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Scientific Report

Diagnosis of Canine Food Sensitivity and Intolerance Using Saliva: Report of Outcomes

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ABBREVIATIONS
Inflammatory bowel disease — IBD
Gastrointestinal — GI
Immunoglobulin A — IgA
Immunoglobulin E — IgE
Immunoglobulin G — IgG
Immunoglobulin M — IgM

Abstract

Objective — To assess the efficacy of a novel saliva-based immunoassay of IgA–and IgM–antibodies in predicting canine food sensitivity and intolerance.

Design — Prospective controlled and clinical trial cohort populations.

Animals — Greyhounds from a closed colony, both healthy (n=29) and with inflammatory bowel disease (IBD) (n=10); clinical samples from dogs of various breeds and mixed breeds, classified as healthy without evidence of IBD (n=208); clinically suspected IBD (n=289); and proven IBD (n=98) cases. Second cohort of clinical samples from dogs suspected to have IBD (n=1008).

Procedures — Saliva was collected with a dental cotton rope from dogs that had not eaten for at least 8 hrs, placed in a double-sleeved saliva collection tube, and transported to the laboratory. Salivary antibodies elicited by 24 foods were measured with goat anti-canine IgA and IgM.

Results — Data distinguished healthy, suspect, and proven IBD cases among Greyhounds and 2 large canine clinical case cohorts. Results were stratified as negative, and as intermediate, medium, and strong reactors against 1 or more of the food antigens tested. The 1–4-year and over 10-year age groups had the highest number of positive food reactors, and the German Shepherd Dog was most represented. Clinical outcome comparisons after eliminating reactive foods (n=50) and follow up saliva re-testing (n=15) demonstrated the clinical accuracy and predictive outcome of this test.

Conclusions and Clinical Relevance — The novel salivary–based food sensitivity and intolerance test described here for canines offers a reliable and clinically predictive alternative to food elimination trials, serum-based food allergy testing, and skin patch testing.

Introduction

The background and rationale for a novel approach to diagnosis of canine food sensitivity and intolerance using saliva was recently published (1) (a). Basically, delayed, latent, or pre-clinical elaboration of IgA and/or IgM antibodies to specific food antigens can be detected in mucosal fluids such as saliva, feces, sweat, and tears (2-6). These antibodies to foods appear in the mucosal biofluids before the clinical or gastrointestinal (GI) tract biopsy diagnosis is made of intestinal biopsy-confirmed inflammatory bowel disease (IBD) and/or or "leaky gut syndrome." A major cause of the leaky gut is known to stem from release of zonulin which physiologically modulates intestinal barrier function and serves as a biomarker of impaired gut function (2-6). Zonulin release is triggered primarily by the gliadin protein of dietary glutens and gut bacteria in the small intestine, thereby creating gradients for the optimal transport of nutrients and balancing the body's tolerance or immunity to

external antigens, including foods (4). Frequently, IgA or IgM antibodies to food ingredients appear in saliva but are not detectable in serum (7). Salivary antibodies thus serve as an indication of a mucosal immune response and can be induced in people and animals without parallel antibodies being detected in serum (7–9). The same test is also available for cats and horses (1).

Materials and Methods

Study Populations

Saliva Diagnostic Testing Clinical Validation Protocol

Healthy adult Greyhounds (n=29) were adopted after retirement from the racing industry in Arizona, Oklahoma, and Texas. They were either neutered males or spayed females of similar adult age (2.5–5 years) and weight (25–35 kg [55–75 lbs] for females; 30–40 kg [65–90 lbs] for males). All had periodic general health examinations and laboratory screening profiles performed quarterly. Laboratory profiles checked CBC, chemistry profile, thyroid profile, vaccine titers, von Willebrand factor antigen, infectious disease screening, urinalysis, and fecal ova and parasites.

The Greyhounds live in the company's licensed, closed colony facility (Biologics License # 84), which is inspected annually by the California Department of Food & Agriculture; this inspection includes a review of the animal blood bank procedures and production program, animal welfare, and inspection of the animal care and laboratory facilities.

Regular Diet

All dogs at Hemopet are fed the same control diet, cereal kibble with some canned food (b). The food is given twice daily in pre-determined amounts to maintain ideal body weight.

Diet for Cohort Group with Food Sensitivity/Intolerance (n=10)

Any resident Greyhound exhibiting 1 or more classical symptoms or signs of food sensitivity/intolerance (inflammatory bowel disease, diarrhea, constipation, flatulence, abdominal cramping, gastritis, anorexia or poor appetite, and/or low-grade chronic skin disease [folliculitis, pyoderma]) is fed novel protein source foods (c).

Initial Clinical Case Cohorts Tested Against 6 Purified Food Extracts

The initial clinical trials involved veterinary clinics throughout the USA and Hemopet's resident rescued Greyhounds. There were 29 healthy control dogs and 81 dogs affected with chronic IBD and/or "leaky gut" syndrome; some cases also had pruritus. Subsequent expansion of these trials included a total of 595 cases (566 new cases plus the 29 healthy greyhounds): Healthy dogs without evidence of IBD (n=208, which included n=122 completely healthy and n=86 healthy with minor non–GI or non-pruritus issues), Suspected cases of IBD based upon the submitting veterinarian's clinical diagnosis (n= 289), and Proven cases by intestinal biopsy and/or food elimination trials to have IBD (n=98).

Sex: There were 455 dogs described by their sex: 244 males and 211 females. Of the males, 195 were intact and 49 neutered; and of the females, 74 were intact and 137 were spayed.

Diet: Fifty dogs of the 566 total cases studied ate raw diets exclusively (Healthy=10; Suspect=34; Proven=6). The majority of dogs studied ate commercial kibbled cereal either dry or with some canned foods and treats. Five of the 6 proven IBD cases ate specialized prescription or homemade elimination diets containing novel proteins and treats.

Larger Clinical Case Cohort Tested Against 24 Purified Food Extracts

Saliva samples submitted by veterinary clinics throughout North America plus some from Australia, Austria, Brazil, France, Germany, Italy, Hong Kong, Japan, Poland, Portugal, Switzerland, and the United Kingdom (n=1008) were tested against 24 affinity-purified (>98% pure by molecular analysis) lyophilized food extracts (d) from the foods listed below.

The raw ELISA-based absorbance data measured to 4 decimal places and run in duplicate were averaged from each of the canine clinical trials and were then transformed from the ELISA O.D. readings into a readily understandable data set (units/mL). Known values of standards for each of the initial 6 and the eventual 24 purified food extracts were used to create a baseline standard curve for each of the canine IgA and IgM antibodies, and for each food allergen (i.e. a total of 48 standard curves).

Saliva was collected from either or both sides of the mouth onto the same simple dental cotton rope, 5 inches long by 3/8 inch diameter (e). The saliva-soaked cotton rope was placed in the inner plastic tube of a special double-sleeved collection tube (f), and the tube was capped and taped for additional security while in transit. The samples were

then shipped by regular or Air Mail post to the Hemopet laboratory for testing. Saliva samples were stable for at least 30 days in this sealed tube system, as established by mailing samples nationally and internationally from and to Hemopet. After centrifugation, the saliva samples could be tested immediately, refrigerated for up to 30 days, or frozen at -20 ° C for later assay; in-house quality control testing of these 3 storage temperatures gave comparable results. For the initial parallel studies comparing results for saliva and serum from Hemopet's resident Greyhounds (n=39; 29 healthy, 10 with IBD), both saliva and blood samples (6 mL whole blood) were collected. After clotting, the serum (~ 2.5 mL) was harvested and used for parallel food antigen testing using serum anti–IgG.

Saliva and Blood Collection

Each of the clinical validation and clinical study cohort dogs had saliva collected with the dental cotton rope. This rope allowed for collection of up to 2 mL of saliva.

Test Methodology

Assays for Salivary anti-IgA and anti-IgM, and Serum anti-IgG were performed using the specific ELISA Food Antigen–Coated Plates containing the 24 affinity-purified food antigens manufactured for this purpose (g). Standard ELISA methodology using a robotic immunoassay autoanalyzer (Tecan [h]) was applied to each of the custom-made food antigen-coated plates. The food antigens were: barley, beef, chicken, corn, duck, egg (hen), lamb, lentil, millet, milk (cow), oatmeal, peanut, pork, potato, quinoa, rabbit, rice, salmon, soy, sweet potato, turkey, venison, wheat, and white-colored fish.

Each of the sealed, refrigerated 96 well ELISA food antigen–coated plates was tested in turn, in duplicate, along with the diluent buffer blanks.

Three antibody conjugates were used purified goat anti–dog IgA, goat anti-dog IgG1, and goat anti–dog IgM, all conjugated to alkaline phosphatase (i).

Quality control was performed by the addition of serum or saliva with low, medium, and high titers of antibodies. In addition, plates were studied for the detection of previously established non-specific reactions to the microwell plates. Without the addition of serum or saliva, the plates underwent the complete ELISA procedure to verify that there was no evidence of non-specific binding. The plates were stored refrigerated.

Analysis of Results

Results were analyzed initially as a Panel of 6 antigens, and then subsequently as 2 Panels of 12 antigens each. Calibration graphs for anti-canine IgG in serum and anti–canine IgA + IgM in saliva were obtained from the O.D. values resulting from the blank and negative reactor control samples. The O.D. readings were then converted to units/ml. The concentration values of anti-canine IgG, IgA, or IgM were compiled for the initial and subsequent sets of dietary antigens for each healthy control dog and canine patient. The degrees of food reactivity were determined from the calibration slopes measured for each of the 24 foods tested and were then converted to units/mL for ease of reporting and comprehension (see examples of standard curves in **Figure 1**). The O.D. values of the duplicate assays for both anti–IgA and anti–IgM were considered acceptable if the coefficient of variation (CV) was not more

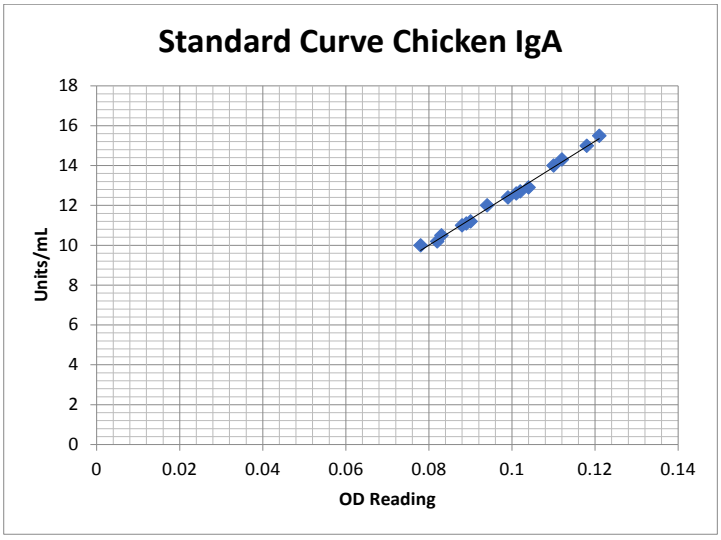

Figure 1a: Nutriscan Standard Dilution Curve for Chicken with IgA

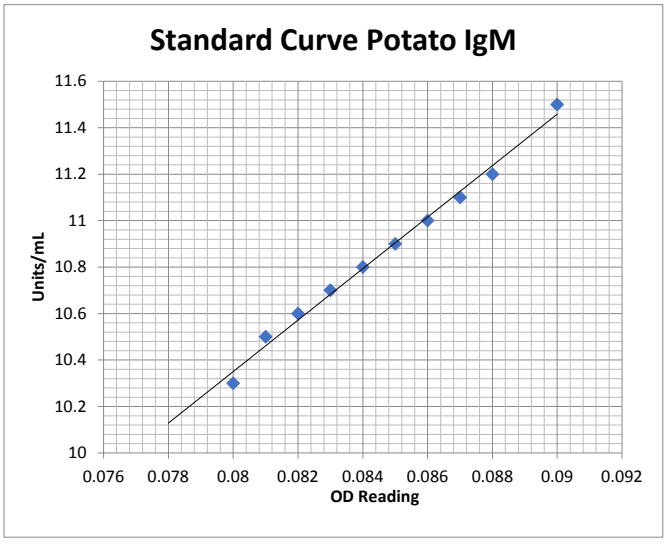

Figure 1b: Nutriscan Standard Dilution Curve for Potato with IgM

than 15% between duplicates (most duplicates had CVs below 5%; any samples with values above 15% were repeated). The sensitivity and specificity of the assay were 95.5% (range 93–99) and 70.7% (range 69–72%), respectively. The likelihood ratios ranged from 3.08-5.30% for positive ratios and 0.63–0.65% for the negative ratios (10).

Samples that tested below the 0.63% negative likelihood ratio cut-off level were clearly negative. Values at or above the 5.30% positive likelihood ratio cut–off level showed varying degrees of reactivity to the foods tested. Because low level antibody concentrations at or just above the cut-off amount of 10 units/mL included some mild or equivocal reactor cases, the lower limit was set at 10 units/mL to avoid the potential misclassification of weak (equivocal) samples as being truly positive. In such cases, the recommendation was made to retest the dog's saliva in 4-6 months. Thus, a 10–11.4 units/mL amount was set as the range for a weak degree of food sensitivity (clinical significance unclear, if any); and any level at or above 11.5 units/mL indicated a positive reaction. The positive reaction was then further classified by degree as being borderline, intermediate, medium, or strong food sensitivity. This classification paralleled what is typically used for food sensitivity testing of humans in Europe (d).

Statistical analyses of results were determined using the standard statistical paired t test formulas on Microsoft Excel.

Results

In the initial clinical validation trials involving 29 healthy Greyhounds and 10 with IBD, anti-IgA and anti–IgM food reactivities were recorded to a varying degree in saliva for the 6 foods tested (beef, corn, cow milk, hen egg, soy and wheat; data not shown). By contrast, none of these dogs had detectable anti-IgG levels with any of the 6 foods tested.

Table 1 shows a typical patient report and illustrates the varying levels of food reactivities for the IgA and IgM antibodies of 24 different foods.

Table 1. Sample Patient Report for Saliva-Based Food Sensitivity Test with 24 Food Antigens

Accession No.	**Doctor**	**Owner**		**Pet Name**	**Received**
Test 00116	**Sample Report**	**Sample Report**		**Sample Report**	**10/21/13**
Species	Breed	Sex	Weight	Pet Age	Reported
Canine	Golden Retriever	FS	45 Lbs	5 Yrs	10/31/13
Diet	Medication	Thyroid Medication	How much medication?	How Often?	Post Pill Timing
Raw diet	None	No			

Test Requested	**Result**	**Remark**	**General Range**	**Units**
Beef Salivary IgA	9.500	Negative Reaction	< 10	U/mL
Beef Salivary IgM	8.125	Negative Reaction	< 10	U/mL
Chicken Salivary IgA	15.698	Strong reaction; Avoid	< 10	U/mL
Chicken Salivary IgM	15.524	Strong reaction; Avoid	< 10	U/mL
Corn Salivary IgA	8.256	Negative Reaction	< 10	U/mL
Corn Salivary IgM	9.635	Negative Reaction	< 10	U/mL
Duck Salivary IgA	7.456	Negative Reaction	< 10	U/mL
Duck Salivary IgM	6.963	Negative Reaction	< 10	U/mL
Lamb Salivary IgA	6.235	Negative Reaction	< 10	U/mL
Lamb Salivary IgM	4.653	Negative Reaction	< 10	U/mL
Milk Salivary IgA	8.563	Negative Reaction	< 10	U/mL
Milk Salivary IgM	8.523	Negative Reaction	< 10	U/mL
Pork IgA	9.636	Negative Reaction	< 10	U/mL
Pork IgM	9.356	Negative Reaction	< 10	U/mL
Soy Salivary IgA	7.562	Negative Reaction	< 10	U/mL
Soy Salivary IgM	6.235	Negative Reaction	< 10	U/mL
Turkey Salivary IgA	11.569	Borderline Reaction; Avoid	< 10	U/mL
Turkey Salivary IgM	12.375	Intermediate reaction, Avoid	< 10	U/mL
Venison Salivary IgA	8.522	Negative Reaction	< 10	U/mL
Venison Salivary IgM	7.563	Negative Reaction	< 10	U/mL

Table continued on page 36.

Table 1. Sample Patient Report for Saliva-Based Food Sensitivity Test with 24 Food Antigens - CONTINUED

Accession No.	Doctor	Owner		Pet Name	Received
Test 00116	Sample Report	Sample Report		Sample Report	10/21/13
Species	Breed	Sex	Weight	Pet Age	Reported
Canine	Golden Retriever	FS	45 Lbs	5 Yrs	10/31/13
Diet	Medication	Thyroid Medication	How much medication?	How Often?	Post Pill Timing
Raw diet	None	No			

Reason for testing: food intolerance, scratching, soft stool

Test Requested	Result	Remark	General Range	Units
Wheat Salivary IgA	7.652	Negative Reaction	< 10	U/mL
Wheat Salivary IgM	9.500	Negative Reaction	< 10	U/mL
White Fish Salivary IgA	8.248	Negative Reaction	< 10	U/mL
White Fish Salivary IgM	7.256	Negative Reaction	< 10	U/mL
Barley Salivary IgA	7.125	Negative Reaction	< 10	U/mL
Barley Salivary IgM	6.359	Negative Reaction	< 10	U/mL
Egg Salivary IgA	7.974	Negative Reaction	< 10	U/mL
Egg Salivary IgM	8.252	Negative Reaction	< 10	U/mL
Lentil Salivary IgA	7.154	Negative Reaction	< 10	U/mL
Lentil Salivary IgM	5.235	Negative Reaction	< 10	U/mL
Millet Salivary IgA	9.256	Negative Reaction	< 10	U/mL
Millet Salivary IgM	10.254	Weak Reaction	< 10	U/mL
Oatmeal Salivary IgA	12.356	Intermediate reaction, Avoid	< 10	U/mL
Oatmeal Salivary IgM	12.457	Intermediate reaction, Avoid	< 10	U/mL
Peanut Salivary IgA	7.281	Negative Reaction	< 10	U/mL
Peanut Salivary IgM	8.643	Negative Reaction	< 10	U/mL
Potato Salivary IgA	10.120	Weak Reaction	< 10	U/mL
Potato Salivary IgM	9.625	Negative Reaction	< 10	U/mL
Quinoa Salivary IgA	9.365	Negative Reaction	< 10	U/mL
Quinoa Salivary IgM	8.453	Negative Reaction	< 10	U/mL
Rabbit Salivary IgA	5.423	Negative Reaction	< 10	U/mL
Rabbit Salivary IgM	4.536	Negative Reaction	< 10	U/mL
Rice Salivary IgA	8.451	Negative Reaction	< 10	U/mL
Rice Salivary IgM	8.263	Negative Reaction	< 10	U/mL
Salmon Salivary IgA	11.258	Weak Reaction	< 10	U/mL
Salmon Salivary IgM	14.653	Medium Reaction; Avoid	< 10	U/mL
Sweet Potato IgA	7.124	Negative Reaction	< 10	U/mL
Sweet Potato IgM	8.364	Negative Reaction	< 10	U/mL

RECOMMENDATIONS

Food reactions were seen to: Chicken, Turkey, Oatmeal and Salmon. **A strong reaction was present for Chicken.**
Please avoid feeding these foods.

Interpretation: Pet should avoid food or treats containing ingredient(s) showing results of 11.5 or greater.
Recommend rechecking salivary food sensitivity or intolerance levels every 6-12 months.

Degree of reactivity:

<10 U/mL indicates a normal food antigen tolerance level = negative result.
10-11.4 U/mL indicates a weak reaction; clinical significance unclear
11.5-11.9 U/mL indicates an borderline reaction
12-12.9 U/mL indicates an intermediate reaction
13-14.9 U/mL indicates a medium reaction
>/= 15 U/mL indicates a strong reaction

Differences between antibodies to IgA and IgM: Antibodies to IgA measure the secretory immunity from body secretions (tears, saliva, feces, urogenital tract). They act as a mechanical barrier or the "first line of defense" to help protect the bowel from invasion by foreign substances, infectious agents, chemicals, and certain foods that it cannot or poorly tolerate. Antibodies to IgM measure the body's primary immune response to a recent exposure within the last 6 months or so (e.g. to a certain food ingredient).

Table 2 summarizes the age and health status for food sensitivity of 345 dogs from the initial 566 clinical case cohort (ages for the remaining cases were either not stated or unknown). The data show that most of the healthy dogs were between 1–2 yrs of age with 2–3 yrs being the next highest age group. For the suspect IBD cases, most of the dogs were between 1–4 yrs of age or over 10 yrs old, whereas in the proven IBD cases, most dogs were over 10 yrs of age.

Table 2. Summary of 345 Cases by Age and Health Status for Food Sensitivity

Age (yrs)	Heath Status for Food Sensitivity			Totals
	Healthy	Suspect	Proven	
	n = 103	n = 165	n = 77	
< 1	3	9	2	14
1-2	28	30	8	66
2-3	16	27	6	49
3-4	10	20	7	37
4-5	8	11	7	26
5-6	9	12	4	25
6-7	3	12	6	21
7-8	3	11	7	21
8-9	5	5	8	18
9-10	8	4	5	17
> 10	10	24	17	51

Table 3 summarizes the breed type of 420 dogs from the initial 566 clinical case cohort (breeds for the remaining cases were unknown). The highest number of cases were in breeds stated on the submission form (111 cases), or in miscellaneous breeds where there were fewer than 10 cases each (99). The German Shepherd Dog had more cases (48) than any other affected breed, and 13 of them were of the white German Shepherd variety. Golden Retrievers (32) ranked second, followed by Labrador Retrievers (22) and mixed breeds (20). There were also 39 Hemopet Greyhounds included in the study. The data for the larger second clinical case cohort (n=1008) showed no differences between the intermediate, medium, and strong anti-IgA or anti-IgM antibody reactivity levels for the following food antigens: lamb, oatmeal, potato, quinoa, rabbit, turkey, wheat, or white-colored fish, so these data were combined for further analyses. For other food antigens, namely barley, beef, hen egg, and venison, anti-IgA reactivity levels were observed higher than those of anti-IgM. Similarly, for the food antigens chicken, corn, cow milk, millet, peanut, rice, and soy, anti-IgM reactivity levels were observed higher than those of anti-IgA.

Table 3. Summary of 420 Cases Tested by Breed	
Breed	**Number of Cases**
Breed Not Stated	111
German Shepherd Dog [13 = White GSD]	48
Golden Retrievers	32
Labrador Retrievers	22
Mixed Breed	20
Bernese Mountain Dog	14
Standard Poodle	14
Doberman Pinscher	11
German Shorthaired Pointer	10
Greyhounds (pre-selected, Hemopet)	39
Miscellaneous Breeds (less than 10 cases)	99

The intermediate, medium, and strong anti-IgA or anti-IgM antibody reactivity levels of the 1008 cases, when analyzed per 100 cases to permit more direct relative comparisons of the number of positive reactions, showed the following: highest number of reactions = white-colored fish (18); turkey (15); venison (13); corn (12); and hen's egg (11). The lowest reacting foods for these positive reacting cases were: wheat (5); peanut (4); rice and lamb (each 3); and beef (2).

In comparison to the intermediate, medium, and strong anti-IgA or anti-IgM antibody positive reactivity levels, negative or weak antibody reactivity levels when analyzed per 100 cases showed the following: highest number = wheat (50); lamb, peanut, and rice (each 48); potato (47); beef, chicken, oatmeal, quinoa, and salmon (each 46). The lowest reacting foods for the non-reactive cases were: turkey and venison (each 43), and white-colored fish (41).

Table 4 summarizes the clinical outcome comparisons of 50 cases selected in sequence from the reported data set both before and after eliminating the reactive foods. This group consisted of a wide spectrum of breed types and sizes, and ages varied from 5 months to 14 years of age. The clinical outcomes after removing the reactive foods, based upon follow up interviews with the client and/or submitting veterinarian, varied from good (2 cases), very good (14 cases) to excellent (33 cases), with one dog showing no improvement (**Table 4**).

Table 4. Clinical Outcomes Before & After Eliminating Reactive Foods (50 Cases)							
Case Breed	**Age (yrs)**	**Sex**	**Clinical History**		**Initial Results * (Reactive Foods)**	**Follow Up Results † Clinical Outcome ‡**	
			GI	**Skin**		**Improved**	**No change**
Lhasa Apso	2	FS		X	Q, SP	E	
Great Dane	1	F	X		BA, P, Q, RI, V, WF	E	
Newfoundland	1.5	M	X	X	BA, MI, O, P, PO, Q, RA, RI, SA	E	
Wire Fox Terr.	8	MN		X	BA, MI, O, P, Q, RA, S	G	
Terrier Mix	3	FS		X	BA, E, MI, O, P, PE, Q, RA, RI, S	VG	
Min. Aussie.	2.5	MN	X		BA, C, CO, M, O, P, RA, SA, T, V, W, WF	E	
Rottweiler	2.5	FS	X		BA, CH, CO, M, MI, O, PE, Q, RI, SO, T, V, W	E	
Toy Fox Terr.	14	MN	X		BA, BE, CH, CO, L, M, MI, O, P, PE, Q, SO, T, V, W, WF	E	
Havanese	3	MN	X	X	CH, M, V	E	
York. Terrier	8	MN	X		CO, M, T, V, WF	E	
Std. Poodle	0.75	MN	X	X	BE, CH, W	VG	
Tibetan Terr.	2.5	FS	X		CO, V, Q	VG	
Tibetan Terr.	1.5	FS	X		CH, CO, M, T, V, W, WF	VG	
Shih Tzu	5	MN	X		CO, P, Q, RI		X
Shih Tzu	4	FS	X		CO, Q, RI, SA, V	VG	
Basenji	8	FS		X	CH, CO, M, T, V, W, WF	E	
Greyhound	11	MN		X	BA, CH, CO, M, MI, O, P, Q, RA, RI, SA, T, V, WF	E	
Cairn Terrier	5	M		X	CH, O, P, SA	E	

Table continued on page 39.

Table 4. Clinical Outcomes Before & After Eliminating Reactive Foods (50 Cases) - CONTINUED

Case Breed	Age (yrs)	Sex	Clinical History GI	Skin	Initial Results * (Reactive Foods)	Follow Up Results † Clinical Outcome ‡ Improved	No Δ
Greyhound	4	FS	X		BA, CH, CO, M, P, RA, T, V, W, WF	E	
Glen of Imal Terr.	3.5	FS	X		BE, M, SO, W	E	
Labradoodle	4	M		X	BA, CH, MI, O, T, V, WF	E	
PONS	1.5	F	X		V	E	
Greyhound	4	FS		X	BE, CO, E, M, SO, W	E	
White Boxer	2.5	MN		X	BE, CH, CO, SO, W, WF	G	
York. Terrier	7.5	MN	X		CH, CO, M, T, V, W, WF	E	
Lab. Mix	10.5	FS	X		CH, CO, E, M, MI, P, RI, T, V, W, WF	E	
Gr. Swiss Mtn. Dog	7.3	FS	X		BA, BE, CO, E, LE, MI, O, P, Q, R, RI, SA, SP, V, WF	E	
Belg. Tervuren	3.25	M	X		BA, BE, CH, M, O, SA, SO, T, V, W, WF	E	
Siberian Husky	8.9	M		X	BE, CH, M, P, T, V	E	
Boxer/Catahoula Leopard Dog	6	FS	X		O, Q, RI, V, W, WF	E	
NSDTR	1	F		X	BE, CO, M, SO, W	E	
Lab/Pointer	12.9	M			MI, O, P, Q, RA, SA, V, WF	VG	
Goldendoodle	2	MN	X		BA, CO, LE, MI, O, P, SA, V, W, WF	E	
Beagle Mix	6	MN	X		V, WF	VG	
Belg. Malinois	1.5	MN	X		PO, V, WF	E	
Wire Fox Terr.	4.5	MN		X	M, PO, T, V, WF	VG	
Brittany Sp.	6.5	MN	X		CH, CO, M, T, V, WF	E	
Flat Coat Retr.	7.7	MN	X		BA, D, E, LE, M, MI, O, PE, PO, Q, RI, SA, SO, SP, T, V, W, WF	E	
York. Terrier	4.9	MN	X		CH, T, V, WF	E	
French Bulldog	0.45	M	X	X	BA, D, LE, M, MI, O, P, PE, PO, RA, RI, SA, T, V, W, WF	E	
German Shepherd	3.75	MN		X	CH, CO, E, M, MI, O, PE, PO, RA, SA, T, V, W, WF	VG	
Golden Retr.	7	FS	X	X	CH, CO, M, SO, T, V, W, WF	VG	
Irish Setter	11	MN		X	CH, CO, MI, O, P, PO, Q, RA, SA, V, W, WF	E	
Irish Setter	9	M	X	X	BA, CH, CO, LE, T, V	VG	
German Shepherd	2.6	FS	X		WF	VG	
Scottish Terr.	1.5	FS		X	CO, E, LE, O, Q, RA, T, WF	VG	
Eng. Bulldog	2	M	X	X	BE, CH, CO, PO, RA, SA, T, V, W	E	
Border Collie	9.5	MN	X		E, SP, T, V	VG	
Bernese Mtn. Dog	3.25	F	X	X	BE, CH, CO, MI, P, RI, SO, T, V, W, WF	E	
Golden Retr.	5.75	M	X		BA, CH, CO, M, SO, T, V, W, WF	E	

*BA = barley, BE = beef, CH = chicken, CO = corn, D = duck, E = egg, L = lamb, LE = lentil, M = milk, MI = millet, O = oatmeal, PE = peanut, PO = pork, P = potato, Q = quinoa, R = rabbit, RI = rice, SA = salmon, SO = soy, SP = sweet potato, T = turkey, V = venison, and W = wheat, WF = white-colored fish.

*† Reactive Foods Removed. ‡ E = excellent, VG = very good, G = good, Δ = change

Table 5 describes results for the 15 cases for which salivary food diagnostic testing was repeated by the owners several months later. Owners of the other cases from **Table 4** elected not to retest their dogs because they stated them to be clinically improved upon removing the prior reactive foods from the diet. A variety of breeds and dog sizes were represented here with ages varying from 10 months to 8.75 years. The follow up saliva-based food test results and clinical outcomes, based again upon follow up interviews with the client and/or submitting veterinarian, varied from very good (5 cases) to excellent (10 cases). The initially reactive foods were mostly non-reactive on retesting, although some newly reactive foods also were identified upon retesting.

Table 5. Initial and Follow Up Test Results After Eliminating Reactive Foods (15 Cases)

Case Breed	Age (yrs)	Sex	Clinical History GI	Clinical History Skin	Initial Results * (Reactive Foods)	Follow Up Results † (Reactive Foods)	Clinical Outcome ‡
Boston Terrier	8.7	M		X	BE, CH, CO, M, SO, T, W	CH, T	VG
Boston Terrier	6	M		X	BA, CH, CO, M, O, P, T, V, W, WF	BE	E
German Shepherd	3	M	X	X	BE, CH, E, Q, RA, SA, T, V	BE, CH, CO, M, PO, T, V, WF	E
German Shepherd	5	FS	X	X	BE, CO, M, SO, W	CO, E, M, MI, SA, WF	E
Irish Setter	5	M	X		BA, CH, CO, E, M, MI, O, PE, PO, P, Q, RA, RI, SA, SO, T, V, W, WF	BA, BE, CO, D, LE, M, O, PE, PO, P, Q, RI, SO, T, V, W, WF	E
Border Collie X	0.8	FS	X		BA, BE. CH, CO, D, E, LE, M, MI, O, PE, PO, P, Q, RA, RI, SA, SO, SP, T, V, W, WF	CH, CO, T, WF	E
Gr. Dane/Dogo	3.75	M	X		BA, BE, CH, CO, D, E, LA, LE, M, MI, O, PE, PO, P, Q, RA, RI, SA, SO, SP, T, V, W, WF	BA, E, LE, MI, O, PE, PO, Q, RA, RI, SA, SP	E
Labrador Retr.	4.25	MN		X	CO, T, V	T, WF	VG
Doberman Pin.	5.5	FS	X		CO, M, SO, T, V, WF	V, WF	E
Min. Poodle	6.5	M	X		BE, M, W	V, WF	VG
Glen of Imal Terr.	3.5	FS	X		BE, M, SO, W	None	E
Basset Hound	7.75	F	X	X	CO, E, LE, M, MI, P, RI, SP, T, V, W	BE, M, W	E
English Setter	7	M	X		BE, M, W §	CH, CO, M, MI, O, SO, T, V, W, WF	VG
English Setter	7	FS	X		BE, M, W §	CH, CO, M, O, SO, T, V, W, WF	VG
NSDTR	2	FS		X	CH, MI, SO, V, WF	CO, WF	E

*BA = barley, BE = beef, CH = chicken, CO = corn, D = duck, E = egg, L = lamb, LE = lentil, M = milk, MI = millet, O = oatmeal, PE = peanut, PO = pork, P = potato, Q = quinoa, R = rabbit, RI = rice, SA = salmon, SO = soy, SP = sweet potato, T = turkey, V = venison, and W = wheat, WF = white-colored fish.
† Reactive Foods Removed; then 2-6 months retesting, reactions were lower or negative.
‡ E = excellent, VG = very good § Only 6 foods tested (BE, CO, E, M, SO, W)

Figures 2 and 3 illustrate the physical differences in 2 dogs shown before and after offending foods identified by the saliva testing had been removed from their diets. These remarkable beneficial effects were clearly seen within 2 weeks of the diet changes, and the original issues were completely resolved within a month.

Figure 2a: Cattle Dog Mix Before Nutriscan

Figure 2a: Cattle Dog Mix One Week After Nutriscan & Reactive Food Removal

Figure 2a: Cattle Dog Mix One Month After Nutriscan & Reactive Food REmoval RevalPicture2

Figure 2b: Cattle Dog Mix Before Nutriscan

Figure 2b: Cattle Dog Mix One Month After Nutriscan & Reactive Food Removal

Figure 3a: Aussie Before Nutriscan

Figure 3a Aussie Before Nutriscan

Figure 3b Aussie Skin & Muscle Before Nutriscan

Figure 3b Aussie After Nutrscan & Reactive Food Removal

Discussion

Saliva is a source of body fluid for detection of an immune response to bacterial, food, and other antigens present in the oral cavity and GI tract (1, 7–9, 11, 12). Indeed, salivary antibody induction has been widely used as a model system to study secretory responses to ingested material, primarily because saliva secretion is simple and easy to collect and analyze (1–3, 7, 12–14).

The results presented here using a novel saliva-based test which quantified the IgA and IgM antibody responses to 24 affinity-purified food antigens convincingly demonstrated the clinical predictability, utility, and efficacy of the assay (**Tables 4 and 5; Figures 2 and 3**). As shown in Table 5, the assay was repeated once more in 15 cases, 2–6 months after the identified offending foods had been completely removed from the diet. In each case, the clinical outcome was stated to be very good (5 cases) and excellent (10 cases). Even though some of the initially reactive foods were still quantified as reactive on retesting (at or above 11.5 units/mL), the degree of reactivity was lower. It is interesting that on repeat testing, some other food antigens were quantified as reactive, suggesting that these dogs could be especially prone to developing food intolerances. Several possible explanations for the ongoing but lowered reactivity of initially reactive food antigens and the appearance of additional reactive foods include: initial reactions were actually to residues in the flesh from what the meat or fish ate before becoming a food source; and reactive foods were still present albeit it in presumed smaller amounts in supplements the dog still ate, such as chicken fat, cornstarch, and fish oils (1, 2, 15–18).

Food intolerance is stated to be the third most commonly recognized syndrome in dogs after flea bite sensitivity and atopy (inhalant allergy), and food intolerance makes up an estimated 10–15% of all allergic skin disease (3). It mimics other skin syndromes. Food intolerance is stated to have no age, sex, or breed predilection, although clinical experience indicates that it can be familial (2, 3). In the author's experience, most affected animals had been eating the offending foods for more than 2 years; the major complaint of their owners was bilateral pruritus, and there was often otitis externa. Secondary skin disease such as seborrhea (both dry or oily) and pyoderma was also common (2, 3).

Delayed food sensitivities in people are extremely common and can be manifested by GI, neurological, pulmonary, dermatologic, ear, nose, throat, musculoskeletal, genitourinary, cardiovascular, and endocrine problems (7, 8). For dogs, in addition to the commonly observed GI tract

signs of food sensitivity, the skin is frequently a concurrent or alternate tissue target (1–3).

Creating a healthy acid-base balance within tissues through optimal nutrition should be the goal of case management and therapy (19). When eaten, different foods produce varying metabolic waste products and by-products. Most foods are acid-forming, with the exception of nuts and seeds, so that when people and animals eat more nuts and seeds, the dietary by-products are alkaline and promote health and prevent disease (19–21). Changing the proportions of macro-nutrients and micronutrients in different nutrient and food products is important in obtaining the right tissue and gut balance (4, 19). To be effective, diets ideally need to be individualized using nutrigenomic principles (2, 4, 20–23). Studies have indicated that specialized nutrient intake extends and improves life, delays onset and slows progression of disease, and enhances the quality of life of animals (2, 4, 20, 24).

Avoiding additives and supplements, as well as avoiding frequent switching from diet to diet, is important too, as up to 20% of cases have concurrent other GI tract issues (2–4). Some canine cases have swollen peripheral lymph nodes, although this sign is more common in affected cats. Affected pets may exhibit tension-fatigue, malaise, and dullness. Effects are usually non-seasonal, and the primary disorder is poorly responsive to steroids (3).

The so-called "gold standard" for food sensitivity or intolerance until now has been either diet elimination trials for 3–12 weeks, micronized or hydrolysed prescription diets, skin patch testing considered by clients to be expensive and unsightly, and allergen provocation (20–34). But, even these specialized, limited ingredient diets have been found to contain ingredients not listed on the label, and there is often poor compliance with the diet elimination trial approach (15–17, 25). The alternative diagnostic approach of performing serum allergy tests for food sensitivity is typically based on measuring IgE, IgG, and immune complexes bound to complement; these tests have high sensitivity but lower individual specificity, and measure only more immediate-type reactions (25–34). Dogs with atopic and GI tract disease have higher levels of serum IgE and IgG antibodies than normal dogs, and the antigen(s) causing the reaction is often contained in the diet (25–27). However, there is generally poor correlation between serum IgE and IgG antibody testing and clinical experience in resolving disease in both humans and dogs (25–34).

Immune complexes containing large food antigens enter the blood from the GI tract and then travel through the liver where most immune complexes are removed. However, if circulating immune complexes pass the liver filtering system, they may cause injury to many body tissues (7, 8, 12). Malabsorption of food particles from the GI tract can also travel by lymphatic drainage to the body (4, 12). The lymph channels in the gut wall converge at the thoracic duct, which drains its contents into the large thoracic veins. This combination of antibody with complement in the blood stream becomes a circulating immune complex. Immune complexes subsequently attach to receptors on red and white blood cells. These altered cells are cleared by the body's liver or spleen (reticuloendothelial system) (5, 7, 8, 12).

Any circulating immune complexes that are not removed by the reticuloendothelial system of the liver (or spleen) can activate the complement cascade. Individuals with more immune complexes on their red blood cells are the ones that can experience chronic food sensitivities or intolerances (5–7). Circulating immune complexes also can damage the integrity of blood vessel capillaries which in turn can trigger inflammatory events (7, 8).

Newer testing for food sensitivity has used serum, saliva or feces [j–for people only] in a simple ELISA format or other immunoassay platform (1–3, 31–35). These methods identify IgG, IgA, or immune complexes to foods in serum, and IgA or IgM antibodies to foods in saliva. As antibodies to foods usually appear in saliva several months before the GI tract diagnosis of IBD or the "leaky gut syndrome" (intestinal dysbiosis), saliva testing can thus reveal the latent or pre-clinical form of food sensitivity (1–3, 5, 7, 13, 33, 35). IgA, especially, but also IgM, are the important antibodies generated by immunological reactions and are expressed as secretory immunity in saliva, as well as other body fluids like tears, sweat, and breast milk (8, 13, 14). IgE serology has been found to offer no advantage for diagnosis when performing dietary trials because it had a sensitivity of 14%, specificity of 87%, positive predictive value of 40%, and negative predictive value of 61% (20–22). Thus, this form of serum food allergy testing is clearly inadequate for clinical diagnostic purposes.

Conclusion

By looking at secretory immune responses to specific food antigens, detected as salivary antibodies to IgA and IgM in humans and with the current saliva-based testing in dogs, a direct correlation between results and clinical allergenic reactivity to foods can be demonstrated (1–3, 14, 35).

Endnotes

a. NutriScan®, Division of Hemolife Diagnostics, Garden Grove, CA 92843; www.nutriscan.org

b. Foundation Formula, Precise Pet Products, Nacogdoches, TX

c. Wellness Pet Food, WellPet, Tewksbury, MA

d. DST, Diagnostic Systems & Technologies GmbH, Schwerin, Germany

e. Patterson Dental Supply Inc, St. Paul, MN

f. Starstedt Inc., Newton, NC

g. Oxford Biomedical Corporation, Rochester Hills, MI

h. Tecan Group Ltd, Männedorf, Switzerland

i. Bethyl Laboratories, Montgomery, TX

j. EnteroLab, Dallas, TX

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7,867,720; 7,892,763; 8,450,072; 8,450,074; Canadian patents 2,743,714; 2,771,948; and European patent 2382469.