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PAPER

Hair and saliva test fails to identify allergies in dogs

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Objectives: Several companies offer saliva and/or hair tests for food and environmental allergies in companion animals, but provide no validation of test accuracy. We examined one such hair and saliva allergy test to determine whether it could reliably differentiate between a normal dog and an allergic dog, and to examine test repeatability.

Materials and Methods: Ten fur and saliva samples were submitted from a known allergic dog and a normal, non-allergic dog. Five fake fur samples and water were also submitted to determine whether the test could differentiate between a real dog and toy animal. The company performed testing for 128 food and environmental allergens. Statistical analyses were performed to determine whether the response distribution differed significantly between dogs, using the Pearson chi-square coefficient, as well as to determine test–retest reliability by calculating Cohen's kappa for each allergen.

Results: The distribution of test results from samples obtained from allergic, non-allergic or fake dogs was not different from that expected due to random chance. Test–retest reproducibility was poor to slight.

Clinical Significance: Hair and saliva testing should not be used to diagnose allergies and is not a substitute for veterinary-directed allergy evaluation and diagnostics.

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INTRODUCTION

Pet owners commonly present dogs to veterinarians for evaluation of pruritic skin disease (Nationwide 2016). There are multiple causes of pruritus and dermatitis in dogs and cats, including parasitic infestation, skin infections due to bacterial and fungal organisms, and hypersensitivity reactions to fleas, food and environmental allergens (atopic dermatitis) (Marsella 2013). Skin parasites and infections can usually be diagnosed by examination, skin scrapings and cytology evaluation, as well as patient response to treatment. However, adverse food reactions and atopic dermatitis require more detailed diagnostic efforts. Adverse food reactions are diagnosed by excluding all other causes of pruritus and by feeding a prescription or home-cooked novel protein diet or a prescription hydrolysed diet with no other foods, treats or supplements for a minimum of 6 to 8 weeks, followed by provocative exposure testing (Kennis 2006, Verlinden *et al.* 2006, Gaschen & Merchant 2011, Marsella 2013, Rosser Jr 2014, Olivry *et al.* 2015). Allergen-specific IgE and IgG serologic tests for food allergy are widely available although false-negative and false-positive results are common (Mueller & Toshalis 1998, Jackson *et al.* 2003, Bethlehem *et al.* 2012, Marsella 2013). Atopic dermatitis is defined as a genetically predisposed inflammatory pruritic allergic skin disease with characteristic clinical features and is associated with IgE, most commonly directed against environmental allergens (Halliwell 2006, Favrot *et al.* 2010, Marsella 2013). Atopic dermatitis has a complicated pathogenesis involving genetics, altered skin barrier function and immunologic abnormalities (Jackson & Mueller 2012, Marsella 2013). No definitive test for atopic dermatitis exists; instead, the diagnosis is based on history, clinical signs and exclusion of all other similar pruritic skin diseases (Jackson & Mueller 2012, Marsella 2013). Intradermal or serologic allergy testing is then performed in order to select ingredients for inclusion in allergen-specific

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immunotherapy, or, occasionally, to identify allergens for avoidance (Jackson & Mueller 2012, Marsella 2013). Recently, several companies have begun offering testing using proprietary analytic methods to identify pets with allergies or pets "prone to allergies" using analysis of fur and/or saliva samples, which pet owners can submit directly to the companies. These tests are undefined and unvalidated.

We therefore evaluated the ability of a hair and saliva allergy test offered by one company (ImmuneIQ™; VetDVM LLC) to correctly identify dogs with and without allergies, by comparing the test results against a veterinary dermatologist's diagnosis. Furthermore, we submitted replicate samples from the same allergic and non-allergic dogs to evaluate the repeatability of the test results. Finally, we submitted sham samples to determine whether the test could differentiate between animal samples and non-animal samples.

MATERIALS AND METHODS

Sampling kits were purchased online from the allergy testing company (ImmuneIQ™; VetDVM LLC), using a discounted online website (Groupon.com). Test kits were mailed by the company to the study participants; test kits consisted of two small plastic zip-lock bags to secure hair samples and a cotton swab to collect saliva samples, as well as a brief submission form for the client to provide the name and species of the pet. Two veterinary dermatologists collected hair and saliva samples from two dogs, one with known allergic dermatitis and the other with no history or evidence of allergies. The allergic dog was a 3-year-old, female spayed Labrador retriever. The diagnosis of food allergies had been obtained using an elimination food trial followed by single ingredient food challenges. Intradermal allergy testing had identified allergic reactions to multiple environmental allergens (Table 1). The normal dog was a 1-year-old male neutered Labrador retriever mixed breed dog. Additionally, fake fur was obtained from a toy animal (furred ear of an animal costume) and cotton swabs saturated with tap water were submitted. One of the investigators (a veterinary dermatologist) microscopically examined the sample from the toy animal to confirm that the fur was synthetic. No institutional approval was obtained because the study posed no risk to dogs or clients. The owners of the two dogs provided informed consent for sampling their dogs.

Ten replicate samples from each dog were divided and then distributed for sample submission to 10 veterinary dermatologists, including the authors, in multiple locations across the United States. Participants submitted an initial sample for analysis and then submitted a second duplicate sample, under a different patient and client name, approximately 4 to 6 weeks later. This provided duplicate data for each dog from each participant, and 10 replicates from each dog. Additionally, six samples of fake fur and water were submitted from the same toy, by multiple veterinarians with six pseudonyms for the "pet," resulting in six replicates for the fake fur and water sample. Therefore, 26 total samples (13 pairs of samples) were submitted for analysis: five pairs for the healthy dog, five pairs for the allergic dog and three pairs for the toy animal (fake fur for hair sample, water as a substitute for saliva sample).

Table 1. Environmental and food allergens identified in the atopic dog. Diagnostic evaluation by the veterinary dermatologist included an elimination food trial followed by single ingredient food challenges, and an intradermal allergy test

Environmental	Dietary
Red alder	Chicken
Elm	Pork
Willow	Beef
Maple	Salmon
Oak mix	Rice
Ash	Corn
KORT grass mix	Carrots
Bermuda	Cheese
Sweet vernal grass	Bread
Velvet grass	
Pigweed	
Dock/Sorrel	
Lambsquarter	
Ragweed	
Cocklebur	
Kochia	
English plantain	
Russian thistle	
Malassezia	
Dermatophagoides farinae	
Tyrophagus putrescentiae	
Acarus siro	
Flea	
Staph	
Juniper	
Tobacco	
Sycamore	
Mulberry	

For each submission, the company then provided results for 128 different potential allergens (117 dietary and 11 environmental) in a list format, identifying each of the tested substances as Good/not a problem" ("Things your pet can have and is attracted to") in green, "Neutral" ("Things that are not good or bad but acceptable") in yellow, and "Bad/Problem" ("Things your pet should avoid and/or has too much of in his/her system (toxicity) or has tested positive for") in red (Fig. 1).

To examine the accuracy of identifying an allergic dog, we first counted the numbers of "Good," "Neutral" and "Bad" for each of the two dogs or the toy animal for each tested allergen and compared the proportions of "Good," "Neutral" and "Bad" results between dogs using a chi-squared test with a Yates correction. Because the numbers of samples submitted for each dog and the toy animal differed, we then corrected for this by dividing each count by the number of samples. To examine the actual proportions, we then summed the corrected proportions to obtain integer values (rounding where necessary). Our hypothesis was that the allergic dog should have a higher proportion of "Bad" results than the healthy dogs or the toy animal, that the healthy dog should have a higher proportion of "Neutral" results than the toy animal and that the toy animal should have exclusively "Good" results, or be identified as fake and not analysed.

To examine test–retest reliability, we examined the proportion of identical results obtained by each participant from their pairs

Customer:	XXXXX	Test Date	XX-XXX-XXXX
Pet Name:	XXXXX	Lab Tech ID	XXXX
Species:	Dog		

OK/ASSISTIVE	NEUTRAL/YIELD TO	NOT OK/OVERWHELMING
Protein		
x Beef	x Chicken	x Cottage cheese
x Buffalo/bison	x Chicken egg	x Dairy
x Duck	x Elk	x Fish meal
x Duck egg	x Kangaroo	x Herring/anchovy
x Moose	x Lamb	x Mackerel
x Pheasant	x Ostrich	x Ocean white fish
x Turkey	x Pork	x Salmon
x Venison	x Quail	x Shrimp
	x Rabbit	x Soy
		x Tuna
		x Whey
		x Yogurt
Carbohydrates		
x Barley	x Chick pea	x Bread (from grains)
x Buckwheat	x Maple syrup	x Corn
x Lentils	x Potato	x Honey
x Molasses	x Quinoa	x Kidney Beans
x Rice, brown	x Sweet potato	x Oat
x Yam	x Tapioca	x Pinto Beans
	x Yucca	x Rice, white
		x Sorghum
		x Wheat
Fruits		
x Apple	x Blackberry	x Lemon juice
x Blueberry	x Cranberry	
x Papaya	x Mango	
x Pineapple	x Peach	
x Strawberry	x Pear	
	x Pomegranate	
	x Raspberry	

OK/ASSISTIVE	NEUTRAL/YIELD TO	NOT OK/OVERWHELMING
Vegetables		
x Artichoke	x Beet	x Celery
x Asparagus	x Broccoli	x Green beans
x Cabbage	x Brussels sprouts	x Kale
x Chard	x Carrot	x Maitake mushroom
x Parsley	x Cauliflower	x Seaweed
x Zucchini	x Cucumber	x Shitaki mushroom
	x Green peas	
	x Kelp	
	x Pumpkin	
	x Spinach	
	x Squash	
Fatty Acids		
x Avocado oil	x Cod liver oil	x Almond oil
x Coconut oil	x Flax oil	x Canola oil
x Lecithin	x Krill oil	x Cotton seed
	x Safflower oil	x Hemp oil
	x Salmon Oil	x Olive oil
	x Sesame oil	x Peanut butter
	x Sunflower oil	
Nutritional Supplements		
x Chlorella	x Alfalfa	
x Ginger	x Carob	
x Green Lipid mussel	x Chicory	
x Green algae	x Green tea extract	
x Nutritional yeast	x Licorice root	
x Turmeric	x MSM (sulfur)	
	x Peppermint	
	x Psyllium	
Environmental		
	x Grass	x Cosmetics
	x Heavy metals	x Fragrance
	x Human dander	x Insect
	x Pet dander	x Petrochemical
	x Plastic / Nylon	x Pollen
		x Rubber/latex

FIG 1. Sample of results provided by hair and saliva allergy testing company

of submissions and compared it with a nominal proportion of 0.5 (being no different from chance).

Finally, we examined whether any potential allergens were more commonly identified as "Bad" or "Good" than others, by counting the number of "Good," "Neutral" or "Bad" responses for each allergen across all 26 samples. We hypothesised that whether the allergic dog had allergies to certain allergens, then these allergens would garner approximately 10 "Bad" ratings on replicate tests. Similarly, the healthy dog and toy animal should garner no bad ratings (with some minor variability) for any allergens. Substantially more than 10 "Bad" ratings for any allergen would therefore be inconsistent with a true allergen identification.

RESULTS

Proportions of "Good," "Neutral" and "Bad" results did not differ between healthy, allergic or fake dogs (P=1.0). Both dogs (hair and salivary samples) and the toy animal (fake fur and water samples) had 26 to 27% of the allergens listed as "Bad," 27 to 28% of the allergens listed as "Good" and 45 to 46% of the results listed as "Neutral."

We had 1664 individual pairs of results for evaluating agreement (test–retest reliability). Of these, 1063 pairs agreed, while 601 pairs did not agree. This proportion of agreements differed from a nominal proportion of 0.5 (P<0.001). Table S1, Supporting Information details proportions of "Good," "Bad" and "Neutral" results for multiple analyses run on one healthy dog, one atopic dog and one fake fur sample.

In the 26 submitted samples, we found that certain potential allergens showed a dramatic deviation from anticipated results. None of the 26 samples, including the 10 samples from the healthy dog and the six samples from the toy animal, had a result of "Good" for any of the potential environmental allergens, such as plants. Conversely, brown rice had a result of "Good" for 24 of the 26 submitted samples, and no sample had a result of "Bad" for brown rice. Certain dietary protein sources had a high number of "Bad" ratings. All of the 26 samples, including the 10 samples from the healthy dog and the six samples from the toy animal, had a result of "Bad" for cottage cheese, dairy, shrimp, tuna, whey and yogurt.

DISCUSSION

Our study demonstrates that hair and saliva testing fails not only to identify allergic dermatitis in dogs, but fails to differentiate between animal and non-animal samples, providing essentially identical results, regardless of the origin of the sample. Furthermore, particular allergens appear to be over-represented as "Bad" across all samples, while others are over-represented as "Good" across all samples.

Our findings are similar to those of previous studies in humans. Claims of accuracy of hair analysis for allergies and health or nutritional status have previously been debunked in human medicines (Niggemann & Gruber 2004). In a British study, hair and blood samples from nine known fish-allergic human subjects and nine non-allergic control subjects were sent under different names to five laboratories providing allergy testing by "alternative" methods

(including hair analysis in three labs) (Sethi *et al.* 1987). The majority of the tests did not correctly identify the fish-allergic patients and found multiple false positive results. In another study, hair samples from two healthy people were sent under assumed names to 13 commercial laboratories performing multi-mineral hair analysis; some of the laboratory claims included being able to use hair analysis results in order to balance body chemistry, reverse ageing, detect predisposition to disease and diagnose metabolic problems (Barrett 1985). Hair analysis results varied considerably between identical samples, and six laboratories recommended a range of 1 to 11 different food supplements with the goal to alleviate or prevent diseases such as goitre, uraemia, depression and sugar/alcohol craving; further unnecessary diagnostic investigations for "possible patient conditions" were also frequently recommended. Interestingly, the company evaluated in this study also recommended supplements for pet owners to purchase based on testing results.

Saliva testing for allergies in humans is also not a published or accepted diagnostic method, and food allergy testing at random often leads to misdiagnosis (Unsworth & Lock 2014, Bird *et al.* 2015). A careful medical and dietary history and targeted use of skin or serologic testing for food allergens can be supportive of a diagnosis of food allergy in humans but the gold standard diagnosis of food allergy is considered to be a physician-supervised oral food challenge (Beyer & Teuber 2005, Boyce *et al.* 2010, Eigenmann *et al.* 2011, Lieberman & Sicherer 2011, Kattan & Sicherer 2015). There are limited data on elevated salivary IgA levels in infants as usually being protective against the later development of allergic asthma and atopy in most studies (Neffen *et al.* 1986, Solé *et al.* 1988, Böttcher *et al.* 2002, Fageras *et al.* 2011, Sandin *et al.* 2011), though in one study, salivary anticasein IgA was significantly higher in infants with elevated cord blood IgE levels or parents with atopic disease and deemed therefore at high risk for future development of allergy (Renz *et al.* 1990). The analysis methods for the company evaluated in our study were proprietary and it is unknown if salivary immunoglobulin was measured. However, in a recent study of atopic and normal dogs, a saliva-based test for food-specific IgA and IgM did not appear to be suitable for diagnosing allergy (Udraite Vovk *et al.* 2017).

A limitation of our study is its small size. Additional sample submission, which we had intended to undertake, is no longer possible because the allergy testing company evaluated in this study has gone out of business. However, our study provides evidence that allergen identification from hair or saliva samples is inaccurate and offers results that are no different from chance. We found that, regardless of the sample submitted, certain potential allergens were more likely to be considered "Good" or "Bad," and some potential allergens were identified as "Bad" in 100% of submitted samples. This suggests a systematic bias in allergen reporting by the company. Additionally, the company's analysis of fake fur and tap water samples provided results that were similar to hair and saliva samples from a healthy dog and from an allergic dog, further suggesting that there is no scientific validity to this approach. Together, our results are important because hair and saliva testing for allergies continues to be offered by other companies to pet owners. Currently, preliminary results (publication pending) by other researchers have confirmed the inaccuracy of these other companies' tests (Lam *et al.* 2017, Udraite Vovk *et al.* 2017).

In summary, the results of this study demonstrate that the hair and saliva testing for allergies in dogs was inaccurate. It neither differentiated between an allergic and non-allergic dog, nor between a real dog and toy animal. Although both veterinarians and pet owners desire a simple and accurate test for allergies in animals, hair and saliva testing is not a substitute for veterinary-directed allergy evaluation and diagnostics.

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Conflict of interest

No conflicts of interest have been declared.

References

Barrett, S. (1985) Commercial hair analysis. Science or scam? *Journal of the American Medical Association* **254**, 1041-1045

Bethlehem, S., Bexley, J. & Mueller, R. S. (2012) Patch testing and allergen-specific serum IgE and IgG antibodies in the diagnosis of canine adverse food reactions. *Veterinary Immunology and Immunopathology* **145**, 582-589

Beyer, K. & Teuber, S. S. (2005) Food allergy diagnostics: scientific and unproven procedures. *Current Opinion in Allergy and Clinical Immunology* **5**, 261-266

Bird, J. A., Crain, M. & Varshney, P. (2015) Food allergen panel testing often results in misdiagnosis of food allergy. *The Journal of Pediatrics* **166**, 97-100

Böttcher, M. F., Häggström, P., Björkstén, B., *et al.* (2002) Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. *Clinical and Experimental Allergy* **32**, 1293-1298

Boyce, J., Assa'ad, A., Burks, A. W., *et al.* (2010) Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *The Journal of Allergy and Clinical Immunology* **126**, S1-S58

Eigenmann, P. A., Oh, J.-W. & Beyer, K. (2011) Diagnostic testing in the evaluation of food allergy. *Pediatric Clinics of North America* **58**, 351-362

Fageras, M., Tomičić, S., Voor, T., *et al.* (2011) Slow salivary secretory IgA maturation may relate to low microbial pressure and allergic symptoms in sensitized children. *Pediatric Research* **70**, 572-577

Favrot, C., Steffan, J., Seewald, W., *et al.* (2010) A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Veterinary Dermatology* **21**, 23-31

Gaschen, F. P. & Merchant, S. R. (2011) Adverse food reactions in dogs and cats. *Veterinary Clinics of North America: Small Animal Practice* **41**, 361-379

Halliwell, R. (2006) Revised nomenclature for veterinary allergy. *Veterinary Immunology and Immunopathology* **114**, 207-208

Jackson, H. A. & Mueller, R. S. (2012) Atopic dermatitis and adverse food reactions. In: BSAVA Manual of Canine and Feline Dermatology. Eds H. A. Jackson and R. Marsella. BSAVA, Gloucester, UK. pp 130-140

Jackson, H. A., Jackson, M. W., Coblentz, L., *et al.* (2003) Evaluation of the clinical and allergen specific serum immunoglobulin E responses to oral challenge with cornstarch, corn, soy and a soy hydrolysate diet in dogs with spontaneous food allergy. *Veterinary Dermatology* **14**, 181-187

Kattan, J. D. & Sicherer, S. H. (2015) Optimizing the diagnosis of food allergy. *Immunology and Allergy Clinics of North America* **35**, 61-76

Kennis, R. A. (2006) Food allergies: update of pathogenesis, diagnoses, and management. *The Veterinary Clinics of North America. Small Animal Practice* **36**, 175-184 vii–viii

Lam, A. T. H., Johnson, L. M. & Heinze, C. R. (2017) Evaluation of clinical accuracy of serological and salivary testing for food allergens in asymptomatic dogs. Abstracts of the North American Veterinary Dermatology Forum. Orlando, FL, USA, April 26-29, 2017.

Lieberman, J. A. & Sicherer, S. H. (2011) Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge. *Current Allergy and Asthma Reports* **11**, 58-64

Marsella, R. (2013) Hypersensitivity disorders. In: Muller and Kirk's Small Animal Dermatology. Eds W. H. Miller, C. E. Griffen and K. L. Campbell. Elsevier Mosby, St. Louis, MO, USA. pp 363-431

Mueller, R. S. & Toshalis, J. (1998) Evaluation of serum allergen-specific IgE for the diagnosis of food adverse reactions in the dog. *Veterinary Dermatology* **9**, 167-171

Nationwide (2016) Nationwide reveals the 10 most common medical conditions in dogs and cats. https://press8.petinsurance.com/articles/2016/march/nationwide-reveals-the-10-most-common-medical-conditions-for-dogs-and-cats. Accessed May 4, 2018

Neffen, H., Crisci, C. D., Busaniche, H., *et al*. (1986) Correlation between serum IgA, secretory IgA and total serum IgE in asthmatic and rhinitic affected patients. *Allergologia et Immunopathologia* **14**, 413-418

Niggemann, B. & Gruber, C. (2004) Unproven diagnostic procedures in IgE-mediated allergic diseases. *Allergy* **59**, 806-808

Olivry, T., Mueller, R. S. & Prélaud, P. (2015) Critically appraised topic on adverse food reactions of companion animals (1): duration of elimination diets. *BMC Veterinary Research* **11**, 225

Renz, H., Vestner, R., Petzoldt, S., *et al*. (1990) Elevated concentrations of salivary secretory immunoglobulin A anti-cow's milk protein in newborns at risk of allergy. *International Archives of Allergy and Applied Immunology* **92**, 247-253

Rosser, E. J. Jr. (2014) Diagnostic workup of food hypersensitivity. In: Veterinary Allergy. Eds C. Noli and A. Foster. Wiley Blackwell, West Sussex, UK. pp 101-123

Sandin, A., Björkstén, B., Böttcher, M. F., *et al*. (2011) High salivary secretory IgA antibody levels are associated with less late-onset wheezing in IgE-sensitized infants. *Pediatric Allergy and Immunology* **22**, 477-481

Sethi, T. J., Lessof, M. H., Kemeny, D. M., *et al*. (1987) How reliable are commercial allergy tests? *Lancet* **330**, 92-94

Solé, D., Zaha, M. M., Leser, P. G., *et al*. (1988) Secretory IgA levels in normal and atopic individuals. Influence of breast and/or bottle feeding. *Allergologia et Immunopathologia* **16**, 385-392

Udraite Vovka, L., Watson, A., Dodds, W.J., Klinger, C.J., Classen, J. & Mueller, R.S. (2017) Testing for food-specific antibodies in saliva and blood of atopic and normal dogs. *Veterinary Dermatology* **28**, 552

Unsworth, D. J. & Lock, R. J. (2014) Food allergy testing. *Advances in Clinical Chemistry* **65**, 173-198

Verlinden, A., Hesta, M., Millet, S., *et al*. (2006) Food allergy in dogs and cats: a review. *Critical Reviews in Food Science and Nutrition* **46**, 259-273

Supporting Information

The following supporting information is available for this article:

Table S1. Proportions of "Good", "Bad" and "Neutral" results for multiple analyses run on one healthy dog, one atopic dog and one fake fur sample.