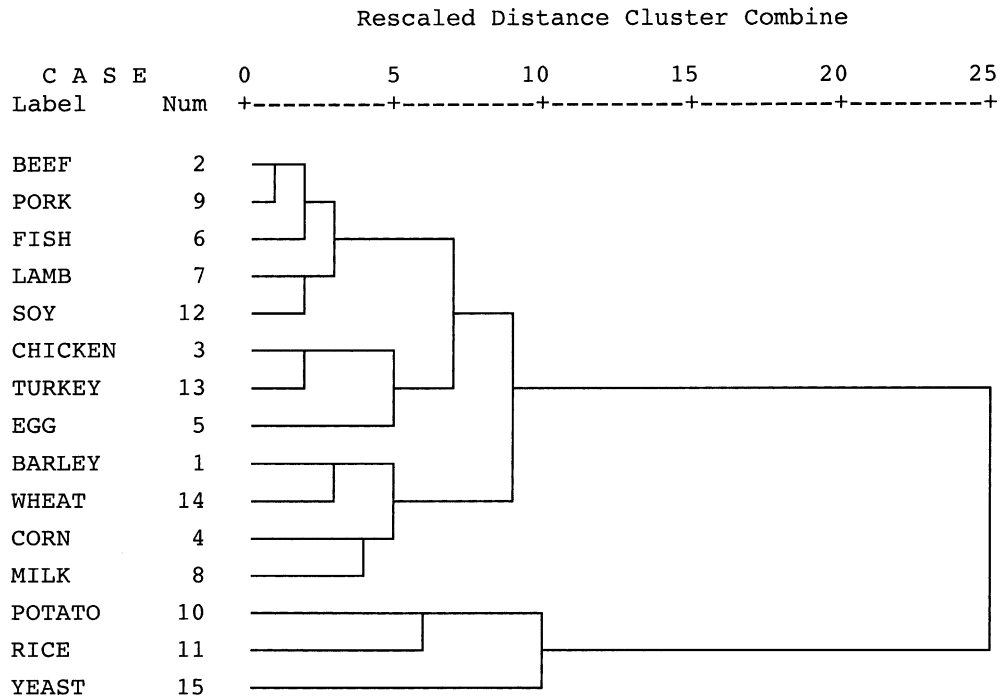


Fig. 1. Dendrogram of the IgG data using the Ward method of clustering to show antigens which elicited similar responses in the dogs.

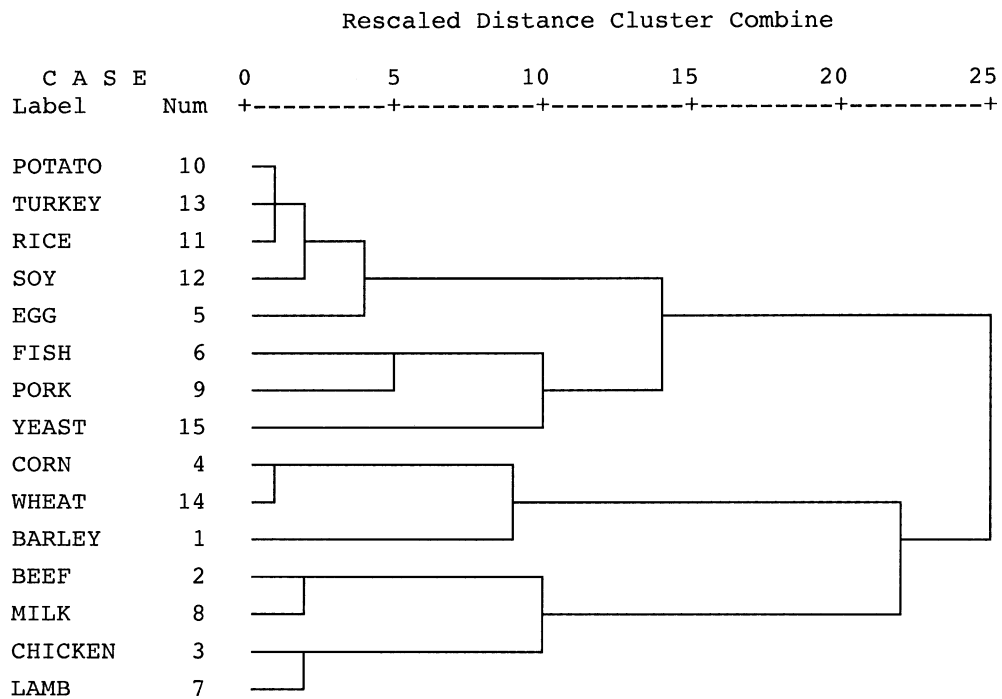


Fig. 2. Dendrogram of the IgE data using the Ward method of clustering to show antigens which elicited similar responses in the dogs.

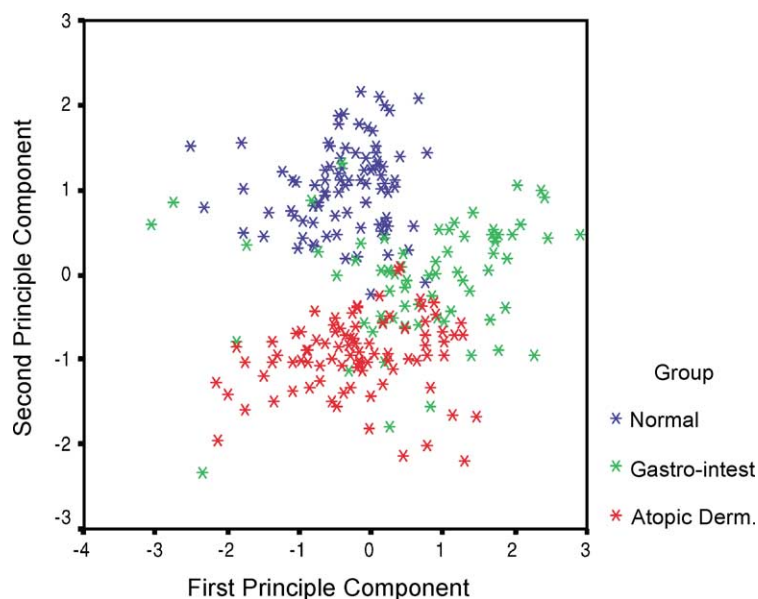


Fig. 3. A scattergram of each dog's score on the first and second principle components of all IgG responses. The three groups of dogs are differentiated by colour.

Multinomial regression of the IgG and IgE data sets for all of the dogs in the present study attempted to produce a predictive model for outcome—dermatitis, gastrointestinal disease or normal. Initial findings suggest that using the IgG data a model, which used beef, chicken, corn, potato, soy, turkey and wheat, assigned 92% of dogs to the correct category. For the IgE data, a model using barley, chicken, fish, lamb, milk, pork, wheat and yeast correctly classified 85% of the dogs. By way of example, Fig. 3 demonstrates that the groups can be differentiated by their antibody responses and shows a plot of each dog's score on the first two principle components formed from all 15 IgG antigen responses. Even in two dimensions the clustering by groups is readily apparent. The analysis and modelling must be refined before the predictive power of the serological assays can be established, and this will be the subject of further studies.

4. Discussion

In human patients with suspected atopic eczema, and/or food allergy, the measurement of serum allergen-specific IgG is not recommended for the investigation of suspected food allergy because both healthy

and diseased patients have IgG antibodies to common dietary antigens (Barnes, 1995). Individual IgG antibody responses in dogs may not have a predictive value but examination of a population response (as in this study) may suggest significant trends.

The serum IgG antibody responses to food antigens in this study were generally greater for dogs with gastrointestinal disease compared with dogs of the other two groups. This may reflect increased antigen exposure due to increased mucosal permeability which is a recognised feature of canine intestinal disease (Hall and Batt, 1990). However, the clinically normal dogs in this study had greater responses to chicken, egg and turkey antigens compared with the other two groups. This may simply reflect exposure to these antigens within the diet, or alternatively indicate the presence of non-specific antibodies cross-reacting with shared epitopes on these, and other environmental antigens. Similarly, the observation that atopic dogs had more IgG antibodies to barley and wheat antigens than dogs of the other two groups, may also reflect dietary exposure or cross-reactivity, rather than being a particular feature of atopic skin disease. Comprehensive information about dietary antigen exposure is usually difficult to obtain for dogs even if fed a single commercial diet as the exact type of food antigens are

not necessarily declared by the manufacturer and the inclusion of table scraps makes this information impossible to obtain.

Further studies of IgG subclass antibodies to food antigens in dogs may be warranted, but still may not necessarily discriminate between dietary exposure and the presence of non-specific, cross-reactive antibodies.

Only four dogs with gut disease in the present series had signs that improved with a change in diet. Alterations in stool frequency and consistency cannot be claimed to be evidence for a food allergy because an exclusion diet is likely to have a different digestibility and content of fat/protein/carbohydrate/micronutrients compared with the previous diet (Hall, 1994). The signs of gut disease by themselves are non-specific, and histopathological changes on gut biopsy are not pathognomonic for food allergy. Objective evidence of histological improvement on an exclusion diet, and relapse on challenge, would be an ideal criterion for diagnosis, but is rarely available. Consequently, caution should be applied when evaluating a positive clinical response to an exclusion diet—this may be the result of the change in composition of the diet, rather than a true benefit of antigen elimination.

None of the atopic dogs were considered to have a food-related skin condition following an elimination diet of 6–8 weeks duration. The prevalence of presumed food allergy (where a dog responds to an elimination diet with recurrence of the skin disease within 7–14 days of challenge with the previous diet) varies widely from study to study (Chesney, 2001; Scott et al., 2001). One of the potential reasons for the difficulty in recruiting food allergic suspects into this study was that, in general, owners preferred to feed a commercial rather than a home-cooked diet. Some authors recommend that where possible a home-cooked diet should be used, although commercial diets have been reported to be useful in the management of dogs with suspected food allergy (Hill, 1999; Chesney, 2002). Yet a series of diets may need to be investigated to maintain or achieve control in some cases (Leistra et al., 2001). Consequently, in this study the reliance upon commercial diets for evaluating dogs with allergic skin disease may have underestimated the number of dogs seen with concurrent food allergy.

Historically, measurement of serum food allergen-specific IgE has not been recommended for establishing a diagnosis of food allergy in the dog. For example,

in a study of 13 dogs with food allergy comparing the diagnostic value of an ELISA, intradermal testing and a food trial; the skin test and ELISA could not replace an elimination diet trial for achieving a diagnosis (Jeffers et al., 1991). Furthermore, using a different ELISA, Mueller and Tsohalis (1998) were unable to recommend this serological test for eight dogs with confirmed adverse reactions to food antigens.

A number of studies have reported on experimental colonies of dogs with food allergy where the exact dietary antigens have been characterised. These studies have facilitated the investigation of the relationship between food antigens, skin disease and allergen-specific serology. For example, a study of a colony of food allergic dogs with spontaneous sensitivity to milk suggested that changes in faecal IgE may reflect a specific gut response to dietary antigens (Jackson and Hammerberg, 2002a). A study of soy-sensitised dogs reported significant differences between sensitised and normal dogs in terms of their soy-specific serum IgE (Kennis et al., 2001). Further studies by these two groups reported on allergen-specific IgE and challenge with diets containing a combination of soy, cornstarch and hydrolysed soy. Yet under such controlled conditions, where clinical signs were stimulated with oral challenge there were no statistically significant changes in serum IgE that could predict the clinical outcome (Jackson et al., 2002; Kennis et al., 2002). Furthermore, food allergic dogs with a clinical response to a flavoured oral heartworm prophylactic medication developed clinical signs of pruritus, otitis and colitis, and there were significant peaks in serum allergen-specific IgE to soy, pork and corn, even though the medication only contained pork and soy proteins (Jackson and Hammerberg, 2002b). Another study of an inbred colony of dogs immunised with food antigen extracts in alum, reported that the dogs developed clinical manifestations of gastrointestinal disease and concurrent increases in antigen-specific serum IgE (Ermel et al., 1997). Olsen et al. (2000) reported a similar study in which dogs were immunised with casein, chicken liver and soy antigens and developed both dermatological and gastrointestinal signs in association with increased serum IgE to milk proteins.

A variety of factors may influence antigen-specific serum IgE production. These include possible genetic variation within and between breeds, the type of

antigen, and the dose, route, frequency and interval of administration. Previous or concurrent gastrointestinal parasitic and microbial infections may influence immune responses (Tada, 1975; Hill et al., 1995; Olsen et al., 2000). Even age and gender may be associated with variation in total serum IgE concentrations (Racine et al., 1999). Furthermore, IgE measurements may be influenced by IgG blocking antibodies (Hammerberg et al., 1997). HogenEsch et al. (2002) suggested that exposure to bovine proteins incorporated in vaccines, might in certain situations cause an intestinal hypersensitivity reaction in dogs. This hypothesis was based, in part, on the observation that rats immunised with ovalbumin and pertussis adjuvant developed antigen-specific IgE and intestinal hypersensitivity when ovalbumin was administered orally. Furthermore, HogenEsch et al. (2002) reported that antigen-specific IgE concentrations may persist in the absence of clinical signs and antigen exposure making the interpretation of the diagnostic value of serological responses a challenge. The studies of food-specific IgE measurements in dog colonies included deliberate immunisation with vaccines including alum. Such adjuvants and vaccines may have profound effects upon serological responses (Carter et al., 1991; Ermel et al., 1997; Olsen et al., 2000; HogenEsch et al., 2002).

An interesting observation in terms of the IgE responses documented in this study was that the atopic dogs were more likely to have greater concentrations of antibodies to food antigens than dogs of the other two groups. This may reflect a non-specific upregulation of IgE antibody production to a variety of antigens with no clinical significance. There is currently considered to be insufficient evidence to confirm or reject a relationship between canine AD and cutaneous adverse food reactions (Hillier and Griffin, 2001). In fact the same authors recommended that studies of large cohorts of dogs with AD and cutaneous adverse food reactions are needed to determine whether an association may exist between the two diseases. In part, the present study has aimed to fulfil that recommendation. In dogs with atopic dermatitis there is thought to be a Th₂ lymphocyte response similar to that which occurs in human AD (Hill and Olivry, 2001; Hayashiya et al., 2002; Nuttall et al., 2002). This may include significant IgE responses to a variety of antigens, but the mere presence of antibo-

dies may not necessarily have clinical significance (Halliwell and DeBoer, 2001).

Studies of human patients with food allergy would suggest that serum IgE concentrations to food antigens may have a clinically useful predictive value (Yunginger et al., 2000). In humans up to 40% of patients with atopic dermatitis may have clinical evidence of food sensitivity, although this association is considered contentious (Leung, 2000; Sampson, 2001). The measurement of serum IgE antibodies to food antigens in dogs may be influenced by a variety of factors, including perhaps, atopic disease. The previous studies of dogs in colonies with food sensitivity, and the studies reported herein, demonstrate that dogs make IgE antibodies to a variety of food antigens. The clinical significance of such antibodies remains to be determined. Further studies of dogs with naturally occurring cutaneous adverse food reactions are warranted to better understand the potential immunological mechanisms involved.

Cluster analysis of the serological responses may suggest possible antigenic relationships between antigens, but this requires further analysis because cross-reactive epitopes may be suggested from serological studies but the clinical significance of such reactivity remains to be demonstrated. Indeed, Jeffers et al. (1996) were unable to demonstrate a relationship between reactivity to beef and milk, or between chicken and egg proteins in 25 dogs with a history and clinical signs consistent with food-allergic dermatitis. Similarly, in human patients with food allergy, serological cross-reactivity has been demonstrated but without clinical cross-reactivity (Sampson, 1999b; Sicherer and Sampson, 1999).

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