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Measurement of allergen-specific IgG in serum is of limited value for the management of dogs diagnosed with cutaneous adverse food reactions

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ABSTRACT

Conflicting results have been reported in the literature in terms of the usefulness of serological testing for IgG against food allergens in dogs with cutaneous adverse food reaction (CAFR). The aim of the present study was to evaluate the suitability of a commercially available IgG ELISA for identifying food allergens in dogs, by challenging dogs with specific food ingredients, selected on the basis of IgG reactivity in serum samples. A total of 24 adult dogs with CAFR were enrolled into the study and 16 healthy dogs were included as a control group. Blood samples were obtained for measurement of specific IgG antibodies against 39 commonly used pet food ingredients by ELISA. Participating owners were surveyed to obtain information on their pet's dietary history. Eleven healthy control dogs and 12 dogs with CAFR were subsequently challenged in a blinded cross-over design experiment with both positive and negative food ingredients, selected on the basis of the ELISA test results.

There was substantial individual variation in ELISA test results to the various food allergens, but no significant difference in IgG reactivity comparing the CAFR and control groups. None of the control dogs developed any clinical signs of an allergic reaction during the dietary challenge study. In the CAFR group, six of 12 dogs developed clinical signs after the negative challenge, and two of nine dogs developed clinical signs after the positive challenge. It was concluded that the ELISA test for dietary allergen-specific IgG is of limited value in the management of dogs with CAFR.

Introduction

An adverse food reaction refers to any clinically abnormal response attributed to the ingestion of a food or food additive (Hillier and Griffin, 2001). Adverse food reactions (AFRs) likely account for 1–6% of companion animal skin disorders seen in first-opinion veterinary practice, and around 10–49% of allergic responses in dogs and cats (Verlinden et al., 2006; Roudebush et al., 2010). However, making a diagnosis of cutaneous adverse food reaction (CAFR) can be challenging, as the clinical signs are frequently indistinguishable from those in other allergic skin diseases, where there is hypersensitivity to other environmental allergens, such as grass pollens or dust mites (Jackson et al., 2005; Picco et al., 2008).

Effective management of CAFR requires allergen avoidance, and therefore discriminating between dietary and environmental causes is crucial and the approach to treatment of CAFR and atopic dermatitis differs markedly (Olivry et al., 2010). The reference standard diagnostic approach for CAFR is based on the performance of an elimination-challenge test, where resolution of clinical signs is expected to occur by feeding a suitable elimination diet for a minimum period of 8 weeks (Olivry et al., 2015), with a subsequent positive response (relapse in clinical signs) following provocation testing, either by feeding a complete diet or multiple/individual dietary ingredients (Olivry and Bizikova, 2010; Hensel et al., 2015). However, this procedure requires a considerable amount of time and effort to identify specific food ingredients as the causative allergens, and owner compliance can be problematic.

Serological testing, for example by enzyme-linked immunosorbent assay (ELISA), is offered commercially to veterinary practitioners to allow them to identify antibody reactivity to individual dietary components. Such tests are designed to measure serum immunoglobulin

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E (IgE) or immunoglobulin G (IgG) against several commonly used food ingredients. However, there are conflicting views as to whether such serology tests are sensitive and specific for identifying dietary allergens and whether they provide useful information that can inform clinical management of CAFR in dogs. Serum IgE testing for dietary allergens is considered to be unreliable as a screening tool for CAFR in humans and numerous animal species (Jeffers et al., 1991; Mueller and Tsohalis, 1998; Guilford et al., 2001; Dupont et al., 2016). Some research studies into canine CAFR suggest that IgG analyses may prove useful in particular circumstances (Halliwell et al., 2004; Bethlehem et al., 2012), while other studies concluded that ELISAs for allergen-specific IgG demonstrate limited value as a diagnostic tool (Hardy et al., 2014; Jeffers et al., 1991; Zimmer et al., 2011).

Previous research studies, designed to investigate serological testing in dogs with CAFR, have not necessarily established whether or not the dietary allergens identified were capable of provoking clinical signs *in vivo*. Therefore, the aim of the present study was to evaluate the usefulness of an IgG ELISA for screening food allergens in dogs, by challenging these dogs with food ingredients, selected on the basis of the presence or absence of serum IgG antibody reactivity.

Materials and methods

Study population

A total of 40 privately owned dogs were recruited into the study (schematic overview of the experimental design and timeline, Appendix: Supplementary Fig. S1>). Dogs with CAFR ($n = 24$) were recruited from the dermatology services of two veterinary referral clinics in the Netherlands. These dogs (Table 1) had been previously diagnosed with CAFR, based on reduction of clinical signs during a dietary elimination trial and recurrence of clinical signs when challenged with the original diet, and their clinical signs were fully controlled with dietary therapy for at least 3 months at the start of the study. Healthy dogs ($n = 16$) were selected from the patient databases of the same veterinary clinics on the basis of being over 1 year of age and having no prior history or clinical signs of skin or gastrointestinal disease.

Blood sampling and IgG analyses

Blood sampling was performed as part of routine veterinary clinical practice, for diagnostic purposes and informed owner consent was obtained for use of residual serum samples for clinical research. Blood was allowed to clot, centrifuged within 20 min of collection and stored at –20 °C until further processing. Serum samples were de-identified and submitted in duplicate to a commercial laboratory in the Netherlands that offers serological screening for food-allergens, using an ELISA as described by Vink (2014) designed to test IgG reactivity against 39 individual dietary ingredients (Table 3). To determine the analytical variation of the commercial assay, the coefficient of variation was determined using the duplicate blood samples ($n = 40$), with an overall CV of <10% being considered satisfactory. IgG values >0.4 U/mL were considered positive, and the recommendation from the laboratory was to exclude any ingredient with IgG reactivity above this value from the diet.

Dietary survey

Owners were surveyed for the purpose of obtaining a dietary history for each dog. The questionnaire collected data on previous and current commercial diets, treats and other sources of food. Owners of the dogs diagnosed with CAFR were also questioned about the composition of any home-cooked elimination diet, the commercial diets that controlled the clinical signs of CAFR in their dogs, and the commercial diets that were suspected or known to provoke clinical signs of CAFR. All owners were asked to also provide specific brand names where applicable.

Food composition by ingredient was determined by consulting the package labels and Internet websites of the specific brands. In case of a closed or inconclusive declaration, manufacturers were contacted to obtain further information on the ingredients used. Dog food manufacturers that were unwilling to provide details of the raw materials used in their diet were asked to provide information on the presence of the 39 allergens in the IgG ELISA panel.

Dietary challenge trial

A total of 12 dogs with CAFR (but free of clinical signs at the start of the trial) and 11 control dogs were included in the dietary challenge study. This was designed as a blinded cross-over experiment to expose dogs to one ingredient (positive challenge), where the serum IgG antibodies were reported to be >10 U/mL and one ingredient (negative challenge), where the IgG antibodies were below the limit of detection. Although the diagnostic laboratory recommended a threshold of 0.4 U/mL for a positive result, we decided to select a potential allergen with a higher value as the positive challenge for the purposes of the study. Although the control dogs

Table 1
Characteristics of the dogs included in the cutaneous adverse food reaction group ($n = 24$).

Dog number	Breed	Sex	Age	Weight (kg)	Age at diagnosis	Clinical signs	Duration of current diet
1	French bulldog	FS	4y 5m	9.8	4y	Erythema, pruritus, otitis externa, interdigital pyoderma	5m
2	Shiba inu × Scottish shepherd	FS	2y 3m	18.0	6m	Cheilitis, pruritus, excessive shedding	1y 6m
3	Beagle	M	7y 5m	16.0	7y	Recurrent malassezia otitis externa, malassezia-paronychia	5m
4	Crossbreed	FS	3y 8m	22.0	3y 4m	Pruritus/erythema abdomen, interdigital pruritus, recurrent otitis externa	4m
5	Spinone Italiano	FS	1y 9m	38.0	6m	Diarrhoea, erythema, pruritus (ears, axillae, inguinal, periocular)	1y 6m
6	American bulldog	M	1y 2m	41.0	11m	Pruritus, papulae on head, dorsum and flanks, periocular swelling, urticae, diarrhea	3m
7	Beagle	M	4y 10m	9.3	4y 3m	Pruritus and brown discoloration tail base	7m
8	Dachshund	FS	5y 2m	9.6	4y 5m	Pruritus, hyperpigmentation, hyperkeratosis (interdigital, axillae, inguinal area)	9m
9	German shepherd	FS	1y 6m	37.8	1y	Pruritus pyoderma (abdomen, dorsum, thorax)	6m
10	German shepherd	FS	2y	36.5	1y 6m	Pruritus axillae inguinal area	6m
11	Rottweiler	FS	2y 11m	47.5	1y	Recurrent otitis externa	1y 10m
12	White shepherd	F	1y 4m	33.5	1y	Diarrhoea, hair loss, pruritus, licking feet	4m
13	Chow chow	FS	4y 1m	20.4	2y	Alopecia/ pruritus dorsum and abdomen	2y 1m
14	Old German shepherd	F	1y 6m	32.6	1y 2m	Otitis, pruritus, pyoderma abdomen	4m
15	French bulldog	M	1y 10m	11.0	1y 6m	Pruritus axillae, ventral thorax	4m
16	Crossbreed	M	7y 5m	10.0	7y	Recurrent pyoderma, otitis externa	5m
17	Polsky owczarek nizinny	M	9y 7m	20.0	9y	Interdigital pruritus, pruritus elbows, groin, otitis externa	7m
18	Soft coated wheaten retriever	M	8y 2m	17.0	7y 9m	Otitis externa, pyoderma, yeast dermatitis inguinal area/abdomen	3m
19	French bulldog	M	2y 6m	9.0	2y	Flank alopecia, scaly dry skin	6m
20	Bullmastiff	M	6y	48.0	4y 6m	Otitis externa, folliculitis, conjunctivitis, interdigital pyoderma	1y 6m
21	Labrador retriever	F	9y 1m	32.5	8y 7m	Interdigital pruritus, pyoderma, otitis externa	4m
22	French bulldog	M	4y	15.2	7m	Demodex, pyoderma, pruritus	3y 6m
23	Bouvier des Flandres	M	1y 6m	45.0	1y 2m	Generalized pruritus	4m
24	Jack Russell terrier	FS	2y 3m	7.2	1y 9m	Interdigital pruritus, otitis externa	6m

F, female; FS, female spayed; M, male; y, year; m, month.