



Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Research paper

Patch testing and allergen-specific serum IgE and IgG antibodies in the diagnosis of canine adverse food reactions

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ARTICLE INFO

Article history:

Received 4 July 2011

Received in revised form 4 January 2012

Accepted 5 January 2012

Keywords:

Food allergy

Adverse food reaction

Dog

Canine

Elimination diet

Patch test

ABSTRACT

Adverse food reaction (AFR) is a common differential diagnosis for pruritic dogs. The only way to diagnose AFR is an elimination diet of 6–8 weeks with a protein and a carbohydrate source not previously fed. In humans, patch testing has been shown to be a useful tool to diagnose food allergies. In veterinary medicine, serum food allergen-specific antibody testing is widely offered to identify suitable ingredients for such diets. The aim of this study was to determine sensitivity, specificity, negative and positive predictability of patch testing with and serum antibody testing for a variety of common food stuffs. Twenty-five allergic dogs underwent an elimination diet and individual rechallenge with selected food stuffs, food patch testing and serum testing for food-antigen specific IgE and IgG. Eleven clinically normal control dogs only were subjected to patch and serum testing. The sensitivity and specificity of the patch test were 96.7 and 89.0% respectively, negative and positive predictability were 99.3 and 63.0%. For IgE and IgG the sensitivity was 6.7 and 26.7%, specificity were 91.4 and 88.3%, the negative predictive values 80.7 and 83.7% and the positive predictive values were 15.4 and 34.8%. Based on these results, a positive reaction of a dog on these tests is not very helpful, but a negative result indicates that this antigen is tolerated well. We conclude that patch testing (and to a lesser degree serum testing) can be helpful in choosing ingredients for an elimination diet in a dog with suspected AFR.

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1. Introduction

Adverse food reactions are defined as unwanted and unpredictable effects caused by ingested dietary allergens. These reactions can be divided into two groups, food allergy and food intolerance. While food allergy is based on immunological mechanisms, food intolerance does not involve the immune system. In most cases however, the pathogenesis remains unknown and AFR is the more accurate term.

In dogs, AFR can affect the skin and gastrointestinal tract. The following information relates to the dermatological symptoms. The exact incidence of canine AFR is

unknown (Verlinden et al., 2006). Reported rates vary from 14% (Denis and Paradis, 1994) to 33% (Vroom, 1995). No clear predilection concerning age or gender is known in the dog (Kennis, 2006; Picco et al., 2008; Verlinden et al., 2006). However, in one study studying allergic dogs, those with clinical signs beginning at less than one year of age were more likely reacting to food than to environmental allergens (Picco et al., 2008). One study did not find breed predilections (Verlinden et al., 2006), in another study West Highland White terriers, pugs, Boxers, Rhodesian Ridgebacks and German Shepherds were predisposed (Picco et al., 2008). A common cutaneous sign is non-seasonal pruritus, that can be generalized or localized to certain regions of the body such as face, ears, paws, axillae, inguinal or perineal region (Denis and Paradis, 1994; Harvey, 1993; Rosser, 1993; Walton, 1967; White, 1986). In some animals, otitis externa (Harvey, 1993; Rosser, 1993) or a recurrent pyoderma with or without pruritus may be the only clinical

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signs (Verlinden et al., 2006). Clinical signs are frequently indistinguishable from those of atopic dermatitis due to environmental allergens (Carlotti et al., 1990; DeBoer and Hillier, 2001; Harvey, 1993; Rosser, 1993). Hence ruling out possible AFR is oftentimes an essential part of a thorough diagnostic evaluation in veterinary dermatology.

In the past, attempts have been made to develop serological tests for food-specific IgE, but none of the assays tested so far accurately predicted AFR (Jeffers et al., 1991; Mueller and Tsohalis, 1998). Currently, the only reliable method to diagnose AFR is a trial with an elimination diet and in case of improvement a subsequent challenge with the previous diet of the patient. The gold standard concerning the type of elimination diet is a home-cooked combination of a protein and a carbohydrate source previously not fed (Kennis, 2006). The recommended duration used to be three weeks (Walton, 1967; White, 1986), but more recently 6–8 weeks are advised (Kennis, 2006). If there is a clear improvement, the dog is subsequently challenged with its previous food. With an AFR deterioration is typically seen within a few hours or days (Jeffers et al., 1991; Rosser, 1993; Walton, 1967). Rarely, a relapse occurs as late as fourteen days into the rechallenge (White, 1986). The reintroduction of the elimination diet in a patient with AFR causes a clinical improvement again. In patients with recurrent pyoderma or coexistent environmental allergies and waxing and waning clinical signs, it is more difficult to evaluate the response to the diet trial (Mueller and Tsohalis, 1998). Furthermore, owner compliance is a huge problem in achieving an accurate diagnosis. The reasons for noncompliance are manifold and include cost, preparation time, inability to limit exposure to other items, low palatability and refusal to eat the diet as well as diarrhoea or constipation (Kennis, 2006; Mueller and Tsohalis, 1998). Therefore, an alternative test in the diagnostic workup of AFR would be of great benefit for veterinarians, owners and patients.

The aim of this paper was to investigate patch testing and food antigen-specific serum antibodies as diagnostic tools in dogs with suspected AFR. Specifically, parameters such as sensitivity, specificity, negative and positive predictability were determined and the combination of serum and patch testing for the diagnosis was evaluated.

2. Materials and methods

2.1. Study participants

The study was approved by the responsible governance of Bavaria under the number Gz. 55.2-1-54-2531-137-09. Owners of all dogs signed an informed consent form prior to inclusion of their dogs. One group of dogs included in the study consisted of eleven healthy dogs with neither history nor clinical signs of skin or gastrointestinal disease. These dogs served as a control group. Eight of these healthy dogs were owned privately, the other three dogs were recruited from an animal facility. Further twenty-five dogs were recruited from patients of the Dermatology Service of the Centre for Clinical Veterinary Medicine, Ludwig Maximilian University Munich. All of these showed a history and clinical signs compatible with nonseasonal

atopic dermatitis. In three dogs AFR were diagnosed via elimination diet and rechallenge before inclusion in the study. Differential diagnoses such as secondary infections or ectoparasite infestations were ruled out with impression smears, skin scrapings and trial therapies as indicated. A strict flea control was prescribed for all animals. Dogs with concurrent diseases such as hyperadrenocorticism or hypothyroidism did not participate in this study.

2.2. Elimination diet trial

The trial to establish or rule out a diagnosis of AFR had three stages. In the first, all atopic dogs were fed an elimination diet. A home-cooked diet with a single meat and single carbohydrate source never fed before was attempted based on the dietary history. Most dogs received cooked horse meat and boiled potatoes or sweet potatoes. If a home-cooked diet was not possible, a commercial hypoallergenic diet was selected. The elimination diets employed are listed in Table 1. If secondary pyoderma or otitis externa was found, it was treated appropriately, but medications were discontinued two weeks before the end of the diet trial to allow assessment of clinical response to the diet change. The second step of the trial was a sequential rechallenge with as many allergens as possible, particularly those protein or carbohydrate sources, to which the dog had shown a positive reaction in the patch test. At least one food allergen was tested in each dog for a period of up to two weeks. If clinical signs improved on the diet and recurred during rechallenge, the elimination diet was fed again as the third step of the trial. Subsequent improvement confirmed the diagnosis of an AFR to the relevant food component. Dogs that did not improve on the diet still underwent a rechallenge and subsequently other diagnostic tests as indicated. Dogs were evaluated by the owners using a 10 point pruritus score. Zero equalled a dog never showing any signs of pruritus such as scratching, licking, biting or rubbing. The maximal score 10 would equal a dog itching constantly, even interrupting eating or playing to scratch or bite at itself. Pruritus was assessed before the diet, before and after rechallenge and (in case of improvement during the dietary trial and subsequent deterioration during rechallenge) after being fed the elimination diet again. In the control group, the daily food of the dogs was analyzed and compared to the patch test results.

2.3. Patch testing

Prior to the elimination diet the patch test was applied. Systemic glucocorticoids were discontinued at least four weeks, topical glucocorticoids and antihistamines at least two weeks prior to patch testing. An area of skin (20 cm × 10 cm) along the lateral thorax was clipped gently with an electric clipper 48 h before testing to minimize irritant reactions. No tape stripping or abrasion was done. Six types of meat (beef, chicken, pork, lamb, turkey and fish) and three types of carbohydrate sources (wheat, corn and potato) were selected as allergens for the patch test. Raw and cooked meat was used separately and mashed in a blender. To facilitate use of the crumbly cooked meat in patch testing, it was mixed

Table 1

Type and duration of elimination diets employed.

Dog	Type of elimination diet	Ingredients
Dog 1	Commercial	Fish, potato (Eukanuba F/P, Procter & Gamble, Schwalbach, Germany)
Dog 2	Commercial	Hydrolyzed chicken liver, cornstarch (Hill's Prescription Diet™ Canine z/d™ ULTRA Allergen-Free, Hill's Pet Nutrition GmbH, Hamburg, Germany)
Dog 3	Commercial	Horse, potato (BauBon® Exclusion® Diet Horse & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 4	Home cooked	Horse, navy beans
Dog 5	1. Home cooked 2. Commercial	1. Horse, millet 2. Rabbit, potato (BauBon® Exclusion® Diet Rabbit & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 6	Home cooked	Horse, sweet potato
Dog 7	Home cooked	Horse, sweet potato
Dog 8	1. Home cooked 2. Commercial	1. Horse, potato 2. Fish, tapioca, hydrolyzed chicken liver (Royal Canin Sensitivity Control Blauer Wittling & Tapioca, Royal Canin, Cologne, Germany)
Dog 9	Home cooked	horse, potato
Dog 10	Commercial	Horse, potato (BauBon® Exclusion® Diet Horse & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 11	Commercial	Horse, potato (BauBon® Exclusion® Diet Horse & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 12	Home cooked	Ostrich, spelt
Dog 13	1. Home cooked 2. Commercial	Horse, navy beans Rabbit, potato (BauBon® Exclusion® Diet Rabbit & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 14	Home cooked	Horse, sweet potato
Dog 15	Home cooked	Horse, potato
Dog 16	1. Home cooked 2. Commercial 3. Commercial	Horse, millet Rabbit, potato (BauBon® Exclusion® Diet Rabbit & Potato, Dorado S.r.l Company, Venice, Italy) Hydrolyzed chicken liver, cornstarch (Hill's Prescription Diet™ Canine z/d™ ULTRA Allergen-Free, Hill's Pet Nutrition GmbH, Hamburg, Germany)
Dog 17	Home cooked	Horse, sweet potato
Dog 18	Commercial	Rabbit, potato (BauBon® Exclusion® Diet Rabbit & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 19	Home cooked	Kangaroo, millet
Dog 20	Commercial	Venison, potato (BauBon® Exclusion® Diet PUPPY-JUNIOR Venison & Potato, Dorado S.r.l Company, Venice, Italy)
Dog 21	Home cooked	Horse, millet
Dog 22	Home cooked	Kangaroo, potato
Dog 23	Commercial	Hydrolyzed chicken liver, cornstarch (Hill's Prescription Diet™ Canine z/d™ ULTRA Allergen-Free, Hill's Pet Nutrition GmbH, Hamburg, Germany)

with petrolatum. Wheat flour, corn meal and cooked potatoes were similarly prepared. Petrolatum was used as negative control. The 12-mm diameter aluminium chambers adhered to a hypoallergenic tape (Finn Chambers on Scanpor®, Allmiral Hermal, Reinbek, Germany) were filled with the allergen–petrolatum mix. A total of sixteen chambers (six varieties of raw and cooked meat, three carbohydrates and one negative control) were fixed onto the previously clipped skin of each dog. To keep the chambers in place, they were covered with a layer of gauze and fixed with another hypoallergenic tape. Then the trunk was wrapped with an elastic bandage. In addition each dog had to wear a body suit to minimize the chance of moving the chambers. After 48 h the patch test was removed and the imprints of the chambers were marked with a pen to facilitate their identification 24 h later. The body suit remained for the complete 72 h to protect the testing field.

The patch test sites were evaluated at 24, 48 and 72 h post-application. They were assessed for the presence of erythema, papules, pustules or vesicles. Scoring details are outlined in Table 2.

2.4. Testing for food allergen-specific serum IgE and IgG

Prior to the elimination diet, a blood sample was obtained by venipuncture. After centrifugation, sera were

Table 2

Evaluation of patch test sites in atopic dogs.

–	No visible reaction or irritation
1+	Mild erythema
2+	Moderate erythema
3+	Severe erythema
++	Erythema and induration or oedema (papules)
+++	Erythema with vesiculation or more severe reactions

frozen at -20°C until simultaneous processing for food allergen-specific IgG and IgE ELISA by Avacta Animal Health, UK. Allergen-specific IgE and IgG against beef, pork, lamb, chicken, turkey, white fish, wheat, potato and corn were measured.

Levels of allergen-specific serum IgE and IgG antibodies were measured by ELISA using modified protocols of previously described assays (Bexley et al., 2009; Foster et al., 2003). Briefly, polystyrene microtitre plates were coated with the antigen extracts of beef, pork, lamb, chicken, turkey, white fish, wheat, potato and corn (1:20 w/v; Greer Laboratories Inc., Lenoir, NC, USA) diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6. Wells were then blocked with 0.2% fish gelatin. Samples were assayed in duplicate at dilutions of 1/10 (IgE) and 1/400 (IgG). Each plate contained a five-point standard curve prepared by assaying serial two-fold dilutions of a dog serum sample with high levels of beef-specific IgE or IgG, as appropriate. Food-specific serum IgE and IgG antibodies were detected using biotinylated monoclonal 5.91 (Bruce Hammerberg, North Carolina State University, Raleigh, NC, USA) and polyclonal goat anti-dog IgG peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA), respectively. Absorbances were determined at 450 nm using an ELISA plate reader (Molecular Devices Co, Sunnyvale, CA, USA), with subtraction of mean blank (un-coated) well optical density (OD). Results were expressed as arbitrary units (AUs) by reference to the standard curve. The measured concentrations of IgE and IgG were evaluated for each type of antibody and the lowest classification group (with less than 20% false-positive results) was used for the calculations. Thus, a result was considered positive if a value of $>15\text{ AU/l}$ (for IgE) and $>25\text{ AU/l}$ (for IgG) was obtained.

2.5. Statistics

Based on the results of the elimination diet and rechallenge trials the sensitivity and specificity as well as positive and negative predictive value of the patch test and the measurement of IgE and IgG concentrations were determined. They were also calculated for the different possible combinations of the single tests. However, the predictive values depend also on the prevalence in the sample population. As the prevalence of AFR in the canine population is not known and the prevalence in the sample populations (allergic dogs and healthy dogs) most likely does not reflect the prevalence in the overall dog population, positive and negative likelihood ratios were also calculated. The gender ratio was compared with a Fisher Exact test, $p < 0.05$ was considered significant. The formulas for calculation of sensitivity and specificity, positive and negative predictive value and positive and negative likelihood ratio are listed in Table 3.

3. Results

3.1. Study participants

Of the 36 dogs included, three dogs did not complete the study. Two of them belonged to the allergic group. In both cases, incomplete cooperation of the owners was the

Table 3

Formulas for calculating the statistical values.

Sensitivity	$\text{TP}/(\text{TP} + \text{FN})$
Specificity	$\text{TN}/(\text{FP} + \text{TN})$
Positive predictive value	$\text{TP}/(\text{TP} + \text{FP})$
Negative predictive value	$\text{TN}/(\text{FN} + \text{TN})$
Positive likelihood ratio	$\text{sensitivity}/1 - \text{specificity}$
Negative likelihood ratio	$1 - \text{sensitivity}/\text{specificity}$

TP, true-positive; FP, false-positive; TN, true-negative; FN, false-negative.

reason for exclusion. One of the owners discontinued the elimination diet without previous consultation and infection control. The other owner did not complete a sequential rechallenge. The third dog was a healthy dog of the control group. To test an alternative application site for the patch test, the test was fixed on the underside abdomen of this dog. Twenty-four hours after application, a severe erythema consistent with an acute contact allergy in the field of the tape was noticed.

There were more female (19/33) than male (14/33) dogs in the study population, but this difference was not significant. The mean age was 4.9 years. Nine different breeds and seventeen crossbreeds participated in the study. All 23 allergic dogs were previously fed commercial dog food and seven owners additionally added home-cooked food. The age of the dogs at onset of clinical signs ranged from 2 months to 8 years, with a mean of 2 years. The primary clinical sign in almost all dogs was nonseasonal pruritus (21/23). Only two dogs did not exhibit pruritus, one of them showed recurrent pyoderma, the other one bacterial pododermatitis. Six dogs had concurrent gastrointestinal signs. The most common locations of pruritus were the paws (15/21) and the ears (7/21).

3.2. Elimination diet trial

Two of the three dogs diagnosed with an AFR prior to inclusion were fed a commercial diet the third dog a home-cooked one. Fourteen of the remaining owners chose a home-cooked elimination diet for their dog, the other six a commercial diet. Three dogs did not tolerate the gradually introduced home-cooked elimination diet and showed diarrhoea and vomiting. The owners of these dogs switched to a commercial diet.

The mean duration of the diet was 8–6 weeks. Twenty of the dogs showed improvement on the elimination diet. In three dogs the pruritus decreased only a little, but in the remaining 17 dogs the pruritus improved by more than 50%. No improvement was seen in one dog with pruritus and in the dogs with recurrent pyoderma and pododermatitis the clinical signs returned on the elimination diet after antimicrobial therapy had been ceased.

After feeding the former food, eighteen dogs, which improved during the elimination diet, deteriorated with subsequent improvement on feeding the elimination diet again.

3.3. Patch testing

Two allergic dogs only underwent the patch test with beef and chicken. As mentioned above, the patch test was

Table 4

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of patch test, specific IgE (≥ 15 AU/l) and specific IgG (≥ 25 AU/l).

	Patch test	Specific IgE	Specific IgG
Sensitivity (%)	96.7	6.7	26.7
Specificity (%)	89.0	91.4	88.3
PPV (%)	63.0	15.4	34.8
NPV (%)	99.3	80.7	83.7
PLR	8.8	0.8	2.3
NLR	0.04	1.0	0.8

removed in one healthy dog because of a severe erythema after 24 h. A patch test with a total of sixteen chambers was conducted for 48 h in the remaining dogs.

Overall, 68 positive and 224 negative reactions were recorded. All but one of the allergic dogs developed at least one positive reaction with a mean of three positive reactions per dog. Only two dogs in the control group showed positive reactions.

Erythema was the only lesion observed, with most reactions being only mild. A positive reaction to the negative control (petrolatum) was not seen. The most frequent positive patch test reactions were against beef and the smallest number against fish. Only six reactions were noted against carbohydrate sources.

Thirty-two of thirty-eight reactions seen after 24 h were also present after 48 h, six were only noted at the first reading. Four of these were confirmed as false-positive reactions by rechallenge, in three instances a rechallenge was not performed against that particular allergen. Almost all reactions noted at 72 h were also seen at previous time points. However, one positive reaction only occurred after 72 h.

By comparing the patch test reactions with the outcome of the rechallenge, 46 positive and 137 negative reactions could be evaluated. Twenty-nine of the positive reactions were true positive (29/46, 63.0%) and 17 false positive (17/46, 37.0%). Of the negative reactions, 136 were true negative (136/137, 99.3%) and only one was false negative (1/137, 0.7%). Based on these results, sensitivity, specificity, positive and negative predictive values and likelihood ratios are listed in Table 4. According to McGee, a negative likelihood ratio of 8.8 increases the likelihood of the event (i.e. adverse reaction against that particular antigen) by at least 40%, and a likelihood ratio of 0.04 decreases the likelihood of such event by at least 50% (McGee, 2002).

The majority of the reactions (71/175, 40.6%) were observed 48 h after the onset of the test. When the reactions

at different reading times (24, 48 and 72 h) and formulations (raw versus cooked) were grouped, 38 of the 68 reactions (55.9%) were noticed for the first time after 24 h. Only one reaction was seen first at 72 h.

The various meats as well as the fish were tested in raw and cooked form in each dog. Of the 62 positive reactions against the various protein sources 28 reactions were seen exclusively to raw protein, 19 were noted to both raw and cooked forms and 15 only to the cooked protein. There was an equal number of true positive reactions to raw protein only, cooked protein only and to both forms (raw and cooked). More reactions to raw protein only (12) were true positive. Three of the false positive reactions were to the cooked version of the antigen, all others to the raw form.

3.4. Testing for food allergen-specific IgE and IgG

The results of the food allergen-specific IgE assays in the allergic dogs were positive in 11 instances and negative in 196. In the healthy group, 7 positive and 56 negative reactions were present. In both groups, beef-specific IgE antibodies were most frequent. No IgE antibodies were detected against chicken, fish and turkey. By means of rechallenge, 76.5% of the positive IgE results (13/17) could be verified. Eleven were false positive, two true positive. Similarly, 145 of the 252 negative results (57.5%) could be evaluated, 28 were false negative, 117 true negative.

In allergic dogs, 24 positive and 182 negative results of specific IgG antibody testing were noted, in the healthy group 5 positive and 58 negative results. Verified by rechallenge, 15 of 29 positive results (51.7%) were false positive and 8 (27.6%) true positive. Twenty-two of the 240 negative results (9.2%) were false-negative and 113 (47.1%) true negative. Sensitivity, specificity, positive and negative predictability for IgG and IgE results are listed in Table 4.

3.5. Combination of patch test and serum test results

Results of patch testing, and serum testing for allergen-specific IgE and IgG were combined to determine a possible increase in specificity, sensitivity, positive and negative predictability. With the AND-combination, the result was considered positive if all involved single tests were positive. In contrast, with the OR-combination it was sufficient if only one of the involved single tests was positive. Table 5 lists the different combinations and their associated statistical values.

Table 5

Combinations of patch test, specific IgE (≥ 15 AU/l) and IgG (≥ 25 AU/l) determinations and their associated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR).

	A	B	C	D	E	F	G
Sensitivity (%)	6.7	96.7	26.7	96.7	6.7	26.7	96.7
Specificity (%)	99.3	82.1	97.9	80.7	99.3	97.9	77.1
PPV (%)	66.7	53.7	72.7	51.8	66.7	72.7	47.5
NPV (%)	83.2	99.1	86.2	99.1	83.2	86.2	99.1
PLR	9.6	5.4	12.7	5.0	9.6	12.7	4.2
NLR	0.9	0.04	0.7	0.04	0.9	0.7	0.04

A, patch test AND specific IgE; B, patch test OR specific IgE; C, patch test AND specific IgG; D, patch test OR specific IgG; E, patch test AND specific IgE AND specific IgG; F, patch test AND specific IgE OR specific IgG; G, patch test OR specific IgE OR specific IgG.

4. Discussion

In this study, patch testing was shown to have an extremely high sensitivity and negative predictability and a very low negative likelihood ratio and thus can be used to select an appropriate elimination diet in dogs with suspected AFR. However, the specificity and positive predictability were much lower, thus patch testing is not suited to diagnose the presence of an AFR in the dog. Similarly the negative predictability of serum IgE and IgG was higher than the positive predictability, although not quite as high as seen in patch testing. However, the positive and negative predictive values are affected by the prevalence of the disease in the tested population (Petrie and Watson, 1999). The dogs in this study were individuals allergic to either environmental or food allergens or both in which differential diagnoses were ruled out by appropriate tests and treatments. Likelihood ratios are alternative measures of diagnostic accuracy (McGee, 2002). A likelihood ratio of greater than 1 indicates the test result is associated with the disease, a ratio less than 1 indicates that the result is associated with absence of the disease. A combination of patch testing and serum testing for food-specific IgE or IgG resulted in an increased positive likelihood ratio compared to the patch test alone; the negative likelihood was not affected by combining the test results.

4.1. Patch testing

In human patients with suspected food allergy, patch testing is not recommended as a routine diagnostic tool for this disease (Cocco and Sole, 2009). However, many studies indicate that this test could be helpful in some patients. In dogs, patch testing, as well as testing for food-specific serum antibodies, does not seem to be suitable to diagnose food allergy without a subsequent elimination diet. Comparing the results of this study with those in human medicine, differences become apparent. In the present study, the sensitivity and negative predictive value are very high; in human medicine, these values are much lower. However, specificity and positive predictability are clearly higher in human studies. Darsow et al. evaluated 314 atopic patients suffering from a diagnosed food allergy (Darsow et al., 2004). Patch tests were conducted with extracts of egg white, celery and wheat flour. In this study, the specificity was 91.0% and the sensitivity between 30.0 and 32.0% (Darsow et al., 2004). The results of a study by Roehr were similar, positive patch test reactions against cows' milk, hens' egg, wheat and soy were verified with a double-blind-placebo-controlled food challenge (Roehr et al., 2001). The specificity of the patch test ranged from 86.0% for soy to 96.0% for cows' milk; sensitivity was lower (cows' milk, 47.0%, and wheat, 89.0%). The positive predictive value was high for cows' milk (95.0%) and low for soy (50.0%). In contrast, the negative predictive value was high for soy (95.0%) and lower for cows' milk (51.0%) (Roehr et al., 2001). Mehl et al. obtained similar results with a specificity of 86% for soy and 95% for cows' milk (Mehl et al., 2006). However, the sensitivity was lower (cows' milk 31.0%, wheat 27.0%). Differing results may be due to a number of reasons such as the allergens employed, their

concentration, or the vehicle used (Canani et al., 2007). No standardized protocols exist regarding the procedure of patch test using food allergens (Cocco and Sole, 2009). In humans, positive patch test reactions are not seen in people without atopic dermatitis (Mehl et al., 2006; Niggemann, 2002). Based on the results of this study, this may apply to dogs as well. Only two healthy dogs had positive reactions in the patch test in contrast to the atopic dogs of which all but one showed at least one positive reaction.

Comparing reactions to raw versus cooked meat and fish, more lesions were noticed against the raw allergens. The allergenicity of a protein can change during cooking due to alteration of the tertiary structure. Thus previously hidden allergenic determinants may be uncovered (Cave, 2006), but non-continuous epitopes may be destroyed. Fresh food is preferred over commercial extracts (Cocco and Sole, 2009), but studies evaluating differences in patch test reactions between raw and cooked food antigens are lacking.

Most reactions in this study were noticed after 48 h and most of the reactions seen at 24 and 72 h were also present after 48 h. Thus an evaluation after 48 h seems to be suited for the patch test with food allergens in dogs. In approximately one tenth of the cases, reactions would have been missed if the patch test only had been read after 48 h. When evaluating only the reactions exclusively positive after 24 h and verified by rechallenge with the individual allergen, all those reactions were false-positive. However, numbers were small and these findings need to be confirmed. In veterinary medicine, other studies with patch testing against food antigens are lacking. However, using environment allergens and similar evaluation times as in this study, the majority of lesions were noticed 48 or 72 h after application (Marsella et al., 2005; Olivry et al., 2006). In human medicine, best results were obtained after 48 h as well (Cocco and Sole, 2009).

4.2. Serum testing

In human medicine, the measurement of allergen-specific IgE is routinely used to diagnose food allergy (Anderson and Lessof, 1983; Berni Canani et al., 2008). Some authors state that an oral provocation test will be negative if no antibodies are measurable (Atkins et al., 1985; Sampson and Albergo, 1984), but others disagree (Asero et al., 2007). For some allergens (such as hens' egg, cows' milk, peanut, fish), recommendations for the interpretation of allergen-specific IgE concentrations exist. With increased concentrations of allergen-specific IgE, the positive predictive value for being allergic against this allergen is greater than 95.0% (Boyano Martinez et al., 2001; Garcia-Ara et al., 2001). The measurement of allergen-specific IgG is not part of the routine diagnostic workup of food allergy in humans (Beyer and Teuber, 2005). In general, allergen-specific IgG against different foods are interpreted as physiological and a mere reflection of exposure to the allergen (Bahna, 2003). However, patients with an irritable bowel syndrome, who for three months consumed a diet excluding all foods to which they had raised IgG antibodies, showed significantly more improvement of clinical signs than patients eating a sham diet excluding the same

number of foods but not those to which they had antibodies (Atkinson et al., 2004). Thus, it cannot be excluded that removal of food components associated with IgG antibodies could possibly improve clinical signs in dogs as well.

In this study, only two true-positive IgE levels could be confirmed; for twenty-eight antigens, the results were confirmed to be false-negative. These results lead to a positive predictive value of 15.4%. The positive predictive value for the measurement of allergen-specific serum IgG was 34.8%. These results are in concordance with a previous study which also failed to detect allergen-specific IgE in eight dogs with confirmed adverse reactions to food (Mueller and Tsohalis, 1998). Several explanations for the high number of false-negative results exist. A reason for this fact could be that the ELISA was not sensitive enough. Alternatively the antigens used did not contain the relevant epitopes, the binding site of the antibodies in these dogs. In some dogs, AFR may not be mediated by IgE, but may be cell-mediated or not be immunologically based at all (Crowe and Perdue, 1992; Halliwell, 2006; Sampson, 1991). Conversely, if antibodies are detected, they may not necessarily reflect a clinically relevant sensitization against that particular antigen (Asero et al., 2007).

In 2005, Halliwell determined food antigen-specific IgG and IgE in sera of normal dogs, atopic dogs and dogs with confirmed AFR. In contrast to the specific IgE, antigen-specific IgG could be measured against almost every allergen in almost all sera (Halliwell et al., 2005). Similarly, in this study many high serum IgG concentrations against food-specific antigens were noted without concurrent clinical reaction upon exposure to that antigen. In another study, the mean level of food-specific IgG was higher in normal dogs than in dogs with a gastrointestinal or atopic disease (Foster et al., 2003). The concentrations of food-specific IgG against most allergens were significantly higher in dogs with confirmed AFR than in normal dogs in another study (Halliwell et al., 2005). In the present study there was no difference between normal dogs and dogs with confirmed adverse reactions to food. The detection of specific IgG antibodies against food antigens could simply demonstrate the exposure to that particular food allergen (Foster et al., 2003). It is important to emphasize that the obtained positive and negative predictive values may change if a random population of dogs is selected for testing, as these values depend on the prevalence of the disease in a population and due to the selection of dogs with confirmed allergic skin disease the prevalence of the tested dogs is likely very different to the prevalence of the general canine population.

Based on the results of this study, it is unlikely that a dog is allergic to a protein, which does not cause a lesion in the patch test. This held true to a lesser degree for the measurement of food-specific IgG and IgE. Thus patch testing (and to a lesser degree serum testing) can be helpful in choosing ingredients for an elimination diet in a dog with suspected AFR.

5. Conclusion

This study evaluated the diagnostic value of patch testing and serum testing of food-specific antibodies in dogs

with AFR. The high negative predictability of the patch test and to a lesser degree of serum testing for food antigen-specific antibodies indicates that if an antigen does not show a reaction on this test, it is a good choice for an elimination diet.

Conflicts of interest

No conflicts of interest are present with Simone Bethlehem and Ralf Mueller, although in the last five years, non-related support was received by Ralf Mueller for studies, lectures or consulting work from Bayer Animal Health, Boehringer Ingelheim, Dechra Veterinary Products, Intervet, Merial, Novartis Animal Health, Pfizer Animal Health, Procter & Gamble, Royal Canin, Selectavet, and Virbac. Jennifer Bexley is employed by Avacta Animal Health, but was not aware of the clinical details of the study until measurement of serum antibodies was completed and evaluated.

Acknowledgements

The authors would like to thank the owners of the dogs included in the study for their cooperation and patience, Prof. Richard Halliwell for helpful suggestions regarding the manuscript and Allmiral Hermal, Reinbek, Germany for providing the Finn chambers.

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