Assessment of the clinical accuracy of serum and saliva assays for identification of adverse food reaction in dogs without clinical signs of disease

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OBJECTIVE

To assess the clinical accuracy of 2 serum-based assays and 1 saliva-based assay for detection of adverse food reaction (AFR) in dogs without clinical signs of disease.

ANIMALS

30 healthy client-owned dogs.

PROCEDURES

Dog owners completed an online survey to collect comprehensive information about their pets' diet history. From each dog, serum and saliva samples were obtained and submitted for AFR testing by means of 3 assays that assessed the immunoglobulin response to 24 foods. Assays A and B measured food allergen–specific IgE concentrations in serum, whereas assay C measured food allergen–specific IgA and IgM concentrations in saliva. Descriptive data were generated, and Fisher exact tests were used to assess the respective associations between positive test results and specific food ingredients to which dogs were exposed.

RESULTS

Assays A, B, and C yielded positive results for 26, 18, and 30 dogs, respectively. All dogs had positive results for at least 1 assay. The median (range) number of foods or ingredients to which dogs tested positive was 10.5 (0 to 24) for assay A, 1 (0 to 13) for assay B, and 12.5 (4 to 22; IgM) and 3 (0 to 24; IgA) for assay C. Positive test results were not significantly associated with prior food exposure.

CONCLUSIONS AND CLINICAL RELEVANCE

Saliva and serum assays for AFR often yielded positive results for apparently healthy dogs and are not recommended for clinical use. Elimination diet trials remain the gold standard for diagnosis of AFR in dogs. (*J Am Vet Med Assoc* 2019;255:812–816)

Food allergy is an immunologic reaction to the ingestion of a normally harmless food component, typically a protein, that elicits an adverse cutaneous or gastrointestinal response. Food allergy is most often associated with a type I hypersensitivity reaction, although type III and IV responses have been reported.[1–3] In clinical practice, immunologic food allergy is indistinguishable from nonimmunologic adverse food responses; therefore, the term AFR has been adopted as a general term to encompass ingredient-specific cutaneous AFR, food-responsive enteropathy, and food intolerance. Food sensitivity is a nonspecific term understood to signify some negative response to food and is considered to be encompassed within AFR for the purpose of the study reported here.

Clinical signs of AFR are varied and nonspecific. Cutaneous clinical signs of AFR can appear similar to those of atopic dermatitis, wherein pruritus can affect any body site. Affected animals often develop aural, perioral, pedal, and perianal pruritus or pruritus of the glabrous regions, although some animals have only signs of otitis externa. Gastrointestinal signs may include vomiting, diarrhea, and flatulence.[2,4–7] Unfortunately, it is not always easy to distinguish AFR from other causes of those signs.

Diagnosis of AFR in dogs can be challenging for veterinarians and pet owners. Food allergy is commonly suspected when a change in diet results in an improvement in clinical signs. However, diet changes involve myriad factors (eg, digestibility, fat content, fatty acid composition, micronutrient content, and fiber content) that can affect cutaneous and gastrointestinal signs but are not specific to a particular ingredient. The current gold standard for diagnosis of AFR is an elimination diet trial, which requires feeding a novel- or hydrolyzed-protein diet for 4 to 12 weeks, followed by a provocation phase with the original diet and subsequent challenge testing with individual foods.[2,8–11] An accurate elimination diet trial requires a thorough diet history and exclusive feeding of an appropriate diet for the entire trial period. Because of the long time

ABBREVIATIONS

AFR Adverse food reaction

and strict adherence required to accurately complete an elimination diet trial, there is great interest in more convenient options for diagnosis of AFR and identification of specific foods of concern.

Several commercial serum- and saliva-based assays purport to identify AFR in dogs, and some can be purchased directly by pet owners and performed without the guidance of a veterinarian. However, results of multiple studies[2,9,12,13] suggest that there is a poor correlation between serum assay results and clinical responses to foods in dogs with suspected AFR, and there is a paucity of published data to support the use of saliva-based assays for diagnosis of AFR in dogs. In our clinical experience, we frequently see dogs for which serum- and saliva-based assay results do not appear to be consistent with the clinical signs or history. Unfortunately, once an assay has been performed, it can be challenging for pet owners to understand and accept the assay's limitations and potential lack of diagnostic usefulness. More information about the diagnostic accuracy of serum- and saliva-based assays for detection of AFR in dogs is needed so that both pet owners and veterinarians can make informed decisions regarding their use and interpretation.

The objective of the study reported here was to assess how frequently serum- and saliva-based assays for identification of AFR yielded positive results in dogs without a history or clinical signs of the condition. One saliva- and 2 serum-based assays were evaluated. On the basis of our clinical experience, we hypothesized that all 3 assays would yield positive results for a high proportion of study dogs and that positive assay results would correspond to prior food exposures.

Materials and Methods

Animals

Dogs belonging to faculty, staff, and students affiliated with the Cummings Veterinary Medical Center at Tufts University were recruited for the study. Dogs > 1 year old that had no clinical evidence of systemic illness or history of gastrointestinal or dermatologic disease were eligible for study inclusion. For dogs that met those initial inclusion criteria, a secondary screening was performed by means of an online survey completed by the owners. Questions were designed to uncover current and previous systemic health issues and to ascertain whether the dog had a history of gastrointestinal or dermatologic disease. Dogs that had a history of vomiting or diarrhea that lasted > 3 days and was not associated with a parasitic infection or dietary indiscretion were excluded from the study. Dogs were also excluded from the study if they had a history of signs associated with allergic skin disease, such as otitis externa, superficial or deep pyoderma, and pruritus, regardless of the diagnosis of record. Dogs without a detailed medical or dietary history were likewise excluded. Consent was obtained from the owners of all dogs prior to study enrollment. The study protocol was reviewed and approved by the Clinical Sciences Review Committee of the Cummings School of Veterinary Medicine at Tufts University and the Tufts University Institutional Animal Care and Use Committee.

Online survey

The online survey was designed to obtain 2 types of dietary exposure information for each dog. Owners were first asked to list the brand names and flavors of all foods, treats, medications, and supplements they were currently feeding or had previously fed to their dogs. Products listed were classified as confirmed diet exposures. Then, owners were provided a list of individual ingredients and asked to indicate whether their pet had ever eaten each ingredient. This list was provided to account for the consumption of table scraps or treats that were not commercially produced as well as instances when the owner forgot the brand name of a particular product that was consumed by their pet. Items indicated as consumed were classified as checklist exposures. Manufacturers' websites and customer service lines were consulted to obtain complete ingredient lists for commercial products that owners reported feeding (ie, confirmed diet exposures). Some products contained nonspecific ingredients, such as meat and poultry. In the United States, nonspecific meat ingredients in pet food must originate from cattle, pigs, sheep, or goats.[14] Because the exact nature of those nonspecific ingredients could not be determined, all dogs that were fed products with meat listed as an ingredient were assumed to have been exposed to beef, pork, lamb, and goat. Similarly, all dogs that were fed products with poultry listed as an ingredient were assumed to have been exposed to chicken, duck, and turkey. Dogs that received antlers as treats were recorded as having been exposed to venison. Consumption of purified fats and oils was not assessed because they have negligible protein content, and food allergies are primarily considered to be a reaction to protein. Consumption of nonspecific ingredients, such as flavor and digest, was not assessed because the species of origin were unknown for those ingredients. Consumption of specific hydrolyzed ingredients was assessed as consumption of the intact ingredient because hydrolyzation reduces but does not eliminate allergenicity.[15–17] The confirmed diet exposure and the checklist exposure items were then combined to provide a more complete overview of historical food exposure (ie, overall food exposure).

Clinical evaluation and sample collection

Owners of study-eligible dogs were instructed to withhold food but not water from their pet for 3 hours prior to examination and sample collection. One of the study investigators (ATHL) performed a brief physical examination on each dog enrolled in the study. Blood and saliva samples were then collected from each dog and submitted for AFR testing

by means of 3 assays (A,[a] B,[b] and C[c]), which were performed by 3 independent veterinary laboratories. For each dog, a blood sample (10 mL) was obtained by jugular venipuncture and placed in a serum separator tube, then a saliva sample (approx 2 mL) was collected by placing a piece of absorbent rope in the cheek pouch and holding it in place for 2 minutes. The saturated rope was enclosed in a plastic case provided by the manufacturer of assay C for sample submission. Blood samples were allowed to clot and then centrifuged. Serum was harvested from each sample and divided into 2 aliquots, which were placed in separate cryovials and stored at –20°C until shipment to the laboratories for assays A and B.

AFR assays

Assays A and B were ELISAs that measured food allergen–specific IgE in serum. Assay C involved the use of proprietary methods to measure food allergen–specific IgM and IgA in saliva. Each assay was purported to evaluate the test subject for AFR to 24 foods; however, the specific foods assessed differed among the 3 assays **(Appendix)**.

Data analysis

Each assay reported positive and negative results differently. Assays A and B provided numeric values with negative and positive cutoff points for each food or ingredient evaluated. Assay C reported numeric values and the following qualitative descriptions for those results: negative, weak, borderline, intermediate, medium, positive, and strong positive. To facilitate statistical analyses, all numeric values were dichotomized as positive or negative on the basis of the cutoff points provided for each assay. For assay C, any results that were not reported as negative were considered positive.

Descriptive statistics were generated for dog variables such as body weight, age, and sex as well as assay results. Originally, it was intended that a Cochran-Mantel-Haenszel test stratified by ingredient would be used to assess the association between food exposure and a positive test result. However, the number of dogs with exposure to or positive assay results for some ingredients was small (< 5), so Fisher exact tests were used for that purpose. All statistical analyses were performed with commercial statistical software,[d,e] and values of $P < 0.05$ were considered significant.

Results

Dogs

Thirty dogs met the inclusion criteria and were enrolled in the study. The study population had a median age of 4 years (range, 1 to 10 years) and body weight of 20.4 kg (44.9 lb; range, 2.2 to 50.8 kg [4.8 to 111.8 lb]) and included 11 castrated males, 4 sexually intact males, and 15 spayed females. There were 13 mixed-breed dogs, 3 Labrador Retrievers, 2 Beagles, and 1 each of the following breeds: American Eskimo, Boxer, Briard, Chihuahua, German Shepherd Dog, Golden Retriever, Nova Scotia Duck Tolling Retriever, pit bull–type dog, Pointer, Portuguese Water Dog, Vizsla, and Yorkshire Terrier.

AFR assay results

Assays were evaluated on the basis of the number of dogs that had a positive result to any of the foods or ingredients evaluated as well as the number of ingredients for which individual dogs had positive test results. All 30 dogs had at least 1 assay yield a positive result. For assay A, positive results were reported for 26 (87%) dogs, and dogs tested positive to a median of 10.5 ingredients (range, 0 to 24 ingredients). Two dogs tested positive for all ingredients assessed in assay A, and 22 (73%) dogs tested positive for kangaroo even though there were no reported exposures to that ingredient. For assay B, positive results were reported for 18 (60%) dogs, and dogs tested positive to a median of 1 ingredient (range, 0 to 13 ingredients).

For assay C, positive results for IgM and IgA were reported for 30 (100%) and 29 (97%) dogs, respectively. Dogs were IgM-test positive to a median of 12.5 ingredients (range, 4 to 22 ingredients) and IgA-test positive to a median of 3 ingredients (range, 0 to 24 ingredients). One dog tested positive for IgA for all ingredients evaluated in assay C. More dogs tested positive for IgM than for IgA for all ingredients except egg, which had an equal number of dogs (n = 4) test positive for IgM and IgA, and sweet potato, which had more dogs test positive for IgA (8) than for IgM (6).

Overall, food exposure was not significantly ($P = 0.98$) associated with a positive assay result. Likewise, confirmed diet exposure was not significantly ($P = 0.98$) associated with a positive assay result.

Discussion

All dogs of the present study were apparently healthy and did not have any clinical signs associated with AFR. Therefore, a positive result on any of the 3 evaluated assays was considered a false-positive result. Given that all dogs had at least 1 false-positive test result for at least 1 of the 3 assays performed and the fact that 3 dogs tested positive for all 24 ingredients assessed in assay A or C, the data suggested that serum and saliva assays contribute to overdiagnosis of AFR in dogs. These findings were in concordance with results of other peer-reviewed studies[2,9,13] that indicate testing for AFR by any means other than a properly executed elimination diet trial is unreliable and should not be used.

The use of unreliable assays for diagnosis and management of medical conditions can have adverse medical and financial consequences. Diagnostic test results affect clinical decisions and time to treatment, influence patient and clinician behaviors, and can alter patient outcomes.[18] Misdiagnosis of AFR in dogs may delay appropriate diagnostic testing and treatment for other underlying medical problems and incur additional costs for owners. The assays evaluated in the present study not only yielded false-positive test results for AFR but also suggested that most dogs

were allergic to many common foods. Owners provided with this misinformation might be compelled to feed their dogs unnecessarily restrictive and expensive diets that contain exotic ingredients, and resultant exposure to uncommon protein sources (eg, rabbit, venison, duck) can make it difficult to perform appropriate elimination diet trials with novel proteins if and when necessary.

The reason for the high rate of false-positive test results for all 3 assays evaluated in the present study remains unknown. Many variables can affect IgE production in dogs including age, sex, breed, and the dose, route, frequency, and interval of antigen exposure.[19–22] Additionally, IgE concentration may be influenced by previous parasitic or microbial infections that alter immune responses.[19] Dogs with a history of resolved parasitic or microbial infections were not excluded from the present study; however, given that the study dogs were representative of a healthy population, it was expected that they would have similar exposure rates to those types of infections as dogs with AFR for which the evaluated assays were designed. It has been reported that AFR may have a cell-mediated rather than an IgE-mediated mechanism.[23,24] Thus, serum food allergen–specific IgE concentration may not be a reliable biomarker for diagnosis of AFR and false-positive reactions may be common. Indeed, in a recent study[25] that used a different IgE assay than those used in the present study, 4 of 16 healthy dogs versus only 2 of 11 dogs with confirmed food allergies had positive responses to antigens.

Assay C reportedly involves the use of proprietary immunoassays to measure food allergen–specific IgM and IgA concentrations in the saliva of dogs and cats. Currently, validation of salivary IgM and IgA concentrations for diagnosis of food hypersensitivity or food intolerance is lacking in both human and veterinary medicine. Two different saliva-based assays have been shown to yield positive results from saliva samples obtained from dogs with no clinical signs of AFR as well as tap water or saline (0.9% NaCl) solution submitted as dog saliva.[26,27] Results of a recent study[25] indicate that assay C has low sensitivity, positive predictive value, and positive likelihood ratio when confirmed allergic dogs are compared with healthy dogs. The investigators of that study[25] also reported weak to strong positive responses for 1 or more antigens in a number of healthy dogs when assay C was used, similar to the results of the present study. According to the company that developed assay C, the assay is not intended to diagnose food allergy in the classical sense but rather to reveal the latent or preclinical form of food sensitivities in both clinically normal dogs and dogs suspected of having AFR.[28] The methodology of assay C is proprietary, and a peer-reviewed validation study for that assay has yet to be published. Unfortunately, without lifelong follow-up and the availability of a reference standard for comparison, it would be difficult to validate or determine the sensitivity and specificity of an assay that purports to predict food sensitivities in dogs without clinical signs.

For the present study, we hypothesized that positive assay results would be correlated with prior food exposures for the evaluated dogs and may be a marker of food exposure rather than allergy. However, positive assay results were not significantly associated with any food exposure in this study. It is possible that the presumed false-positive assay results were detecting subclinical disease as suggested by the developer of assay C. However, that seems unlikely given that all dogs had positive results for at least 1 assay and the overall prevalence of AFR in dogs is low[2,5,8]; thus, most study dogs would not be expected to develop clinical AFR. Even if AFR could be detected during its subclinical stage, more research is necessary to determine the appropriate course of action for subclinically affected dogs. To our knowledge, there is no evidence to suggest that avoiding exposure to certain foods will effectively circumvent the development of clinical disease in dogs with suspected subclinical AFR.

The present study had multiple limitations. A major limitation was the potential for recall bias by dog owners during completion of the diet history survey. Owners were asked to report all foods, treats, and supplements that their dogs had consumed in their lifetimes. There was potential for uncounted food exposures through ingredients like animal digest or flavorings, incomplete diet histories, or cross-contamination during product manufacturing. This bias could have resulted in altered associations between exposure and positive assay results but was unlikely to have affected the overall study conclusion because apparently healthy dogs tested positive for multiple ingredients regardless of prior exposure. Future studies that evaluate food exposures in dogs will benefit from a prospective design in which owners keep detailed journals of foods introduced to their pets' diets. Alternatively, future studies could use laboratory dogs for which complete diet histories are available and food exposures can be more stringently controlled and recorded. Published guidelines[29] for evaluation of diagnostic tests recommend comparison of the results for the assay or test being evaluated to those of an established reference standard. None of the dogs of the present study had clinical signs of AFR, so we were able to characterize only false-positive assay results. A high rate of false-positive test results in apparently healthy dogs might suggest a similarly high rate of false-positive test results in dogs with clinical signs of AFR. However, this study was not designed to address that possibility, and the results might not be applicable to dogs with clinical signs of AFR.

In the present study, all 3 assays evaluated yielded positive, and presumably erroneous, results for a high proportion of dogs without clinical signs of AFR. Therefore, use of serum- and saliva-based assays for diagnosis of AFR in dogs is not recommended. Properly performed elimination diet trials remain the gold standard for diagnosis of AFR in dogs. Veterinarians are encouraged to use diagnostic tests that have been validated and are supported by evidence-based methods to avoid misdiagnosis and potential adverse medical and financial consequences.

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Footnotes

a. Food Allergen panel, Idexx Laboratories Inc, Westbrook, Me.
b. Allercept Serum IgE assay, Heska Veterinary Diagnostic Laboratories, Loveland, Colo.
c. Nutriscan Food Sensitivity and Intolerance Test, Hemopet, Garden Grove, Calif.
d. SAS, version 9.4, SAS Institute Inc, Cary, NC.
e. IBM SPSS statistics, version 24, IBM, Armonk, NY.

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Appendix

List of the 24 foods or ingredients evaluated in each of 3 assays for AFR, the clinical accuracy of which was assessed in a study involving 30 dogs without clinical signs of the condition.

Assay A[a]	Assay B[b]	Assay C[c]
Barley, **beef**, beet pulp, brewer's yeast, **chicken**, **corn**, duck, **eggs**, fish mix, flaxseed, kangaroo, **lamb**, liver, **milk**, **oats**, pinto beans, **pork**, **potato**, **rabbit**, **rice**, **soybean**, **turkey**, venison, **wheat**	Apple, **barley**, **beef**, brewer's yeast, carrot, **chicken**, **corn**, **egg**, fish mix, flax, green bean, **lamb**, **milk**, **oats**, pea, peanut, **pork**, **potato**, **rabbit**, **rice**, **soybean**, sweet potato, **turkey**, **wheat**	**Barley**, **beef**, **chicken**, **corn**, duck, **egg**, **lamb**, lentil, **milk**, millet, **oatmeal**, peanuts, **pork**, **potato**, quinoa, **rabbit**, **rice**, salmon, **soy**, sweet potato, **turkey**, venison, **wheat**, white fish

Assays A and B measured food allergen–specific IgE concentrations in serum, whereas assay C measured food allergen–specific IgA and IgM concentrations in saliva. Foods or ingredients common to all assays appear in bold font.