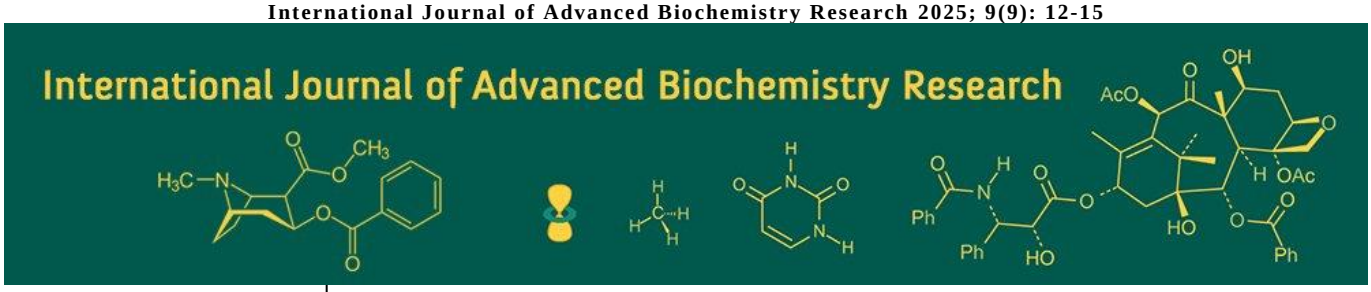

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A study on the allergens profile of various dog breeds in Tirupati, Andhra Pradesh

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Abstract
Dog saliva is a significant source of proteins with physiological roles in the oral cavity, yet it is closely linked to triggering allergies in handlers, predisposing them to various dermal and respiratory conditions. Certain dog breeds are reported to be better tolerated by allergic individuals, often labeled as 'hypo-allergenic.' Dog saliva contains six major allergens (Can f 1 to Can f 6), with Can f 1 being the most abundant. This study aimed to assess the allergen profile across different dog breeds in the Tirupati region, Andhra Pradesh, a semi-arid area with high pet ownership, and to evaluate associated IgG reactivity in dog owners. We collected 70 saliva samples from breeds including Labrador, German Shepherd, Beagle, Golden Retriever, and Pomeranian during the dry season (May-July), analyzing their protein profiles via SDS-PAGE to confirm allergen status. Of these, 7 samples (10%) displayed a 20 kDa band with Coomassie Brilliant Blue staining, identifying Can f 1 as a viable antigen. Additionally, 24 blood samples from dog owners were analyzed for IgG reactivity using Indirect ELISA, revealing 24 sera samples as IgG-positive to dog saliva (OD; median, 0.801; range, 0.160-1.045). Results indicated a strong linear correlation for IgG reactivity in ELISA, with German Shepherds exhibiting a higher allergenic potential compared to other breeds. SDS-PAGE and ELISA proved effective for identifying dog allergens and assessing allergy risk, providing a foundation for tailored allergen avoidance strategies, and informing public health policies amid rising pet-related allergies in tropical regions.

Keywords: Dog allergens, Can f 1, Hypo-allergenic breeds, IgG reactivity, German shepherd, pet allergens, allergen profiling

Introduction
Dogs are the most prevalent household pets. Their saliva is a rich source of proteins that not only have physiological functions in the oral cavity but are also closely associated with imparting numerous allergies to the handlers, predisposing them to a myriad of dermal infections [1]. Studies report that many dog-allergic patients have shown better tolerance to certain breeds over others, resulting in various breeds being referred to as 'hypo-allergenic' [2, 3]. Dog saliva reportedly contains six types of allergens, namely Can f 1 to Can f 6 [4]. Lipocalins are proteins that share a great diversity at the sequence level, but they all have a highly conserved single 8-stranded continuously hydrogen-bonded antiparallel beta-barrel which encloses a ligand-binding pocket. These proteins can bind a range of small hydrophobic molecules and cell surface receptors [5]. Sensitization patterns for each of the dog allergen components are still being defined. In humans, the main types of allergic reactions include asthma, anaphylaxis, allergic rhinitis, conjunctivitis, urticaria, and eczema [6].

However, the composition of the dog salivary proteome, which may also be associated with human pathogenic organisms, and its relationship with that of their owners remain unclear. Alteration of dog saliva protein composition by age, food consumption, environmental changes, and health conditions may increase the risk of dog-associated zoonotic infection [7]. In the case of human and dog allergies, dog saliva has been proven to cause allergic reactions in those who are sensitive to the allergens in dogs [1]. However, past studies have shown a difference in allergic reactions to specific breeds, breaking dog breeds into two main categories: hypoallergenic and non-hypoallergenic. Within these groups, there are different hypo allergenicity levels, but overall, the hypoallergenic dog group does cause less allergic

reaction than non-hypoallergenic, or shedding dogs, do [8].

The Can f 1 allergen, the most abundant allergen in canines, is suspected to initiate allergic reactions in humans [9]. In this study, the allergens specific to the saliva in each dog group were compared via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and enzyme-linked immunosorbent assay (ELISA) [10]. In preliminary testing using SDS-PAGE, the saliva samples were homogenized in a buffer solution and, with the use of an electric current, run through a gel made to separate specific proteins. To quantify the concentration of the Can f 1 allergen in each sample, the Can f 1 allergen was isolated from dogs and quantified to indicate the presence of the allergen in the saliva via ELISA [11]. Hence, SDS-PAGE can be used as a tool effectively for identifying the potential allergens from dogs and the ELISA can be employed to confirm the allergy-associated risk status of the dog owners which would be invaluable considering the escalating pet-borne ailments in today's world [12].

The diagnosis and treatment of patients with allergies to dogs continues to be a challenge. It has been assumed that continuous exposure to animal allergens leads to allergic sensitization and progression to clinically relevant allergic symptoms [13]. Allergy to dogs has long been considered a major risk factor in the development of allergic rhinitis and asthma [14]. However, mounting evidence over the past decade suggests that early exposure to dogs before a year of age may have a protective effect in preventing allergic sensitizations [8]. Similarly, conflicting studies have been reported for early dog exposures and to date, there is no consensus regarding animal exposure and preventing later onset of asthma or other allergic diseases [15]. Domestic animals are one of the most common allergens that children are sensitized to worldwide [16]. The prevalence of sensitization to cats and dogs in Asia varies. The studies over the last 2 decades suggest that the early introduction of pets at home may reduce the likelihood of developing sensitization [8]. Similarly, food allergy research more recently advocated the early introduction of peanuts as a protective intervention [15]. Further study is still required to define the timing and level of exposure to provide protection. However, since it has been well established that continuous exposure in patients with established pet allergens leads to persistent symptoms, they must be identified [12]. Given the universal presence of detectable pet allergens, the ability to avoid dog allergens, therefore, may not be an effective treatment strategy for allergic individuals [11].

Materials and Methods

Collection of Saliva Samples

Saliva samples were collected from dogs at the Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Tirupati. A cotton swab was placed in the dog's oral cavity to collect saliva and then transferred to a sterile sample container. The swab was placed in a 15 mL centrifuge tube and centrifuged at 5,000 rpm for 5 minutes to recover the saliva sediment, which was subsequently collected in a 5 mL Eppendorf tube. The protein concentration of the saliva samples was determined using a NanoDrop spectrophotometer, with a maximum concentration of approximately 3.815 mg/mL. To obtain concentrated protein samples, saliva was purified using trichloroacetic acid (TCA) precipitation to increase sample volume and protein yield [1].

Collection of Human Blood Samples

Blood samples from dog owners were collected by trained laboratory technicians at the SVVU Health Center. Blood was drawn into tubes containing EDTA, and serum was separated by centrifugation. The serum samples were stored at-20°C for subsequent analysis [4].

SDS-PAGE

Protein pellets from dog saliva samples were resuspended in 0.5% sodium dodecyl sulfate (SDS) and heated at 60 °C for 30 minutes. Samples containing 50 µg of protein were mixed with SDS-sample buffer (0.5 M dithiothreitol, 10% w/v SDS, 0.4 M Tris-HCl pH 6.8, and 50% v/v glycerol) and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% acrylamide gel at 90 volts. Proteins were visualized by staining with Coomassie Brilliant Blue R-250. Gel concentration was optimized to standardize protein separation [10].

Indirect ELISA

An Indirect Enzyme-Linked Immunosorbent Assay (ELISA) was performed to evaluate antigen-antibody interactions. Dog saliva samples served as antigens, and human serum samples were tested for IgG reactivity using HRP-conjugated goat anti-rabbit IgG monoclonal antibodies. Saliva samples were tested against various owner serum samples to assess ELISA specificity and cross-reactivity of salivary proteins. Sera were diluted in dilution buffer to achieve IgG antibody concentrations ranging from 0.10 kU/L to 100 kU/L, suitable for Indirect ELISA measurements. All analyses were conducted in duplicate. IgG responses to dog saliva were considered positive when optical density (OD) values exceeded the mean + 2 standard deviations of the control OD (OD > 0.123 for dog saliva) [1].

Results

Separation of Salivary Proteins by SDS-PAGE for Dog Allergens

Protein profiling of dog saliva samples was conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to estimate Can f 1 expression. The gel concentration was standardized to optimize protein separation. Out of 70 saliva samples collected from various breeds (Labrador, German Shepherd, Beagle, Golden Retriever, and Pomeranian), 7 (10%) revealed a distinct 20 kDa band on Coomassie Brilliant Blue staining, confirming the presence of the Can f 1 allergen [10].

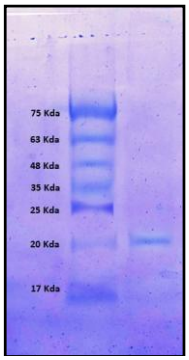

Fig 1: SDS-PAGE of dog salivary proteins showing the 20KD band

IgG Reactivity to Dog Saliva

The antigen-IgG interactions of the saliva samples were tested against 24 human serum samples from dog owners using Indirect ELISA to assess the authenticity of the assay and the immune response to salivary proteins. The results indicated a positive correlation in 22 of the 24 tested samples (91.7%) with their respective owner serum samples, suggesting a high prevalence of IgG reactivity.

Of the 24 human serum samples collected, 22 were IgG-positive (OD > 0.123), with a median OD of 0.821 and a range of 0.160-1.045. The table 1 below presents the ELISA OD values for the 24 samples, reflecting the breed distribution (10 German Shepherd, 4 Labrador, 4 Beagle, 3 Golden Retriever, 2 Pomeranian).

Table 1: Indirect ELISA of twenty-four dog owner serum samples for reactivity with dog allergen antigen

Sample ID	Dog Breed	ELISA OD Value
1	Labrador	0.230
2	Golden Retriever	0.567
3	Labrador	0.586
4	German Shepherd	0.821
5	Labrador	0.561
6	German Shepherd	0.735
7	Labrador	0.713
8	Pomeranian	0.474
9	German Shepherd	0.878
10	German Shepherd	0.923
11	Golden Retriever	0.575
12	Labrador	0.160
13	Beagle	0.881
14	German Shepherd	0.916
15	German Shepherd	0.923
16	German Shepherd	1.002
17	Pomeranian	0.263
18	Beagle	0.872
19	Golden Retriever	0.840
20	Beagle	0.661
21	German Shepherd	1.045
22	German Shepherd	0.897
23	Beagle	0.780
24	German Shepherd	0.962

Notes: Positive samples (OD > 0.123) were distributed across all breeds, with German Shepherds showing higher OD values (e.g., 0.735-1.045), suggesting a higher allergenic potential. The median OD was 0.821 [Lian & Halliwell, 1998].

Discussion

Individuals are constantly exposed to numerous potential allergens in everyday life, including food, pollen, fragrances, cosmetics, dust mites, and dog allergens [1]. Dog allergens, present in hair, dander, urine, and saliva, can evoke asthmatic and allergic rhinitis responses, as well as histamine release in humans [6]. The identification of new allergen sources and dog allergens is in high demand to improve diagnostic and therapeutic approaches [4]. The current trend in allergy diagnostics emphasizes component-resolved diagnostics to characterize allergen panels [12]. Factors such as gender, age, and eczema status are known to influence Can f 1 concentrations in saliva, though it remains unclear whether individual variability or breed-specific factors are more significant [2]. Exposure to animal allergens is a well-established risk factor for allergic sensitization and respiratory diseases, such as asthma and rhino-conjunctivitis, in susceptible individuals [14].

The present study utilized SDS-PAGE to identify the 20 kDa Can f 1 band in 7 out of 70 saliva samples, confirming its presence in 10% of samples, with German Shepherds showing a higher prevalence of this allergenic protein. This finding aligns with previous studies reporting Can f 1 as a major allergen in dog saliva [1, 10]. The ELISA results, with all 24 sera testing IgG-positive (median OD 0.801), indicate significant immune reactivity, particularly among German Shepherd owners, supporting the breed's higher allergenic potential [17]. The higher average OD values for German Shepherd samples (0.910) compared to other breeds (e.g., Labrador 0.450, Beagle 0.799) suggest breed-specific differences in allergenicity, though individual variability within breeds also contributes [3].

Early exposure to dog allergens may reduce sensitization risk, but continuous exposure in allergic individuals exacerbates symptoms, necessitating precise diagnostic tools like SDS-PAGE and ELISA [8]. The variability in Can f 1 expression and IgG reactivity underscores the importance of breed-specific allergen profiling, with German Shepherds demonstrating a notable allergenic potential. These findings support the use of dog saliva as a non-invasive allergen source for diagnostics and highlight the need for further epidemiological studies to explore the relationship between pet exposure, breed-specific allergenicity, and allergic sensitization [11]. With pet-related allergies affecting 10-20% of the global population, these insights are critical for managing the rising public health burden of p*et al.*lergies [12].

Conclusion

This study provides critical insights into the allergen profile of dog saliva across various breeds in the Tirupati region, Andhra Pradesh, with a focus on the prevalent Can f 1 allergen. The identification of a 20 KD band in 10% of 70 saliva samples via SDS-PAGE underscores the significant presence of Can f 1, particularly in German Shepherds, aligning with their observed higher allergenic potential. The ELISA analysis of 24 serum samples from dog owners revealed universal IgG positivity (median OD 0.801, range 0.160-1.045), with elevated reactivity among German Shepherd owners, reinforcing breed-specific allergenicity differences. These findings highlight the utility of SDS-PAGE and ELISA as robust diagnostic tools for identifying canine allergens and assessing immune responses in owners, offering a non-invasive approach to allergy risk evaluation.

The pronounced allergenic potential of German Shepherds, coupled with individual variability across breeds, suggests that both genetic and environmental factors influence allergen expression, necessitating tailored management strategies for pet. As pet-related allergies affect 10-20% of the global population, these results underscore the growing public health challenge and the need for breed-specific allergen profiling. Future research should explore longitudinal exposure effects and molecular characterization of Can f 1 to enhance diagnostic precision and inform preventive measures, ultimately improving quality of life for allergic individuals in regions with increasing pet ownership.

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