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Evaluation of a Spontaneous Canine Model of Immunoglobulin E-Mediated Food Hypersensitivity: Dynamic Changes in Serum and Fecal Allergen-Specific Immunoglobulin E Values Relative to Dietary Change

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The purpose of the pilot study reported here was to evaluate serum and fecal total and allergen-specific immunoglobulin E (IgE) responses to dietary change in five Maltese x beagle dogs with suspected food hypersensitivity, compared with those of five clinically normal dogs. Clinical parameters (pruritus, otitis, and diarrhea) improved in the Maltese x beagle dogs during feeding of a novel diet, and signs were exacerbated by oral allergen provocation. Relative concentrations of serum and fecal wheat-, corn-, and milk-specific IgE were determined by use of an ELISA. The onset of clinical signs of disease was accompanied by an increase in serum allergen-specific IgE concentrations. In contrast, changes in clinical signs of disease or allergen-specific IgE values were not seen in the control group undergoing the same regimen. Total serum IgE concentration was measured by use of the ELISA, and comparison with known quantities of a monoclonal IgE allowed absolute values to be reported. Values were high in the Maltese x beagle colony (7 to 34 μg/ml), compared with those in the control dogs (0.7 to 6 μg/ml). Total serum and total fecal IgE concentrations did not change in either group during the study. Although allergen-specific IgE was detected in the feces of both groups, significant interassay variability made interpretation of the results difficult. The authors concluded that these Maltese x beagle dogs satisfied the currently recognized clinical criteria for the diagnosis of canine food hypersensitivity. Furthermore, the clinical and serologic responses seen in these dogs in response to oral allergen provocation suggest that this may be a useful model for the study of spontaneous food hypersensitivity.

Food hypersensitivity is an important clinical problem in humans, being more prevalent in infants. In most instances, it is believed to be mediated by immunoglobulin E (IgE). Clinical signs of IgE-mediated reactions typically develop within 24 h of the ingestion of allergen and involve the gastrointestinal tract, skin, and sometimes, the respiratory system (1). Intradermal prick testing and serum IgE concentration are often used as an aid to diagnosis in people, but results can be unreliable. A double-blinded, placebo-controlled, oral food challenge is considered the optimal method of diagnosis. Because of the potential for anaphylactic reactions, particularly in infants, this must be carried out in a controlled setting (2). As a consequence of the practical difficulties surrounding diagnosis and management of food hypersensitivity in people, there has been considerable interest in development of an animal model. Although rodent and canine models have been described, in all instances, these animals have been sensitized with the allergen of interest, using alternative routes of administration plus adjuvants to provoke an immunologic response (3-5).

Food hypersensitivity is recognized as an important clinical problem in the client-owned canine population by practicing veterinarians and, similar to the situation in people, confirmation relies on documentation of a relapse of clinical signs of disease in response to oral allergen challenge after elimination of the suspected allergen from the diet.

Intradermal skin testing and measurement of allergen-specific serum IgE concentration have been evaluated in the dog as an aid to diagnosis, but the results correlate poorly with the offending allergen identified by use of oral challenge (6-8).

Immunoglobulin E has been documented to be excreted in the feces of people, and total fecal IgE concentration is high in those with intestinal parasites or food hypersensitivity, compared with that in healthy controls (9). There also is poor correlation between serum and fecal IgE values in affected patients, supporting the belief that this IgE is locally produced (10, 11). It also has been proposed that food-specific fecal IgE measurements in infants might be predictive of development of allergic disease in later life. Allergen-specific fecal IgE concentration has been measured in the dog, and was found to fluctuate with dietary change in soft-coated wheaten terriers with suspected food hypersensitivity, and protein-losing nephropathy and enteropathy (12).

The purpose of the study reported here was to evaluate the clinical response to dietary allergy restriction and oral allergen provocation in Maltese x beagle dogs from a colony housed at North Carolina State University suspected of manifesting ad-

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verse clinical signs of disease related to dietary components. The response was compared with that of a group of clinically normal dogs housed in the same facility and undergoing the same regimen.

Additionally, we measured total and dietary allergen-specific IgE concentration in the serum and feces of both groups of dogs after dietary change. Although food-specific serum IgE concentration previously has been measured in dogs with suspected food hypersensitivity, association with current dietary intake has not been made either in the suspected allergic animals or healthy dogs.

Our hypothesis was that dynamic changes in serum concentration of allergen-specific IgE may be an aid in the diagnosis of food hypersensitivity in individual animals, and that fecal IgE concentration may reflect local gastrointestinal tract immunogloblin production in response to allergen. Thus, it may provide a useful tool for clinical diagnosis of food hypersensitivity.

Materials and Methods

Dogs. At North Carolina State University, we have a colony of crossbred Maltese x beagle dogs that manifest pruritus, otitis, and intermittent colitis from four to six months of age. The colony was established five years previously, and has an inbreeding coefficient of < 0.5. Dogs included in this study were F2 generation. The owners of six dogs previously adopted from this colony and not included in this study reported that their pets experienced pruritus, diarrhea, and/or vomiting when given cows milk or commercial pet foods containing dairy products. The colony dogs have not been orally exposed to cows' milk proteins. They are fed a diet containing chicken, corn, barley, and soy (Canine Growth, Hills Pet Nutrition, Topeka Kans.) until 12 months of age when the diet is changed to that containing corn, pork, and whole soybean protein (Prolab Canine 2000, Labdiet, Richmond, Ind.). A few treats containing corn, wheat, and soybean mill run are occasionally given (Science Diet Light dog treats, Hills Pet Nutrition Inc., Topeka, Kans.). All dogs also receive flavored milbemycin heartworm prophylaxis monthly, containing pork liver and soy (Interceptor, Novartis Animal Health, US Greensboro, N.C.).

Five Maltese x beagles from the colony were included in this study, three sexually intact females and two sexually intact males. Female dogs were not included if they were in estrus or pregnant. Age ranged from 15 to18 months, and all the dogs had the same sire. Their dietary history has been described previously. Mean body weight was 4.1 kg.

Control dogs. Five non-colony dogs of mixed breed were selected on the basis of not having previous history of pruritic skin disease, otitis, or colitis. All dogs had been housed in the laboratory animal research facility for a minimum of three years, during which they received the previously described diet (Prolab Canine 2000). Age ranged from three and a half to seven years, and the group comprised three sexually intact males and two neutered females. Mean body weight was 24.3 kg.

Study design. This was an open controlled pilot study. At the time of entry to the study, all dogs were fed the same diet, Prolab Canine 2000, containing corn, pork, and whole soybean protein. This was mixed with a novel diet containing a soy hydrolysate and cornstarch (HA Formula, Purina CNM, St. Louis, Mo.) for three days prior to feeding of the hydrolysate diet (HA) exclusively for eight weeks (56 days). Whole milk was then fed on two occasions, 24 h apart, at a dosage of 10 ml/kg of body weight. After 48 h, milk consumption was discontinued and the hydrolysate was fed for a further 10 days before re-introducing the Prolab diet gradually by mixing with the HA as described previously.

Table 1. Sample collection times during the study

Day of study	Diet	Serum	Feces	Clinical score
0	Prolab	Yes	Yes	Yes
7	HA diet introduced			
27	HA			Yes
63	HA	Yes	Yes	Yes
64 & 65	Milk /HA			
66	HA	Yes	Yes	Yes
72	HA			Yes
77	Prolab diet introduced			
80	Prolab	Yes	Yes	Yes

Prolab = Prolab Canine 2000 Labdiet; HA = HA Canine formula; milk = vitamin D milk, pasteurized, homogenized.(Hunter Farms, Charlotte, N.C.).

Each dog was examined and was assigned a cutaneous clinical score, using a modification of a previously validated system (13). Briefly, we assessed three criteria; erythema, excoriations, and evidence of infection (pustules or papules), which were graded on a scale of 0 to 3, 0 representing normal skin, in 35 areas of the skin including the ear canal. A maximal score of 315 could be achieved. This also included an otic examination on each occasion.

Scoring was performed by the principal investigator blinded to previous individual scores. Diarrhea or vomiting was recorded as present or absent; however, due to the fact that the dogs were group housed, changes in feces quality for individual dogs could not be consistently recorded. Physical examinations were performed immediately before and three to five days after each diet change. Serum and fecal samples also were obtained as indicated in the timeline (Table 1). Due to the quantity of feces required, a naturally passed sample was collected. This was obtained within eight hours of passage on three occasions at 24-h intervals. Dogs were temporarily housed in individual cages for this procedure. Serum was frozen at -70°C for batch analysis. The research protocol was approved by North Carolina State University Animal Care and Use Committee.

Measurement of total and allergen-specific IgE concentration. Relative concentrations of allergen-specific IgE against wheat, corn, and milk extracts (Greer Laboratories, Lenoir, N.C.) were determined. Corn proteins were specific ingredients in the Prolab diet, which all the dogs were fed on a regular basis. Wheat had been fed intermittently, and milk was chosen with regard to the dietary history obtained from client-owned Maltese x beagles.

Glycerin was removed from the allergen extracts by dialysis in 0.1*M* carbonate buffer, pH 9.0. Frozen fecal samples were lyophilized, ground to a powder, and stored at -20°C until analysis. For quantification of antigen-specific IgE, 0.2 g of powdered feces was mixed with 1.5 ml of phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Sigma Chemical Co., St Louis, Mo.) and soy bean trypsin inhibitor (10 mg/ml; Sigma Chemical Co.), and was allowed to stand at room temperature for 30 min before centrifugation at 16,000 ×*g* for 4 min, followed by collection of the supernatants.

Food extracts at a concentration of 50 µg/ml in carbonate buffer were coated onto polystyrene microtitration plates (Dynex, Chantilly, Va.), incubated overnight at 4°C, and blocked

with 0.5% gelatin for two hours at room temperature before 50 µl of fecal supernatants was applied in triplicate. Fecal IgE concentration was detected by addition of 50 µl of biotinylated mouse monoclonal antibody 5.91 (2 µg/ml) in PBS/Tween 20 containing 10% normal mouse serum, which is specific for a papain-sensitive site on the canine and feline epsilon chain of IgE (14). Plates were incubated for two hours before washing and development with avidin peroxidase and peroxidase substrate (Kirkegaarde and Perry Labs, KPL, Gaithersburg, Md.). Five washes with PBS-Tween 20 were done between each step.

For quantification of total IgE concentration, microtitration plates were coated with unlabeled anti-IgE monoclonal antibody 5.91 at a concentration of 10 µg/ml. Fecal supernatants were incubated on these plates for two hours, then were treated with biotinylated anti-IgE monoclonal antibody 5.91 and developed with avidin peroxidase as described previously.

Serial dilutions of serum samples, from 1:5 to 1:40, were assayed for food extract-specific and total IgE concentrations, using the techniques described previously. In this system, each dog acted as its own control for extract-specific IgE as we were concerned with the dynamic changes rather than absolute values of allergen-specific IgE. Total IgE concentration was quantified against standard curves of absolute quantities of monoclonal canine IgE generated for each plate, using purified products of the heterohybridoma cell line 2.39 (15) (Bethyl Labs, Montgomery, Tex.).

Efficacy of detection of fecal IgE. To examine the efficacy of the ELISA system in detecting IgE in fecal samples, the following procedure was performed. Microtitration plates were coated with *Brugia phangi* antigen (10 µg/ml) overnight, as described previously for food extracts. Canine monoclonal IgE 2.39 (1 µg/ml), specific for a *B. phangi* allergen (15), was added to 26 fecal supernatants. These were allowed to stand for 30 min before detection was done as previously described. Comparison was made with serial dilutions of canine monoclonal IgE 2.39 in PBS/Tween 20 on the same plates. Each assay was performed in triplicate.

Analysis. Due to the small number of subjects in this pilot study, absolute and median values are reported. Clinical score, and total and allergen-specific fecal and serum IgE concentrations were plotted for each group over the time course of the dietary changes.

Results

Clinical evaluation. Scores for the control group did not change substantially over the time course of the study. The Maltese x beagle dogs, however, had evidence of pruritic skin disease at the time of entry to the study. The pruritic skin disease was ameliorated by feeding the novel protein diet and was exacerbated by feeding milk or the Prolab diet. Absolute values for the cutaneous scores are given in Table 2.

Four of the Maltese x beagles and two of the control dogs developed diarrhea after challenge with milk. One of these Maltese x beagles also vomited profusely. This dog's reaction was so severe that milk was given on only one occasion. Otitis externa with secondary yeast infection developed in two Maltese x beagles after challenge with milk and the Prolab diet.

Total and allergen-specific IgE concentrations. Total serum IgE concentration ranged between 7 and 34 µg/ml for the Maltese x beagles, which was significantly higher than that for the control group (0.7 to 6 µg/ml; one outlying value at 21 µg/ml). The concentration of IgE detected in the fecal samples was similar for both groups: Maltese x beagles, 0.04 to 1.6 µg/ml, and controls, 0.3 to 2 µg/ml. The highest value obtained in each individual dog was detected in the fecal sample collected 24 to 48 h after dietary change. There was no marked change in the concentration of total serum or fecal IgE concentration in either group during the study period (data not shown).

The changes in corn-specific serum and fecal IgE concentration in each group of dogs are seen in Fig. 1-4. Corn-specific se-

Table 2. Individual cutaneous clinical scores during the course of the study

Day of study	Diet	Maltese X beagle dogs					Control dogs
0	Prolab	16.0	14.0	4.0	12.0	16.0	2 1 2 0 0
27	HA	15.0	12.0	4.0	8.0	12.0	2 0 2 0 0
63	HA	12.0	8.0	15.0	0.0	2.0	0 0 2 0 0
66	Milk	14.0	13.0	20.0	4.0	14.0	0 0 2 0 0
72	HA	2.0	13.0	9.0	0.0	4.0	0 0 0 0 0
80	Prolab	24.0	11.0	16.0	3.0	9.0	0 1 1 0 0

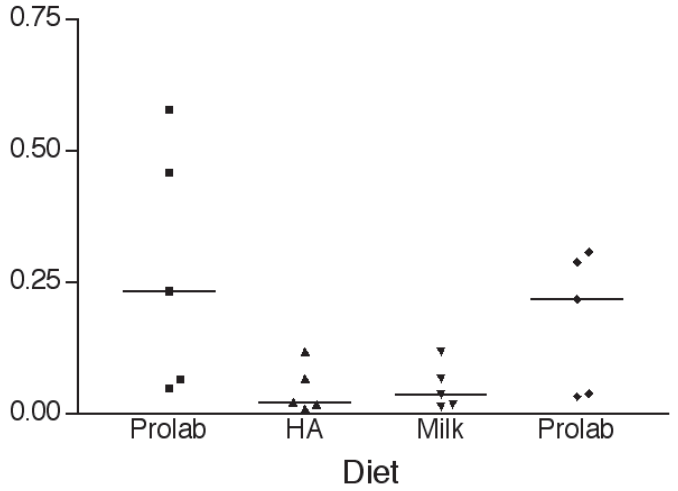

Figure 1. Serum corn-specific IgE concentrations in Maltese x beagles. Values on the y-axis represent optical density and on the x-axis, the diet. Median values are represented by the horizontal lines. Samples were taken on days 0, 63, 66, and 80.

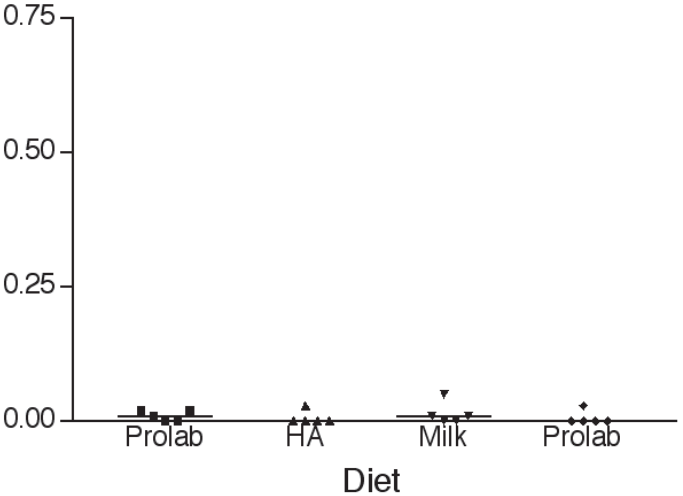

Figure 2. Serum corn-specific IgE concentrations in control dogs. *See* Fig. 1 for key.

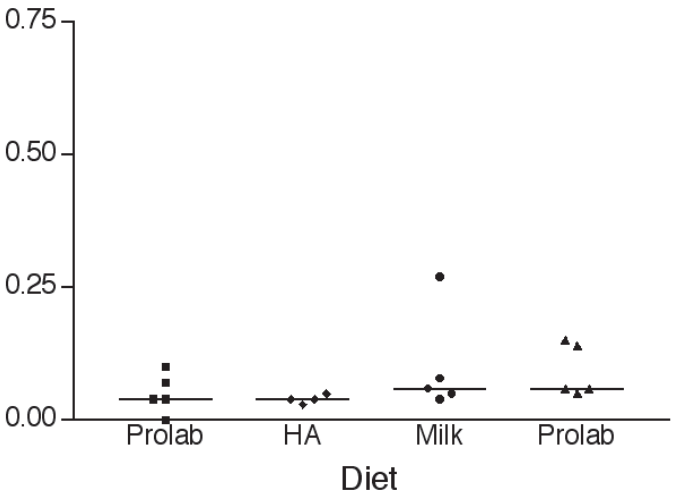

Figure 3. Fecal corn-specific IgE concentrations in Maltese x beagles. *See* Fig. 1 for key.

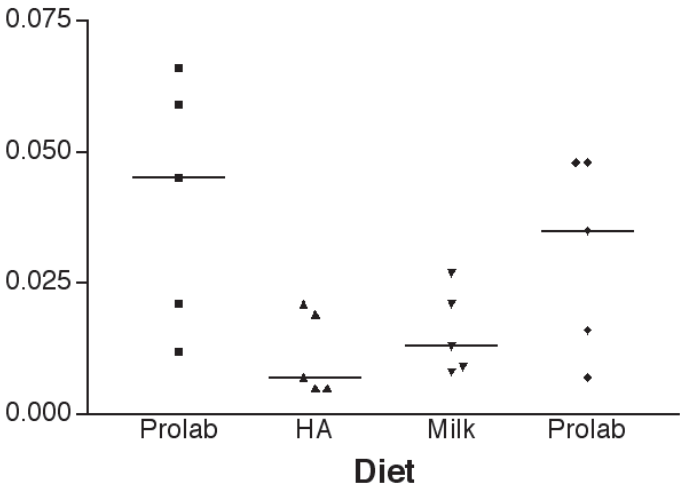

Figure 5. Serum milk-specific IgE concentrations in Maltese x beagles. *See* Fig. 1 for key.

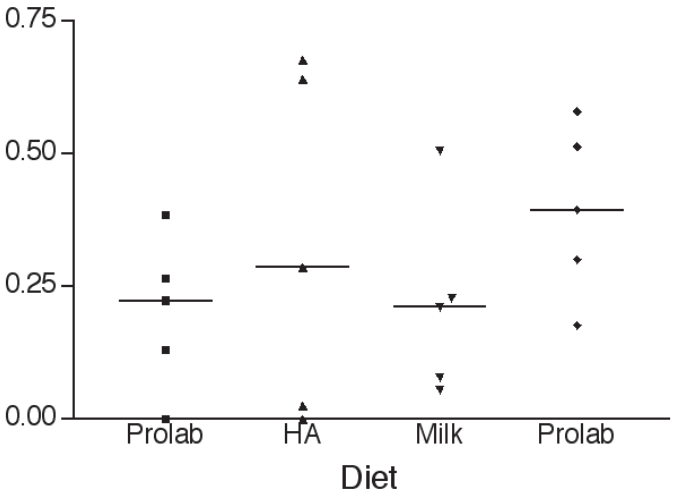

Figure 4. Fecal corn-specific IgE concentrations in control dogs. *See* Fig. 1 for key.

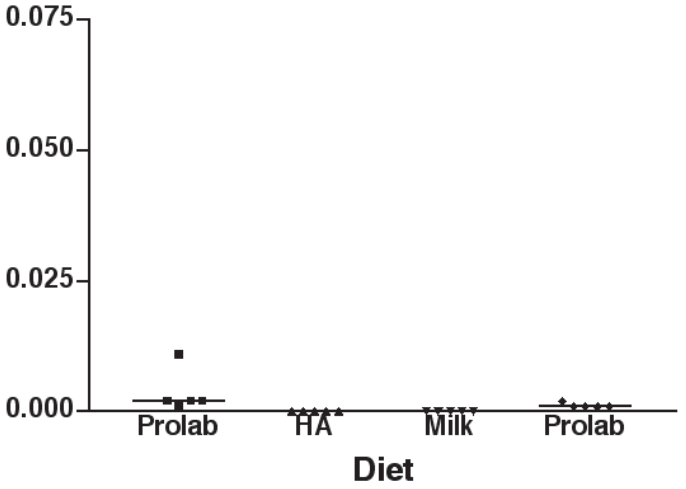

Figure 6. Serum milk-specific IgE concentrations in control dogs. *See* Fig. 1 for key.

rum IgE was present at the limits of detection of our assay in the control dogs, and concentration did not change during the study. In contrast, initial concentration of corn-specific serum IgE was high in the Maltese x beagles, then decreased during dietary allergen restriction before increasing again with introduction of the Prolab diet. There was no marked change after oral challenge with milk. Corn-specific fecal IgE was detected in both groups of dogs and was higher in the control group.

All Maltese x beagles had serum IgE antibodies that recognized milk proteins, in contrast to that in control dogs (Fig. 5 and 6). Concentrations were higher in dogs consuming the Prolab diet. Serum milk-specific IgE concentration did not increase after oral challenge with milk in any dog. Fecal milk-specific IgE concentration only increased in the dog that vomited, and interestingly, this was accompanied by an increase in corn- and wheat-specific fecal IgE concentration. Although wheat-specific IgE was detected in all dogs, there was no change in its concentration in serum or feces during the study period.

Efficacy of detection of fecal IgE. A mean value of 75% of the added canine IgE was detected in fecal supernatants. The amount of IgE detected varied by 18.5% among individual fecal samples.

Discussion

The observed improvement in clinical signs of disease in dogs consuming the novel diet and relapse in response to challenge with the previous diet in the Maltese x beagle dogs of this study satisfy the current criteria for diagnosis of canine food hypersensitivity (16). Clinical diagnosis of food hypersensitivity is characterized by pruritus and/or signs of gastrointestinal tract dysfunction and can develop at any age in the dog. Thus, although there was a difference in mean age between the groups, it was not considered an important factor in the study design by the authors. Selection of a control population without previous history of pruritic skin disease that had additionally been in the same environment and received the same diet for the preceding three years was considered more important.

Non-specific responses to dietary change are supported by failure of the control population to manifest clinical cutaneous disease while subjected to the same regimen. Diarrhea developed in

two of five controls and four of five Maltese x beagles after introduction of milk. This may be due to lack of intestinal lactase in the adult dog (17). Concurrent vomiting or otitis externa in one and two Maltese x beagles, respectively, suggests systemic rather than local response, consistent with hypersensitivity.

Random allergen-specific IgE measurements have failed to be of value in the diagnosis of canine food hypersensitivity (6, 8). However this is the first time, to the authors' knowledge, that dynamic changes in allergen-specific IgE concentration have been followed in individual animals and associated with dietary change and the advent of clinical signs of disease. Of particular note in this study were the rapid changes in serum concentration of corn-specific IgE. Although both groups of dogs had previous long-term exposure to this dietary component, the Maltese x beagles had significantly higher baseline corn-specific serum IgE concentration that decreased in response to elimination of corn protein from the diet. This was accompanied by improvement in clinical signs of disease. Re-challenge with a corn protein-containing diet resulted in evidence of pruritus and a concurrent increase in corn-specific IgE concentration after three days of challenge. In contrast, the control population had lower serum concentration of corn-specific IgE and no change was detected in response to changes in diet. Clearly this study is limited by the number and timing of serum samples obtained, and further studies are underway, using larger numbers of dogs and more frequent sample collection after challenge to characterize the nature of the IgE response over time.

Fecal corn-specific IgE concentration was lower in the Maltese x beagles, compared with controls, and did not change during the time course of the study in either group. Due to the problems encountered with this assay, specific conclusions cannot be drawn from this observation at this time.

Of further interest is that the HA diet contains 56% cornstarch. It is recognized that in humans, most food allergens are proteins, and therefore, one would expect the carbohydrate moiety of a food source to be tolerated. Further studies are underway in this colony of dogs to examine their clinical and immunologic reactions to cornstarch and whole corn proteins.

Wheat-containing treats had been fed intermittently to both groups of dogs. Although allergen-specific IgE was present in canine serum, there was no significant change over time in individual animals.

Of particular interest is the clinical hypersensitivity of the Maltese x beagles to bovine milk and the presence of IgE-specific antibodies in serum that recognize milk proteins when none of these dogs had been previously exposed to bovine milk. Two explanations are possible. Canine milk proteins share epitopes with bovine milk, and the dogs were sensitized while suckling their dam or, and perhaps less likely, there are cross reacting epitopes on proteins in the Prolab diet.

The Maltese x beagles studied here had significantly higher concentration of serum IgE than that previously reported (14). Various authors have documented that there is no significant difference in IgE concentrations between atopic, parasitized, and clinically normal dogs (18-20). However evidence is increasing to suggest that canine serum IgE concentration is genetically determined (4, 5), and it is possible that individuals of this colony have been previously selected for this trait. Evidence is conflicting as to whether age and sex have an effect on total serum IgE concentration in the dog (21, 22), but with the small number of dogs studied here, conclusions cannot be made as to how these factors influenced our results.

In contrast to that in humans, fecal IgE was detectable in both groups of dogs in similar amounts. As for other proteins, fecal IgE is subject to proteolytic degradation by digestive enzymes. The time of collection and fast processing of samples is, therefore, imperative. Samples obtained in this study were typically freshly voided feces, but a delay in collection of up to eight hours was possible, during which time, degradation could occur. However, the monoclonal antibody used in this study is specific for a region on the constant portion of the molecule (Fc). In people, most fecal IgE is believed to consist of Fc fragments resulting from proteolytic degradation (23). If this is also the case in dogs, our detection system should be an accurate reflection of total IgE excreted. Apart from the fact that preparation of fecal samples for assay is time consuming, the results we obtained indicated much interassay variation. This is almost certainly due to other substances in the fecal supernatants interfering with the assay, and precludes use of this system as a sensitive measure of local gastrointestinal tract IgE production without further modification.

The following conclusions may be drawn from this pilot study. The concentration of allergen-specific serum IgE appears to vary with oral allergen exposure in the allergic dog, and of specific note, is the rapid increase in niamounts that can be measured after oral challenge concurrent with evidence of pruritic skin disease. This is in contrast to the control population, which had no evidence of pruritus and no change in allergen-specific serum IgE during the same period.

Although allergen-specific IgE can be detected in canine feces, modifications need to be made to the assay described here before any conclusions can be drawn regarding the relevance of its presence in this species. Evidence from this pilot study suggests that this colony of Maltese x beagle dogs at NCSU has spontaneous IgE-mediated hypersensitivity to dietary allergens and has potential as a model for the study of canine food hypersensitivity in the future.

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References

1. **Sampson, H.** 1998. Adverse reactions to food, p. 1162-1182. *In* E. Middleton, C. Reed, E. Ellis, N. Adkinson, J. Yunginger, and W. Busse (ed.), Allergy: principles and practice, 5th ed., vol. II. Mosby, St. Louis.
2. **Sampson, H. A.** 1999. Food allergy. Part 2: diagnosis and management. J. Allergy Clin. Immunol. **103:**981-989.
3. **Xiu-min, L., B. H. Schofield, H. Chih-Kang, G. I. Kleiner, and H. A. Sampson.** 1999. A murine model of IgE mediated cow's milk hypersensitivity. J. Allergy Clin. Immunol. **103:**206-214.
4. **deWeck, A., P. Mayer, B. Stumper, B. Schiessl, and L. Pickart.** 1997. Dog allergy, a model for allergy genetics. Int. Arch. Allergy Immunol. **113:**55-57.
5. **Ermel, R., M. Kock, S. Griffey, G. Reinhart, and O. Frick.** 1997. The atopic dog: a model for food allergy. Lab. Anim. Sci. **47:**40-49.
6. **Jeffers, J., K. Shanley, and E. Meyer.** 1991. Diagnostic testing of dogs for food hypersensitivity. J. Am. Vet. Med. Assoc. **198:**245-250.

7. **Kunkle, G. and S. Horner.** 1992. Validity of skin testing for the diagnosis of food allergy in dogs. J. Am. Vet. Med. Assoc. **200:**677-680.
8. **Mueller, R. and J. Tsohalis.** 1998. Evaluation of serum allergen specific IgE for the diagnosis of food adverse reactions in the dog. Vet. Dermatol. **9:**167-171.
9. **Kolmannskog, S., J. Florholmen, and T. Floegstad.** 1986. The excretion of IgE with feces from healthy individuals and from others with allergy and diseases affecting the gastrointestinal tract. Int. Arch. Allergy Appl. Immunol. **79:**357-364.
10. **Kolmannskog, S. and B. Haneberg.** 1985. Immunoglobulin E in feces from children with allergy: evidence of local IgE production in the gut. Int. Arch. Allergy Appl. Immunol. **76:**133-137.
11. **Andre, F., C. Andre, and L. Colin.** 1995. IgE in stools as an indicator of food sensitisation. Allergy **50:**328-333.
12. **Vaden, S. L., B. Hammerberg, D. J. Davenport, S. M. Orton, M. M. Trogdon, L. T. Melgarejo, S. D. VanCamp, and S. A. Williams.** 2000. Food hypersensitivity reactions in soft coated wheaten terriers with protein-losing enteropathy or protein losing nephropathy or both: gastroscopic food sensitivity testing, dietary provocation, and fecal immunoglobulin E. J. Vet. Intern. Med. **14:**60-67.
13. **Olivry, T., E. Guaguere, and D. Heripret.** 1997. Treatment of canine atopic dermatitis with misoprostol, a prostaglandin E1 analogue: an open study. J. Dermatol. Treatment **8:**243-247.
14. **Hammerberg, B., D. Bevier, D. DeBoer, T. Olivry, S. Orton, D. Gebhard, and S. Vaden.** 1997. 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. Vet. Immunol. Immunopathol. **60:**33-46.
15. **Gebhard, D., S. Orton, D. Edmiston, K. Nagagaki, D. DeBoer, and B. Hammerberg.** 1995. Canine IgE monoclonal antibody specific for a filarial antigen: production by a canine x murine heterohybridoma using B cells from a clinically affected node. Immunology **85:**429-435.
16. **Rosser, E. J.** 1998. Food allergy in dogs and cats: a review. Vet. Allergy Clin. Immunol. **6:**21-24.
17. **Roudebush, P., W. G. Guildford, and K. J. Shanley.** 2000. Adverse reactions to food, p. 439. *In* M. S. Hand, C. D. Thatcher, R. L. Remillard, and P. Roudebush (ed.), Small animal clinical nutrition, 4th ed., vol. 1. Mark Morris Institute, Topeka, Kans.
18. **Jackson, H. A., H. R. P. Miller, and R. E. W. Halliwell.** 1996. Canine leucocyte histamine release: response to antigen and to anti-IgE. Vet. Immunol. Immunopathol. **53:**195-206.
19. **Hill, P., K. Moriello, and D. DeBoer.** 1995. Concentrations of total serum IgE, IgA and IgG in atopic and parasitized dogs. Vet. Immunol. Immunopathol. **44:**105-113.
20. **Wilkie, J. S. N., J. A. Yager, P. Eyre, and W. M. Parker.** 1990. Morphometric analysis of the skin of dogs with atopic dermatitis and correlations with cutaneous and plasma histamine and total serum IgE. Vet. Pathol. **27:**179-186.
21. **Griot-Wenk, M.E., A. Busato, M. Welle, B. P. Racine, R. Weilenmann, P. Tschudi, and A. Tipold.** 1998. Total serum IgE and IgA antibody levels in healthy dogs of different breeds and exposed to different environments. Res. Vet. Sci. **67:**239-243.
22. **Racine B. P., E. Marti, A. Busato, R. Weilenmann, S. Lazary, and M. E. Griot-Wenk.** 1999. Influence of sex and age on serum total immunoglobulin E concentration on beagles. Am. J. Vet. Res. **60:**93-97.
23. **Kolmannskog, S.** 1987. Similarities between IgE in human feces and a chymotrypsin digest of an IgE myeloma protein. Int. Arch. Allergy Appl. Immunol. **82:**100-107.