





## Evaluation of skin prick test, exclusion diet and dietary challenge in the diagnosis of food allergy in dogs with chronic pruritus<sup>1</sup>

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**ABSTRACT.**- Alcalá C.O.R., Possebom J., Ludwig L.A., Cerdeiro A.P., Gaertner R. & Farias M.R. 2023. **Evaluation of skin prick test, exclusion diet and dietary challenge in the diagnosis of food allergy in dogs with chronic pruritus.** *Pesquisa Veterinária Brasileira* 43:e07196, 2023. Departamento de Medicina Veterinária, Escola de Medicina e Ciências da Vida, Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição 1155, Curitiba, PR 80215-901, Brazil. E-mail: [camilla\\_alcala@hotmail.com](mailto:camilla_alcala@hotmail.com)

Allergic dogs are commonly sensitized to food allergens. We evaluated the use of the prick test to diagnose food allergies in dogs with pruritus and the efficacy of an exclusion diet based on the test. The prick test was performed in 10 healthy dogs and 34 dogs with pruritus, of which 25 received an exclusion diet for 60 days and was challenged with positive food on the test. pVAS and CADESI-4 were assessed on days 0, 30, and 60 after re-exposure. As a result, two control group dogs reacted to a single food allergen, milk and wheat. Of the 25 dogs with pruritus that reacted to food allergens, 24 (96%) reacted to more than one food allergen, and only one (4%) reacted to a single food protein: pork. In the test group (n=25), there was a significant improvement of pVAS and CADESI-4 after 30 and 60 days of dietary exclusion, with significant worsening of the scores with food allergen challenge. In conclusion, the prick test can be used for screening food allergens to make an exclusion diet.

INDEX TERMS: Food allergy, allergens, dogs, allergy test, skin prick test, exclusion diet.

### RESUMO.- [Avaliação do teste de puntura, dieta de exclusão e desafio dietético no diagnóstico de alergia alimentar em cães com prurido crônico.]

Cães alérgicos são comumente sensibilizados a alérgenos alimentares. Nós avaliamos o uso do teste de puntura para o diagnóstico de alergias alimentares em cães com prurido e a eficácia de uma dieta de exclusão baseada no teste. O teste de puntura foi realizado em 10 cães saudáveis e em 34 cães com prurido, dos quais 25 receberam uma dieta de exclusão por 60 dias e depois desafiados com o alimento que foi positivo no teste. pVAS e CADESI-4 foram realizados nos dias 0, 30, 60 e depois da reexposição. Como resultados, dois cães do grupo controle reagiram a um único alérgeno alimentar, leite e trigo. Dos 25 cães com prurido que reagiram aos alérgenos alimentares, 24 (96%) reagiram a mais de um alérgeno alimentar e apenas um (4%) reagiu a uma única proteína, que foi porco. No grupo teste (n=25),

houve uma significativa melhora do pVAS e CADESI-4 depois de 30 e 60 dias de dieta de exclusão, com significante piora dos scores com o desafio alimentar. Como conclusão, o teste de puntura pode ser usado para selecionar alérgenos alimentares para fazer uma dieta de exclusão.

TERMOS DE INDEXAÇÃO: Alergia alimentar, alérgenos, cães, teste alérgico, teste de puntura, dieta de exclusão.

### INTRODUCTION

Atopic dermatitis in dogs is a chronic, inflammatory and pruritic allergic skin disease with epidermal barrier dysfunction which enhances sensitization to environmental, microbial and food allergens (Nuttall et al. 2019, Possebom et al. 2022). In dogs, adverse skin reactions to food can occur in up to 30% of dogs with atopic dermatitis (Roudebush et al. 2010, Bhagat et al. 2017). They may be associated with a dependent IgE response, although 90% of dogs with atopic dermatitis have a lymphocyte (type IV) reaction (Mueller & Olivry 2017, Nuttall et al. 2019, Possebom et al. 2022).

Investigation of food hypersensitivity is based on history, clinical signs and an exclusion diet for eight weeks (Carlotti

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2011, Pucheu-Haston et al. 2015). The exclusion diet must contain only ingredients with no previous exposure by the dog (Olivry et al. 2015). A presumptive diagnosis can be made if clinical signs improve or partially resolve during the exclusion diet, and the confirmation requires recurrence of clinical signs after oral provocation tests (OPT) (Proverbio et al. 2010, Olivry et al. 2014, 2015).

Poor compliance and dropouts highlight the importance of the research on new tests to identify food allergens involved in adverse reactions in dogs (Mueller & Olivry 2017).

An isolated prick test for food sensitivity in dogs with allergic dermatitis is not previously reported. The main objective of this study was to evaluate the use of the prick test in the diagnosis of food allergen sensitization in dogs with chronic pruritus related to allergic dermatitis.

## MATERIALS AND METHODS

**Animals.** This study included 34 dogs (Group 1), representing a variety of breeds, and ages, predominantly living in private households, presented at the Veterinary Teaching Clinic that had a history of chronic, not seasonal, severe, continuous pruritus, mainly in the interdigital, ear, abdominal, axillary, inguinal, perioral, periorcular and perianal regions, and responsive to glucocorticoids and/or oclacitinib 0.4-0.6mg/kg once-daily (Apoquel, Zoetis). Before inclusion, infectious and parasitic dermatopathies were excluded, and all dogs included in the study were regularly treated for ectoparasites.

Exclusion criteria included: treatment with topical and/or systemic corticosteroids and antihistamines in the 30 days before inclusion in the study; the presence of superficial pyoderma and/or malasseziosis; severe erythroderma or lichenification that made it impossible to perform the prick test; aggressive patients and those with any other systemic and/or immunosuppressive disease.

A control group (Group 2) was used to investigate the potential irritation caused by food-allergenic extract concentrations. This group comprised 10 healthy dogs, regardless of breed and gender, with no dermatological lesions nor history of previous dermatopathies and use of antihistamine and immunosuppressive medications.

**Skin prick test.** All dogs in the study groups were submitted to the prick test. A prick test was performed using food allergen extracts in both groups. Saline and histamine (10mg/mL) solutions were used as negative (CN) and positive (CP) controls, respectively. The food extracts were bovine, chicken, pork, milk, egg, fish, wheat and soybean proteins at a concentration of 1:20w/v (weight/volume). All extracts were manufactured by Vet Allergenics (FDA Laboratory, Rio de Janeiro, Brazil) and comprised sodium chloride, phosphates, phenol, glycerol and 5% of the determined food protein.

Five drops of each extract were instilled into individual Eppendorf tubes in an allergen tray. A plastic Duotip Test double-ended puncture tip device (Lincoln Diagnostics, Decatur) was placed over the allergen tray, with the tips entirely immersed in the allergen extract. Between tests, the allergen tray was refrigerated at 6 to 10°C.

An area, approximately 7.5cm x 13.5cm, was clipped on the lateral thoracic wall, the skin was cleaned with saline, and 16 dots were marked on the skin with a demographic pen, approximately 1cm apart (Fig.1). A puncture next to each marking was performed with a Duotip-Test II® (Lincoln Diagnostics, Decatur, Illinois) which had been immersed in the specific allergen to be tested. The tip was pressed against the skin surface at 45 to 60° to enable percutaneous penetration of allergens (Fig.2).

The test site was examined 15 minutes later to identify erythematous papules (Fig.3), which were demarcated using a dermatographic pen. The diameter of the papule was measured using a caliper (Fig.4).

The animals were sensitized to the tested allergens when the average diameter of the papule was  $\geq 3$ mm larger than the mean diameter of the papular reaction to the negative control.

**Exclusion diet and dog evaluation.** After the prick test, all dogs in Group 1 were placed on a homemade exclusion diet for 60 days. This was based on rice or potatoes as the carbohydrate source and a protein source to which the patient had tested negative in the prick test. All dogs were evaluated on the 0th, 30th and 60th days, and their lesion scores were established by the CADESI-4 scale (Canine Atopic Dermatitis Extent and Severity Index) elaborated and validated by Olivry et al. (2014) and the pruritus intensity from the Visual Pruritus Scale (pVAS) (Rybníček et al. 2009).

After 60 days, all dogs included in Group 1 were submitted to OPT for up to 14 days, according to each patient's response, and lesion and pruritus scores were again measured. The challenge was performed with a single protein, producing the largest prick test reaction intensity.

Animals with severe pruritus requiring medication during the exclusion diet period were treated with oclacitinib 0.4-0.6mg/kg once daily, which was always suspended one or two weeks before the reassessment on day 30, and establishment of CADESI-4 and pVAS scores. If deemed unnecessary, medication was no longer performed until the assessment on day 60. During the OPT period, no medications were used to control pruritus before patient evaluation (Fig.5).

**Statistical analysis.** The response to the tests performed and the estimate of their clinical improvement was presented descriptively using percentage, mean and standard deviation. The included variables were a reaction to environmental or food allergens, clinical signs, decrease in medication use, frequency of pruritus or dermatological improvement observed by the owner.

The repeated measures of the ANOVA test were used to compare the pVAS and CADESI-4 results of days 0, 30, 60 and after re-exposure. The independent variable was the time of dietary exclusion and reintroduction, and the response variable was the ordinal data of the CADESI-4 and pVAS scales. The percentages of animals requiring medication were analyzed using a generalized mixed model. Data were expressed as means, with a confidence interval of 95% and  $p < 0.05$ .

## RESULTS

### Skin prick test

Of the 34 dogs in Group 1, four (12%) did not react to food allergens and were excluded from the study. In addition, five more dogs were withdrawn from the study, two with severe gastrointestinal disorders with the introduction of the home diet and three by their owners for other reasons.

Of the 25 dogs remaining in the study group, 20 (80%) reacted to wheat, 16 (64%) to egg, 14 (56%) to pork, 13 (52%) to beef, 11 (44%) to soybean, 10 (40%) to milk, 9 (36%) to fish and 7 (28%) to chicken (Table 1). Of these, 24 (96%) reacted to more than one food allergen, and only one (4%) reacted to a single food protein (pork).

Of the 10 dogs in Group 2, only one reacted to milk protein and another to wheat.

### Clinical aspects

Regarding the main clinical signs of the 25 dogs in Group 1, 21 (84%) had interdigital pruritus, 18 (72%) perioral

pruritus, 17 (68%) periorbital pruritus and seven (28%) perianal pruritus. Of these 25 dogs, 14 dogs (56%) had a history of, or had, bacterial or fungal dysbiosis at the time of consultation, which was confirmed by cytology, 11 (44%) had

bilateral otitis externa, seven (28%) had partially lichenified skin and three (12%) had gastrointestinal disorders, diarrhea or soft stools, all meeting the diagnostic criteria for atopic dermatitis.



Fig.1-4. (1) Skin marking with a demographic pen. (2) Duotip Test II<sup>®</sup> lancet at an angle of 45° against the skin surface. (3) Formation of papular reactions 15 minutes after the puncture. (4) Demarcation of papular reactions.

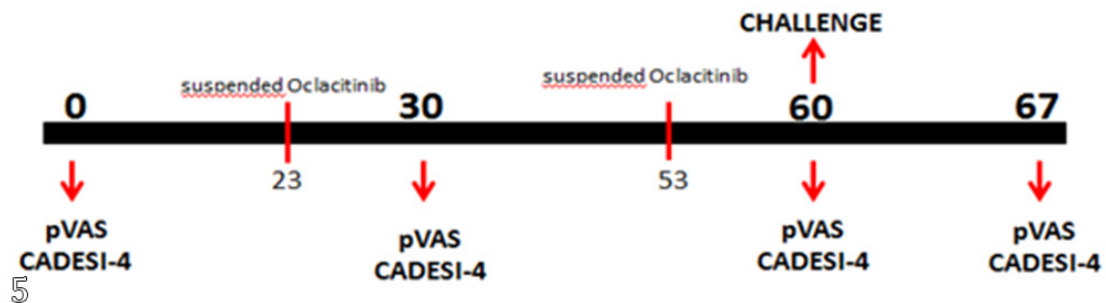


Fig.5. Restrictive diet assessment flowchart.

**Table 1. Percentage of dogs which reacted to different food extracts**

Food	Percentage of dogs
Wheat	20/25 (80%)
Egg	16/25 (64%)
Pork	14/25 (56%)
Beef	13/25 (52%)
Soybean	11/25 (44%)
Milk	10/25 (40%)
Fish	9/25 (36%)
Chicken	7/25 (28%)

## Exclusion diet and relationship to visual itching scale and CADESI-4

All 25 dogs showed decreased pruritus during the period of dietary exclusion. Four (16%) had improvement of less than 50%, 15 (60%) of dogs showed improvement of 50 to 80% and six (24%) dogs had more than 90% reduction in pruritus.

There were significant differences between the mean pVAS and CADESI-4 score results on days 0, 30, 60 and after the food challenge (PRA) ( $p=0.0001$ ).

Mean pruritus scores decreased between days 0 and 30 ( $p=0.0001$ ). However, between days 30 and 60, there was no significant mean reduction ( $p=0.281$ ) (Fig.6).

After OPT, four dogs (16%) showed no significant increase in pruritus, and in 21 (84%) dogs, the pVAS increased after the challenge with positive proteins. Of these 21, pVAS scores increased by 50-80% in 15 dogs (60%), in four (16%) increased by >80%, and in two (8%) increased by 25-50%. Following exposure to a protein that had tested positive in the prick test, there was a significant increase in mean pruritus, compared to days 30 and 60 ( $p=0.0001$ ), and all dogs returned to present clinical signs in an average of 48 hours after re-exposure.

The pruritus increased after the challenge test in all six dogs in which pVAS was reduced by >80% on the exclusion diet. In four (67%) of these, the pVAS increased more than 80% from the value recorded on the exclusion diet, and in two (33%), the pVAS increased by 50-80%.

The mean CADESI-4 values were reduced between days 0 and 30 following the introduction of an exclusion diet ( $p<0.0001$ ). Between days 30 and 60, no significant mean lesion score reduction ( $p=0.655$ ). However, there was a worsening of the average lesion scores after introduction to the challenge diet ( $p=0.039$ ) (Fig.7).

## DISCUSSION

Most previous studies highlight the importance of environmental allergens as triggers of atopic eczema. However, recent studies have observed the importance of food allergens in the etiopathogenesis of AD in dogs, demonstrating that approximately 67% of dogs with pruritus are co-sensitized to

environmental and food allergens, and up to 81% of them may present significant or partial improvement with restrictive diets (Possebom et al. 2022).

In this study, excluding the removed five dogs and four dogs that did not have their symptoms aggravated after OPT, 21 (70%) of the dogs had confirmed adverse reactions to food. All of them met the diagnostic criteria for atopic dermatitis. Of these, 96% of dogs were positive for more than one tested food, suggesting that dogs with AD may sensitize to food allergens they are frequently exposed to.

The prick test with food extracts had low irritant potential since only two non-allergic dogs reacted positively to a tested allergen. The positive reactions observed may be related to the puncture's action and, less commonly, the reaction to the extracted vehicle (Ling et al. 2016, Matricoti & Noli 2018).

Positive reactions to the food prick test suggest that these dogs may have an immediate IgE-dependent reaction to dietary proteins, which may be responsible for erythematous and pruritic skin reactions. When the lancet superficially injures the skin during the procedure, the food allergen disperses in the adjacent tissue. In sensitized patients, specific IgE on the surface of mast cells identifies the allergens causing the release of pro-inflammatory substances, leading to a positive reaction (Hill et al. 2004). The hypothesis of a type I hypersensitivity reaction to food allergens in dogs is also supported by a good response to oclacitinib, a selective Janus kinase 1 inhibitor that blocks the action of Th2 cytokines, such as IL4, IL13 and IL31, which participate in the immediate allergen-dependent IgE response (Collard et al. 2014, Gonzales et al. 2014, Banovic et al. 2019).

However, the prick test does not detect late cell-mediated reactions that can occur in food adverse reactions (Johansen et al. 2017), which may be a limitation for food selection for restricted diets. In addition, cross-reactions between food allergens such as egg and chicken; milk and beef; fish and chicken and chicken and pork limit the specificity of the prick test (Suto et al. 2015, Pali-Schöll et al. 2017).

Home elimination diets with protein and carbohydrate sources are traditionally used for up to eight weeks to diagnose

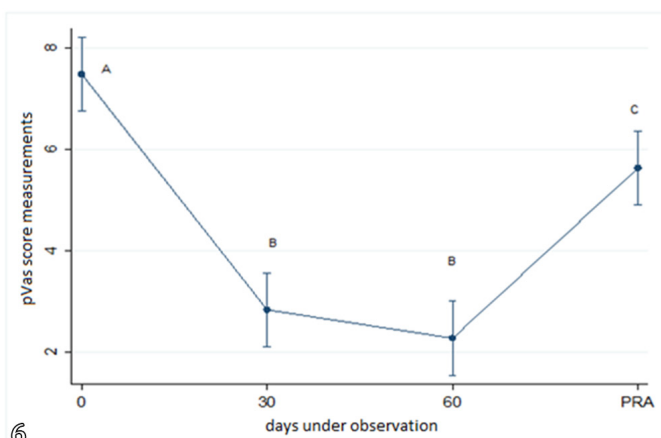


Fig.6. Mean linear distribution of the pVAS score between days 0, 30 and 60 days of food exclusion and 7 days after food reintroduction (PRA). Different letters represent significant statistical differences in results ( $p=0.001$ ). Identical letters represent no statistically significant difference in results ( $p=0.281$ ).

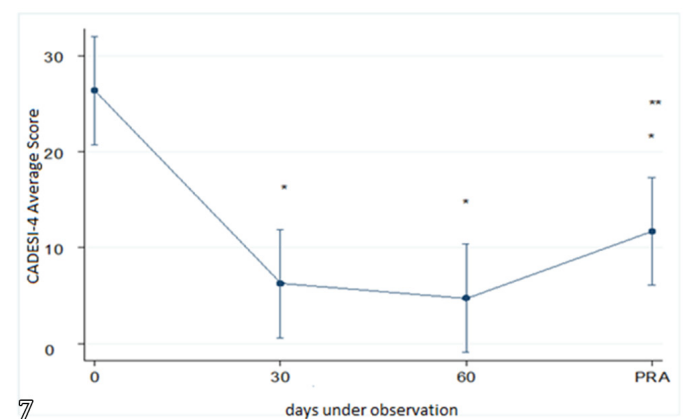


Fig.7. Mean linear distribution of the CADESI-4 score between days 0, 30 and 60 days of food exclusion and 7 days after food reintroduction (PRA). Significant difference between days 0 and 30, 0 and 60 and 0 and 90 ( $p=0.0001$ ). Significant difference between 60 days and PRA ( $p=0.039$ ).

dog food allergies (Bethlehem et al. 2012, Olivry et al. 2015). However, these are laborious, nutritionally insufficient and require full cooperation from the owner, which can reduce compliance or lead to their abandonment (Mueller & Olivry 2017). In addition, contamination during the preparation and cross-reactions between foods reduce its effectiveness and prevent diagnosis (Solé et al. 2018).

Clinical signs improved when exclusion diets were constructed based on the allergic prick test. After OPT, 21 of 30 (70%) dogs showed worsening signs, suggesting that the prick test for food allergens may be useful for selecting exclusion diets in dogs with suspected food-induced pruritus. These findings differ from those who selected foods for an exclusion diet based on the owner's history when 38% to 54% of dogs showed reduced pruritus with dietary exclusion (Salzo & Larsson 2009, Vandresen & Farias 2018).

These better results of this study, when compared with an empirical food choice for exclusion diets, may be due to the exclusion diet being based on negative results, which can be assumed that these dogs did not have specific cutaneous IgE against these proteins. Thus, the formulation of exclusion diets based on allergic tests *in vivo* can lead to an early exclusion diet response and increase the owner's support. The reduction of pruritus during the exclusion diet occurred significantly in the first 30 days, and there was no significant difference in pruritus between days 30 and 60. This is in line with Olivry et al. (2015), which reported that remission of clinical signs may occur in up to five weeks in about 85% of the cases.

However, in humans, studies show that the patch test for food allergens seems to be an alternative tool for patients with negative prick test and positive TPO (Resende & Segundo 2010, Edwards & Martinez 2014, Mansouri et al. 2018). In other studies, the prick test for diagnosing food allergy in humans with atopic dermatitis showed high sensitivity but low specificity, with positive predictive values of 60 to 75% and negative predictive values of 95%. When combined with the patch test, sensitivity and specificity are increased, and this combination may be helpful in the diagnosis of food allergy (Chung et al. 2010, Hammond & Lieberman 2018)

In dogs, the association between prick and patch tests seems to improve the individual accuracy values of each test, enabling a better food selection for a restrictive diet. A recent study showed a sensitivity of 80%, a specificity of 66.7%, a positive predictive value of 66.7% and a negative predictive value of 80% when both tests are combined (Possebom et al. 2022).

A significant reduction in pruritus and lesion scores occurred in 84% of the dogs on an exclusion diet. In addition, in 84% of dogs, pruritus increased by >50% after the dietary challenge, confirming the sensitization to diet proteins and the test's usefulness in selecting foods for a restrictive diet.

After exposure to a positive food, there was a significant increase in the mean pruritus compared to days 30 and 60 during diet ( $p=0.0001$ ), and all dogs showed clinical signs in an average of 48 hours after re-exposure. This is probably because the prick test only identifies immediate reactions.

Another important fact is that 60% of the dogs in this study developed clinical signs before two years of age and 40% before one year. Sensitization of food allergens in dogs can start early due to exposure to protein-rich diets containing numerous animal protein sources (Possebom et al. 2022). In

people, it has been shown that industrialized hyperosmotic foods containing dyes, flavorings and preservatives can cause dysbiosis, leading to the growth of intestinal bacteria with the production of superantigens that can stimulate a Th2 inflammatory response and break the immunological tolerance (Tordesillas et al. 2014, Chinthrajah et al. 2016). Hemida et al. (2021) demonstrated that puppies exposed to heat-processed foods had an increased risk of developing canine atopic dermatitis. In addition, the authors showed that early exposure to raw meat seems to determine a protective factor against developing the disease.

## CONCLUSION

The present study demonstrated that the prick test with food protein extracts is fast and viable and can guide the formulation of exclusion diet protocols in dogs with chronic allergic skin disease.

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**Conflict of interest statement.** - The authors have no conflicts of interest.

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