



Original Article

Testing for food-specific antibodies in saliva and blood of food allergic and healthy dogs

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ABSTRACT

An elimination diet (ED) followed by re-challenge has been the reference standard to diagnose adverse food reactions (AFR) in dogs, but can be challenging to conduct. This study investigated the accuracy of a saliva-based test for food-specific IgA and IgM and an ELISA serum test for food-specific IgE. Three groups of dogs were tested. Group 1 ($n = 11$) included dogs with previously diagnosed and controlled AFR; group 2 ($n = 15$) comprised dogs with allergic dermatitis at the beginning of their ED; and group 3 ($n = 16$) was composed of clinically healthy research dogs. Saliva samples were collected from all groups and blood samples from group 1 and group 3. The results of clinical re-challenges with individual food components were compared with the test results. Specificity, sensitivity, positive and negative predictive values and likelihood ratios were determined.

Forty-one dogs completed the study; one dog was lost to follow up. There was a total of 163 re-challenges. Sensitivity, positive predictive value and likelihood ratio, specificity, negative predictive value and likelihood ratios were unsatisfactory for both tests in most instances, except for IgM testing in group 2, which had moderate specificity. There was no clear difference in the number of positive reactions between the allergic dogs and healthy dogs from a research population. Based on these results, the saliva test for food specific IgA and IgM and the ELISA serum test for food specific IgE were not reliable to diagnose adverse food reactions in dogs. Until more data are available, elimination diets remain the reference standard in the diagnosis of this disease.

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Introduction

Dermatology is an important part of small animal practice and allergic dermatitis caused by flea, food and environmental antigens is common in dogs and cats (Lund et al., 1999). Based on the current practice guidelines for atopic dermatitis (AD), the clinical signs of AD induced by environmental allergens can resemble those seen with food-induced AD, and every non-seasonal atopic dog should undergo an elimination diet (ED) as the reference standard to diagnose an adverse food reaction (AFR; Olivry et al., 2007; Favrot et al., 2010; Bethlehem et al., 2012; Mueller and Olivry, 2017). However, this usually takes time and effort, and client compliance can be difficult to achieve (Mueller and Tsohalis, 1998; Kennis, 2006). Most pet owners would prefer faster and more easily performed diagnostic tests, such as intradermal, salivary or blood testing. In previous studies,

intradermal tests with food components (Jeffers et al., 1991; Kunkle and Horner, 1992) or tests for food-specific serum IgE (Mueller and Tsohalis, 1998; Foster et al., 2003; Wilhelm and Favrot, 2005; Bethlehem et al., 2012; Hardy et al., 2014) did not reliably identify dogs with AFR and thus cannot be recommended for the diagnosis of AFR in practice (Mueller and Olivry, 2017). More recently, food patch testing was reported to be reliable as a tool to identify suitable ingredients for EDs, due to its high negative predictive value (Bethlehem et al., 2012). However, patch testing is time-consuming, expensive, and because of its low positive predictive value, cannot be used for the diagnosis of adverse food reactions.

In human studies, salivary IgA did not differ between atopic and non-atopic patients (Sole et al., 1988; Bootcher et al., 2003). However, in atopic dogs, cutaneous IgA concentrations were increased compared to normal controls (Mueller et al., 1997). Currently a test for food-specific IgA and IgM in saliva (Hemopet Nutriscan, Garden Grove) is available in the United States. The test is easy to perform, noninvasive, and thus is very acceptable to pet owners. Furthermore, an ELISA test for food-specific IgE is

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advertised as an improved and reliable serum test¹ to diagnose AFR in many countries (SpotPlatinum, Spectrum Labs).

The aim of this study was to assess the accuracy, sensitivity and specificity, positive and negative predictive values and likelihood ratios of the saliva-based test for canine AFR and a new commercial ELISA serum test for food-specific IgE.

Materials and methods

This study was submitted to the Royal Canin Ethical Committee and approved under the number C2EA-90 on November 4th, 2013. Owner consent was obtained prior to inclusion from the privately-owned allergic dogs.

Study populations

Three groups of dogs were included in the study; groups 1 and 2 came from a clinic in Germany, group 3 came from France.

Group 1 consisted of 11 client-owned dogs with previously diagnosed AFR. Those dogs had clinical signs of AD with non-seasonal pruritus >2/10 on a validated pruritus visual analog scale (PVAS; Hill et al., 2007). The dogs improved on an ED of 8 weeks with either a protein and a carbohydrate source not previously fed, or a diet containing hydrolysed proteins; they deteriorated on re-challenge and improved again when fed the ED. The improvement on an ED was confirmed if skin lesions measured with the validated Canine Atopic Dermatitis Extend and Severity Index (CADESI; Olivry et al., 2014) improved by >50% and pruritus was <2/10 on the PVAS. Each dog was then challenged with up to seven individual food components in a sequential provocation. Every individual food component was fed for up to 2 weeks. If during the 2 weeks the dog's condition did not deteriorate, we assumed that the individual foodstuff was tolerated well. A positive re-challenge was defined as deterioration within those 2 weeks with increased pruritus of >2/10 PVAS (with or without increased CADESI-3 scores) and subsequent improvement when fed the elimination diet again. These dogs were controlled on restricted diets and had pruritus ≤2/10 on the PVAS for >8 months up to the time of sampling.

Group 2 included 15 client-owned dogs which were presented with clinical signs compatible with non-seasonal atopic dermatitis (Hensel et al., 2015). Those dogs were sampled at the beginning of the ED and the diet and re-challenges were performed as described above for group 1.

Finally, group 3 included 16 healthy control dogs with no history or clinical evidence of AFR or allergic dermatitis. These dogs were selected from a research population that was fed a variety of diets over time.

Specimen collection

A saliva sample was collected from each dog using a saliva collection kit (Hemopet, Garden Grove). All saliva samples were collected by a veterinarian. Dogs were fasted overnight and sampled in the morning. After lifting the upper lip, a cotton dental rope was placed in the corner of the dog's mouth and kept in place for 2 min. Dogs were presented with a treat to stimulate saliva production. After collection, the saliva-moistened cotton dental rope was placed in the special double-sleeved collection tube, capped, and sealed tightly with tape. Samples were labeled and shipped in a styrofoam mailer after cooling.

Blood samples were obtained by venipuncture of the jugular or cephalic vein from 11 dogs in group 1 and 16 dogs in group 3, placed in a tube without anticoagulant, centrifuged, and serum was collected. The serum was frozen at –20 °C and stored until shipment. Batches of serum and saliva specimens were sent by courier to Hemopet (Garden Grove), where saliva specimens were processed for IgA and IgM testing and the serum samples were forwarded to another laboratory (Spectrum Labs, Phoenix) for routine food-specific serum IgE testing. Both laboratories use whole extracts of individual food components to bind the serum or salivary IgA, IgM and IgE. The tested food antigens are listed in Table 1.

Statistics

The results of the known clinical re-challenges with individual food components were compared to the results of the tests for food-specific salivary IgA and IgM and for food-specific serum IgE. In groups 1 and 2 only antigens with a known individual challenge were evaluated; in group 3 all feeding was considered a negative challenge of fed antigens. The salivary testing was defined by the laboratory to have three possible outcomes – elevated or positive (>11.5 units/mL), normal or negative (<10 units/mL) and weakly reactive (≥10.0 to ≤11.5 units/mL). These weak reactions were assumed to reflect the 'background noise' of the assay reagents and the autoanalyser instrument. Three analyses of the data were performed – with concentrations in the weakly reactive (questionable) grey zone

Table 1

Salivary IgA and IgM and serum-IgE antibodies against food antigens tested in this study.

	Saliva testing	Serum testing
Beef	x	x
Lamb	x	x
Pork	x	x
Chicken	x	x
Turkey	x	x
Duck	x	x
Fish	White fish, Salmon	Catfish, Cod, Herring, Mackerel, White Fish
Milk	x	x
Eggs	x	x
Venison	x	x
Rabbit	x	x
Soy	x	x
Corn	x	x
Wheat	x	x
Sorghum		x
Rice	x	x
Oatmeal	x	x
Barley	x	x
Potato	x	x
Tomato		x
Carrot		x
Pea		x
Kelp		x
Brewer's Yeast		x
Lentil		x
Peanut		x
Quinoa		x
Sweet Potato		x

considered as (1) positive, (2) negative or (3) not considered at all. Specificity, sensitivity, positive and negative predictive values, and positive and negative likelihood ratios were determined for all dogs and for the individual groups.

Results

In group 2, six dogs improved on an elimination diet, but did not deteriorate on the re-challenge with their original food and were subsequently diagnosed as dogs with atopic dermatitis due to environmental allergens with clinical signs all year round. The dogs were not receiving immunomodulatory medications during the last 4 weeks of the ED. Six dogs were diagnosed with an AFR. Those dogs improved on an ED of 8 weeks, deteriorated on re-challenge, and improved again when refed the ED. Subsequent individual provocations were conducted with up to seven food antigens. In two dogs, the elimination diet failed due to poor owner compliance. The last dog in group 2 was lost to follow up.

Overall, there were 37 re-challenges with individual food components in group 1, 46 re-challenges in group 2, and 80 re-challenges in group 3. In the latter group, dogs were fed diets containing chicken, beef, corn, wheat and rice and did not have any gastrointestinal or cutaneous signs prior to sampling or during the 12 months follow-up after sampling. Because of the 12-month follow-up, adverse reactions to those food antigens were assumed to have been ruled out. Individual positive and negative re-challenges in all tested dogs are listed in Table 2. There were 19 positive re-challenges in nine dogs and 144 negative re-challenges in 41 dogs. These results reflect the fact that not all dogs on the ED were proven to have AFRs and that healthy dogs from group 3 had no positive re-challenges.

Salivary food antigen-specific IgA and IgM testing

Specificity, sensitivity, positive and negative predictive values, and positive and negative likelihood ratios calculated based on positive and negative results of antigen-specific salivary IgA and

¹ See: Spectrum Lab, direction insert: <http://www.vetallergy.com/vet/all.pdf>. (Accessed 8 December 2018).

Table 2

Positive and negative re-challenges with individual antigens in tested dogs.

Allergen	Negative re-challenges (n = 41)	Positive re-challenges (n = 9)
Beef	16	3
Chicken	18	4
Corn	18	1
Duck	2	
Goat	2	
Horse	8	1
Kangaroo	8	
Lamb	4	
Millet	2	
Oats		1
Ostrich	1	
Parsnip	8	
Pork	1	
Potato	7	3
Pumpkin	2	
Quinoa	1	
Rabbit	2	
Rice	17	2
Reindeer	2	1
Sweet potato	4	
Turkey		1
Venison	3	
Wheat	18	2

IgM are summarized in Table 3. For sensitivity, specificity, and positive and negative predictive values, most values were between 16.7% and 63.7%, with two notable exceptions; a specificity of 87.5% with IgM testing in dogs of group 2 (paired with a sensitivity of 0%) and a high negative predictive value of 100% in group 3. Likelihood ratios were generally between 0.43 and 2.99, except for the positive likelihood ratio of 0 with IgM testing in group 2 dogs. The results of tests for salivary antigen-specific IgA and IgM in the individual groups are summarized in Supplementary Tables 1 and 4, respectively, for the dogs on the restricted diets for >8 months; in Supplementary Tables 2 and 5 for the dogs with recent food allergen exposure; and in Supplementary Tables 3 and 6 for the healthy research dogs.

A large number of reactions (between 37.7% and 55.5%, depending on group and antibody type) were weakly reactive and considered in the grey zone. Calculations in which the weakly reactive questionable results were variably considered as negative, positive or not considered at all are summarized in Supplementary Tables 7 and 8. None of the three calculations yielded acceptable specificity, sensitivity, negative or positive predictive values, or negative or positive likelihood ratio.

Serum food antigen-specific IgE testing

Results of the tests for serum antigen-specific IgE are summarized in Supplementary Table 9 for the dogs on the

Table 3

Specificity, sensitivity, positive and negative predictive values and positive and negative likelihood ratio of dogs tested for food antigen-specific salivary (Sal) IgA and IgM.

		Spec	Sens	PP	NP	PLR	NLR
Sal IgA	Group I	16.7%	50%	28.6%	33.3%	0.6	2.99
	Group II	53.8%	20%	14.3%	63.6%	0.43	1.49
	Group III	57.9%	N/A	0%	100%	N/A	N/A
Sal IgM	Group I	50%	60%	75%	33.3%	1.20	0.8
	Group II	87.5%	0%	0%	58.3%	0	1.73
	Group III	34.2%	N/A	0%	100%	N/A	N/A

Spec, specificity; Sens, sensitivity; PP, positive predictive value; NP, negative predictive values; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

restricted diets for >8 months and in Supplementary Table 10 for the healthy dogs. Specificity, sensitivity, positive and negative predictive values, and positive and negative likelihood ratios for dogs tested for food antigen-specific serum IgE are summarized in Table 4. Specificity of tests for food antigen-specific IgE varied between 70% and 75%, sensitivity was 10%, positive and negative predictive values were between 4% and 25% and between 44% and 88%, respectively; likelihood ratios varied between 0.3 and 1.3.

Healthy and allergic dogs

Between 10 and 30% of all of the dogs had positive reactions, there was no consistent pattern differentiating healthy from food-allergic individuals. There were more positive IgM reactions in healthy dogs than in allergic dogs; for IgA this was reversed. One quarter of the healthy dogs and 20% of allergic dogs had positive IgE test reactions. The number and proportion of positive and questionable reactions in salivary antigen-specific IgA and IgM and positive reactions in serum antigen-specific IgE tests in the healthy research and client-owned allergic dogs are summarized in Table 5.

Discussion

Sensitivity, specificity, positive and negative predictive value and positive and negative likelihood ratios were assessed in our study for both the saliva-based test for food-specific IgA and IgM and the ELISA serum test for food-specific IgE. Results of the saliva-based test demonstrated moderate IgM specificity of 87.5% in the six dogs with AFR from group 2 only. Sensitivity, positive and negative predictive value, and positive and negative likelihood ratio of IgM testing in group 2 was low. The saliva-based test for food-specific IgA and ELISA serum-based test yielded unsatisfactory results for all of these calculated statistics. The study did not show any clear difference in the number of positive reactions between allergic and healthy research dogs.

Three groups of dogs were tested. In the first group, dogs with confirmed AFR controlled using diet for >8 months were evaluated. In those dogs, it could be speculated that the lack of food-specific antibodies reflected the absence of offending allergens, or subclinical immunostimulation by such antigens. Serum IgE concentrations in dogs can be increased up to 12 months after allergen exposure (Schiessl et al., 2003). To our knowledge, the duration of persistence of salivary IgA and IgM in dogs is unknown, although experience by one of the authors with this novel saliva-based test revealed food-related IgA but not IgM antibodies that persisted for as long as 2 years (WJD data not shown).

In group 2, sampling occurred at the beginning of the elimination diet, at a time when the dogs were clinically affected and antibody production was expected to be high. Thus, in this group, the number of positive results was expected to be higher than in the other two groups. However, the number of positive reactions for IgM, IgA and IgE was approximately the same in groups 1 and 2.

Table 4

Specificity, sensitivity, positive and negative predictive values and positive and negative likelihood ratio of dogs tested for food antigen-specific serum IgE.

		Spec	Sens	PP	NP	PLR	NLR
Serum IgE	Group 1	70.0%	10%	25%	43.8%	0.33	1.29
	Group 2	75%	N/A	0%	100%	N/A	N/A
	All dogs	74.4%	10%	4.2%	88.2%	0.39	1.21

Spec, specificity; Sens, sensitivity; PP, positive predictive value; NP, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Table 5

Results of the number (percentage) of positive and questionable reactions in tests for food-specific salivary IgA and IgM and serum IgE based on positive or negative re-challenge with specific antigens in healthy and allergic dogs.

	Strong positive reactions	Weak positive reactions	Total number of reactions
Healthy dogs, IgA	16 (19.5%)	44 (53.6%)	82 (100%)
Allergic dogs, IgA	14 (31.1%)	17 (37.7%)	45 (100%)
Healthy dogs, IgM	25 (30.5%)	44 (53.6%)	82 (100%)
Allergic dogs, IgM	5 (11.1%)	25 (55.5%)	45 (100%)
Healthy dogs, IgE	20 (25%)		80 (100%)
Allergic dogs, IgE	4 (20%)		20 (100%)

The third group consisted of research dogs without any gastrointestinal or cutaneous signs of AFR prior to sampling and in the 12 months after sampling. A test to differentiate diseased from healthy dogs should elicit a lower number of positive reactions in healthy dogs than in affected dogs. However, the number of positive reactions in the healthy research dogs was approximately the same or (for serum IgE and salivary IgM) even slightly higher than in the allergic dogs. This is consistent with the published literature for serum IgE and IgG. [Hardy et al. \(2014\)](#) also reported no correlation between different laboratories testing for food-specific serum IgE and IgG in healthy dogs and dogs with skin disease. In that study, the tests did not distinguish between healthy and affected dogs.

The laboratory used in this study reports that the test detects elevated salivary IgA and IgM antibody concentrations to certain foods and thus allows identification of specific food ingredients causing the clinical signs of allergy. IgA is produced by plasma cells mainly at mucosal surfaces and is responsible for mucosal first line adaptive immunity. IgM is mainly found in the circulation and also participates in the early immune response to initial antigen presentation ([Woof and Ken, 2006](#); [Day and Schultz, 2011](#); [Abbas et al., 2018](#)). Decreased IgA concentrations in allergic dogs could conceivably facilitate penetration of allergens through skin or mucous membranes. Previous investigations do not support this hypothesis. Increased cutaneous IgA concentrations were reported two decades ago in haired skin washings of atopic dogs compared to normal controls ([Mueller et al., 1997](#)). If IgA and IgM played a major role in the pathogenesis of AFR, these antibodies could be detectable in saliva and serum, respectively, of dogs with AFR. [Frossard et al. \(2004\)](#) reported that food tolerant mice had more beta-lactoglobulin-specific IgA-secreting cells and higher fecal beta-lactoglobulin-specific IgA than mice with food allergy. The results of our study do not support the statement of the laboratory that elevated salivary IgA and IgM concentrations to certain foods could help diagnose AFR. This finding is similar to those in human medicine, where laboratories offer saliva testing for detecting elevated IgA, but studies have shown that those results are not clinically relevant. Some studies have evaluated specific immunoglobulins in the saliva of normal dogs ([German et al., 1998](#); [Lam et al., 2017](#)), but to the authors' knowledge there are no published data investigating the accuracy of saliva-based tests for food-specific antibodies and correlating those to clinical re-challenges. The immune system is exposed to a large variety of food antigens, and only a small percentage of individuals develop food allergies due to oral tolerance to dietary proteins ([Chehade and Mayer, 2005](#)). Allergen-specific salivary immunoglobulins may simply reflect exposure to food antigens or subclinical food intolerance reactions, rather than being causally associated with the development of clinical signs, or even indicating food allergy. AFR could be IgE-mediated, cell-mediated, or there could be mixed reactions, with or without the participation of immunoglobulins ([Burks et al., 2012](#); [Noli, 2014](#)). Alternatively, other immunopathological pathways can be activated, as non-IgE-mediated mechanisms may involve immune complex or delayed hypersensitivity reactions ([Foster et al., 2003](#)).

There were three possible outcomes of the saliva-based test – strong positive, clear negative, and weakly reactive reaction which has been termed the 'grey zone'. Approximately half of all saliva test results were weak positive. If counting grey zone results as either positive or as negative would have increased their reliability, then the test cut-off could have been adjusted accordingly, but this was not the case. One possible interpretation is that positive results were due to a developing AFR, which would lead to future development of clinical signs, but this is considered unlikely. This is based on the fact that none of the healthy research dogs that had numerous weakly reactive and strongly positive results developed any clinical signs of allergy to antigens that they had reacted to and which they continued to consume for the 12-month follow-up observation period.

Published studies failed to detect food-specific IgE in dogs with confirmed adverse reactions to those antigens ([Mueller and Tsohalis, 1998](#)) and serum IgE responses did not correlate with specific dietary exposures ([Ricci et al., 2010](#)). When clinical signs of AFR were stimulated in research dogs with AFR through an oral challenge, serum IgE changes did not predict the clinical outcome ([Kennis et al., 2002](#); [Jackson et al., 2003](#)). In human medicine, antigen-specific IgE may persist in the absence of antigen exposure and clinical signs, or conversely, antigen-specific IgE may not necessarily reflect clinically relevant sensitisation against a particular allergen ([Asero et al., 2007](#)). The relatively high number of false negative and false positive reactions in our study concurs with these findings and provides further evidence that many clinically atopic dogs have negative test results and that healthy dogs can have increased concentrations of IgE antibodies. The sensitivity of serum testing for food-specific IgE in both groups was 10%, and a true positive reaction was only seen in one instance. The specificity was slightly >70% in the healthy and the allergic dogs, and this also supports previously published results. Although the higher specificity is explained by a larger number of true negative reactions with this test, a quarter of the results in healthy dogs were false positives, indicating that the serum IgE test cannot differentiate between healthy and allergic dogs. The positive predictive value of serum-specific IgE in our study was low, but the negative predictive value was higher. This correlates well with the previously reported results of serum IgE testing in a recent study, where results also correlated with clinical provocation ([Bethlehem et al., 2012](#)). AFR may be IgE-mediated, but cell-mediated or mixed reactions, with or without the participation of immunoglobulins, have also been described ([Foster et al., 2003](#); [Burks et al., 2012](#)), and this probably explains these varied results.

The likelihood ratio (LR) is a very sensitive and independent measure of diagnostic accuracy ([McGee, 2002](#)). It has a big advantage compared to other methods (such as sensitivity/specificity or positive and negative predictive values) in that its numeric value does not depend on patient selection. Likelihood ratios <1 decrease the probability of the tested disease being present, while LR >1 increases this probability. A LR of 0.4 decreases the probability of the disease being present by 20%, a LR

of 3 increases the probability of disease by 20%. In order to say a test is clinically relevant, the positive LR should be >12 , and the negative LR <0.2 . Tests with LRs close to 1 have no diagnostic value (McGee, 2002). The LRs of salivary IgA and IgM were 0.4–3.3 and 0.33–1.29, respectively. Thus, while a positive salivary test result increased the chance of a correct diagnosis by 20%, a negative result decreased it by approximately the same amount. Since serum IgE testing has a LR close to 1, it has no diagnostic value.

One limitation of our study is the comparatively small number of positive re-challenges performed in tested dogs. All dogs with clinical signs of AFR in our study were client-owned animals, and it is difficult to obtain optimal compliance with numerous sequential re-challenges in daily practice. In published studies, the average number of re-challenges was between 1 and 9 (Mueller et al., 2016) and the mean number of suspected allergens per dog was 2.4 (Jeffers et al., 1996). In our study, feeding an individual allergen led to allergic deterioration 19 times. Although this is not sufficient to evaluate the reliability of the tests with specific food antigens, these results suggest that re-challenge tests have low accuracy, making them unsuitable for the diagnosis of adverse food reactions in dogs.

Conclusions

This study demonstrated that positive serum and saliva test results could not be used to reliably confirm adverse food reaction in dogs. Negative results did not reliably rule out a reaction to the allergen in question. The large number of positive reactions on salivary testing in the group of healthy dogs without any clinical signs of AFR and a similar number of positive reactions in normal dogs and dogs with confirmed adverse reactions to food in this study provides evidence that until further data is obtained, these tests cannot be recommended for the diagnosis of cutaneous adverse food reactions. Elimination diets remain the reference standard in the diagnostic work-up of food-related allergic disease.

Conflict of interest

RM has consulted and lectured for Royal Canin and has received funding for research studies from Royal Canin amongst others. AW is an employee of Royal Canin and WJD is the owner of Hemopet Nutriscan. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.tvjl.2018.12.014>.

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1 **Supplementary Table 1**

2 Results of testing for salivary food antigen-specific IgA in dogs with known adverse food
3 reaction to individual allergens on a restricted diet for > 8 months.

4

	Re-challenge positive	Re-challenge negative
Saliva positive	2	5
Saliva negative	2	1
Saliva weakly reactive	6	5

5

6

7 **Supplementary Table 2**

8 Results of testing for salivary food antigen-specific IgA in allergic dogs sampled at the start of an
9 elimination diet.

10

	Re-challenge positive	Re-challenge negative
Saliva positive	1	6
Saliva negative	4	7
Saliva weakly reactive	2	4

11

12

13 **Supplementary Table 3**

14 Results of testing for salivary food antigen-specific IgA in healthy research dogs.

15

	Re-challenge positive	Re-challenge negative
Saliva positive	0	16
Saliva negative	0	22
Saliva weakly reactive	0	44

16

17

Supplementary Table 4

Results of testing for salivary food antigen-specific IgM in dogs with known adverse food reaction to individual allergens on a restricted diet for >8 months.

	Re-challenge positive	Re-challenge negative
Saliva positive	3	1
Saliva negative	2	1
Saliva weakly reactive	5	9

24 **Supplementary Table 5**

25 Results of testing for salivary food antigen-specific IgM in dogs sampled at the start of an
26 elimination diet.

27

	Re-challenge positive	Re-challenge negative
Saliva positive	0	1
Saliva negative	5	7
Saliva weakly reactive	2	9

28

29

Supplementary Table 6

Results of testing for salivary food antigen-specific IgM in healthy research dogs.

	Re-challenge positive	Re-challenge negative
Saliva positive	0	25
Saliva negative	0	13
Saliva weakly reactive	0	44

Supplementary Table 7

Specificity, sensitivity, positive and negative predictability and positive and negative likelihood ratio of dogs tested for food antigen-specific salivary IgA with results in weak questionable zone considered negative, positive or not considered at all.

			Spec	Sens	PP	NP	PLR	NLR
Sal IgA	Group I	+/- = +	9,1 %	80 %	44,4 %	33,3 %	0,88	2,20
		+/- = -	54,5 %	20 %	28,6 %	42,9 %	0,44	1,47
		No +/-	16,7 %	50 %	28,6 %	33,3 %	0,60	2,99
	Group II	+/- = +	41,2 %	42,9 %	23,1 %	63,6 %	0,73	1,39
		+/- = -	64,7 %	14,3 %	14,3 %	64,7 %	0,41	1,32
		No +/-	53,8 %	20 %	14,3 %	63,6 %	0,43	1,49
	Group III	+/- = +	26,8 %	N/A	0 %	100 %	N/A	N/A
		+/- = -	80,5 %	N/A	0 %	100 %	N/A	N/A

	No +/-		N/A	0 %		N/A	N/A
		57,9 %			100 %		
All dogs	+/- = +	27,3 %	64,7 %	12,1 %	83,3 %	0,89	1,29
	+/- = -	75,5 %	17,6 %	10 %	85,6 %	0,72	1,09
	No +/-	52,6 %	33,3 %	10 %	83,3 %	0,70	1,27

40

41 Spec = specificity, Sens = sensitivity, PP = positive predictability, NP = negative predictability,

42 PLR = positive likelihood ratio and NLR = negative likelihood ratio

43

44 **Supplementary Table 8**

45 Specificity, sensitivity, positive and negative predictability and positive and negative likelihood
 46 ratio of dogs tested for food antigen-specific salivary IgM with results in weak questionable zone
 47 considered negative, positive or not considered at all.

48

			Spec	Sens	PP	NP	PLR	NLR
Sal IgM	Group I	+/- = +	9,1 %	80 %	44,4 %	33,3 %	0,88	2,20
		+/- = -	90,9 %	30 %	75,0 %	58,8 %	3,30	0,77
		No +/-	50 %	60 %	75 %	33,3 %	1,20	0,80
	Group II	+/- = +	41,2 %	28,6 %	16,7 %	58,3 %	0,49	1,73
		+/- = -	94,1 %			69,6 %		1,06
				0 %	0 %		0 %	
		No +/-	87,5 %			58,3 %		1,14
				0 %	0 %		0 %	
	Group III	+/- = +	15,8 %	N/A		100 %	N/A	N/A
					0 %			
		+/- = -	69,5 %	N/A		100 %	N/A	N/A
					0 %			

	No +/-	34,2 %	N/A		100 %	N/A	N/A
				0 %			
All dogs	+/- = +	19,1 %	58,8 %	10,1 %	75 %	0,73	2,16
	+/- = -	75,5 %	17,6 %	10 %	85,6 %	0,72	1,09
	No +/-	43,8 %	30 %	10 %	75 %	0,53	1,60

49

50 Spec = specificity, Sens = sensitivity, PP = positive predictability, NP = negative predictability,

51 PLR = positive likelihood ratio and NLR = negative likelihood ratio

52

Supplementary Table 9

Results of testing for serum food antigen-specific IgE in dogs with known adverse food reaction to individual allergens on a restricted diet for >8 months.

	Re-challenge positive	Re-challenge negative
Serum positive	1	3
Serum negative	9	7

Supplementary Table 10

Results of testing for serum food antigen-specific IgE in healthy research dogs.

	Re-challenge positive	Re-challenge negative
Serum positive	0	20
Serum negative	0	60



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Corrigendum

Corrigendum to: “Testing for food-specific antibodies in saliva and blood of food allergic and healthy dogs” [Vet. J. 245 (2019) 1–6]

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During the revision of the above paper, one of the authors requested that the findings of the following article be discussed in the manuscript text:

- Dodds, W.J. 2017. Diagnosis of Canine Food Sensitivity and Intolerance Using Saliva: Report of Outcomes. *Journal of the American Holistic Veterinary Medical Association* 49, 32–43.
- https://www.nutriscan.org/wp-content/uploads/documents/DODDS-_1.PDF.

The purpose of this corrigendum is to inform readers.

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