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Sensitization to environmental allergens by skin test in dogs with atopic dermatitis in Ourinhos/SP, Brazil, and region[1]

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ABSTRACT.- Rosa AAS, De Souza FP, De Almeida BFM, Alcalá COR, Zanutto MS. **Sensitization to environmental allergens by skin test in dogs with atopic dermatitis in Ourinhos/SP, Brazil, and region**. *Pesquisa Veterinária Brasileira 46:e07782, 2026.* Departamento de Medicina Veterinária, Universidade Estadual de Londrina, Rodovia Celso Garcia Km 380, Campus Universitário, Londrina, PR 86057-970, Brazil. E-mail: andresalla@outlook.com

Canine atopic dermatitis (CAD) is a hereditary and multifactorial disease caused by an immediate hypersensitivity response to one or more allergens, leading to a specific and chronic immune response. Pruritus and inflammatory conditions are frequently observed, ranging from moderate to severe. Clinically relevant allergens include house dust mites and grass pollens. Currently, the skin prick test is used as a method to identify immune-specific allergic reactions mediated by immunoglobulin E. Therefore, the present study aimed to identify the incidence of sensitization to environmental allergens by skin prick test in 200 dogs with atopic dermatitis in Ourinhos (SP, Brazil) and the region. The study had a retrospective character and analyzed data collected from 2022 to 2024, selecting atopic dogs that lived exclusively indoors and underwent the skin prick test. Of these, 53% (106/200) were sensitized for *Dermatophagoides farinae* (Df), 53.50% (107/200) for *Dermatophagoides pteronyssinus* (Dp), 54% (108/200) for *Blomia tropicalis* (Bt), 31.50% (63/200) for *Cynodon dactylon* (Cd), 26.50% (53/200) for *Lolium perenne* (Lp), and 27.50% (55/200) for *Paspalum notatum* (Pn). Regarding mosquitoes (*Culex* sp. and *Aedes aegypti*), 45% (90/200) showed sensitization, and 55% (110/200) showed absence of sensitization. The results demonstrated a higher frequency of sensitization to house dust mites, suggesting relevance in the sensitization profile of dogs in this region. However, these findings reflect sensitization and do not indicate a clinically relevant allergic condition. Environmental control measures to reduce exposure to house dust mites may be considered in clinical management, although further studies are required to determine the impact on disease expression.

INDEX TERMS: Allergies, prick test, household dust mites, grass pollens, environmental control, immunotherapy.

RESUMO.- [**Sensibilização a alérgenos ambientais por meio de teste cutâneo em cães com dermatite atópica em Ourinhos/SP, Brasil, e região.**] A dermatite atópica canina (DAC) é uma doença hereditária e multifatorial, causada por uma resposta de hipersensibilidade imediata a um ou mais alérgenos, resultando em uma resposta imune específica e crônica. Prurido e inflamações cutâneas são frequentemente observados, variando de moderadas a graves. Os alérgenos clinicamente relevantes incluem ácaros da poeira domiciliar e pólens de gramíneas. Atualmente, o teste cutâneo de puntura é utilizado como método para identificar reações alérgicas específicas mediadas pela imunoglobulina E. Assim, o presente estudo teve como objetivo identificar a incidência de sensibilização a alérgenos ambientais por meio do teste cutâneo de puntura em duzentos cães com dermatite atópica

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em Ourinhos (SP, Brasil) e região. O estudo teve caráter retrospectivo e analisou dados coletados de 2022 a 2024, selecionando cães atópicos que viviam exclusivamente em ambiente interno e foram submetidos ao teste cutâneo de puntura. Dos animais avaliados, 53% (106/200) apresentaram sensibilização para *Dermatophagoides farinae* (Df), 53,5% (107/200) para *Dermatophagoides pteronyssinus* (Dp), 54% (108/200) para *Blomia tropicalis* (Bt), 31,5% (63/200) para *Cynodon dactylon* (Cd), 26,5% (53/200) para *Lolium perenne* (Lp) e 27,5% (55/200) para *Paspalum notatum* (Pn). Em relação aos mosquitos (*Culex* sp. e *Aedes aegypti*), 45% (90/200) apresentaram sensibilização e 55% (110/200) não foram sensibilizados. Os resultados demonstraram maior frequência de sensibilização aos ácaros da poeira doméstica, sugerindo relevância no perfil de sensibilização dos cães nesta região. No entanto, esses achados refletem sensibilização e não indicam condição alérgica clinicamente relevante. Medidas de controle ambiental para reduzir a exposição aos ácaros da poeira doméstica podem ser consideradas no manejo clínico, embora estudos adicionais sejam necessários para determinar impacto na expressão da doença.

TERMOS DE INDEXAÇÃO: Alergias, teste cutâneo de puntura, ácaros de poeira domiciliar, pólens de gramíneas, controle ambiental, imunoterapia.

INTRODUCTION

Canine atopic dermatitis is a chronic and multifactorial disease involving alterations in the skin barrier and an exaggerated immune response (De Caro Martins et al. 2018, Cobiella et al. 2019). Classified as an allergic, inflammatory, and intensely pruritic dermatopathy, it manifests as hyperreactivity of lymphocytic subpopulations and reaction mediated by immunoglobulin E (IgE) upon exposure to environmental allergens (Nuttall et al. 2019, Marsella 2021). In humans, it is considered the most common chronic inflammatory skin condition worldwide, with a prevalence of approximately 13% in children and 7% in adults (Boguniewicz et al. 2018).

In veterinary medicine, atopic dermatitis is recognized as one of the most frequent dermatopathies in clinical practice. However, epidemiological data determining the percentage of affected animals remains controversial, as the incidence is heavily influenced by factors such as genetic predisposition, environmental conditions, and regional differences (Hillier & Griffin 2001, Drechsler et al. 2024). In Brazil, studies have demonstrated variable prevalence rates of canine atopic dermatitis, ranging from 25.65% in dogs attended at a veterinary hospital in Belém to approximately 36.1% in animals evaluated at a veterinary hospital in Rio de Janeiro, reinforcing the influence of geographic and environmental factors on disease occurrence (Amarante et al. 2015, Couceiro et al. 2021).

In humans, clinically relevant allergens responsible for allergic diseases with an environmental component include house dust mites, grass pollens, fungi, cockroaches, animal epithelia, mosquitoes and ectoparasites (specifically fleas and ticks). These allergens are associated with the development or exacerbation of clinical signs and hypersensitivity conditions in diseases such as allergic asthma and rhinitis, as well as atopic dermatitis (Gabet et al. 2019, Lei & Grammer 2019).

In dogs with atopic dermatitis, pollen and mites are the most frequent causes of sensitization, occurring through exposure and absorption via the skin or respiratory route in genetically predisposed and sensitized animals (Cerdeiro et al. 2021, Ludwig et al. 2021).

Skin tests are used in medicine as the primary method for identifying sensitization to allergens, confirming IgE-mediated sensitization (Gedon et al. 2019). In veterinary practice, a test that is being studied is the prick test, which is minimally invasive and easy to perform (Possebom et al. 2022). Positive results indicate the presence of allergen-specific IgE and, therefore, sensitization; however, this finding does not necessarily confirm a clinically relevant allergic condition, as it may reflect immunological sensitization without associated clinical signs. Furthermore, there is still limited information regarding the sensitivity and specificity of this test for diagnosing allergic conditions in dogs (Marsella 2021). Although genetic factors are strongly implicated in the predisposition to the disease, the impact of environmental factors has also been widely recognized. It is believed that approximately 50% of the variability in pathogenesis can be attributed to genetic components, while the other 50% is related to environmental influences (Harvey et al. 2019).

Epidemiological studies demonstrate the importance of the environment in the progression of a patient with canine atopic dermatitis. A retrospective study in Switzerland showed that dogs in the developmental phase of atopic dermatitis (AD) spend most of their time indoors, suggesting that atopic dogs are more sensitized to indoor allergens than humans, and living indoors may be a predisposing factor for CAD (Nødtvedt et al. 2007). When compared to another study, it was observed that dogs living in rural areas and with contact with other animals had a lower likelihood of developing AD (Prélaud 2013).

Therefore, reducing contact with environmental allergens can be a determining factor in the treatment of canine atopic dermatitis. Consequently, the present study aims to characterize the profile of sensitization to environmental allergens such as house dust mites, grass pollen, and mosquitoes through prick testing in dogs with atopic dermatitis in Ourinhos (SP, Brazil) and the surrounding region.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Animal Ethics Committee of the "Centro Universitário das Faculdades Integradas de Ourinhos" (CEUA-UNIFIO) in a meeting held on February 17, 2023 (protocol number 06/2023). The study was conducted in accordance with institutional requirements and national legislation for animal research.

The study conducted is retrospective in nature and analyzed data collected from 2022 to 2024, including animals diagnosed with canine atopic dermatitis (CAD) based on Favrot's criteria (2010). An elimination diet comprising novel or hydrolyzed proteins was conducted for a minimum of eight weeks to identify and exclude dogs exhibiting reactions to food. Dogs weighing < 3 kg and aged < 12 months, as well as those with a history of neoplasia, demodicosis, leishmaniosis or chronic systemic infections and those receiving immunosuppressive medications, were also excluded.

The research was carried out in the city of Ourinhos, located in the state of São Paulo, Brazil (latitude: -22.9785, longitude: -49.8707). This was a multicenter study involving animals from

multiple veterinary clinics in Ourinhos and the surrounding region. The animals were selected regardless of breed, gender, or age and presented a history of pruritus, ranging from moderate to severe, which was responsive to corticosteroids or oclacitinib, with clinical signs consistent with CAD. These signs were primarily observed in the auricular pavilion and ear canal, periocular and perioral regions, abdominal area, axillary and inguinal regions, distal parts of the limbs, especially the paw area, flexural surfaces, and the perianal region. The pruritus persisted even after the exclusion of infectious and parasitic dermatoses or allergic origin was ruled out through tests such as skin scraping, cutaneous cytological evaluation and strict ectoparasite control.

Patients who had used corticosteroids and antihistamines, whether topical and/or systemic, for less than 21 days before the prick test were not included in the study group. Additionally, dogs with hyperkeratosis, lichenification or hyperpigmentation; aggressive and/or agitated patients who required sedation or anesthesia for the test; and patients with other systemic or dermatological diseases were also excluded. Furthermore, all the dogs in the study were indoor dogs, meaning they lived exclusively inside their owners' homes, with only occasional contact with the external environment, mainly during supervised walks.

For the prick test, standardized compounds were used with saline-based solutions as the negative control (NC) and histamine (10 mg/mL) as the positive control (PC). The environmental allergen extracts used for testing were *Dermatophagoides farinae* (Df), *Dermatophagoides pteronyssinus* (Dp), *Blomia tropicalis* (Bt), *Cynodon dactylon* (Cd), *Lolium perenne* (Lp), *Paspalum notatum* (Pn), and mosquitoes (*Culex* spp. and *Aedes aegypti*), containing 10 HEP of potency (equivalent to 10,000 UBE/mL). All extracts and controls were prepared by the Veterinary Allergenics Laboratory at a concentration of 50% glycerol and 0.45% phenol to ensure their preservation and stability during application on the skin of dogs and cats. Although it is well established that dogs require higher allergen concentrations for reliable skin test responses due to their lower cutaneous reactivity, in Brazil, only the concentrations formulated for human use are available, which may affect the sensitivity and accuracy of the test results.

The allergen drops are placed in individual standardized capsules on an allergen tray. They are perfectly occluded with a double-tipped Duotip Test II® pricker (Lincoln Diagnostics, Decatur, USA) for skin testing, which remains immersed in the extract. The tray is stored under refrigeration at temperatures between 6 °C and 10 °C. However, this method may result in lower repeatability and reproducibility of the test, as variations in the amount of allergen transferred, differences in pressure applied during pricking, and potential inconsistencies in extract concentration over time can affect test outcomes. To minimize these issues, the same veterinarian performs all the tests, ensuring consistency in technique and application. Additionally, the veterinarian undergoes specific training to standardize the procedure and reduce the likelihood of variability in the results.

For the test, a trichotomy of approximately 12 x 15 cm was performed on the lateral thoracic region, followed by cleaning with a saline solution and marking the skin with a dermographic pen, with points spaced 1.5 cm apart. The prick was made next to or below each marking, using prickers immersed in the specific allergens to be tested. These were pressed at an angle of 45 to 60 degrees against the skin surface to allow the allergen to penetrate the epidermis. The test was read 15 minutes after the prick, where erythematous papules were visible, marked, and measured (average of the largest diameter) using a caliper. Animals were considered positive/sensitized to the allergens when the average orthogonal diameter of the papule produced by the allergen was at least 3 mm greater than the average diameter of the papular reaction formed by the negative control. The illustration of the prick test procedure is shown in Figure 1.

The results for animals that underwent the prick test, according to the inclusion and exclusion criteria described above, were collected from medical records.

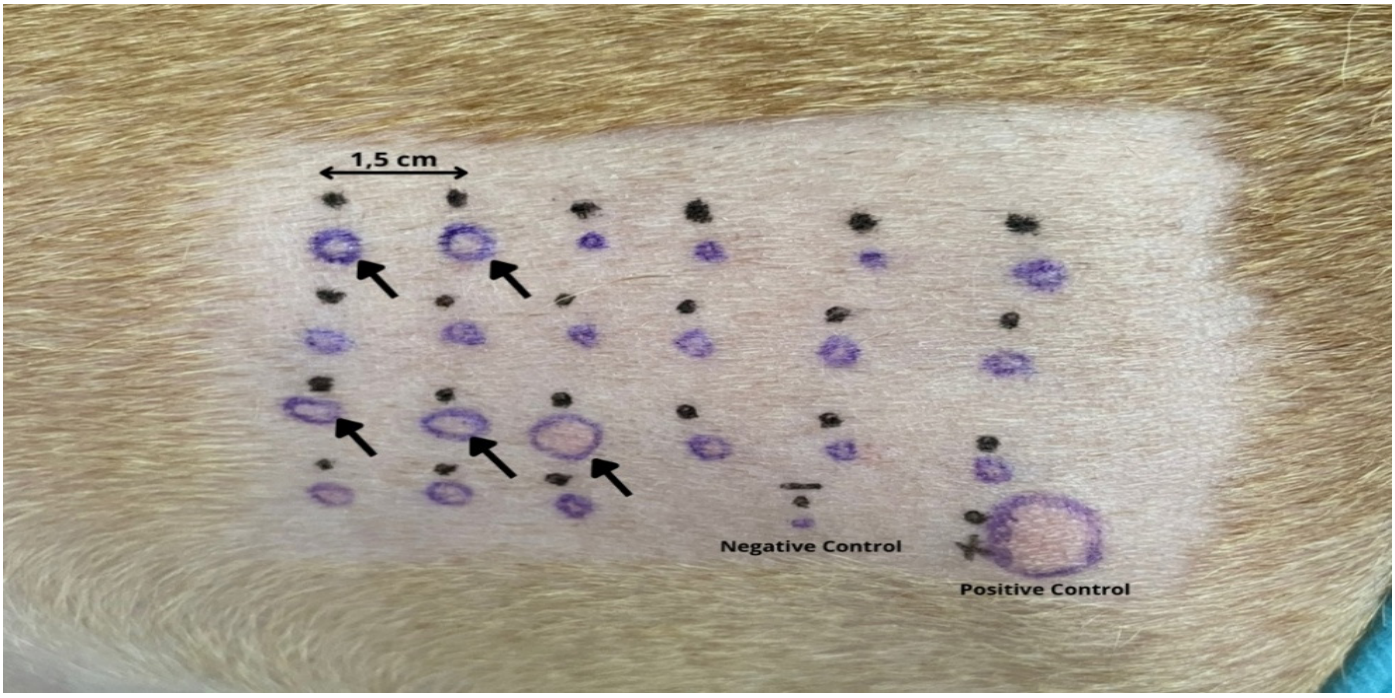


Fig. 1. Prick test performed on the lateral thorax of a canine patient, showing the points used for the prick, with a 1.5 cm distance between them, positive and negative controls, as well as the positive papules indicated by arrows.

The epidemiological profile, including data such as breed, sex, and age, was presented descriptively. The positivity of environmental allergens was also presented descriptively, in terms of percentages and absolute values. Statistical analysis was performed to evaluate the association between sensitizations to different environmental allergens. The association between categorical variables was assessed using the chi-square test. Statistical significance was set at $p < 0.05$. Additionally, odds ratios (OR) were calculated as a descriptive measure of the strength of association between sensitizations within the same population; however, no causal inference or risk estimation was intended. Statistical analysis was performed using OpenEpi version 3.01.

RESULTS

A total of 200 dogs met the study's inclusion criteria over a two-year period (2022–2024), with 68.5% (137/200) being females and 31.5% (63/200) being males. Among these, 75.18% (103/137) of the females and 68.25% (43/63) of the males were neutered. The ages ranged from 1 to 15 years, with an average of 5.23 years. Regarding breeds, 33% (66/200) were Shih Tzu, followed by 17.5% (35/200) mixed breed dogs, 8% (16/200) Lhasa Apso, 7.5% (15/200) Golden Retriever, 5.5% (11/200) French Bulldog, and 4% (8/200) Pug and Pinscher. The overall frequency of these breeds and others with lower representation is included in Table 1.

The Shih Tzu breed, despite having a higher number of positive reactions (66/200) than other breeds, did not show statistical significance by the chi-square test ($p > 0.05$), indicating that allergen positivity was similar across breeds.

All animals used in the study had an indoor lifestyle. Among these, 66% (132/200) had access to grasses during walks or within their own residence.

Frequency of sensitization to environmental allergens

The mean wheal diameter induced by mite extracts ranged from 4 to 5 mm, while pollen extracts produced wheals measuring from 3 to 4 mm. In contrast, the negative control (NC) resulted in minimal to no wheal formation, with diameters ranging from 0 to 1 mm. The interpretation of wheal diameter was not a limiting factor in this study, as the observed reactions were consistent and distinguishable among allergen groups, ensuring the reliability and reproducibility of the intradermal test in evaluating hypersensitivity responses.

Table 1. Epidemiological data of dogs in the present study (Ourinhos/SP, Brazil, 2024)

Characteristic	Subgroup	N	%
Sex	Female	137	68.5%
	Male	63	31.5%
Neutered dogs	Neutered females	103	75.18%
	Neutered males	43	68.25%
Age	Average (years)		5.23
	Age range	1–15	
Breeds	Shih Tzu	66	33%
	Mixed breed	35	17.5%
	Lhasa Apso	16	8%
	Golden Retriever	15	7.5%
	French Bulldog	11	5.5%
	Pug	8	4%
	Pinscher Doberman	8	4%
	Yorkshire	7	3.5%
	Poodle	5	2.5%
	Maltese	4	2%
	Schnauzer	3	1.5%
	Poodle Toy	2	1%
	Jack Russel	2	1%
	Daschund	2	1%
	Boxer	2	1%
	Border Collie	2	1%
	American Bully	2	1%
	German Spitz	2	1%
	Pitbull	1	0.5%
	Shetland Sheepdog	1	0.5%
	Pekingese	1	0.5%
	Labrador	1	0.5%
	Fox Paulistinha	1	0.5%
	Cocker Spaniel	1	0.5%
	English Bulldog	1	0.5%
	American Staffordshire	1	0.5%
TOTAL		200	100%

Of the total cases that participated in the study, 91% (182/200) showed a positive reaction to one or more of the tested house dust mites, with 10.5% (21/200) showing a positive reaction to all mites and 9% (18/200) showing no positivity to any of them. The positivity rates were as follows: 53% (106/200) for *Dermatophagoides farinae* (Df), 53.5% (107/200) for *Dermatophagoides pteronyssinus* (Dp), and 54% (108/200) for *Blomia tropicalis* (Bt).

Regarding grass pollen, 59% (118/200) showed a positive reaction to one or more of the tested pollens, with only 3% (6/200) showing a positive reaction to all pollens tested and 41% (82/200) showing no positivity to any of them. The positivity rates were: 31.5% (63/200) for *Cynodon dactylon* (Cd), 26.5% (53/200) for *Lolium perenne* (Lp), and 27.5% (55/200) for *Paspalum notatum* (Pn).

For mosquitoes (M), 45% (90/200) showed a positive reaction, and 55% (110/200) showed a negative reaction. The prevalence of positive and negative reactions was grouped individually and in groups in Table 2 and 3.

Comparison of sensitization among environmental allergens

Associations between sensitizations to different allergens were evaluated using the chi-square test (Table 4).

Odds ratios (OR) were calculated as a descriptive measure of the strength of association between sensitizations (Table 5), without implying causality or risk.

The findings in Table 5 show that Df and Dp exhibited no significant associations with most other allergens, with OR values close to 1 (0.98 and 0.96, respectively). However, Df showed a strong co-sensitization with other allergens such as Cd, Lp and Pn, with OR values ranging from 2.45 to 3.13. Similarly, Dp exhibited a positive co-sensitization with grasses (Cd, Lp, Pn) and mosquitoes, with OR values between 2.50 and 3.19, suggesting a significant link between these allergens.

In contrast, certain comparisons, such as between Cd and Lp or Cd and Pn, demonstrated a weak co-sensitization (OR values of 1.28 and 1.21, respectively), while others, such as the comparison between Cd and mosquitoes, showed a moderate negative co-sensitization (OR = 0.56).

Regarding the occurrence of reactions after the prick test, none of the 200 tested animals exhibited systemic anaphylactoid reactions. However, two individuals did present with eczema-like plaques and pruritus at the site of the prick. These animals were treated with prednisone at a dose of 0.5 mg/kg every 12 hours for three days, with no further complications.

DISCUSSION

The epidemiological profile analysis revealed that 68.5% of the dogs were females, a finding that aligns with previous studies (Halliwell 1971, Zur et al. 2002). However, other authors suggest a higher predisposition in males, indicating that the influence of sex remains controversial in AD (Harvey et al. 2019, Favrot et al. 2010).

The higher frequency of females in this study may reflect a greater representation of female dogs in the hospital

Table 2. Frequency of dogs with positive and negative reactions to each allergen (Ourinhos/SP, Brazil, 2024)

Allergen	Positive		Negative	
	n	%	n	%
*Blomia tropicalis**	108	54	92	46
*Dermatophagoides pteronyssinus**	107	53.5	93	46.5
*Dermatophagoides farinae**	106	53	94	47
Mosquitoes *(Culex* spp. and *Aedes aegypti)*	90	45	110	55
*Cynodon dactylon***	63	31.5	137	68.5
*Paspalum notatum***	55	27.5	145	72.5
*Lolium perenne***	53	26.5	147	73.5

* House dust mites, ** grass pollen.

Table 3. Prevalence of dogs with positive and negative reactions (Ourinhos/SP, Brazil, 2024)

Allergen	Positive to at least one	Positive to all	Negative to all
House dust mites	182 (91%)	21 (10.5%)	18 (9%)
Grass pollens	118 (59%)	6 (3%)	82 (41%)

Table 4. Significance coefficient (*p*-value) using the chi-square method for comparisons between reactions (Ourinhos/SP, Brazil, 2024)

Allergen	Df	Dp	Bt	Cd	Lp	Pn	M
Df	-	0.92	0.84	< 0.01	< 0.01	< 0.01	0.1
Dp		-	0.87	< 0.01	< 0.01	< 0.01	0.09
Bt			-	< 0.01	< 0.01	< 0.01	0.07
Cd				-	0.37	0.28	< 0.01
Lp					-	0.82	< 0.01
Pn						-	< 0.01
M							-

Df = *Dermatophagoides farinae*, Dp = *Dermatophagoides pteronyssinus*, Bt = *Blomia tropicalis*, Cd = *Cynodon dactylon*, Lp = *Lolium perenne*, Pn = *Paspalum notatum*, M = mosquitoes.

population or a potential preference for female dogs in household environments. Factors such as owner preference, breeding practices, and differences in lifestyle or exposure to allergens could contribute to this observed distribution. Therefore, while epidemiological data suggest a predominance of females, this may not necessarily indicate a true sex predisposition for atopic dermatitis but rather a bias related to population dynamics.

Regarding age, the average was five years, consistent with previous studies that indicate clinical signs typically start in puppies or young adults, although they can also appear at older ages. However, many animals had a history of clinical signs starting at younger ages, with owners delaying veterinary visits, suggesting that the onset of clinical signs occurred below this average (Marsella & Olivry 2003, Olivry et al. 2010, Outerbridge & Jordan 2021).

Breed predisposition is well documented, suggesting that genetic factors play a significant role in AD (Nødtvedt et al. 2007, Hensel et al. 2024). In Brazil, the Shih Tzu is frequently reported as one of the most common breeds among domestic dogs, with a significant prevalence in veterinary cases (Paula 2019). Although the Shih Tzu was the most affected breed in this study (33% of cases), there was no statistical significance regarding allergen positivity when compared to other breeds.

The lack of statistical significance regarding allergen sensitization among different breeds suggests that, although genetic predisposition influences the development of atopic dermatitis (AD), environmental exposure plays a crucial role in determining allergen reactivity (Olivry 2011, Marsella et al. 2012). Factors such as geographic location, climate, and household conditions contribute to the types and levels of allergen exposure, potentially homogenizing sensitization patterns across breeds (Pali-Schöll et al. 2021). Additionally, while certain breeds may have a genetic susceptibility to AD, the immune response to specific allergens is complex and multifactorial, involving not only genetic predisposition but also individual immune tolerance and epigenetic factors (Nødtvedt et al. 2007, Hensel et al. 2024). Therefore, the high prevalence of Shih Tzus in this study likely reflects their overall representation in the population rather than an inherent difference in allergen sensitization.

Sensitization to dust mites was high (91%), with no statistical difference between tested species, corroborating previous findings (Carmona-Gil et al. 2019). Studies show that dogs with AD have higher concentrations of mites both on their skin and in their environment, reinforcing the role of environmental control in disease management (Assunção et al. 2017). The studied population, predominantly upper-middle-class, frequently interacts with upholstered furniture and bedding, favoring mite proliferation (Ludwig et al. 2021). The humid tropical climate of Ourinhos (SP), with average temperatures of 26.5 °C and high humidity, creates ideal conditions for these allergens, explaining the high sensitization observed, similar to studies in regions with comparable climates, such as Botucatu (Mazziero 2024).

The fact that all study dogs lived indoors supports the "hygiene hypothesis", which suggests that dogs raised predominantly in closed environments have a higher predisposition to AD due to reduced exposure to external immunomodulatory factors (Flohr & Yeo 2011). This correlation is evident in breeds such as the Shih Tzu, which live in close contact with mite reservoirs, increasing exposure to these allergens (Harvey et al. 2019,

Table 5. Odds ratios (OR) as a descriptive measure of association between sensitizations to environmental allergens (Ourinhos/SP, Brazil, 2024)

Compared allergens	Odds ratio (OR)	Interpretation
Df x Dp	0.98	No significant association
Df x Bt	0.96	No significant association
Df x Cd	2.45	Positive association
Df x Lp	3.13	Positive association
Df x Pn	2.97	Positive association
Df x M	1.38	Moderate positive association
Dp x Bt	0.98	No significant association
Dp x Cd	2.50	Positive association
Dp x Lp	3.19	Positive association
Dp x Pn	3.03	Positive association
Dp x M	1.41	Moderate positive association
Bt x Cd	2.55	Positive association
Bt x Lp	3.26	Positive association
Bt x Pn	3.09	Positive association
Bt x M	1.43	Moderate positive association
Cd x Lp	1.28	Weak positive association
Cd x Pn	1.21	Weak positive association
Cd x M	0.56	Moderate negative association
Lp x Pn	0.95	No significant association
Lp x M	0.44	Negative association
Pn x M	0.46	Negative association

Df = *Dermatophagoides farinae*, Dp = *Dermatophagoides pteronyssinus*, Bt = *Blomia tropicalis*, Cd = *Cynodon dactylon*, Lp = *Lolium perenne*, Pn = *Paspalum notatum*, M = mosquitoes.

Hensel et al. 2024). Studies in Switzerland indicate that dogs with AD spend more time indoors and are more sensitized to household allergens than humans (Nødtvedt et al. 2007).

Findings reinforce the interaction between the exposome, genome, endotypes, and phenotypes in canine AD, demonstrating that environmental factors modulate immune response (Marsella 2021). Prolonged exposure to household allergens appears to intensify the Th2-mediated inflammatory response characteristic of AD (Hensel et al. 2015). Genetic predisposition determines susceptibility, but clinical manifestation depends on environmental exposure, revealing different clinical phenotypes and allergen reactivity patterns (Ferreira et al. 2023).

Sensitization to grass pollen was lower than to mites but still significant (59%). In comparison, a study in Paraná reported 25.6% positivity, with *Cynodon dactylon* (Cd) as the most relevant species (Cerdeiro et al. 2021). In the present study, Cd showed 31.5% positivity. In France, sensitization was 21.5% (Roussel et al. 2013), suggesting regional variations. The humid subtropical climate of Ourinhos favors grass proliferation, increasing environmental allergen load and sensitization. Additionally, 66% of dogs had access to grassy areas, increasing pollen exposure.

A notable observation in this study was the identification of dogs with polysensitization, meaning concurrent sensitization to both mites and grass pollen. Sensitization to house dust mites is known to activate a type 2 inflammatory response, characterized by Th2 cell activity and cytokine release (IL-4, IL-5, and IL-13), which promotes IgE production and mast cell degranulation, leading to inflammation and pruritus (Pereira et al. 2015). In cases of polysensitization, the presence of multiple allergens may amplify this response, exacerbating the clinical severity of AD.

Polysensitization also has implications for skin barrier integrity. The type 2 inflammatory response impairs the production of filaggrin, a key protein essential for maintaining epidermal function. As a result, the skin becomes more permeable, facilitating further allergen penetration and worsening inflammation (Kang et al. 2014). This cyclical process can contribute to chronic disease progression, making management more challenging.

In this study, statistical analyses revealed significant co-sensitization ($p < 0.05$) between house dust mites (Df and Dp) and grass pollens (Cd, Lp, and Pn), further underscoring the role of mites as primary environmental allergens influencing sensitization patterns in atopic dogs (Nødtvedt et al. 2007, Assunção et al. 2017, Carmona-Gil et al. 2019, Ludwig et al. 2021). The associations observed between sensitizations to different allergens suggest patterns of co-sensitization, which may be related to shared environmental exposure or possible cross-reactivity between allergens (Kang et al. 2014). The evaluation of these associations aimed to explore such patterns within the studied population, providing insights into the complexity of allergen sensitization in dogs with atopic dermatites (Carmona-Gil et al. 2019, Marsella 2021). However, these findings should be interpreted with caution, as they do not imply causality.

Positivity for mosquito allergens was 45%, a relevant finding considering the ongoing debate about their role in AD (Sousa & Halliwell 2001). Studies indicate possible cross-reactivity between mosquito, flea, and tick allergens (Pucheu-Haston et al. 1996). In tropical regions, where insect exposure is higher, mosquito allergen sensitization may be associated with allergic reactions to ectoparasites (Cantillo et al. 2015). This underscores the need for regional studies to assess the influence of insects on the pathogenesis of canine AD.

It is important to emphasize that the results obtained through the skin prick test indicate sensitization to the tested allergens, reflecting the presence of allergen-specific IgE. However, sensitization does not necessarily correspond to a clinically relevant allergic condition. Therefore, these findings should be interpreted with caution, as they do not confirm that the identified allergens are directly responsible for triggering clinical signs of atopic dermatitis in the evaluated dogs (Carmona-Gil et al. 2019. Marsella 2021).

Study limitations included the exclusion of patients due to improper corticosteroid use and the need for sedation. The prick test showed 66% sensitivity and 100% specificity in identifying allergens in dogs with AD (Alcalá et al. 2023). Studies indicate that it can detect sensitizations more frequently than intradermal testing while maintaining similar specificity (Santoro 2019). However, the accuracy of these tests may be influenced by extract quality, application technique, and patient clinical status.

Despite these limitations, the tests were practical and effective in identifying relevant allergens. Allergen-specific immunotherapy (ASIT) is the only approach capable of modulating the immune response and reducing long-term Th2 pathway activation, making it essential for highly sensitized patients like those in the present study (Favrot et al. 2010, Santoro 2019, Fischer et al. 2020).

CONCLUSIONS

This study highlighted the highest frequency of sensitization to house dust mites, with particular emphasis on *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp), followed by grass pollen and mosquitoes in dogs with atopic dermatitis in the region of Ourinhos (SP, Brazil).

These findings highlight the diversity of environmental allergens to which dogs may be sensitized in this geographic area and suggest that house dust mites may represent an important component of the sensitization profile in these patients. However, the results of the skin prick test reflect sensitization and do not necessarily indicate a clinically relevant allergic condition. Therefore, while environmental control measures, particularly those aimed at reducing exposure to house dust mites, may be considered as part of the clinical management of atopic dogs, further studies are required to determine the clinical relevance of these sensitizations and their direct impact on disease expression.

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Data availability statement.- All data supporting the findings of this study are available from the corresponding author upon reasonable request.

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