



Grass pollen sensitization in dogs in Paraná, Brazil: comparison between percutaneous and intradermal testing

[Sensibilização a polens em cães no Paraná, Brasil: comparação entre o teste percutâneo e o teste intradérmico]

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ABSTRACT

Canine atopic dermatitis is an inflammatory, genetic, pruritic and chronic dermatosis that affects between 10 and 30% of dogs and one of the most important allergens is grass pollen. The objective of this study was to evaluate the sensitization to grass pollen allergens in dogs with canine atopic dermatitis and to compare intradermal skin test (IDT) with percutaneous test (PT). For this study, ten healthy dogs and 39 dogs with atopic dermatitis were tested. Dogs were submitted to IDT and PT for *Lolium multiflorum*, *Cynodon dactylon* and *Paspalum notatum*. The IDT and PT tests were compared using the Proportion Test. All healthy dogs were negative to both tests. Ten atopic dogs (25.6%) responded positively to the PT and none were positive in IDT. *C. dactylon*, *L. multiflorum* and *P. notatum* were responsible for positive reactions in 70%, 70% and 30% of positive dogs, respectively. The number of positive reactions in PT were statistically higher than IDT ($P < 0.05$). In conclusion, grass pollen can be important source of allergens for dogs in Paraná state (Brazil) and the PT showed higher sensitization to grass pollen in dogs with atopic dermatitis than IDT.

Keywords: dogs, grass pollen, atopic dermatitis, prick test

RESUMO

A dermatite atópica canina é uma dermatose inflamatória, genética, prurítica e crônica que afeta entre 10% e 30% dos cães, e um dos alérgenos mais importantes são os polens de gramíneas. O objetivo deste estudo é avaliar a sensibilização a alérgenos de polens de gramíneas em cães com dermatite atópica e comparar o teste intradérmico (TID) com o teste percutâneo (TP). Para o estudo, 10 cães hígidos e 39 cães com dermatite atópica foram testados. Estes foram submetidos ao TID e ao TP para *Lolium multiflorum*, *Cynodon dactylon* e *Paspalum notatum*. TID e TP foram comparados usando-se o teste de proporção. Todos os cães hígidos foram negativos em ambos os testes. Dez cães atópicos (25,6%) responderam positivamente ao TP e nenhum ao TID. *C. dactylon*, *L. multiflorum* e *P. notatum* foram responsáveis por reações positivas de 70%, 70% e 30% dos cães positivos, respectivamente. O número de reações positivas no TP foi estatisticamente maior que no TID ($P < 0,05$). Foi concluído que os polens de gramíneas podem ser importantes fontes de alérgenos para cães no estado do Paraná (Brasil) e que o TP mostrou maior sensibilização a polens em cães com dermatite atópica que o TID.

Palavras-chave: cães, polens de gramíneas, dermatite atópica, teste percutâneo

INTRODUCTION

Canine atopic dermatitis (CAD) is a pruritic, inflammatory, and chronic dermatopathy, with a genetic predisposition, affecting between 10 and

30% of the canine population (Carlotti, 2012). CAD results in the loss of the integumentary physical barrier function and increased immunoreactivity. It is estimated that approximately 80% of dogs with CAD have

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increased levels of the specific antibody immunoglobulin E (IgE), mainly as a response to environmental, microbial and food allergens (Pucheu-Haston *et al.* 2015). The environmental major allergens inducing CAD come from house dust mite allergens, weeds, shrubs, and grasses (Prélaud, 2014). In people with atopic dermatitis IgE mediated by extrinsic factors, it was found that exposure to grass pollens induces a significant worsening of cutaneous symptoms (Werfel *et al.*, 2016).

The major allergenic grasses in south Brazil are *Lolium multiflorum* (LM) or Ryegrass, *Cynodon dactylon* (CD) or Bermuda grass, and *Paspalum notatum* (PN) or Bahia Grass. The subfamily Pooideae, to which belongs the LM, has the major allergens in groups 1 and 5 and may confer cross-reactivity with other species of the same subfamily and with species of other subfamilies (Vieira, 2012). Among them, *Lolium multiflorum* is considered to be the most important allergen in humans in south Brazil (Rosário Filho, 2012). Veterinary studies to verify the incidence of pollinosis in Brazil are rare. A recent study that used intradermal skin test (IDT) in 58 dogs with atopic dermatitis in Rio Grande do Sul state, demonstrated a positive response to CD (24.13%), and PN (12%) (Pereira *et al.*, 2015). Sensitization to LM was not evaluated.

Percutaneous test (PT) has been used in medicine since its first description by Jack Pepys in 1975 as an alternative with lower risk of systemic reactions compared to IDT. Studies performed in cats and dogs showed papule reactions easier to interpret, no cutaneous and systemic reactions and good specificity (Rocha, 2012; Rossi *et al.*, 2013, Gentry e Messinger, 2016). The objective of this study was to evaluate the sensitization to grass pollen allergens in dogs with CAD and to compare IDT with PT.

MATERIAL AND METHODS

The present study was approved by the Ethics Committee for the Use of Animals at the Pontifical Catholic University of Paraná (registry number: 1027/2016). It was a cross-sectional, non-randomized and controlled study. Ten healthy dogs, 5 females and 5 males, average age of 5 years, and 39 dogs with atopic dermatitis, 26 females, average age of 5 years, irrespective of breed, from the Pontifical Catholic University of

Paraná Veterinary Clinic School were included. There were not included in this study dogs that received antihistamines (1 week), oral and topical glucocorticoids (2 weeks) or long-term injectable glucocorticoids (4 weeks), according to Olivry's study of anti-allergic drugs optimal and minimal withdrawal times before allergic tests in dogs (2013). Females in estrus, gestation or lactation and dogs with chronic systemic diseases or with any other dermatopathy were also not included. All dogs in this study were submitted to the IDT and PT sequentially in the same day by the same examiner, who previously did a proficiency test as described by Cunha *et al.* (2015).

For IDT, the skin was shaved using a grooming machine and blade 40 in the lateral region of the thorax. After careful cleaning of the skin with a physiological solution, 10 application points were marked at an interval of 3cm with a dermatographic pen. For both groups, 0.9% phenolated saline and a histamine solution (0.05 mg/ml) were used as a negative and positive controls, respectively. Sensitization to pollen was evaluated using standardized extracts of LM, CD and PN at the concentration of 100 UBE /ml. All extracts were supplied by FDA Allergenic (Rio de Janeiro, Brazil). The intradermal applications were performed using 0.5-ml insulin syringes, attached to hypodermic needles (13 x 4,5mm), containing 0.05ml of each grass pollen extract. Fifteen minutes after application, the papules were marked with a dermatographic pen to facilitate the reading. The cut-off value was established by calculating the mean for the negative control (CN) and positive control (CP) values. The responses to the tested antigens were considered positive when the mean diameter of the papules was \geq the calculated cut-off value.

In the PT, histamine hydrochloride (10 mg/ml) and saline (containing 0.45% phenol) were used as the positive and negative control, respectively. Glycerinated allergenic extracts of LM, CD and PN were used at the concentration of 10.000 UBE/mL. All the extracts were supplied by FDA Allergenic (Rio de Janeiro, Brazil). After applying a drop of each extract or control solutions on the skin, the device (Duotip-test II®, Multi-test Brazil) was passed through the drop at a 45° to 60° angle to the skin. The skin was then gently lifted, creating a small break in the epidermis through which the suspected allergen solution penetrates. Then an absorbent paper was

used to remove the excess of solutions. After 15 minutes, the test sites were observed, and the papules were evaluated. Papules that were at least 3mm larger than the negative control were considered positive (Van der Valk *et al.*, 2016). The results of the PT and IDT were compared in a descriptive way and then by the proportion test using the Minitab16 software (Minitab Inc., State College, Pennsylvania), with significance level set at $\alpha=0.05$ ($P\leq 0.05$).

RESULTS

All dogs in Group 1 were negative to the allergen extracts in the IDT and PT. The mean histamine papule diameter in the IDT and PT was 15 and 7.45mm, respectively. The positive reactions in

PT ranged from 3 to 6mm, with formation of pseudopods in some cases. The comparison between papular histamine reaction in IDT and positive reaction to grass pollen in PT is shown in Figure 1. In DAC group, none of the dogs were positive for the pollen tested in IDT. Ten dogs (25.6%) reacted to grass pollen with PT. Seven dogs (18%) were positive to CD and LM, while three dogs (7.7%) were positive to PN. Two dogs (5.1%) reacted to three species tested and three dogs (7.7%) to two species. Monosensitization was observed in five dogs (12.8%) as shown in Table 1. The statistical analysis with Proportion Test showed that the PT was more effective in identifying sensitization to CD and LM ($p=0,002$), and PN ($p=0,036$) compared to IDT.

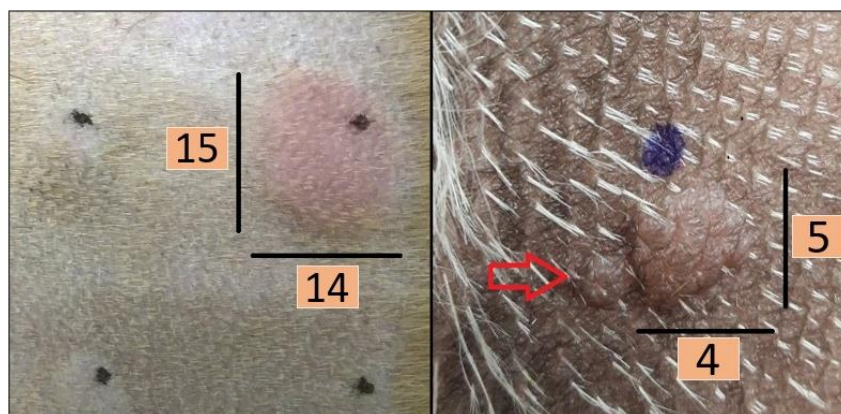


Figure 1. Comparison of papular histamine reaction in the intradermal test (left) and positive for grass pollen in the prick test (right) in millimeters. Note the formation of a pseudopod (arrow).

Table 1. Results of sensitization among ten reactive dogs in to grass pollen through the-Percutaneous Test

Pollen/Dog	1	2	3	4	5	6	7	8	9	10
CD	+	-	+	+	-	+	+	+	-	+
LM	-	+	+	-	+	+	+	+	+	-
PN	-	-	-	-	-	+	+	-	-	+

CD – Cynodon dactylon; LM – Lolium multiflorum; PN – Paspalum notatum.

DISCUSSION

In the present study, the PT and IDT showed high specificity, justified by the lack of positive animals in the control group; these solutions were tested previously for irritative threshold in healthy dogs (unpublished data) and were tested again in this study to prove these findings. It is believed that IDT based on the irritation threshold have high specificity in the diagnosis of allergic sensitization (Hensel *et al.*, 2004). It is estimated that the pollen sensitization in allergic people in

Brazil varies from 16% to 34%, being more prevalent in the South and Center-West of the country (Vieira *et al.*, 2011; Vieira, 2014; Sopelete *et al.*, 2014). In dogs, the prevalence of grass pollen sensitization ranges from 10 to 27.7% (Masuda *et al.*, 2000; Roussel *et al.*, 2013). In Brazil, one study using IDT identified 10.3% of sensitization in atopic dogs using grass polens extracts (Pereira *et al.*, 2015). In the present study using PT, the prevalence of sensitization to grass pollen was (25.6%) and CD and LM were the most important species.

This could be explained by the wide distribution of these species in the South region of Brazil, which can be found along roads, backyards, and parks (Viera, 2012). LM may provoke rhino conjunctivitis and allergic asthma in people (Rosário Filho, 2012) due to its wide distribution and an increase in its cultivation over the years. It has high allergenic potential due to its large-scale production and rapid spread in the wind (Bonissoni and Neto, 2014). In Curitiba, South of Brazil, a study done in 1999 found positive results using PT for LM in 4.7% of children aged 13 to 14 years and 15.4% of adults over 20 years of age (Esteves *et al.*, 1999). A study conducted in 2002 found positivity for *Lolium perene* of 16.5% in skin allergic tests (Rosário Filho, 2002). In veterinary medicine, the incidence of sensitization to LM has not been evaluated so far and the present study revealed a positive response to this grass among atopic dogs (12.8%) similar to previous findings.

Additionally, other species such as CD are allergenic and produce allergy symptoms beyond September and December (Rosário Filho, 2012). Sensitization to CD was detected in 16.8 and 80% of patients with rhinitis in Curitiba (Araújo *et al.*, 2015) and Rio Grande do Sul (Vieira *et al.*, 2011), respectively. A previous study using IDT in Brazil found that 24.13% of atopic dogs were sensitized to CD (Pereira *et al.*, 2015). In the present study, the positive response to this grass was similar to the other studies cited. The lowest incidence of positive response was observed with PN. Positive reactions to PN were accompanied by positive reactions to CD, suggesting the possibility of cross-reaction between these species. In dogs, the major allergens of grasses are yet to be identified; hence we could not test the hypothesis of cross-reaction between and PN in this study.

Among the ten animals with positive pollen responses through PT, 2 were positive only to CD, 2 to CD and LM, 2 to CD, LM and PN, 3 only LM and 1 to CD and PN. Therefore, there was no pattern of response suggestive of cross reactivity. The exception was the 3 PN positive dogs that were also CD positive. However, 4 dogs were CD positive but not PN positive. These results may indicate true sensitization to both species and cross reactivity between species, however suggesting that CD extracts may contain their own antigens. Due to the small number of positive tests

(n=10) and the heterogeneous pattern of responses, it was not possible to perform an adequate statistical analysis for evaluation of cross-reactivity among the species tested. For cross-reactivity studies, the ideal would be the use of immunochemical methods such as Cross-Inhibition ELISA, which is beyond the scope of this study.

The negative results in the present study can be explained by the sensitization to other environmental allergens, mainly from house dust mites species, species, as *Dermatophagoides farinae*, *D. pteronyssinus* and *Blomia tropicalis*, as shown by Cunha *et al.* in studies done in Brazil (Cunha *et al.*, 2007; Cunha, 2012). Besides that, it is estimated that about 30% of patients with clinical diagnosis of atopic dermatitis have negative skin tests (Bauer *et al.*, 2010). This suggests an innate, cellular inflammatory component that mediates pruritus development and symptoms related to atopic dermatitis in some patients, with no clear relation with sensitization and IgE formation (Halliwell, 2006). These findings could also explain some negative results observed. No systemic or local adverse reactions were observed in the PT and IDT, suggesting the safety of these tests in dogs. Some dogs presented only local effects characterized by pruritus and erythema, which disappeared in a few hours. In humans, the PT generates fewer adverse reactions than IDT, and fatal reactions can occur in the latter (Bernstein *et al.*, 2008).

CONCLUSION

The prick test was more effective in identifying grass pollen sensitization in dogs with atopic dermatitis than the intradermal skin test. Sensitization to grass pollen occurred in about a quarter of the subjects and was more common to CD and LM through the prick test. Future studies are recommended to evaluate the efficacy of allergen-specific immunotherapy in dogs sensitized to these grass species.

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