

Evaluation of colonoscopic allergen provocation as a diagnostic tool in dogs with proven food hypersensitivity reactions

OBJECTIVE: To evaluate the colonoscopic allergen provocation (COLAP) test as a new tool for the diagnosis of IgE-mediated food allergy.

METHODS: Oral food challenges as well as COLAP testing were performed in a colony of nine research dogs with proven immediate-type food allergic reactions. In addition, COLAP was performed in five healthy dogs.

RESULTS: When compared with the oral challenge test, COLAP accurately determined 18 of 23 (73 per cent) positive oral challenge reactions (73 per cent) in dogs with food allergies and was negative in the healthy dogs.

CLINICAL SIGNIFICANCE: The accuracy of this new test may be higher than that for gastric sensitivity testing. Therefore, COLAP holds promise as a new test to confirm the diagnosis of suspect IgE-mediated food allergy in dogs.

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INTRODUCTION

A significant number of dogs with chronic diarrhoea, vomiting, and weight loss respond favourably to treatment with elimination diet and are therefore suspected of having a food allergy (Jergens and others 1992, Guilford 1994, Paterson 1995, German and others 2001). In true food allergy, an immunological reaction takes place in the intestinal mucosa and causes the associated clinical signs. The exact pathogenesis of food allergy in dogs remains unclear and is believed to involve type I as well as late-type hypersensitivity reactions (type II and IV) (Rosser 1993, Hall 1994).

To date, reaching a definitive diagnosis of food allergy in dogs is hampered by the lack of appropriate diagnostic tests (Guilford 1994). The current gold standard is the oral food challenge test (August 1985, Rosser 1993, White 1998) during which dogs suspected of food allergy are

fed an elimination diet for six to 14 weeks, leading to resolution of clinical signs if the diagnosis is confirmed. Thereafter, challenge with single proteins from the original diet are performed until the clinical signs recur.

Several recent studies support the hypothesis that type I hypersensitivity reactions are, at least in part, responsible for the clinical signs in dogs with food allergies. One study reported higher mast cell numbers and increased IgE levels in dogs with chronic enteropathies implying participation of type I hypersensitivity reactions in the pathogenesis of the disease (Locher and others 2001). In addition, measurement of allergen-specific IgE in the faeces correlated well with the results of oral food challenges in food hypersensitive dogs (Vaden and others 2000a). Using Doppler ultrasound of cranial mesenteric and coeliac arteries in some of the dogs in this study, allergens leading to a clinical reaction one to four days after oral challenge elicited a strong vascular response in the first 24 hours after starting oral challenges. These results reproduce the findings reported in humans with food hypersensitivity (Kircher and others 2004). The only procedure currently available for direct assessment of immediate IgE-mediated reactions in the gastrointestinal mucosa of dogs is the gastroscopic food sensitivity test (GFST).

The use of this test to diagnose canine IgE-mediated food allergy has been described in healthy dogs and dogs with suspected food allergies (Elwood and others 1994, Guilford and others 1994), as well as in a colony of soft coated wheaten terriers (SCWT) with clinically proven food allergy (Vaden and others 2000a). During this procedure, allergen solutions are dropped around rugal folds in the most ventral portion of the stomach while performing gastroscopy. The test is technically difficult to perform, and the mucosal response is inconsistent and dif-

difficult to interpret because allergen extract may run off the application site (Guilford and others 1994, Vaden 2000a). The GFST has been reported to have a low sensitivity and variable specificity when compared with oral challenge tests (Elwood and others 1994, Guilford and others 1994, Vaden and others 2000a).

In human beings, type I hypersensitivity is thought to be the most common form of food allergy (Bischoff and others 2000). The diagnostic elements include patient history, serological and skin tests, as well as double-blind placebo-controlled food challenges with the suspect protein (Bischoff and Manns 2001). The colonoscopic allergen provocation (COLAP) has been reported to help in the diagnosis of food allergy in adults (Bischoff and others 1997b). During this procedure, the food allergens are injected into the colonic mucosa and a mucosal wheal and flare reaction is evaluated to assess the possibility of an immediate type I reaction. COLAP has also been shown to correlate very well with the history of food allergy in human beings (Bischoff and others 1997a). Histological evidence of mast cell degranulation in biopsies taken after allergen injection at sites that showed a wheal and flare reaction confirm the involvement of an IgE-mediated reaction in human beings as well as in dogs (Bischoff and others 1997b, Puhl and others 1999). The COLAP technique has already been applied in dogs during a study using atopic dogs as a model for human allergies (Puhl and others 1999).

The purpose of the current study was therefore to evaluate the COLAP procedure in a group of dogs with proven IgE-mediated food hypersensitivity by comparing it with the results of an oral food challenge test as the gold standard. The dogs of this colony are affected by a familial form of protein-losing enteropathy (PLE), protein-losing nephropathy (PLN), or both (Littman and others 2000). Previous studies on these animals have shown that daily gluten administration led to a significant decrease in serum globulin concentrations (Vaden and others 2000b). In addition, clinically manifest food hypersensitivity reactions accompanied by decreased albumin levels were observed

during previous oral food challenges (Vaden and others 2000a).

The protocol used during these oral challenges was shortened compared with published protocols from clinical trials. The oral challenges were performed over one to four days during which period all of the dogs showed clinically visible reactions to the offending feed, that is, pruritus as well as vomiting and diarrhoea (Vaden and others 2000a). Since this protocol has worked well in the same dogs before, it was used as a gold standard to which to compare the results of the COLAP.

MATERIALS AND METHODS

Dogs with food allergies

All investigations were performed on research dogs from a colony maintained at the College of Veterinary Medicine, North Carolina State University.

The animals were cared for according to the principles outlined in the *National Institute of Health Guide for the Care and Use of Laboratory Animals*. The Study was approved by the North Carolina State University Institutional Animal Care and Use Committee. Three purebred food allergic SCWT (one female and one male, each six years old and one female, eight years old), as well as six food allergic SCWT × beagle crosses (all seven years old, three male and three female) were included into the study.

Control dogs

Five healthy beagles (four females, five years old and one male, six years old) that were sacrificed for an unrelated study were used as controls. Immediately before euthanasia, the dogs were anaesthetised to perform the COLAP test. Preparation of the dogs and anaesthetic procedures were identical to the ones used for the clinically allergic dogs.

Laboratory tests and histology

A complete blood count (CBC), biochemical profile, urinalysis, urine protein:creatinine ratio, and faecal flotation for intestinal parasites were performed two

months before the oral challenge tests at the North Carolina State University, College of Veterinary Medicine Pathology Laboratory, using standard laboratory methods. Faecal α 1-proteinase inhibitor concentration was evaluated as the mean of three samples collected over three consecutive days two months before the oral challenges using previously reported methodology (Melgarejo and others 1998).

Biopsies of the duodenal and the colonic mucosa were obtained from all dogs included into the study during gastroduodenoscopy and colonoscopy. The biopsies were stored in 10 per cent neutral buffered formaldehyde for no longer than 24 hours before being further processed.

COLAP test

The COLAP test was conducted using the method first described by Bischoff and others (1997b) and applied to atopic dogs by Puhl and others (1999). A liquid elemental diet (Vivonex RTF; Novartis Nutrition) was fed for three days. Thereafter, the dogs were fasted for 48 hours before endoscopy. In addition, an electrolyte solution (30 ml/kg, orally [Golytely; Braintree Labs]) was given to the dogs by stomach tube the day before anaesthesia to cleanse the colon. Anaesthesia was performed using atropine (0.02 mg/kg subcutaneously) as premedication and isoflurane for induction and maintenance. Oxymorphone (0.05 mg/kg intravenously) was given during recovery from anaesthesia. A flexible gastroscope (GIF XQ230 flexible videoendoscope; Olympus Keymed) connected to a video processor (EVIS CL 100; Olympus America) via an attached camera (OVC 100; Olympus America) was used for all procedures. After gastroduodenoscopy with sampling of duodenal mucosal biopsies, a colonoscopy was performed. Injection sites for the allergens were selected in a clockwise fashion around the ileocolic valve. Allergens were injected into the colonic mucosa using an instrument similar to that used for endoscopic sclerotherapy of oesophageal varices in human beings (Kobi GMBH). This device consists of a 150 cm-long flexible metallic tube connected to a fine needle at its distal end and a syringe containing the allergen extract at its prox-

imal end. The tube was washed between each injection with 5 ml of 0.9 per cent sodium chloride and 10 ml of air. This procedure has previously been performed in human beings during colonoscopy and has been shown to suffice for cleaning of the endoscope channels between injections (Bischoff and others 1997b). Allergen extracts (chicken, wheat, lamb, milk, egg, corn: intracutaneous allergen solutions, Allergomed; soybean: intracutaneous allergen solution, Greer Laboratories) of wheat, lamb, egg, chicken, soybean, corn, and milk were used at a concentration of 1 mg/ml, injecting 0.3 ml per site and allergen. In addition, 0.9 per cent sodium chloride (0.3 ml) and histamine (intracutaneous allergen solutions, Allergomed) 0.3 ml were used as negative and positive controls, respectively. In dog 8, corn was injected at a reduced concentration of 0.2 mg/ml instead. In this dog, strong paroxysms of stomach motility as well as a decrease in mean arterial blood pressure and an increase in heart rate had been noticed during a previously performed GFST procedure with this allergen, which was interpreted as a strong hypersensitivity reaction. All injection sites were observed for 15 minutes after application of the allergen. The reactions were interpreted as positive when clearly demarcated swelling, oedema, and hyperaemia were observed (Figs 1 and 2). The investigator who evaluated the reaction site (K. A.) was blinded as to which allergens were being injected. The COLAP procedures were also videotaped to be reviewed in a blinded fashion by two investigators (K. A. and F. G.) for confirmation of reactions.

After the COLAP test, the colonoscope was withdrawn to the level of the descending colon and biopsies were taken for subsequent histological examination.

Oral food challenge tests

Oral food challenge tests were performed in dogs with food allergies approximately six months after the COLAP. The abbreviated protocol of the oral challenge test used in this study has been previously published (Vaden and others 2000a). Elevated faecal IgE levels during these tests have clearly shown the involvement

of a type I hypersensitivity reaction in the mucosa of the dogs (Vaden and others 2000a). All proteins tested with the COLAP were also fed during the oral challenge. Before starting the oral food trials, the dogs were fed a hypoallergenic liquid elemental diet for three days (Vivonex RTF; Novartis Nutrition). In the months before starting the study, none of the dogs exhibited more than occasional vomiting, diarrhoea, or pruritus. Seven test meals were fed, each for two days, and cooked white rice was added to the proteins to fulfil basic calorie needs. After two days of testing, the dogs were fed cooked white rice as a sole diet during the washout period of 24 to 72 hours. The test meals were composed of chicken (canned, pre-cooked chicken, white and dark meat, Valley Fresh), wheat (wheat farina, Bob's red Mill Natural Foods), lamb (cooked shoulder), soybean (precooked, firm-style tofu, The Soy Shop), milk (lactose-free 2 per cent milk, Lactaid 100, H. P. Hood), egg (cooked whole chicken eggs, 14 g of protein per day, remaining calories to fulfil requirements were added as cooked white rice), and corn (boiled yellow corn, Harris Teeter; 6 g of protein per day). Dogs were observed for vomiting, diarrhoea, and pruritus throughout the one-hour postpran-

dial period and at 2, 4, 8, 10, 15, 24, 29, and 35 hours later. During the wash-out days, none of the dogs showed any signs of vomiting, diarrhoea, or pruritus. During challenge days, loose stools, vomiting, or excessive pruritus were interpreted as a reaction against the ingested food ingredient.

RESULTS

Laboratory results and histology

Dogs with food allergies CBCs were within normal limits for the nine dogs before starting the study. Two out of nine dogs had evidence of PLE as manifested by hypoalbuminaemia or hypoglobulinaemia (2.2 mg/dl albumin, 2.2 mg/dl globulin, reference range for both >2.5 g/dl). The rest of the chemistry profile was within normal limits. Faecal α_1 -proteinase inhibitor concentration was increased in two of nine dogs (6.85 and 5.85 μ g/g, reference range 0.23 to 5.67 μ g/g). There was evidence of PLN in two of nine dogs as defined by urine protein: creatinine ratios >1.0 (2.08 and 2.9).

Histology of mucosal biopsies was interpreted by one board-certified patholo-

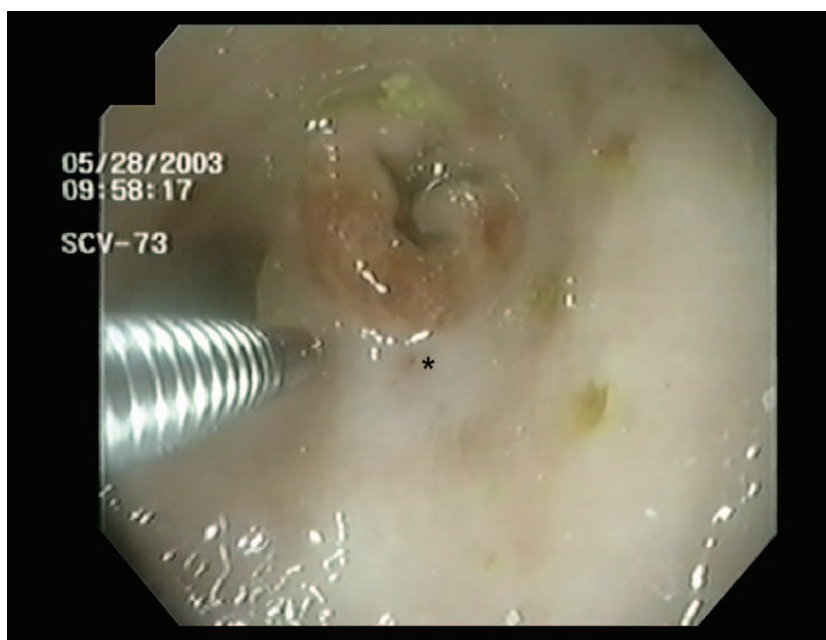


FIG 1. Example of positive test results as seen during the colonoscopic allergen provocation testing immediately after injection of the allergen solution. The asterisk marks the injection site. There is a clearly demarcated swelling visible on the mucosa

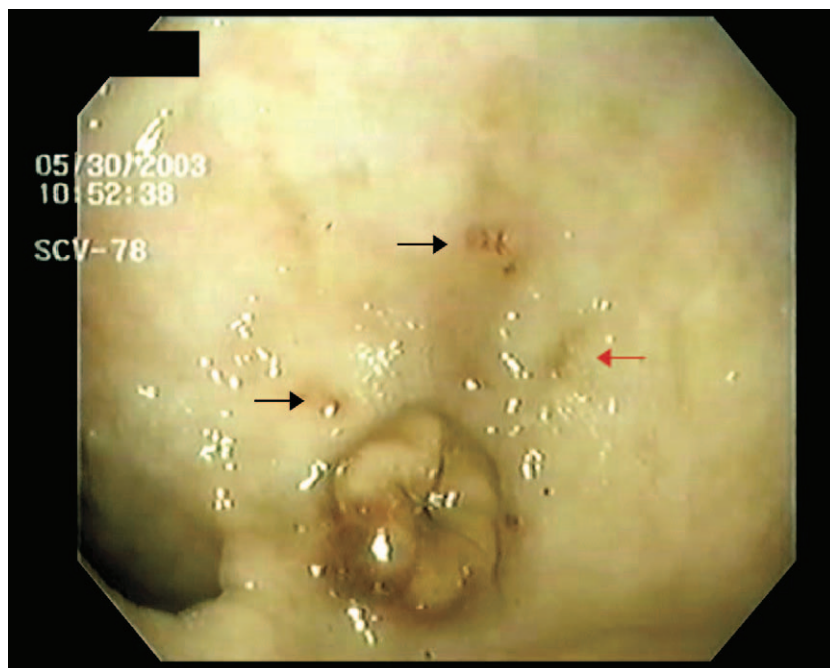


FIG 2. Colonoscopic allergen provocation test showing two positive reactions (black arrows: wheat and soybean) and a negative reaction (red arrow: lamb) approximately 15 minutes after injection. The positive reactions show erythema around the injection site, whereas the negative reaction does not. In the lower part of the image, the ileocolic valve and the entrance into the caecum are visible

gist (A. G.) according to a published scoring scheme for inflammatory bowel disease (IBD) in dogs (Jergens and others 1992). On the duodenal biopsies, mild to moderate lymphoplasmacytic inflammation of the duodenum was seen in six of nine dogs. Additionally, mild to moderate eosinophilic duodenal inflammation was noticed in two dogs. In one dog, severe lymphoplasmacellular infiltration with lymphangiectasia was diagnosed. In the colon, most biopsies revealed mild lymphoplasmacellular inflammation (eight of nine), and only one dog showed a moderate lymphoplasmacellular infiltrate.

Control dogs None of the control dogs showed any signs of gastrointestinal or dermatological disease. A CBC, chemistry profile, urinalysis, measurement of trypsin-like immunoreactivity as well as folate and vitamin B₁₂ measurements, faecal parasitology and faecal α_1 -proteinase inhibitor concentration were within normal limits. An abdominal ultrasound examination showed no evidence of gastrointestinal or other diseases. Histological examinations of duodenal and

colonic biopsies were interpreted as normal in all control dogs.

COLAP in control dogs During the COLAP, none of the control dogs showed positive reactions against any of the injected allergen extracts of wheat, lamb, egg, chicken, soybean, corn, and milk. In every COLAP procedure, the positive and negative control injections were interpreted appropriately.

COLAP in dogs with food allergies (Table 1) A total of 63 allergen injections were performed, and 29 (46 per cent) positive reactions for food allergens were observed. Each dog had positive reactions for at least two allergens. The proteins most commonly eliciting reactions were soybean (six of nine), followed by corn (five of nine), chicken, wheat, and milk (each four of nine). Reactions to egg and lamb were seen more rarely (three of nine).

Oral challenge tests (Table 1) In the dogs with food allergies, all proteins used in the COLAP also were tested in the oral challenge. None of the dogs showed any signs of vomiting, diarrhoea, and pruritus, before the test was started or during the

washout days. Eight of the nine dogs showed at least one positive reactions to food. One dog did not have any positive reactions during the oral food challenge test, even though the COLAP was positive for milk and corn. A total of 63 protein challenges were performed, and 23 positive reactions were observed (37 per cent). Positive reactions were elicited by feeding wheat (six of nine), soybean (five of nine), corn (four of nine), milk and lamb (each three of nine), and egg (two of nine). No reactions were observed in any dog after feeding chicken.

The results of the oral challenge test were used as a gold standard to which COLAP results were compared.

Concordance of food sensitivity studies

Concordance of COLAP and oral challenge test results in the nine dogs of the present study are listed in Table 1. When compared with the oral challenge test, COLAP accurately identified 17 of 23 positive reactions (74 per cent). Specificity of the test was high, as none of the five healthy control dogs showed any reactions to injected allergens.

DISCUSSION

The results of COLAP were compared with the current gold standard, the oral food challenge, in a group of nine research dogs with clinically proven food allergic reactions. In addition, the specificity of the test was evaluated in five healthy, research dogs. GFST has been previously described as a test to measure immediate-type hypersensitivity reactions in healthy as well as dogs with food allergies (Elwood and others 1994, Guilford and others 1994, Vaden and others 2000a). In one study, in which some of the dogs in the present study were also used, GFST and oral challenges were directly compared, and sensitivity (33 per cent, two of six dogs) as well as specificity (50 per cent, three of six dogs) were mediocre (Vaden and others 2000a). In another study, the GFST detected three of six positive reactions that were confirmed by oral challenges (Guilford and others 1994).

Table 1. Concordance of positive (+) and negative (–) results in nine research dogs with clinical food allergy: COLAP versus oral challenge testing

Dog number	Test	Wheat	Lamb	Egg	Chicken	Soybean	Corn	Milk
1	COLAP	+	+	+	–	+	+	–
	Oral challenge	+	+	–	–	+	–	–
2	COLAP	+	+	–	–	–	+	+
	Oral challenge	+	+	–	–	–	+	–
3	COLAP	–	–	–	–	–	+	+
	Oral challenge	–	–	–	–	–	–	–
4	COLAP	–	–	–	+	+	–	+
	Oral challenge	–	–	–	–	+	–	+
5	COLAP	–	–	–	+	+	–	+
	Oral challenge	+	+	+	–	+	+	+
6	COLAP	+	–	–	+	+	–	–
	Oral challenge	+	–	–	–	–	–	–
7	COLAP	+	–	+	+	+	–	–
	Oral challenge	+	–	+	–	+	–	–
8	COLAP	–	–	–	–	+	+	–
	Oral challenge	–	–	–	–	+	+	+
9	COLAP	–	+	+	–	–	+	–
	Oral challenge	+	–	–	–	–	+	–

COLAP Colonoscopic allergen provocation

However, the specificity could not be calculated in that study because only dogs that had a positive reaction in the GFST were subsequently tested by oral challenge (Guilford and others 1994). In yet another study, the GFST identified seven positive reactions in 17 dogs (40 per cent), but only those seven dogs were subsequently tested by oral challenge tests (Elwood and others 1994). In human beings, GFST was shown to be very sensitive in one study: 14 of 14 tested patients showed positive reactions. However, the test proved to be technically difficult to perform and difficult to interpret (Reimann and Lewin 1998). The GFST has also been studied in 55 cats with clinical food allergy, and proved to be unreliable for establishing a diagnosis in that species (0 positive reactions of 55 cats) (Guilford and others 2001).

In human beings with food allergies, direct mucosal allergen provocation in the colon has been shown to correlate better with double-blind food challenges and the patient history than the GFST (Bischoff and others 1997a,b). In 75 individuals with suspect food allergy, the COLAP detected 77 per cent of sensitive people when compared with the history of foods that elicited positive reactions (Bischoff and others 1997a).

To the authors' knowledge, this is the first study in dogs evaluating a new test for direct assessment of mucosal hypersensitivity reactions in the gastrointestinal

mucosa compared with oral challenge tests. Based on these results, COLAP may be more accurate in correctly identifying positive reactions of oral challenges than GFST. The accuracy of COLAP reported in the present study was comparable with that seen in human beings (Bischoff and others 1997a). COLAP therefore holds promise in dogs as a new tool with potentially higher accuracy for the diagnosis of immediate-type hypersensitivity to food allergens.

Even though the oral challenge test has been advocated as the gold standard for diagnosing food allergy in practice, this test is neither 100 per cent sensitive nor 100 per cent specific (August 1985, Rosser 1993). False-positive reactions are probably frequent, because non-immunological reactions, such as food intolerance, will also elicit clinical signs. False-negative reactions may also occur if the allergenic food was not given long enough or if subtle reactions such as bloating or minor pruritus are overlooked. In addition, the test is tedious and time-consuming to perform; and dog owners generally show low compliance when performing this test on their animals (Rosser 1993). However, in spite of these considerations, the oral challenge test is still believed to be the best clinically available test to diagnose food allergy in dogs.

In the GFST, it has been described that the allergen solution can run off the application site, which could account for false-

negative results (Guilford and others 1994). Additionally, catheter injuries to the mucosa, gastric rugal folds, or marking the injection site by taking a biopsy may be misread as mucosal swelling, producing false-positive results (Guilford and others 1994, Vaden and others 2000a). Both of these situations are less likely to occur during the COLAP, because the injection is performed directly into the colonic mucosa around the landmark of the ileocolic valve. In the authors' experience, peristaltic contractions during the procedure do not frequently interfere with performance of the test, and the specific injection sites can easily be located again after a contraction. These facts may account for both higher sensitivity and higher specificity rates when compared with the GFST.

False-negative reactions may theoretically occur during both GFST and COLAP because only part of the gastrointestinal mucosa is naturally exposed to the allergen during digestion. It is also possible that some ingredients may expose new epitopes and become allergenic only after being processed or digested (Guilford 1994). In addition, some allergens will elicit unspecific histamine release in the mucosa, leading to false-positive interpretation of test results (Guilford and others 2001). Finally, allergens might unmask subclinical food allergies, which are not yet causing overt clinical signs. All these situations would lead to false-positive interpretations, probably occurring with comparable frequency in both GFST and COLAP.

A drawback of both GFST and COLAP is that they only assess immediate-type hypersensitivity reactions that can be appreciated during the time endoscopy is performed. However, it has been reported that some dogs experience delayed type reactions that take up to several weeks to develop (White 1998). These reactions will be missed by both tests.

It is possible that, in the present study, the time period chosen for the oral challenges (48 hours) was too short to elicit any symptoms. This would decrease the sensitivity of the gold standard or increase the sensitivity of the COLAP. However, oral challenges performed previously on

the same dogs have shown that reactions can be seen as early as 12 hours after feeding the offending diet (Vaden and others 2000a). Another obvious disadvantage of this new technique compared with the GFST is the longer fasting time for the dogs and the necessity to give an oral laxative solution before colonoscopy. In the authors' experience, it is important to clean the colon as well as possible before performing the procedure. In case of faeces contaminating the area around the ileocolic orifice, flushing the area with warm water through the endoscope channel has been shown to be helpful. In clinical cases of suspected food allergy, it might not be a disadvantage to perform colonoscopy, since a majority of clinically allergic dogs were described as showing symptoms of large intestinal involvement anyway (Paterson 1995, Elwood 1994, Guilford 1994, Hall 1994).

In the setting of this study, no conclusions can be drawn on the utility of this test in clinical cases. However, in certain clinical cases with strong suspicion of a type I hypersensitivity reaction to food allergens, it may, in the future, be an option to perform this test in addition to an oral challenge.

Conclusions

A novel method for the clinical diagnosis of food allergy in dogs is described in this study, which shows better agreement with oral challenge studies than GFST in SCWT with food allergy. Larger studies including clinical cases of dogs with suspected food allergies with follow-up information on elimination diet trials and re-challenge studies will be necessary to more precisely delineate the clinical utility of this new test.

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