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# Combined prick and patch tests for diagnosis of food hypersensitivity in dogs with chronic pruritus

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**Background –** Previous studies have shown that patch testing with food extracts can assist formulation of elimination diets (ED) in human patients with suspected adverse food reactions (AFR). Little is known about the use of these tests in dogs.

**Objectives –** To evaluate the effectiveness of a combination of prick and patch testing in current protocols, and food challenge (FC) tests in dogs with AFR.

**Methods and materials –** Prick and patch tests were performed on 21 dogs with chronic, nonseasonal pruritus. Dogs then were fed an ED formulated on the basis of the results. All dogs with improved clinical signs then were challenged with a food to which there had been a positive reaction in the tests. Six dogs subsequently were challenged with a food to which they had been negative on testing. Pruritus Visual Analog Scale (pVAS) and Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) were evaluated on Day (D)0, D30 and D60 of the ED. Sensitivity (SE), specificity (SP), positive (PPV) and negative (NPV) predictive values, and the Kappa (κ)-value were calculated.

**Results –** Of the 21 dogs, there was a significant mean improvement in pVAS and CADESI-04 scores in 16 (76%) dogs after D30 ($P < 0.01$) and D60 ($P < 0.01$) of the ED. There were no statistical differences between D30 and D60. The combination of tests had SE, SP, PPV, NPV and κ values of 80%, 66.7%, 66.7%, 80% and –0.17, respectively.

**Conclusions and clinical relevance –** The combination of prick and patch testing reached high values of SE and NPV. A diagnosis of AFR was made in 76% of the dogs, and test results were useful for the selection of an ED.

## Introduction

Adverse food reaction (AFR) in dogs includes allergy (or hypersensitivity) and food intolerance. Food allergy is described as an adverse health effect, resulting from a specific immune response, which occurs after exposure to a component of the food. It can be immunoglobulin (Ig) E-mediated, cell-mediated or a combination of both.[2,3]

In dogs presenting with chronic and nonseasonal pruritus, AFR should be considered as a differential diagnosis, whether or not gastrointestinal (GI) signs are present. GI signs may include diarrhoea, vomiting, tenesmus, flatulence, distension, visceral colic and increased intestinal peristalsis.[4-8]

An AFR is suspected when complete resolution or reduction of clinical signs occurs after feeding of a home-prepared or commercial elimination diet (ED), containing novel or hydrolysed proteins. The average recommended trial period is eight weeks, although in some cases feeding of an ED for ≤13 weeks may be required.[4,9] In dogs with an AFR, clinical signs will recur after food challenge (FC) with the previous diet. In some dogs, elimination of more than one dietary component may be necessary to obtain better control of clinical signs.[4]

To date, other than an ED trial, there is no effective test for diagnoses of AFR.[2,10] In people, serological tests that measure total and specific IgE (sIgE), and the skin prick test for immediate hypersensitivity, may indicate sensitisation to a food, yet do not confirm the diagnosis of food allergy.[11,12] Patch tests for use in dogs with chronic pruritus have been developed recently.[2,10] These tests have been shown to have a strong negative predictive value – thus, foods to which the patient does not react on the test are probably appropriate for inclusion in an ED.

There are no previous reports in the veterinary literature on the correlation between the prick and patch tests in the evaluation of AFR. Thus, the objective of the present study was to evaluate the sensitivity (SE), specificity (SP), and negative and positive predictive values (NPV, PPV) of a combination of prick and patch tests in the diagnosis of AFR in dogs with chronic and nonseasonal pruritus.

## Methods and materials

### Animals

The study was approved by the Animal Use Ethics Committee of the Pontifícia Universidade Católica do Paraná –

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**Conflicts of Interest:** None declared.

PUCPR (protocol no. 01252). Thirty-eight dogs, representing a variety of breeds and ages, and both sexes were selected. All had chronic, primary and nonseasonal pruritus, predominantly in the interdigital, abdominal, axillary, inguinal, perioral or perianal regions. Secondary bacterial or *Malassezia* spp. infections were treated before inclusion in the study and parasitic conditions were ruled out by direct inspection and skin scrapings. Mite and flea preventatives were administered at least three weeks before the start of the study and maintained with formulas for oral (not flavoured) or topical use according to the manufacturers' indications throughout the study.[2]

For inclusion, dogs were required to test positive to at least one food using at least one of the two tests (prick or patch). Antihistamines and topical glucocorticoids were withheld for two weeks and systemic glucocorticoids for four weeks before testing was performed.[2] Tests were performed on the same day, on contralateral sides of the thoracic region.

### Prick test

The lateral thoracic region was clipped and 2.0 cm equidistant points marked on the skin with a dermographic pen.[13] Saline and histamine-based solutions (10 mg/mL) were used as negative and positive controls, respectively. The food extracts tested were from uncooked beef, chicken, fish, pork, egg, milk, soy and wheat proteins, in addition to rice, potato and yucca, in concentrations of 1:20 w/v (weight/volume). All extracts and controls used were prepared by the Veterinary Allergenics Laboratory (Rio de Janeiro, Brazil), in a solution of 50% glycerol and 0.45% phenol, and were subjected to standardisation and potency analyses.

A double-tipped prick test instrument, Duotip-Test (Lincoln Diagnostics; Decatur, IL, USA) was used to perform the test. The tip of the instrument holds a drop of extract by capillary action, which then is placed against the skin at a 45°–60° angle as pressure is applied, in order to puncture the skin and deliver the drop of extract percutaneously.[14] Test sites were evaluated for reactivity 15 min after application. Erythematous wheals were demarcated and measured with a calliper (using the mean of the largest and its perpendicular diameter). The reaction was considered positive if the mean diameter was 3 mm greater than the negative control (Figure 1).[13,15]

### Patch test

The extracts for patch testing were made from raw beef, pork, chicken, whitefish, fresh milk, whole chicken egg (white and yolk), fresh soy, wheat flour, rice flour, cooked potato and cooked yucca. The allergens were weighed (approximately 500 mg) and homogenised with 0.2 mL petrolatum.[2] For the contact testing, an area measuring approximately 20 × 10 cm was clipped in the lateral thoracic region and cleansed with saline solution. All extracts (and petrolatum as a negative control) then were placed into 8 mm plastic chambers (Alergochambers, Neoflex; São Paulo, Brazil) and adhered to the test area with hypoallergenic adhesive tape strips. To minimise the risk of chamber movement, a bandage was placed over the tape and dogs were dressed in a surgical recovery suit. After 48 h the adhesive tape was removed and positive reactions defined by the presence of erythema, papules, erythematous plaques and microvesicles, as established previously (Figure 2).[2]

### CADESI-04 and pVAS

Skin lesions were evaluated using the Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) score,[16] and the intensity of pruritus scored using a pruritus Visual Analog Scale (pVAS).[17] The evaluations were carried out before instituting the elimination diet on Day (D)0, and on D30 and D60 of the ED trial. Dogs with severe pruritus were treated either with a glucocorticosteroid (prednisone, 0.5 mg/kg once daily) or oclacitinib (Apoquel, Zoetis; São Paulo, Brazil), at a dose of 0.4–0.6 mg/kg once daily. Antipruritic therapy was suspended at least seven days before each reassessment (e.g. D23

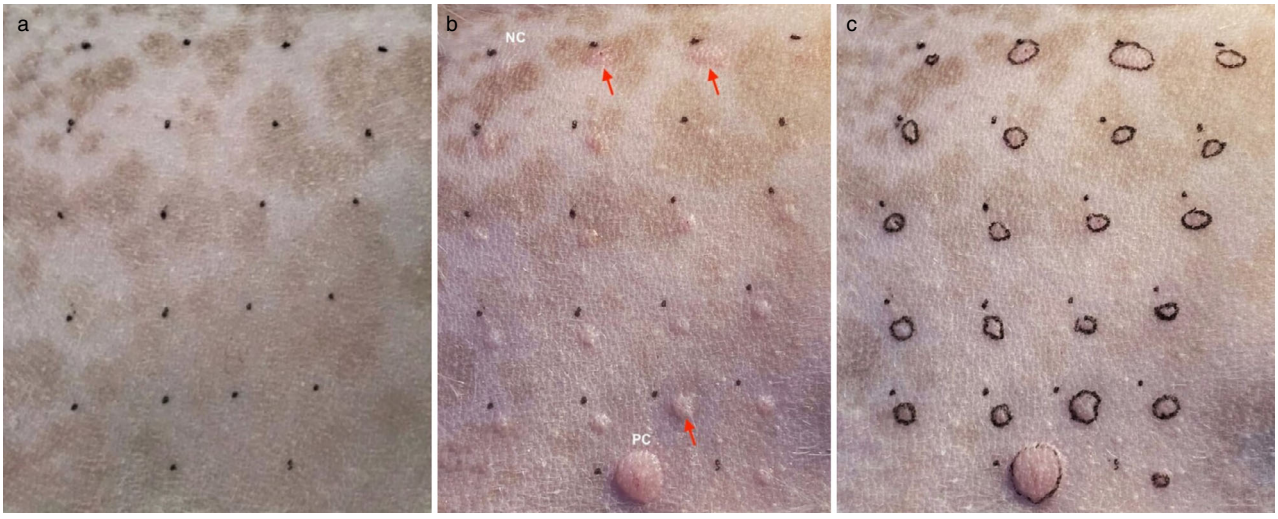


**Figure 1.** Prick test performed on a dog with adverse food reaction.
(a) Lateral thoracic region showing the skin markings made with a dermographic pen immediately after extract application. (b) Reactions 15 min after application, arrows indicate the positive reactions. (c) Demarcation of reactions 15 min after application for measurement of wheal diameter. PC, positive control; NC, negative control.

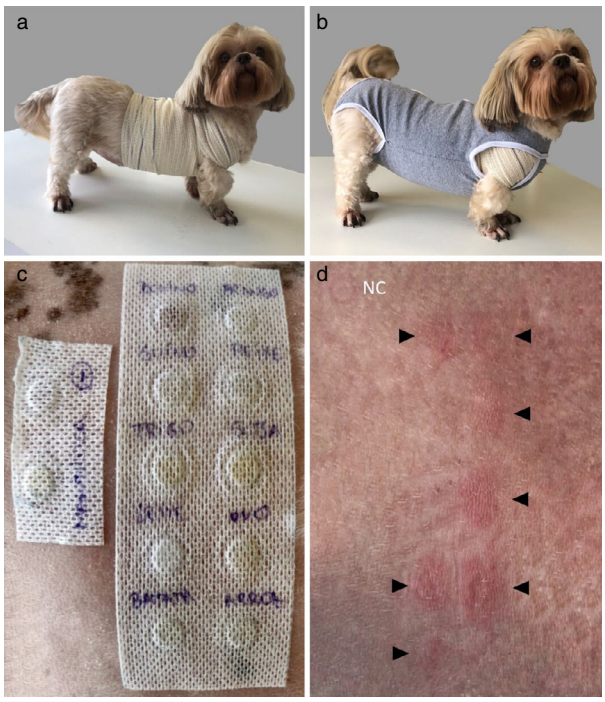


**Figure 2.** Patch test with food allergens performed on a dog with adverse food reaction.
(a) After the adhesive tapes were fixed, a bandage was wrapped around the dog. (b) Dog dressed in surgical recovery suit. (c). Hypoallergenic adhesive tapes (Alergochambers) with multiple food extracts. (d) After 48 h the adhesive was removed and the positive reactions were recorded. The arrows indicate erythematous plaques considered to be positive reactions. NC, negative control.

and D53), to allow measurement of CADESI-04 and pVAS scores. In dogs where pruritus showed significant improvement on D30, antipruritic therapy was discontinued.

### Elimination diet

A homemade elimination diet was formulated (50 g/kg),[18] composed of a protein and a carbohydrate source that had not caused a reaction in either of the tests. The diet was started immediately after testing and maintained exclusively for 60 days.[4] Dogs with a reduction in the CADESI-04 scale (value < 10 points) and at least three points on the pVAS scale, underwent FC. To avoid bias in the interpretation of the pVAS scale during the FC process, the owners were blinded to the results of the tests.

In order to perform the FC, a food which caused reactions (positive food challenge, PFC) in both prick and patch tests was selected. In cases where this was not possible, the food producing the most severe reaction in the patch test was selected. When there was no reaction elicited by patch testing, the food with the most severe reaction elicited by prick testing was used. Each FC was continued for up to 14 days, and recurrence of clinical signs confirmed the AFR diagnosis. A single FC was performed in 20 dogs, and one dog was challenged with two foods (chicken and egg) for a total of 22 FC.

After the initial FC, the dogs were fed the ED again and clinical signs were treated as needed with prednisone (0.5 mg/kg once daily) or oclacitinib (0.4–0.6 mg/kg once daily). Once clinical signs were controlled, and seven days after withdrawal of antipruritic drugs, six owners agreed to an additional FC using a food to which their dog had not reacted on either of the tests (negative food challenge, NFC).

### Statistical analysis

Demographic data – which included breed, age, sex, comorbidities, and age at onset of clinical signs – are presented as proportions or medians. Further descriptive data such as reactions to foods upon prick and patch tests also are described as proportions. CADESI-04 and pVAS scores obtained before the ED, and at D30 and D60 are presented as mean, standard deviation, maximum and minimum values. The scores at each time point were compared using repeated measures ANOVA.[19]

Performance of prick and patch screening tests were evaluated for SE, SP, PPV and NPV as compared to the gold standard of a positive FC, using 2 x 2 tables.[20] The agreement between FC and prick or patch test results, individually or in combination, was analysed using Cohen's Kappa ($\kappa$) test. Results were evaluated based on Landis and Koch criteria.[21]

All analyses were conducted using STATA 14 software (College Station, TX, USA) and the threshold for statistical significance of all comparisons set at $P < 0.05$.

## Results

### Animals

Of the 38 dogs, 10 (26.3%) were withdrawn from the study by their owners. Another four (10.5%) dogs did not complete the patch test, and three dogs (7.8%) developed vomiting and/or diarrhoea during ED. Thus, 21 dogs completed the study. Of these 21 dogs, 14 (66.8%) were male and seven (33.3%) were female. The median age of participants was five years, with a minimum age of one year and a maximum of 10 years. Prevalence data for breed, sex, age and clinical signs are shown in Table 1.

### Cutaneous tests

Four dogs (19%) reacted to food extracts only upon prick testing, while another four reacted only to patch tests. However, the majority of dogs (61.90%) reacted to extracts used in both tests, and to different foods. The analysis of agreement between tests showed an index of 38.45%, with $\kappa = -0.17$, which defines a lack of correlation ($\kappa$ 95% CI = –0.51–0.18; $P = 0.84$; Table S1).

Positive prick test reactions most commonly occurred to pork protein, followed by egg, soy, fish, beef, wheat, chicken and milk. There were fewer reactions to carbohydrates (most commonly to rice, followed by potato). There were no reactions to cassava (Table 2).

Positive patch test reactions most commonly occurred to chicken, followed by soy, fish, beef, milk, pork, egg and wheat. The carbohydrate associated with the most reactivity was potato. Rice and cassava provoked the same number of reactions (Table 2).

The values of SE, SP, PPV and NPV for prick and patch tests, individually and combined, are shown in Table 3. Using the PFC and NFC results, absolute numbers of false positive, false negative, true positive and true

**Table 1.** Breed, age, clinical signs and comorbidities of dogs with adverse food reaction (AFR) and non-AFR

| | AFR | Non-AFR | Total |
|---|---|---|---|
| Number of dogs | 16 (76.1%) | 5 (23.8%) | 21 |
| Breed | | | |
| French bulldog | 3 (14.3%) | 1 (4.8%) | 4 (19.0%) |
| Lhasa apso | 5 (23.8%) | 0 | 5 (23.8%) |
| Shih tzu | 2 (9.5%) | 2 (9.5%) | 4 (19.0%) |
| Mixed breed | 2 (9.5%) | 0 | 2 (9.5%) |
| Yorkshire terrier | 2 (9.5%) | 1 (4.8%) | 3 (14.3%) |
| Labrador retriever | 0 | 1 (4.8%) | 1 (4.8%) |
| Maltese terrier | 1 (4.8%) | 0 | 1 (4.8%) |
| Pug | 1 (4.8%) | 0 | 1 (4.8%) |
| Age at onset of clinical signs (years) | | | |
| <1 | 5 (23.8%) | 4 (19.0%) | 9 (42.8%) |
| 1 to 3 | 6 (28.6%) | 1 (4.8%) | 7 (33.3%) |
| 4 to 10 | 5 (23.8%) | 0 | 5 (23.8%) |
| Pruritus | | | |
| Perioral | 11 (52.4%) | 4 (19.0%) | 15 (71.4) |
| Perianal | 4 (19.0%) | 0 | 4 (19.0%) |
| Periocular | 1 (4.8%) | 1 (4.8%) | 2 (9.5%) |
| Interdigital | 16 (76.1%) | 5 (23.8%) | 21 (100%) |
| Axilla | 6 (28.6%) | 0 | 6 (28.6%) |
| Inguinal / abdomen | 7 (33.3%) | 2 (9.5%) | 9 (42.8%) |
| Antecubital flexure | 3 (14.3%) | 2 (9.5%) | 5 (23.8%) |
| Gastrointestinal signs | | | |
| Vomiting | 4 (19.0%) | 2 (9.5%) | 6 (28.6%) |
| Diarrhoea | 5 (23.8%) | 1 (4.8%) | 6 (28.6%) |
| Flatulence | 2 (9.5%) | 1 (4.8%) | 3 (14.3%) |
| Chronic otitis externa | 10 (47.6%) | 4 (19.0%) | 14 (66.6%) |
| Bacterial infection | 8 (38.0%) | 3 (14.3%) | 11 (52.3%) |
| *Malassezia* spp. infection | 2 (9.5%) | 0 | 2 (9.5%) |

**Table 2.** Demonstration of the number of reactions to the different foods tested in prick and patch test in dogs with adverse food reactions

| | Prick test | Patch test |
|---|---|---|
| Chicken | 23.8% | 57.1% |
| Soy | 28.6% | 52.4% |
| Beef | 28.6% | 47.6% |
| Fish | 28.6% | 47.6% |
| Milk | 23.8% | 42.8% |
| Pork | 38.0% | 38.0% |
| Egg | 33.3% | 23.8% |
| Wheat | 28.6% | 4.8% |
| Potato | 4.8% | 38.0% |
| Yucca | 0 | 4.8% |
| Rice | 14.3% | 4.8% |

negative protein reactions were calculated, and are detailed in Tables 4 and 5.

### Assessment of response to diet based on allergic tests

Comparing the pVAS values between D0 and D60, there was clinical improvement in 16 (76%) of the 21 dogs that completed the study. All 16 of these dogs also had positive FC defined as recurrence of pruritus following food exposure. As defined by CADESI-04 score values, clinical improvement occurred in 16 (76%) dogs. All 16 dogs responded to FC with pruritus, and only 13 developed signs that led to an increased CADESI-04 score before intervention was provided. Of the 16 dogs confirmed with AFR, five (31.25%) required use of oclacitinib at the beginning of the ED, and four still needed it after 30 days. However, none of these dogs required oclacitinib for pruritus control after 60 days on ED. Of the five dogs that did not improve with ED, antipruritic therapy was required throughout the study by four of them, with one needing prednisone to control clinical signs.

**Table 4.** Summaries of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) reactions to food allergens used for prick testing of dogs with adverse food reactions (AFR)

| Allergen | TP | FP | TN | FN | Food challenge |
|---|---|---|---|---|---|
| Protein (dogs with AFR) | 9 | 2 | 6 | 11 | 28 |
| Beef | 2 | 0 | 3 | 0 | 5 |
| Chicken | 3 | 1 | 2 | 5 | 11 |
| Egg | 1 | 0 | 0 | 1 | 2 |
| Fish | 2 | 0 | 0 | 3 | 5 |
| Soy | 0 | 0 | 1 | 0 | 1 |
| Pork | 1 | 1 | 0 | 2 | 4 |

**Table 5.** Summaries of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) reactions to food allergens used for patch testing of dogs with adverse food reactions (AFR)

| Allergen | TP | FP | TN | FN | Food challenge |
|---|---|---|---|---|---|
| Protein (dogs with AFR) | 14 | 4 | 4 | 6 | 28 |
| Beef | 0 | 1 | 2 | 2 | 5 |
| Chicken | 6 | 2 | 1 | 2 | 11 |
| Egg | 1 | 0 | 0 | 1 | 2 |
| Fish | 4 | 0 | 0 | 1 | 5 |
| Soy | 0 | 0 | 1 | 0 | 1 |
| Pork | 3 | 1 | 0 | 0 | 4 |

### Pruritus visual scale (pVAS) and CADESI-04

The means and standard deviations for pVAS and CADESI-04 on D0, D30 and D60 are reported in Table 6. The single-factor ANOVA applied to the 16 dogs with confirmed AFR showed that there was a significant decrease in the mean pVAS ($P < 0.01$) and CADESI-04 ($P < 0.01$) at D30, D60 and D75 (Figure 3). At D30, pVAS had decreased by 3 points in 14 dogs and CADESI-04 had decreased to <10 in 10 dogs.

## Discussion

In this study 76% of the dogs with nonseasonal pruritus improved during feeding of an ED, demonstrating that AFR is an important cause of chronic pruritus in dogs. AFR can be classified as an IgE-mediated (Th2) or cell-

**Table 3.** Sensitivity (SE), specificity (SP), positive (PPV) and negative (NPV) predictive values for prick and patch tests for food allergens

| | SE (95%CI) | SP (95%CI) | PPV (95%CI) | NPV (95%CI) |
|---|---|---|---|---|
| Prick test | 45 (23.1–68.5) | 75 (34.9–96.8) | 81.8 (48.2–97.7) | 35.2 (14.2–61.7) |
| Patch test | 70 (45.7–88.1) | 50 (15.7–84.3) | 77.8 (52.4–93.6) | 40 (12.2–73.8) |
| Prick / patch tests combined | 80 (28.4–99.5) | 66.7 (22.3–95.7) | 66.7 (22.3–95.7) | 80 (28.4–99.5) |

**Table 6.** Means of pruritus Visual Analog Scale (pVAS) and Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) scores at each evaluation time in dogs with (AFR) and without (non-AFR) adverse food reactions

| | pVAS | | | | CADESI-04 | | | |
|---|---|---|---|---|---|---|---|---|
| Study day (D) | Mean | SD | Min | Max | Mean | SD | Min | Max |
| AFR (N = 17) | | | | | | | | |
| D0 | 7.82 | 1.67 | 4 | 10 | 16.71 | 13.57 | 4 | 63 |
| D30 | 3.65 | 1.62 | 2 | 6 | 8.23 | 5.37 | 0 | 18 |
| D60 | 2.47 | 1.18 | 1 | 5 | 5.47 | 4.10 | 0 | 14 |
| D75 | 6.11 | 1.99 | 3 | 9 | 14.17 | 12.84 | 0 | 59 |
| Non-AFR (N = 3) | | | | | | | | |
| D0 | 6.67 | 1.15 | 6 | 8 | 13.33 | 10.97 | 7 | 26 |
| D30 | 6 | 3.46 | 2 | 8 | 6.33 | 5.69 | 0 | 11 |
| D60 | 6.67 | 1.53 | 5 | 8 | 6 | 2 | 4 | 8 |

Max, maximum; Min, minimum; SD, standard deviation.

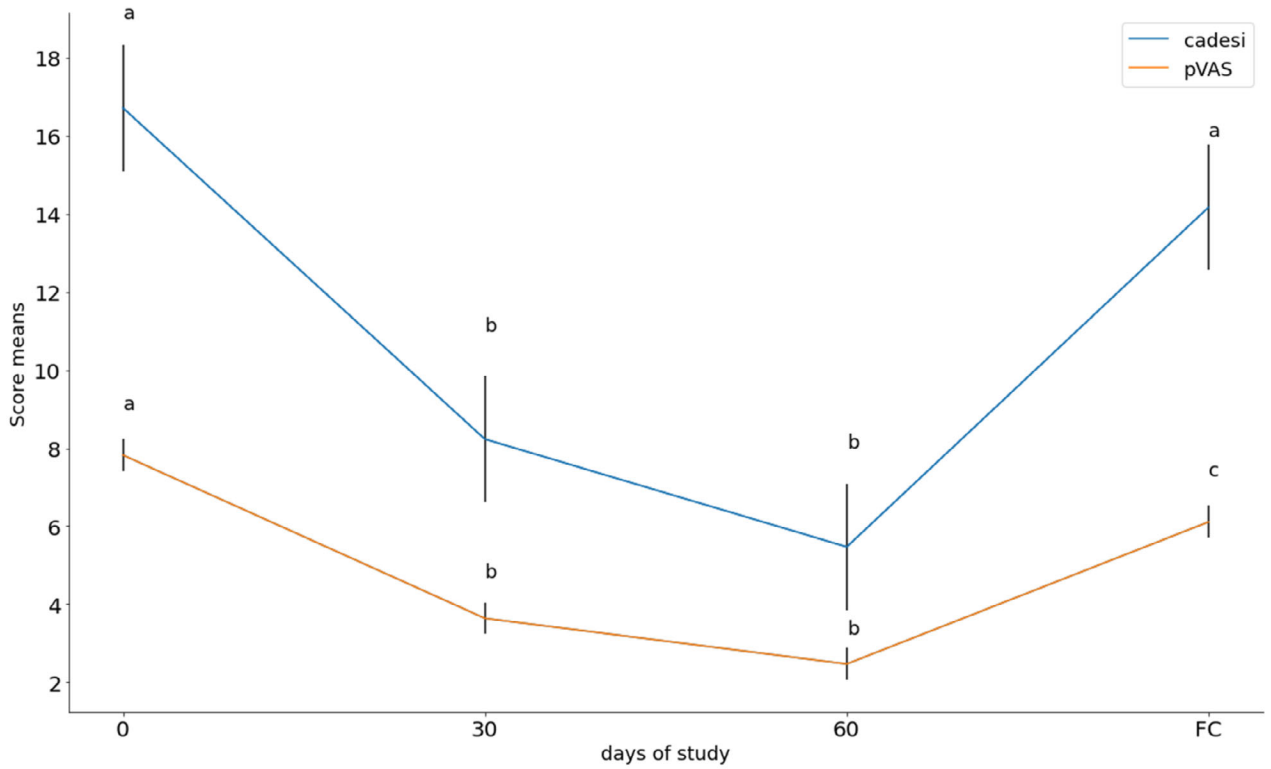


**Figure 3.** Means of Pruritus Visual Analog Scale (pVAS) and Canine Atopic Dermatitis Extent and Severity Index, 4th iteration (CADESI-04) scores at baseline [Day (D)0], during an elimination diet (D30 and D60) and after food challenge, in dogs with adverse food reactions.
*Different letters represent a statistically significant difference in the results ($P < 0.01$).
Bars show confidence intervals.

mediated (Th1) disorder. However, in human patients with AD a mixed immune response is common.[12] In cases of suspected IgE-mediated reaction in man, the prick test can be performed to detect sensitisation to food.[12]

In our study, the prick test was positive in 81% of the dogs, with a specificity of 75% and a positive predictive value of 80%. This makes it a useful tool in the presumptive diagnosis of AFR in dogs with chronic and nonseasonal pruritus, particularly because the test is easy to perform in an outpatient setting. In addition, these findings underline the importance of the IgE-mediated immunoallergic response to food components in dogs with AFR. In addition, the patch test also was positive in 81% of the dogs and had a PPV of 77.8%. Thus, the patch test can assist in the presumptive diagnosis of delayed reactions to food, which usually are cell-mediated. A delayed reaction may be partially responsible for the chronic pruritus noted in these animals and, possibly, for the development of erythroderma, tendency to dyskeratosis, and cutaneous lichenification.[22] However, while the patch test showed 70% sensitivity, it was only 50% specific, suggesting that test extracts may induce irritant contact dermatitis resulting in false positive results. The results of the Kappa index indicate that there is a low level of agreement between these tests. This probably occurs because the tests identify different mechanisms of an allergic reaction (immediate versus

delayed hypersensitivity). Thus, results of these tests may be complementary, and if all positive responses are considered, the sensitivity and negative predictive values increase to 80%. This information may assist in the selection of foods for use in an ED and for challenging dogs that improve on the ED.

The prevalence of AFR varies between 3% and 34% in people,[23] and has been reported to range from 10% to 20% in dogs with chronic pruritus.[24] These data vary according to the population studied and the criteria evaluated. Studies evaluating the improvement of chronic pruritus in dogs with atopic dermatitis, with the use of an ED and challenge, diagnosed AFR in 30–62% of cases.[8,25,26] In this study we demonstrated a prevalence of 76% in dogs with nonseasonal pruritus. Thus, AFR may be underestimated as a cause of chronic pruritus, and when EDs are formulated based on tests of allergenicity – rather than patient history – AFR may be identified more frequently. Pre-diet testing may allow a more appropriate ED choice owing to the fact that the owner often is unaware of the animal's previous feeding history. This can make it difficult to select diets with proteins that are truly novel to the pet, based on history alone. Furthermore, there can be cross-reactivity between foods which cannot be predicted by diets based on history alone.

Pork and chicken protein produced the most reactions in the prick and patch tests, respectively, with 59% of dogs with AFR reacting to chicken. This is in contrast to the study by Roudebush,[27] in which only 15% of dogs were reactive to chicken. Chicken and pork are widely used as protein sources in dog food in Brazil, and continuous and early exposure to these foods may favour sensitisation and the development of AFR.

Currently it is recommended that an ED be fed for at least five weeks, and up to 13 weeks, to identify the greatest number of dogs with AFR.[9] Although we found no statistically significant differences between pVAS and CADESI-04 scores assessed on D60 compared to D30, we did note that extension of the ED until D60 resulted in a greater overall proportion of dogs (76%) showing improvement in both scales, compared to baseline. At D30, only 66.7% of dogs had significantly improved pVAS and 47.6% had significantly improved CADESI-04 scores compared to baseline. Therefore, we recommend that an ED ideally should be continued for at least eight weeks to identify as many patients with AFR as possible.

In conclusion, this study demonstrates that AFR is an important cause of chronic/nonseasonal pruritus in dogs. The prick and patch tests are useful tools to aid in the selection of foods for an ED and subsequent FC, allowing the more accurate diagnosis of AFR. The low level of agreement between the tests is probably reflective of the allergic mechanisms that they assess, and shows the importance of carrying out the tests together for the elaboration of an ED.

## Acknowledgements

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## AUTHOR CONTRIBUTIONS

**Juliane Possebom:** Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; writing – original draft. **Ariane Cruz:** Data curation; investigation. **Vanessa Cunningham Gmyterco:** Writing – review and editing. **Marconi Farias:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing – review and editing.

## References

1. Burks AW, Tang M, Sicherer S et al. ICON: food allergy. *J Allergy Clinical Immunol* 2012; 129: 906–920.
2. Johansen C, Mariani C, Mueller RS. Evaluation of canine adverse food reactions by patch testing with single proteins, single carbohydrates and commercial foods. *Vet Dermatol* 2017; 28: 473-e109.
3. Boyce JA, Assa'ad A, Burks AW et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *J Allergy Clin Immunol* 2010; 126: S1–S58.
4. Hensel P, Santoro D, Favrot C et al. Canine atopic dermatitis: Detailed guidelines for diagnosis and allergen identification. *BMC Vet Res* 2015; 11: 196.
5. Nuttall TJ, Marsella R, Rosenbaum MR et al. Update on pathogenesis, diagnosis, and treatment of atopic dermatitis in dogs. *J Am Vet Med Assoc* 2019; 254: 1,291–1,300.
6. Picco F, Zini E, Nett C et al. A prospective study on canine atopic dermatitis and food-induced allergic dermatitis in Switzerland. *Vet Dermatol* 2008; 19: 150–151.
7. Favrot C, Steffan J, Seewald W. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet Dermatol* 2010; 21: 23–31.
8. Proverbio D, Perego R, Spada E et al. Prevalence of adverse food reactions in 130 dogs in Italy with dermatological signs: a retrospective study. *J Small Anim Pract* 2010; 51: 370–374.
9. Olivry T, Mueller RS, Prélaud P. Critically appraised topic on adverse food reactions of companion animals (1): Duration of elimination diets. *BMC Vet Res* 2015; 11: 225.
10. Bethlehem S, Bexley J, Mueller RS. Patch testing and allergen-specific serum IgE and IgG antibodies in the diagnosis of canine adverse food reactions. *Vet Immunol Immunopathol* 2012; 145: 582–589.
11. Lieberman JA, Sicherer SH. Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge. *Curr Allergy Asthma Rep* 2011; 11: 58–64.
12. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol* 2016; 16: 751–765.
13. Bousquet J, Heinzerling L, Bachert C et al. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy* 2012; 67: 18–24.
14. Carnett MJH, Plant JD. Percutaneous prick test irritant threshold concentrations for eight allergens in healthy nonsedated dogs in the USA. *Vet Dermatol* 2018; 29: 117-e47.
15. van der Valk JPM, Gerth van Wijk R, Hoorn E et al. Measurement and interpretation of skin prick test results. *Clin Transl Allergy* 2016; 6. https://doi.org/10.1186/s13601-016-0092-0
16. Olivry T, Saridomichelakis M, Nuttall T et al. Validation of the Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4, a simplified severity scale for assessing skin lesions of atopic dermatitis in dogs. *Vet Dermatol* 2014; 25: 77–85.
17. Rybnícek J, Lau-Gillard PJ, Harvey R et al. Further validation of a pruritus severity scale for use in dogs. *Vet Dermatol* 2009; 20: 115–122.
18. Rosser EJ. Diagnostic workup of food hypersensitivity. In: Noli C, Foster A, Rosenkrantz W, eds. *Veterinary Allergy*. 1st edition. Oxford: John Wiley & Sons, 2014; 119–123.

19. Moore DS, McCabe GP, Craig BA. Inference for proportion. In: *Introduction to the Practice of Statistics*, 7th edition. New York, NY: W.H. Freeman and Company, 2012; 473–510.
20. Dohoo I, Wayne M, Stryhn H. Chapter 5: Screening and diagnostic tests. In: *Veterinary Epidemiologic Research*, 2nd edition. Charlottetown, Canada: University of Prince Edward Island, 2010; 91–127.
21. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 1977; 33: 363–374.
22. Edwards KP, Martinez BA. Atopy patch testing for foods: A review of the literature. *Allergy Asthma Proc* 2014; 35: 435–443.
23. Madsen C, Prevalence of food allergy: an overview. *Proc Nutr Soc* 2005; 64: 413–417.
24. Chesney CJ. Food sensitivity in the dog: a quantitative study. *J Small Animl Pract* 2002; 43: 203–207.
25. Biourge VC, Fontaine J, Vroom MW. Diagnosis of adverse reaction to food in dogs: efficacy of a soy-isolated hydrolyzate-based diet. *J Nutr* 2004; 134: 2,062S–2,064S.
26. Vandresen G, de Farias MR. Efficacy of hydrolyzed soy dog food and homemade food with original protein in the control of food-induced atopic dermatitis in dogs. *Pesq Vet Bras* 2018; 38: 1389–1393.
27. Roudebush P. Ingredients and foods associated with adverse reactions in dogs and cats. *Vet Dermatol* 2013; 24: 293–294.

## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Table S1.** Results of the prick (Pr) and patch (Pa) tests for each allergen, the elimination diet (ED), the food challenge (FC) and the outcome of FC.

**Résumé**

**Contexte –** De précédentes études ont montré que les tests épicutanés avec des extraits alimentaires peuvent aider à la formulation de régimes d'éviction (DE) chez les patients humains présentant des réactions indésirables alimentaires suspectées (AFR). On sait peu de choses sur l'utilisation de ces tests chez les chiens.

**Objectifs –** Évaluer l'efficacité d'une combinaison de prick et patch tests dans les protocoles actuels et de tests de provocation alimentaire (FC) chez les chiens atteints de RFA.

**Matériels et méthodes –** Des prick-tests et des patch-tests ont été réalisés sur 21 chiens atteints de prurit chronique non saisonnier. Les chiens ont ensuite été nourris avec un ED formulé sur la base des résultats. Tous les chiens présentant des signes cliniques améliorés ont ensuite été confrontés à un aliment auquel il y avait eu une réaction positive dans les tests. Six chiens ont ensuite été confrontés à un aliment auquel ils avaient été négatifs lors des tests. L'échelle visuelle analogique du prurit (pVAS) et l'indice d'étendue et de gravité de la dermatite atopique canine, 4e itération (CADESI-04) ont été évalués aux jours (J)0, J30 et J60 de l'urgence. La sensibilité (SE), la spécificité (SP), les valeurs prédictives positives (PPV) et négatives (NPV) et la valeur Kappa (κ) ont été calculées.

**Résultats –** Sur les 21 chiens, il y avait une amélioration moyenne significative des scores pVAS et CADESI-04 chez 16 (76 %) chiens après J30 ($P < 0,01$) et J60 ($P < 0,01$) de la DE. Il n'y avait pas de différences statistiques entre J30 et J60. La combinaison de tests avait des valeurs respectives de SE, SP, VPP, VPN de 80 %, 66,7 %, 66,7 %, 80 % et –0,17.

**Conclusions et pertinence clinique –** La combinaison de prick-tests et de patch-tests a atteint des valeurs élevées de SE et de VPN. Un diagnostic d'AFR a été posé chez 76% des chiens, et les résultats des tests ont été utiles pour la sélection d'un DE.

**Resumen**

**Introducción –** estudios anteriores han demostrado que las pruebas alérgicas de parche con extractos de alimentos pueden ayudar a la formulación de dietas de eliminación (ED) en pacientes humanos con sospecha de reacciones adversas a los alimentos (AFR). Se sabe poco sobre el uso de estas pruebas en perros.

**Objetivos –** evaluar la eficacia de una combinación de pruebas de punción y parche en los protocolos actuales y pruebas de reexposición alimentaria (FC) en perros con AFR.

**Métodos y materiales –** se realizaron pruebas de punción y parche en 21 perros con prurito crónico no estacional. Luego, los perros fueron alimentados con una ED formulada en base a los resultados. Todos los perros con signos clínicos mejorados fueron reexpuestos con un alimento al que había habido una reacción positiva en las pruebas. Posteriormente, seis perros fueron reexpuestos a un alimento al que habían dado negativo en las pruebas. La escala análoga visual de prurito (pVAS) y el índice de gravedad y extensión de la dermatitis atópica canina, cuarta revisión (CADESI-04) se evaluaron en los días (D) 0, D30 y D60 de la dieta de eliminación. Se calcularon los valores predictivos de sensibilidad (SE), especificidad (SP), positivos (PPV) y negativos (NPV) y el valor de Kappa (κ).

**Resultados –** de los 21 perros, hubo una mejora media significativa en las puntuaciones de pVAS y CADESI-04 en 16 (76%) perros después de D30 ($P$ <0,01) y D60 ($P$ <0,01) de la ED. No hubo diferencias estadísticas entre D30 y D60. La combinación de pruebas tuvo valores SE, SP, PPV, NPV y κ de 80%, 66,7%, 66,7%, 80% y –0,17, respectivamente.

**Conclusiones y relevancia clínica –** La combinación de pruebas de punción y parche alcanzó valores altos de SE y NPV. Se hizo un diagnóstico de AFR en el 76% de los perros y los resultados de las pruebas fueron útiles para la selección de una ED.

**Zusammenfassung**

**Hintergrund –** Frühere Studien haben gezeigt, dass Patch Testen mit Futterextrakten bei der Formulierung von Eliminationsdiäten (ED) beim Menschen mit Verdacht auf Futtermittelnebenwirkungen (AFR) behilflich sein kann. Bisher ist bei Hunden wenig über die Verwendung dieser Tests bekannt.

**Ziele –** Eine Evaluierung der Wirksamkeit der Kombination aus Prick und Patch Test mit den derzeitigen Protokollen, sowie Futterprovokationstests (FC) bei Hunden mit AFR.

**Methoden und Materialien –** Es wurden Prick und Patch Tests an 21 Hunden mit chronischem, nicht saisonalem Juckreiz durchgeführt. Den Hunden wurde dann eine ED basierend auf diesen Ergebnissen zusammengestellt und gefüttert. Danach wurden alle Hunde, die sich klinisch besserten, mit einem Futter provoziert, auf welches eine positive Reaktion in den Tests auftrat. In der Folge wurden sechs Hunde mit einem Futter provoziert, auf welches sie im Test negativ reagiert hatten. An den Tagen (D)0, D30 und D60 der ED wurde der Juckreiz mittels Pruritus Visual Analog Scale (pVAS) und Canine Atopic Dermatitis Extent and Severity Index, 4te Auflage (CADESO-04) beurteilt. Die Sensitivität (SE), Spezifität (SP) und der negative prädiktive Wert (NPV) sowie die Kappa (ƙ) Werte wurden kalkuliert.

**Erebnisse –** Von den 21 Hunden zeigten 16 (76%) nach D30 ($P < 0,01$) und D60 ($P < 0,01$) der ED eine signifikante durchschnittliche Verbesserung im Bezug auf die pVAS und CADESI-04 Werte. Es gab keinen statistischen Unterschied zwischen D30 und D60. Die Kombination dieser Tests zeigten SE, SP, PPV, NPV und ƙ Werte von 80%; 66,7%; 66,7; 80% bzw -0,17.

**Schlussfolgerungen und klinische Bedeutung –** Die Kombination von Prick und Patch Tests erreichte hohe SE und NPV Werte. Die Diagnose einer AFR wurde bei 76% der Hunde gestellt und die Testergebnisse waren bei der Selektion einer ED hilfreich.

**要約**

**背景** – これまでの研究で、食物有害反応(AFR)が疑われるヒトの患者において、食物抽出物によるパッチテストが除去食(ED)の策定に役立つことが示されている。しかし、犬におけるこれらの試験の使用についてはほとんど知られていない。

**目的 –** 本研究の目的は、犬のAFRにおいて、現在のプロトコールにあるプリックテストおよびパッチテストの組み合わせ、および食物負荷試験(FC)の有効性を評価することであった。

**材料と方法 –** 非季節性慢性掻痒を有する犬21頭に対してプリックテストおよびパッチテストを実施した。その結果に基づいて処方された抗炎症薬を投与した。臨床症状が改善したすべての犬に、試験で陽性反応を示した食品を摂取させた。その後、6頭の犬には、試験で陰性であった食品を与えた。Pruritus Visual Analog Scale(pVAS)およびCanine Atopic Dermatitis Extent and Severity Index, 4th iteration(CADESI-04)は、除去食試験の0日(D)、D30およびD60に評価した。感度(SE)，特異度(SP)，陽性的中率(PPV)，陰性的中率(NPV)およびKappa(κ)値を算出した.

**結果 –** 21頭中，16頭(76%)で除去食試験のD30(P<0.01)およびD60(P<0.01)後にpVASおよびCADESI-04スコアに有意な平均改善がみられた。D30とD60の間に統計的な差はなかった。検査の組み合わせは，SE，SP，PPV，NPV，κ値がそれぞれ80%，66.7%，66.7%，80%，-0.17であった.

**結論と臨床的意義 –** プリックテストおよびパッチテストの組み合わせは、SEおよびNPVが高い値に達した。76%の犬でAFRと診断され，検査結果は除去食試験の選択に有用であった.

**摘要**

**背景** – 先前的研究表明，在疑似食物副反应(AFR)的病人中，食物提取物的斑贴试验可辅助食物排查的配方(ED)。对这些试验在犬中的使用知之甚少。

**目的 –** 评价当前方案中点刺和斑贴试验以及AFR犬食物激发(FC)试验组合的有效性。

**方法和材料 –** 对21只慢性、非季节性瘙痒犬进行点刺和斑贴试验。然后给根据结果犬饲喂ED配方。然后用试验中呈阳性反应的食物对所有临床症状改善的犬进行激发。随后用检测结果为阴性的食物对6只犬进行激发试验。在ED第(D)0天、第30天和第60天评价瘙痒视觉模拟量表(pVAS)和犬特应性皮炎程度和严重指数第4版(CADESI-04)。计算敏感性(SE)、特异性(SP)、阳性(PPV)和阴性(NPV)预测值、Kappa(κ)值。

**结果 –** 在21只犬中，16只(76%)犬在ED的D30($P < 0.01$)和D60($P < 0.01$)后pVAS和CADESI-04评分有显著的平均改善。D30和D60之间无统计学差异。联合检测SE、SP、PPV、NPV和κ值分别为80%、66.7%、66.7%、80%和-0.17。

**结论和临床相关性 –** 点刺和斑贴试验组合达到了较高的SE和NPV值。76%的犬被诊断为AFR，试验结果有助于ED的选择。

**Resumo**

**Contexto –** Estudos anteriores demonstraram que o teste de contato (*patch test)* com extratos alimentares pode auxiliar na formulação de dietas de eliminação (DE) em pacientes humanos com suspeita de reações adversas a alimentos (AFR). Pouco se sabe sobre o uso desses testes em cães.

**Objetivos –** Avaliar a eficácia de uma combinação de testes de puntura (*prick test)* e *patch test* em protocolos atuais e testes de desafio alimentar (DA) em cães com AFR.

**Métodos e materiais –** Os testes *prcik test e patch test* foram realizados em 21 cães com prurido crônico não sazonal. Os cães foram então alimentados com uma DE formulada com base nos resultados. Todos os cães com melhora nos sinais clínicos foram então desafiados com um alimento para o qual houve uma reação positiva nos testes. Posteriormente, seis cães foram desafiados com um alimento para a qual haviam sido negativos no teste. A Escala Visual Analógica de Prurido (pVAS) e o Índice de Gravidade e Extensão da Dermatite Atópica Canina, 4ª iteração (CADESI-04) foram utilizados no Dia (D) 0, D30 e D60 da DE. Foram calculados os valores preditivos de sensibilidade (SE), especificidade (SP), valores preditivos positivo (PPV) e negativo (VPN) e o valor Kappa (κ).

**Resultados –** Dos 21 cães, houve uma melhora média significativa nos escores pVAS e CADESI-04 em 16 (76%) cães após D30 ($P$ <0,01) e D60 ($P$ <0,01) da DE. Não houve diferenças estatísticas entre D30 e D60. A combinação de testes teve SE, SP, PPV, NPV e valores de κ de 80%, 66,7%, 66,7%, 80% e –0,17, respectivamente.

**Conclusões e relevância clínica –** A combinação do prick test e patch test atingiu altos valores de SE e NPV. O diagnóstico de AFR foi feito em 76% dos cães, e os resultados dos testes foram úteis para a formulação de uma DE.

# Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main.

**Background** – Previous studies have shown that patch testing with food extracts can assist formulation of elimination diets (ED) in human patients with suspected adverse food reactions (AFR). Little is known about the use of these tests in dogs. **Objectives** – To evaluate the effectiveness of a combination of prick and patch testing in current protocols, and food challenge (FC) tests in dogs with AFR. **Conclusions and clinical relevance** – The combination of prick and patch testing reached high values of SE and NPV. A diagnosis of AFR was made in 76% of the dogs, and test results were useful for the selection of an ED.