

Western blot analysis of sera from dogs with suspected food allergy

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Background – Food allergy is often suspected in dogs with clinical signs of atopic dermatitis. This diagnosis is confirmed with an elimination diet and a subsequent challenge with regular food. Laboratory tests for the diagnosis of food allergy in dogs are unreliable and/or technically difficult. Cyno-DIAL[®] is a Western blot method that might assist with the selection of an appropriate elimination diet.

Hypothesis/Objectives – To evaluate the performance of Cyno-DIAL[®] for the selection of an elimination diet and diagnosis of food allergy.

Animals/Methods – Thirty eight dogs with atopic dermatitis completed an elimination diet. Combining the results of the diet trials and the challenges, 14 dogs were classified as food allergic (FA), 22 as nonfood-allergic and two as ambiguous cases.

Results – Amongst all dogs and amongst dogs with a clinical diagnosis of FA, 3% and 7% (respectively) were positive to Royal Canin Allergen[®], Vet-Concept Kanguru[®] or Vet-Concept Dog Sana[®]; 8% and 7% to Hill's d/d Duck and Rice[®]; 8% and 21% to Hill's z/d Ultra Allergen Free[®]; 53% and 64% to Eukanuba Dermatitis FP[®]; and 32% and 43% to a home-cooked diet of horse meat, potatoes and zucchini. The specificity and sensitivity of Cyno-DIAL[®] for diagnosing food allergy were 73% and 71%, respectively.

Conclusions and clinical importance – Although Cyno-DIAL[®] was considered potentially useful for identifying appropriate foods for elimination diet trials, it cannot be recommended for the diagnosis of food allergy. The Cyno-DIAL[®] test performed better than some previously evaluated ELISA-based tests.

Introduction

Food allergy (FA) is often suspected in dogs with clinical signs of atopic dermatitis (AD). Food proteins may cause or induce flares of AD and other AD-unrelated conditions, such as urticaria, angioedema, anaphylaxis and gastrointestinal disturbances.¹ Clinically, it can be difficult to differentiate FA from other forms of adverse food reactions (AFRs).² AFRs encompass both immunological and non-immunological reactions, with the former being immunoglobulin (Ig)- or cell-mediated.²

The diagnosis of AFRs is based on clinical improvement during an elimination diet (ED) trial, followed by relapse during a challenge with the previous or suspected diet.³ The ED is considered to be the gold standard for the diagnosis of AFR because other tests lack sensitivity and/or specificity.^{4,5,6–10}

Food selection for an ED is an area of debate. They may be home-cooked or dry/wet commercial foods. Many authors recommend the use of a home-cooked formulation,⁴ because this approach avoids feeding dogs with unexpected food components (potential allergens not mentioned on the label),¹¹ additives or preservatives. Nevertheless, many owners prefer commercially manufactured products. Commercial foods for EDs may incorporate rarely used protein sources such as exotic animal meat (ostrich, kangaroo) or horse meat. Or they may use hydrolysed proteins; however, some relatively large proteins may persist even after hydrolysis because the hydrolysis process is considered to be incomplete.¹² One systematic review compared home-cooked and commercial foods as EDs and did not show any differences between the diets in terms of diagnostic efficacy.¹³

Accepted 16 October 2016

This study was presented in part as an abstract at the 8th World Congress of Veterinary Dermatology, 2016, Bordeaux, France. *Vet Dermatol* 2016; 27 (Suppl 1): 67 (abstract).

Sources of Funding: The study has been self-funded.

Conflicts of Interest: Nicolas Couturier, Sandrine Jacquenet and Bernard E. Bihain are employees of Gencelis SA, a company that develops and commercializes the Cyno-DIAL[®] test through its trademark Galileo Diagnostics. Claude Favrot is a scientific consultant for Gencelis SA. The remaining authors declare no conflict of interests.

Veterinary clinicians must collect a thorough food history before choosing the food for an ED. This process is relatively easy in dogs that are fed one unique or several well-identified foods, but might be very challenging in dogs that receive multiple foods, treats or leftovers. For these reasons, some authors recommend that AFRs can only be excluded after two unsuccessful ED trials.⁴ The success and interpretability of an ED depend largely on the owners and the communication skills of the veterinarian. Little is known about the possible cross-reactivity between foods, or between food allergens and some environmental allergens, such as mites, helminths and pollens.^{14–16}

Cyno-DIAL[®] (Galileo Diagnostics by Genclis, Vandoeuvre-lès-Nancy, France) is a Western blot (WB)-based test for the detection of food-specific IgE in dog serum. As IgE antibodies may be present in nonfood-allergic dogs, the test is designed mainly to select the food for an ED. The test is carried out on the dog's regular diet and several possible alternative foods for the ED. Then, foods not targeted by the dog's test results are recommended for the ED procedure.

The purpose of this study was to evaluate, first, the results from the Cyno-DIAL[®] test as a guide to choosing an appropriate food for an ED and, secondly, the value of the test for diagnosing food allergy in dogs with AD. Consequently, the study evaluated the frequency of detecting IgE antibodies to food extracts from commonly used EDs, including those empirically chosen by the vet; then correlating positive test results with the clinical response to the ED. The positive IgE results with extracts of the dog's regular diet were compared with the diagnosis of food allergy in dogs with AD.

Material and methods

Case selection

Dogs with clinical signs of nonseasonal AD were enrolled in the study. Dogs were excluded if they had any other disease resembling AD, including ectoparasites, and bacterial and yeast infections. The diagnosis of AD was based on published criteria.¹⁷ Serum was collected at the time of study enrolment, before starting the ED. Disease severity and pruritus were assessed using the Canine Atopic Dermatitis Extent and Severity Index, 4th edition (CADESI-04) and a validated Visual Analog Scale, respectively.^{18,19} Data and serum samples were collected in the context of routine clinical practice. Dog owners gave written approval for their dog to be enrolled in the study.

Elimination diet

Dogs were put through an 8-week ED, with the choice of food for the ED being based on the dog's food history and the preferences of the investigators and owners. Seven EDs were used, including Royal Canin Anallergenic (Royal Canin; Aimargues, France), Hill's z/d Ultra Allergen Free and Hill's d/d Duck and Rice (Hill's Pet Nutrition; Sophia-Antipolis, France), Eukanuba Dermatitis FP (IAMS Europe; Coevorden, the Netherlands), Vet-Concept Kanguru and Vet-Concept Dog Sana (Vet-Concept GmbH & Co. KG; Föhren, Deutschland) and a homemade mix containing cooked horse meat, potatoes and zucchini (See Table S1 in Supporting Information). Regular diets before the ED of most dogs included multiple foods, treats and, sometimes, leftovers.

Dogs improving during the ED trial were challenged with their previous regular diet. Clinical improvement was defined as reductions of AD severity and pruritus of at least 50%. The challenge was

considered positive if the dog relapsed within 10 days. Dogs relapsing during the challenge and improving a second time when ED was reintroduced were classified as food allergic (FA). NFA cases (Non-Food-Allergic dogs) did not show improvement during the ED. Cases were considered ambiguous when the dog's pruritus and lesions improved by less than 50% and/or when the challenge was not associated with relapse. Ambiguous cases were included in analyses of positive test reactions to ED, but not in other analyses. Cases were excluded from all analyses when the dog did not strictly follow the ED or when the owners declined challenge after initial improvement.

Cyno-DIAL[®] test

The Cyno-DIAL[®] test was used to detect IgE antibodies in the dog's sera to food extracts from the seven EDs and selected food items that were most consistently fed, except treats, from each dog's regular diet. The Cyno-DIAL[®] test results were reported only after the ED was completed, to ensure blinding of investigators during the whole ED process.

Cyno-DIAL[®] is a form of Western blot. Briefly, each food item was mechanically homogenized in a saline buffer. The soluble protein part was collected in the supernatant after centrifugation and the protein concentration was determined by the Bradford assay. Food protein extracts were separated by SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane (GE Healthcare; Buckinghamshire, UK) for immunoblotting. The membrane was blocked, incubated with the dog's serum (diluted in blocking buffer), washed with a Tris-buffered saline and Tween buffer, and incubated with an enzyme-labelled anti-dog IgE antibody (Editor's note: the authors have not provided information about the sensitivity or specificity of the anti-canine IgE reagent). The IgE-reactive bands were revealed by enhanced chemiluminescence (GE Healthcare) on autoradiography films. A negative control was carried out with the anti-dog IgE antibody alone to identify nonspecific IgE-reactive bands. A positive test result was defined as the presence of at least one clearly visible and weakly reactive band on the film, corresponding to at least one IgE-targeted food protein.

When the Cyno-DIAL test reported antibodies to the ED, a second diet trial, using a food source with no evidence of IgE antibodies by Western blot test, was recommended for those dogs that had not improved clinically with the first ED.

Data analysis

The sensitivity and specificity of the Cyno-DIAL[®] test were calculated for the final clinical diagnosis of FA or NFA, based on the presence of at least one test positive result for the dietary extract representing the regular diet used before the ED. Differences in positive IgE test rates between the FA and NFA groups were analysed statistically by creating a contingency table and using Fisher's exact test and SPSS Statistics, v22 (SPSS, IBM; Somers, NY, USA).

Results

Of the 48 dogs that were enrolled in the study, 10 dogs did not complete the study because the ED was not strictly followed or was stopped early (eight dogs), or because the owners declined the challenge after initial improvement during the ED (two dogs). Therefore, 38 dogs completed the study and were included in the final analysis (Table 1). No differences between the FA and NFA groups were identified in terms of age, gender, breed, disease severity or pruritus intensity at inclusion (Table 1).

The first goal of this study was to assess if Cyno-DIAL[®] could be an aid in the selection of a diet for an ED. The specific ED for each dog was selected by considering both the dog's food history and owner preference. Accordingly, one of the following was chosen: Royal Canin Anallergenic (14 dogs), Hill's z/d Ultra Allergen Free

Table 1. Demographic information and disease status at study enrolment for 38 dogs with atopic dermatitis and suspected food allergy

Dog	Age (years)	Sex	Breed	Disease severity*	Pruritus intensity†	Diagnosis
1	1	MC	Chihuahua	3	5	FA
2	6.5	FC	Pug	38	5.5	FA
3	0.5	M	French bulldog	8	5.5	FA
4	8	MC	Lhasa apso	18	4.5	FA
5	1.5	MC	Golden retriever	5	5.5	FA
6	9	FC	Mongrel	27	5	FA
7	2	FC	French bulldog	24	5.3	FA
8	1	M	Rhodesian ridgeback	31	4	FA
9	1	M	English bulldog	4	3	FA
10	5	F	Sheltie	4	10	FA
11	8	F	Matino Espanol	15	8	FA
12	13	F	Labrador	18	8	FA
13	5	M	Rhodesian ridgeback	8	5.5	FA
14	3	M	German shepherd dog	0	5	FA
15	1	FC	French bulldog	16	5.3	NFA
16	6	M	Pug	7	8	NFA
17	2	FC	French bulldog	32	7.3	NFA
18	7	M	French bulldog	19	7	NFA
19	1	FC	French bulldog	29	6.3	NFA
20	3	MC	West Highland white terrier	30	5	NFA
21	7	FC	German shepherd	26	8	NFA
22	0.5	F	French bulldog	12	2.3	NFA
23	6	FC	Mongrel	42	8	NFA
24	1	F	French bulldog	39	7.5	NFA
25	5	MC	Shih tzu	32	6.8	NFA
26	3	M	French bulldog	5	6	NFA
27	3	FC	Beagle	42	10	NFA
28	4	M	Labrador retriever	12	8	NFA
29	2	M	Romagnolo	12	3	NFA
30	10	F	French bulldog	127	9	NFA
31	5	MC	Bichon frise	6	8	NFA
32	7	F	German shepherd dog	12	8	NFA
33	7	FC	Mongrel	0	5	NFA
34	2	FC	Poodle	11	8	NFA
35	7	NA	Picardy shepherd	20	7.5	NFA
36	5	M	Labrador retriever	12	4	NFA
37	9	MC	Mongrel	25	4	A
38	9	MC	French bulldog	14	5	A

F female, FC female castrated, M male, MC male castrated, NA unknown, FA food allergy, NFA nonfood allergy, A ambiguous.

*Results from CADESI-04.

†Results from Visual Analog Scale.

(seven dogs), Vet-Concept Dog Sana (three dogs), another commercial diet, not specified above (three dogs) and home-cooked diet (nine dogs). Cyno-DIAL[®] analyses revealed test-positive results to the seven specified chosen EDs in only three cases (Table 2), including two dogs on Hill's z/d Ultra Allergen Free (cases 1 and 9) and one dog on home-cooked diet (Case 12). These three dogs underwent a second ED trial with a diet not associated with positive test results (Royal Canin Anallergenic or Vet-Concept Kanguru). In the three cases, the second diet led to clinical improvement and a final diagnosis of FA.

Combining the results of the first and second ED trials and the challenges, 14 dogs were classified as FA and 22 as NFA. Two additional dogs improved markedly during the trial but failed to relapse during the challenge and, therefore, were classified as ambiguous cases. Ambiguous cases were included only in analyses testing the adequacy of the food chosen for the ED.

As shown in Table 2, substantial differences in antibody responses were found among the tested EDs. Royal

Canin Anallergenic, Vet-Concept Kanguru and Vet-Concept Dog Sana rarely were associated with positive antibody responses (one of 38 dogs), whereas positive responses to the home-cooked diet and Eukanuba Dermatitis FP were relatively frequent [12 of 38 (32%) and 20 of 38 (53%), respectively]. In addition, dogs with a final diagnosis of FA were more often test-positive to EDs than NFA dogs, although this was not statistically significant (Tables 2 and 3).

The second goal of the study was to determine the value of Cyno-DIAL[®] for the diagnosis of food allergy in dogs. When the final clinical diagnosis of FA or NFA was compared to the test result of "at least one weak positive test to an extract of the dog's regular food before the ED", the specificity and sensitivity of Cyno-DIAL[®] for diagnosing food allergy were 73% and 71%, respectively. Significant differences between the FA and NFA groups were observed for the Cyno-DIAL[®] test result (i.e. positive test results to extracts of the regular diet were 10 of 14 for FA and 6 of 22 for NFA; $P = 0.018$, Fisher's exact test).

Table 2. Cyno-DIAL[®] positive test results in 38 dogs to seven common elimination diets and the dog's regular original diet

Dog	Diagnosis	Regular diet	Elimination diet						
			RC	z/d	d/d	FP	Kang	Sana	HC
1	FA	NS	NS	S	NS	S	NS	NS	S
2	FA	S	NS	NS	NS	S	NS	NS	S
3	FA	S	NS	NS	NS	NS	NS	NS	NS
4	FA	S	NS	NS	NS	S	NS	NS	S
5	FA	S	NS	NS	NS	NS	NS	NS	NS
6	FA	NS	NS	NS	NS	NS	NS	NS	NS
7	FA	S	NS	NS	S	S	NS	NS	NS
8	FA	S	NS	S	NS	S	NS	NS	S
9	FA	S	S	S	NS	S	NS	S	S
10	FA	S	NS	NS	NS	NS	NS	NS	NS
11	FA	NS	NS	NS	NS	S	NS	NS	NS
12	FA	S	NS	NS	NS	S	S	NS	S
13	FA	S	NS	NS	NS	S	NS	NS	NS
14	FA	NS	NS	NS	NS	NS	NS	NS	NS
15	NFA	NS	NS	NS	NS	NS	NS	NS	NS
16	NFA	S	NS	NS	NS	S	NS	NS	S
17	NFA	NS	NS	NS	NS	NS	NS	NS	NS
18	NFA	S	NS	NS	NS	S	NS	NS	NS
19	NFA	NS	NS	NS	S	S	NS	NS	NS
20	NFA	NS	NS	NS	NS	S	NS	NS	NS
21	NFA	S	NS	NS	NS	NS	NS	NS	NS
22	NFA	S	NS	NS	NS	NS	NS	NS	NS
23	NFA	NS	NS	NS	NS	S	NS	NS	NS
24	NFA	NS	NS	NS	NS	NS	NS	NS	NS
25	NFA	NS	NS	NS	NS	NS	NS	NS	NS
26	NFA	S	NS	NS	NS	S	NS	NS	S
27	NFA	NS	NS	NS	NS	NS	NS	NS	NS
28	NFA	NS	NS	NS	NS	NS	NS	NS	NS
29	NFA	NS	NS	NS	NS	S	NS	NS	S
30	NFA	NS	NS	NS	NS	S	NS	NS	S
31	NFA	NS	NS	NS	NS	NS	NS	NS	NS
32	NFA	NS	NS	NS	NS	NS	NS	NS	NS
33	NFA	NS	NS	NS	NS	NS	NS	NS	NS
34	NFA	NS	NS	NS	NS	NS	NS	NS	NS
35	NFA	NS	NS	NS	NS	NS	NS	NS	NS
36	NFA	S	NS	NS	NS	S	NS	NS	S
37	A	S	NS	NS	S	S	NS	NS	NS
38	A	NS	NS	NS	NS	S	NS	NS	S

RC Royal Canin Anallergenic, z/d Hill's z/d Ultra Allergen Free, d/d Hill's d/d Duck and Rice, FP Eukanuba Dermatitis FP, Kang Vet-Concept Kanguru, Sana Vet-Concept Dog Sana, HC home cooked horse meat, potatoes and zucchini, NS not sensitized (test negative), S sensitized (test positive).

Bold face, result of the Cyno-DIAL test to the food chosen for ED.

All diet extracts were a mixture of food proteins used for the Cyno-DIAL[®] test.

Table 3. Summary of test positive dogs to elimination diets reported in Table 2

Positive test result	Elimination diet (%)						
	RC	z/d	d/d	FP	Kang	Sana	HC
FA group	7	21	7	64	7	7	43
NFA group	0	0	5	41	0	0	23

FA food allergy group, NFA nonfood allergy group, RC Royal Canin Anallergenic, z/d Hill's z/d Ultra Allergen Free, d/d Hill's d/d Duck and Rice, FP Eukanuba Dermatitis Fish and Potato, Kang Vet-Concept Kanguru, Sana Vet-Concept Dog Sana, HC home-cooked horse meat, potatoes and zucchini.

All diet extracts were a mixture of food proteins used for the Cyno-DIAL[®] test.

Discussion

This study reports the results of a Western blot method for the diagnosis of canine FA. The results of the present

study indicate that although useful for assessing positive reactions for EDs, Cyno-DIAL[®] is not sufficiently accurate to warrant its recommendation as a diagnostic test for FA.

There are several possible reasons for the insufficient accuracy of Cyno-DIAL[®] in diagnosing food allergy. First, not all foods eaten by the dogs were tested. Although the most frequently fed foods were tested, the possibility that reactions were caused by less frequently fed foods cannot be excluded. Secondly, many atopic dogs are polyallergic. Some atopic dogs classified as NFA may have had some degree of food allergy that contributed to their clinical signs, but the ED was not sufficiently sensitive to detect the food allergy. Finally, at least in some cases, a cell-mediated response cannot be ruled out.

This study showed that in most cases, dogs that improved during an ED trial were not sero positive to

the food chosen. The high rate of positive test reactions to home-cooked horse and potato may be due to the more frequent presence of potatoes in regular commercial canine food products. Interestingly, however, no positive test reactions to Vet-Concept Dog Sana, a product with similar ingredients, was shown. This could be due to the different processing of primary ingredients. Commercial diets are usually processed at high temperature and pressure, which may lead to allergen modifications. Detecting antibodies may help, at least on some occasions, to avoid the use of inappropriate ED. Detection of IgE does not confirm the existence of a food allergy; however, it seems safer to choose an ED that is not associated with a positive test result.

WB presents several advantages and drawbacks. On the one hand, each protein is separated in the electrophoresis step, which increases the protein exposure and the sensitivity. On the other, electrophoresis requires denaturation of the molecules, which precludes any identification of conformational epitopes. Allergenic proteins usually present several IgE-targeted epitopes, some of which are conformational whereas others are not. Consequently, it may be that the allergenic proteins are still identified, even if some of the epitopes are not recognized.²⁰ Additionally, persistent allergic problems in humans, especially to milk and egg, are mostly associated with sequential epitopes.²¹ Previous studies in dogs have consistently shown very low sensitivities for ELISA-based IgE serology tests.^{7,22,23} Although still insufficient, the sensitivity of Cyno-DIAL® (71%) was markedly higher than that reported for some ELISA-based techniques; in one ELISA study, sensitivity was recorded at 7.2%.⁷ Therefore, one could conclude that the advantages of WB-based tests may exceed their drawbacks.

A major drawback of any IgE-based allergen test, including ELISA-based tests, is that the food-specific IgE may not be associated with any clinically significant allergic reaction. There are two reasons for this lack of an association. First, cross-linking of the IgE receptor requires binding to two adjacent epitopes on the inducing protein.²⁰ Secondly, there are numerous cross-reactions between allergens, leading to the synthesis of pathogenic and nonpathogenic allergen-specific IgE molecules.²⁴ Thus, the only proof of food allergy is positive challenge with the suspected protein or food. Finally, any IgE-based allergen test can only identify IgE-driven reactions. In humans, for instance, although most food-associated allergic skin reactions are IgE-mediated, some are not.²⁵ The proportion of food reactions in dogs that are IgE-mediated reactions is unknown.²

Cyno-DIAL® may assist the veterinary clinician with the selection of an appropriate ED, but this test cannot be currently regarded as a diagnostic test for food allergy. Further developments of WB-based tests may permit “component-resolved diagnosis” by enabling more appropriate food challenges, as well as more accurate identification of foods and component proteins that elicit IgE-driven reactions in dogs.

Acknowledgements

The authors thank Aurélien Gustin, Marie-Christine Sancier and Sylvianne Faron (Genclis SA employees) for their technical expertise.

References

- Olivry T, DeBoer DJ, Prelaud P et al. Food for thought: pondering the relationship between canine atopic dermatitis and cutaneous adverse food reactions. *Vet Dermatol* 2007; 18: 390–391.
- Verlinden A, Hesta M, Millet S et al. Food allergy in dogs and cats: a review. *Crit Rev Food Sci Nutr* 2006; 46: 259–273.
- Rosser EJ. Diagnosis of food allergy in dogs. *J Am Vet Med Assoc* 1993; 203: 259–262.
- Rosser EJ. Diagnostic work-up of food hypersensitivity. In: Noli C, Foster A, Rosenkranz W, eds. *Veterinary Allergy*. Chichester, West Sussex, UK: John Wiley & Sons, 2014; 119–123.
- Zimmer A, Bexley J, Halliwell RE et al. Food allergen-specific serum IgG and IgE before and after elimination diets in allergic dogs. *Vet Immunol Immunopathol* 2011; 144: 442–447.
- Hardy JI, Hendricks A, Loeffler A et al. Food-specific serum IgE and IgG reactivity in dogs with and without skin disease: lack of correlation between laboratories. *Vet Dermatol* 2014; 25: 447–e470.
- Bethlehem S, Bexley J, Mueller RS. Patch testing and allergen-specific serum IgE and IgG antibodies in the diagnosis of canine adverse food reactions. *Vet Immunol Immunopathol* 2012; 145: 582–589.
- Fujimura M, Masuda K, Hayashiya M et al. Flow cytometric analysis of lymphocyte proliferative responses to food allergens in dogs with food allergy. *J Vet Med Sci* 2011; 73: 1309–1317.
- Ishida R, Masuda K, Kurata K et al. Lymphocyte blastogenic responses to inciting food allergens in dogs with food hypersensitivity. *J Vet Intern Med* 2004; 18: 25–30.
- Guilford WG, Strombeck DR, Rogers Q et al. Development of gastroscopic food sensitivity testing in dogs. *J Vet Intern Med* 1994; 8: 414–422.
- Ricci R, Granato A, Vascellari M et al. Identification of undeclared sources of animal origin in canine dry foods used in dietary elimination trials. *J Anim Physiol Anim Nutr (Berl)* 2013; 97 (Suppl 1): 32–38.
- Roitel O, Maurice D, Douchin G et al. High molecular-weight proteins in hydrolysed dog foods. *Vet Dermatol* 2015; 26(Suppl 1): 304.
- Olivry T, Bizikova P. A systematic review of the evidence of reduced allergenicity and clinical benefit of food hydrolysates in dogs with cutaneous adverse food reactions. *Vet Dermatol* 2010; 21: 32–41.
- Sánchez-Borges M, Suárez-Chacón R, Capriles-Hulett A et al. An update on oral anaphylaxis from mite ingestion. *Ann Allergy Asthma Immunol* 2005; 94: 216–221.
- da Costa Santiago H, Ribeiro-Gomes FL, Bennuru S et al. Helminth infection alters IgE responses to allergens structurally related to parasite proteins. *J Immunol* 2015; 194: 93–100.
- Sidenius KE, Hallas TE, Poulsen LK et al. Allergen cross-reactivity between house-dust mites and other invertebrates. *Allergy* 2001; 56: 723–733.
- Favrot C, Steffan J, Seewald W et al. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet Dermatol* 2010; 21: 23–31.
- Hill PB, Lau P, Rybníček J. Development of an owner-assessed scale to measure the severity of pruritus in dogs. *Vet Dermatol* 2007; 18: 301–308.
- Olivry T, Saridomichelakis M, Nuttall T et al. Validation of the Canine Atopic Dermatitis Extension and Severity Index (CADESI)-4, a simplified severity scale for assessing skin lesions of atopic dermatitis in dogs. *Vet Dermatol* 2014; 25: 77–85.
- Huby RDJ, Dearman RJ, Kimber I. Why are some proteins allergens? *Toxicol Sci* 2000; 55: 235–246.

21. Wang J, Sampson HA. Food allergy. *J Clin Invest* 2011; 121: 827–835.
22. Mueller RS, Toshiaki J. Evaluation of serum allergen-specific IgE for the diagnosis of food adverse reactions in the dogs. *Vet Dermatol* 2003; 9: 167–171.
23. Ricci R, Hammerberg B, Paps J et al. A comparison of the clinical manifestations of feeding whole and hydrolysed chicken to dogs with hypersensitivity to thenative protein. *Vet Dermatol* 2010; 21: 358–366.
24. Foster AP, Knowles TG, Moore AH et al. Serum IgE and IgG responses to food antigens in normal and atopic dogs, and dogs with gastrointestinal disease. *Vet Immunol Immunopathol* 2003; 92: 113–124.
25. Spergel J. Nonimmunoglobulin E-mediated immune reactions to foods. *Allergy Asthma Clin Immunol* 2006; 2: 78–85.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Composition of commercial EDs.

Résumé

Contexte – L’allergie alimentaire est souvent suspectée chez les chiens présentant des signes cliniques de dermatite atopique. Ce diagnostic est confirmé par un régime d’éviction suivi d’un test de provocation avec l’alimentation habituelle. Les tests de laboratoire pour le diagnostic d’allergie alimentaire ne sont pas fiables ou sont techniquement difficiles. Cyno-DIAL[®] est une méthode de Western blot qui peut aider la sélection d’un régime d’éviction approprié.

Hypothèses/Objectifs – Evaluer l’efficacité de Cyno-DIAL[®] pour la sélection d’un régime d’éviction et le diagnostic d’allergie alimentaire.

Sujets/Méthodes – Trente-huit chiens atteints de dermatite atopique ont suivi un régime d’éviction. Les résultats des régimes d’éviction et des tests de provocation ont été regroupés, 14 chiens ont été classés comme allergiques alimentaires (FA), 22 comme non allergiques et deux cas ambigus.

Résultats – Parmi tous les chiens et parmi les chiens atteints de FA, 3% et 7% (respectivement) étaient positifs à Royal Canin Anallergenic[®], Vet-Concept Kanguru[®] ou Vet-Concept Dog Sana[®]; 8% et 7% à Hill’s d/d Duck and Rice[®]; 8% et 21% à Hill’s z/d Ultra Allergen Free[®], 53% et 64% à Eukanuba Dermatitis FP[®]; et 32% et 43% à un régime ménager de viande de cheval, pommes de terre et courgettes. La spécificité et la sensibilité de pour le diagnostic d’allergie alimentaire étaient respectivement 73% et 71%.

Conclusions et importance clinique – Bien que Cyno-DIAL[®] soit considéré comme potentiellement utile pour identifier les aliments appropriés pour les régimes d’éviction, il ne peut pas être recommandé pour le diagnostic d’allergie alimentaire. Le test est plus efficace que certains tests ELISA précédemment évalués.

Resumen

Introducción – Se sospecha a menudo alergia a los alimentos en perros con signos clínicos de dermatitis atópica. Este diagnóstico se confirma con una dieta de eliminación y un posterior desafío con alimentos regulares. Las pruebas de laboratorio para el diagnóstico de la alergia alimentaria en perros son poco fiables y /o técnicamente difíciles. Cyno-DIAL[®] es un método de Western blot que puede ayudar con la selección de una dieta de eliminación adecuada.

Hipótesis/Objetivos – Evaluar la utilidad de Cyno-DIAL[®] para la selección de una dieta de eliminación y el diagnóstico de alergia alimentaria.

Animales/Métodos – Treinta y ocho perros con dermatitis atópica completaron una dieta de eliminación. Combinando los resultados de las pruebas dietéticas y las reexposiciones, 14 perros fueron clasificados como alérgicos alimenticios (FA), 22 como alérgicos no alimenticios y dos como ambiguos.

Resultados – Entre todos los perros y entre los perros con un diagnóstico clínico de FA, 3% y 7% (respectivamente) fueron positivos para Royal Canin Anallergenic[®], Vet-Concept Kanguru[®] o Vet-Concept Dog Sana[®]; 8% y 7% a Hill’s d/d Pato y Arroz[®]; 8% y 21% a Hill’s z/d Ultra Allergen Free[®]; 53% y 64% a Eukanuba Dermatitis FP[®]; y 32% y 43% a una dieta casera de carne de caballo, patatas y calabacín. La especificidad y sensibilidad de Cyno-DIAL[®] para el diagnóstico de la alergia alimentaria fue de 73% y 71%, respectivamente.

Conclusiones e importancia clínica – Aunque Cyno-DIAL[®] se consideró potencialmente útil para la identificación de alimentos apropiados para ensayos con dieta de eliminación, no puede recomendarse para el diagnóstico de alergia alimentaria. La prueba Cyno-DIAL[®] fue mejor que algunas pruebas previamente basadas en ELISA.

Zusammenfassung

Hintergrund – Eine Futtermittelallergie wird bei Hunden mit klinischen Anzeichen einer atopischen Dermatitis häufig vermutet. Die Diagnose dafür wird mittels Eliminationsdiät und einer nachfolgenden Provokation mit Normalfutter bestätigt. Laboruntersuchungen zur Diagnose einer Futtermittelallergie bei Hunden sind nicht verlässlich und/oder technisch schwierig. Cyno-DIAL[®] ist eine Western Blot Methode, die bei der Selektion einer passenden Ausschlussdiät helfen kann.

Hypothese/Ziele – Eine Evaluierung der Effektivität des Cyno-DIAL[®] zur Auswahl einer Eliminationsdiät und zur Diagnose einer Futtermittelallergie.

Tiere/Methoden – Achtunddreißig Hunde mit atopischer Dermatitis beendeten eine Ausschlussdiät. Nach Kombination der Ergebnisse der Eliminationsdiäten und der Provokationen wurden 14 Hunde als Futtermittel-allergisch (FA), 22 als nicht futtermittelallergisch und zwei als nicht eindeutig klassifiziert.

Ergebnisse – Von allen Hunden sowie von den Hunden mit einer klinischen Diagnose FA waren 3% bzw 7% positiv mittels Royal Canin Anallergenic®, Vet-Concept Kanguru® oder Vet-Concept Hund Sana®; 8% und 7% waren positiv mittels Hill's d/d Duck and Rice®; 8% und 21% mittels Hill's z/d Ultra Allergen Free®; 53% und 64% mittels Eukanuba Dermatitis FP®; und 43% mittels einer selbstgekochten Pferd und Kartoffeldiät mit Zucchini. Spezifität und Sensibilität des Cyno-DIAL® zur Diagnose einer Futtermittelallergie lag bei 73% bzw 71%.

Schlussfolgerungen und klinische Bedeutung – Obwohl Cyno-DIAL® für möglicherweise sinnvoll zur Identifizierung geeigneter Futtermittel für Ausschlussdiäten gehalten wurde, kann es nicht zur Diagnose einer Futtermittelallergie empfohlen werden. Der Cyno-DIAL® war insgesamt besser als früher evaluierte ELISA-basierte Tests.

要約

背景 – 食物アレルギーはアトピー性皮膚炎の臨床所見を持つ犬でしばしば疑われる。その診断は、除去食試験とそれに引き続いて実施される通常食による負荷食試験によって確定される。犬の食物アレルギー診断のための臨床検査は信頼性に欠け、および/あるいは技術的に困難である。Cyno-DIALは、適切な除去食試験食を選択するための補助となる可能性のあるウェスタンブロット法である。

仮説/目的 – 除去食試験の選択および食物アレルギーの診断におけるCyno-DIALの有用性を評価すること。

供与動物/方法 – 除去食試験を完遂したアトピー性皮膚炎の犬38頭。除去食試験と負荷食試験の結果を総合し、14頭が食物アレルギー(FA)、22頭が非食物アレルギー、2頭が不確かな症例と分類された。

結果 – すべての犬およびFAと臨床診断された犬におけるそれぞれの陽性率は以下の通りである。Royal Canin AnallergenicとVet-Concept KanguruとVet-Concept Dog Sanaに対して3%および7%、Hill's d/d Duck and Riceに対して8%および7%、Hill's z/d Ultra Allergen Freeに対して8%および21%、Eukanuba Dermatitis FPに対して53%および64%、馬肉とイモとズッキーニを使ったホームメイド食に対して32%および43%であった。食事アレルギー診断に対するCyno-DIALの特異性および感度はそれぞれ73%および71%であった。

結論および臨床的な重要性 – Cyno-DIALは除去食試験のための適切な食事の選択に有用である可能性があるが、食物アレルギーの診断には推奨されない。Cyno-DIALは、これまでに評価されたいくつかのELISAに基づく臨床検査よりも優れていた。

摘要

背景 – 犬の异位性皮膚炎常被怀疑存在食物过敏。确诊需要食物排除和随后的常规食物激发试验。实验室试验诊断食物过敏不可靠,并且有技术难度。Cyno-DIAL是西方的一种蛋白质印迹法,也许可以帮助挑选适宜的低敏食物。

目的 – 评价Cyno-DIAL检测在挑选低敏食物和诊断食物过敏的表现

方法 – 38只患有异位性皮膚炎の犬,全部做食物排除试验。结合食物试验和激发试验的结果,有14只犬被证明有食物过敏(FA),22只犬非食物过敏,另有两只犬结果模稜两可。

結果 – 在所有犬和诊断有食物过敏的犬中,3%和7%(分别)对Royal CaninAnallergenic,Vet-Concept Kanguru 或者Vet-Concept Dog Sana呈阳性; 8% 和 7% 对Hill's d/d Duck and Rice 阳性; 8% 和 21% 对Hill's z/d Ultra Allergen Free阳性;53% and 64% to Eukanuba Dermatitis FP阳性;另有32% 和 43%对自制的马肉食物,马铃薯和西葫芦阳性。Cyno-DIAL诊断食物过敏的特异性和敏感性分别是73% 和 71%。

结论和临床意义 – 尽管Cyno-DIAL可识别合理的低敏食物,用于食物排除试验,但是并不推荐用于食物过敏的诊断。Cyno-DIAL 检测比以前基于ELISA方法检测效果更好。

Resumo

Contexto – Frequentemente, suspeita-se de dermatite trofoalérgica em cães com sinais clínicos de dermatite atópica. Este diagnóstico é confirmado com a dieta de eliminação e subsequente desafio com o alimento regular. Testes laboratoriais para o diagnóstico da dermatite trofoalérgica em cães não são confiáveis e/ou são tecnicamente complicados. Cyno-DIAL® é um método de Western blot que pode auxiliar na seleção de uma dieta de exclusão adequada.

Hipótese/Objetivos – Avaliar a performance de Cyno-DIAL® para a seleção de uma dieta de eliminação e diagnóstico de dermatite trofoalérgica.

Animais/métodos – Trinta e oito cães com dermatite atópica completaram a dieta de eliminação. Associando os resultados das dietas hipoalergênicas e com os desafios, 14 cães foram diagnosticados com hipersensibilidade alimentar (HA), 22 como não alérgicos a alimentos e dois casos como ambíguos.

Resultados – Dentre todos os cães e dentre todos os com diagnóstico de HA, 3% e 7% (respectivamente) foram positivos para Royal Canin Anallergenic®, Vet-Concept Kanguru® ou Vet-Concept Dog Sana®; 8% e

7% a Hill's d/d Duck and Rice[®]; 8% e 21% to Hill's z/d Ultra Allergen Free[®]; 53% e 64% to Eukanuba Dermatitis FP[®]; e 32% e 43% a uma dieta caseira a base de carne de cavalo, batatas e abobrinha. A sensibilidade e especificidade de Cyno-DIAL[®] para o diagnóstico de dermatite trofoalérgica foram 73% e 71%, respectivamente.

Conclusões e importância clínica – Apesar de Cyno-DIAL[®] ter sido considerado potencialmente útil para a identificação de alimentos apropriados para a dieta de eliminação, não pode ser recomendado para o diagnóstico de hipersensibilidade alimentar. O Cyno-DIAL[®] obteve resultados melhores que outros testes de ELISA previamente avaliados.