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Original Research

Diagnosis of Canine Atopic Dermatitis in Pugs by Intradermal Test

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Abstract

Present study was undertaken to identify the common aeroallergens associated with atopic dermatitis in Pugs. Total of 50 Pugs were selected for the study based on history, clinical signs and after fulfilment of criteria for the diagnosis of canine atopic dermatitis and were subjected to intradermal test. Pruritus was the outstanding clinical sign observed in all the cases followed by licking of paws (92%), rubbing of face (70%), otitis externa (40%), scratching of the ears (34%) and rubbing of eyes (20%). In the present study majority of the Pugs showed positive reactions to house dust mites, Dermatophagoides farinae (61.11%) and Dermatophagoides pteronyssinus (55.56%), followed by human dander (55.56%), cockroach (Periplanata americana) (41.67%), House Dust (27.78%) and Amaranthus spinosus (19.44%). In conclusion indoor allergens were associated with atopic dermatitis in pugs compared to outdoor allergens.

Keywords: Intradermal Test, Canine Atopic Dermatitis

Introduction

Canine atopic dermatitis (CAD) is a common skin disease that small animal practitioners will see on an almost daily basis. Atopic dermatitis is a complex, multifactorial disease (Olivry *et al*., 2005). It is defined as "a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens" (Halliwell, 2006). Environmental allergens involved in atopic dermatitis include, house dust mite proteins, plant pollen, mold spores, and various other allergens. There is no single specific test to diagnose atopic dermatitis. A sub-group of the International Committee for Allergic Diseases in Animals (ICADA) developed a set of practical guidelines for the diagnosis of canine AD and these includes ruling out of other skin conditions with clinical signs that can resemble, or overlap with canine AD, detailed interpretation of the historical and clinical features of the condition by application of "Favrot's criteria" (Favrot *et al*., 2010) and assessment of skin reactivity by intradermal testing (IDT) or detection of IgE by allergen-specific IgE serology (ASIS) testing.

Favrot's set 1 criteria for diagnosis of CAD include, age at onset should be less than 3 years, mostly indoor, corticosteroid-responsive pruritus, chronic or recurrent yeast infections, affected front feet, affected ear pinnae, non-affected ear margins and non-affected dorsolumbar area and set 2 criteria include, age at onset less than 3 years, mostly indoor, pruritus sine materia (ie, without lesions) at onset, affected front feet, affected ear pinnae, non-affected ear margins and non-affected dorsolumbar area (Favrot *et al*. 2010).

Intradermal testing is the method most commonly used and the most reliable for the diagnosis of atopic dermatitis (Willemse, 1986). IDSTs have been accepted as the 'gold standard' for the diagnosis and management of atopic skin disease because it is believed to most closely reflect the natural disease pathogenesis in the dog (Willemse, 1986; Codner and Lessard, 1993). IDT is an indirect measure of cutaneous mast cell reactivity due to presence of IgE (Hiller and DeBoer, 2001).

Materials and Methods

Total of 50 Pugs presented to Veterinary College Hospital, Bengaluru showing one or more clinical signs of atopic dermatitis were selected as subjects for the study. Pugs were considered as atopic mainly based on history, clinical signs and after fulfilment of criteria for the diagnosis of canine atopic dermatitis (Favort *et al*., 2010) and were subjected to intradermal test.

Twenty-two aqueous allergens were selected based on geographical prevalence, previous study (Vaseem, 2008) and on information collected from human allergy testing centres. All twenty-two allergens including positive and negative control solutions were procured from All Cure's Pharma Pvt. Ltd., New Delhi (Table 1). The dilution of the allergens used was as per the recommendations ascribed for intradermal test (Reedy *et al*., 1997; Scott *et al*., 2001). The allergens were used in the concentration of 1:1000 w/v, except dust mites (1:5000 w/v) (Vaseem, 2008) and the allergens were stored at 2-8ºC when not in use. Control agents for intradermal tests were used as per the standard procedure. Histamine phosphate (1:10000 w/v) was used as positive control and 0.9% phosphate buffered saline was used as negative control (Reedy *et al.,* 1997; Scott *et al.,* 2001).

Intradermal Test Procedure

Before performing intradermal test antihistamines and oral glucocorticoids were withdrawn for 10 days and 3 weeks respectively. Intradermal test was performed in 50 Pugs suspected for CAD. Test was conducted as per the standard procedure given by Reedy *et al*. (1997) and Scott *et al*. (2001).

Hair was clipped in a rectangular shape on the lateral aspect of the thorax using electrical clipper with number 40 blade and the area was not scrubbed and washed. Injection sites were marked with waterproof marker pen 2cm apart. A volume of 0.05ml of each allergen and positive and negative control solutions were injected intradermally. The results were interpreted at 25- 30 minutes post injection and results were evaluated objectively by measurement of diameter or area of wheal or erythema by using measuring scale. Positive reactions were designated as reactions that are at least equal to or greater than halfway between the reactions observed for negative and positive controls or reactions that are at least 3mm greater in diameter than the negative control (Scott *et al*., 2001).

Table 1: List of allergens used in intradermal test

S. No.	Name	Group
1.	*Dermatophagoides farinae*	House Dust Mite
2.	*Dermatophagoides pteronyssinus*	House Dust Mite
3.	*Albizzia lebbeck*	Pollen
4.	*Amaranthus spinous*	Pollen
5.	*Azadirachta indica*	Pollen
6.	*Cassia occidentalis*	Pollen
7.	*Cynodon dactylon*	Pollen
8.	*Partenuim hysterophrous*	Pollen
9.	*Pennisetuim typhoides*	Pollen
10.	*Typha angustata*	Pollen
11.	Cockroach (Male) (*Periplanata americana*)	Insect
12.	*Aspergillus flavus*	Fungi
13.	*Candida albicans*	Fungi
14.	*Pencillium spp.*	Fungi
15.	*Trichoderma spp..*	Fungi
16.	House dust	Dust
17.	Paper dust	Dust
18.	Cat dander	Epithelia
19.	Human dander	Epithelia
20.	Kapok cotton	Fabrics
21.	Wool mix	Fabrics
22.	*Parthenium* leaves	Miscellaneous

Results and Discussion

Pruritus was the outstanding clinical sign observed in 100 per cent of the cases. Other clinical signs recorded in atopic Pugs were, licking of paws (92%), rubbing of face (70%), otitis externa (Plate 1&2) (40%), scratching of the ears (34%) and rubbing of eyes (20%). Clinical signs recorded in the present study correlate with the findings of Scott *et al*. (1981), Willemse (1986), Umesh (1992), Saridomichelakis *et al.* (1999), Vaseem (2008) and Favrot *et al*. (2010). In canine atopic dermatitis (CAD) the clinical manifestations are due to the effect of vasoactive amines and in the early stages, clinical signs are characterized by edema, erythema, and pruritus (Chamberlain, 1974).

Canine mast cells are known to produce a variety of inflammatory mediators that are either stored in the granules or produced de novo following activation. These include histamine (De Mora *et al*., 1993), tryptase (Myles *et al*., 1995), chymase (Schechter *et al*., 1988), leukotrienes (Marsella and Nicklin, 2001) and tumor necrotic factor ά (Thomas *et al*., 1996). These mediators are responsible for the complex interplay between the microvasculature and other inflammatory cells that results in the many of the clinical signs of atopic dermatitis. The most important of the vasoactive molecules released by mast cells is histamine. The effects of histamine is mediated through several different receptors. H1 and H2 receptors are expressed on nerve cells, smooth muscle cells, endothelial cells, neutrophils, eosinophils, monocytes, T and B cells. Histamine binding to H1 receptors stimulates endothelial cells to convert L- arginine to nitric oxide, a very potent vasodilator. At the same time histamine causes vascular leakage, leading to fluid accumulation and local edema (Tizard, 2004). Otitis externa was recorded in 40 per cent of the atopic dogs, this is slightly lesser than Saridomichelakis *et al.* (1999) and Favrot *et al*. (2010) reports, who have reported 47.3% and 50% of otitis externa in atopic dogs respectively. The most common primary disease resulting in otitis externa is atopic dermatitis. Although the precise incidence of atopy is unknown, an estimated 10% of dogs have clinically significant atopy. Of these up to 80% exhibit otitis externa as part of their disease (Angus, 2005).

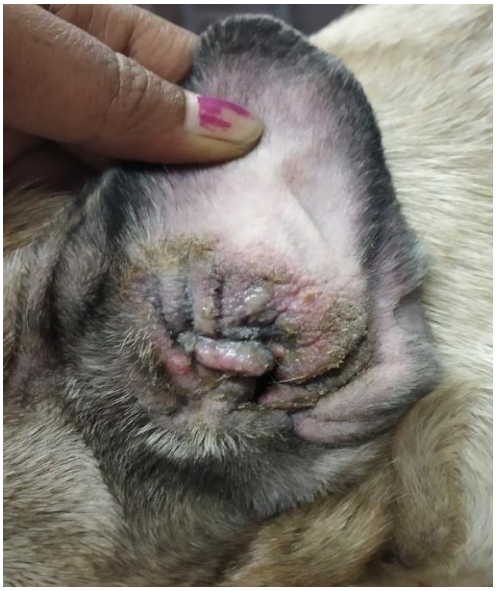

Plate 1: Otitis externa

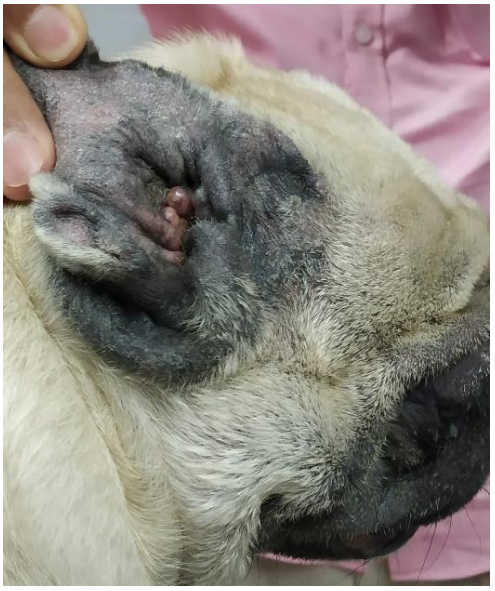

Plate 2: CAD with Otitis externa

In atopic dermatitis a variety of abnormal immunologic events occur, resulting in inflammation, vasodilation, edema, erythema, and pruritus. In the ear canal, early pathologic changes include alteration of epidermal barrier, changes in cerumen composition, dermal edema, and glandular hyperplasia. Narrowing of the canal coupled with accumulation of ceruminous debris provides a better environment for microorganism overgrowth (Angus, 2005).

In the present study, intradermal test was performed in 50 Pugs. Out of which 36 animals showed positive reactions to allergens tested in IDT. Fourteen Pugs did not show any reactions to allergens tested on IDT even though they were clinically showing signs of CAD. Majority of the Pugs showed positive reactions to house dust mites, *Dermatophagoides farinae* (61.11%) and *Dermatophagoides pteronyssinus* (55.56%), followed by human dander (55.56%), cockroach (*Periplanata americana*) (41.67%), house dust (27.78%) and *Amaranthus spinous* (19.44). 8.33% of animals showed positive reactions to *Albizzia lebbeck, Trichoderma sps.,* paper dust and kapok cotton. 5.56% of Pugs showed positive reactions to *Cynodon dactylon, Pennisetuim typhoides, Typha angustata, Aspergillus flavus, Candida albicans* and cat dander. 2.78% of Pugs showed positive reactions to *Azadirachta indica* and *Cassia occidentalis*. None of the Pugs showed positive reactions to *Pencillium sp.* wool mix and *Parthenium* leaves on IDT (Plate 3&4, Fig.1).

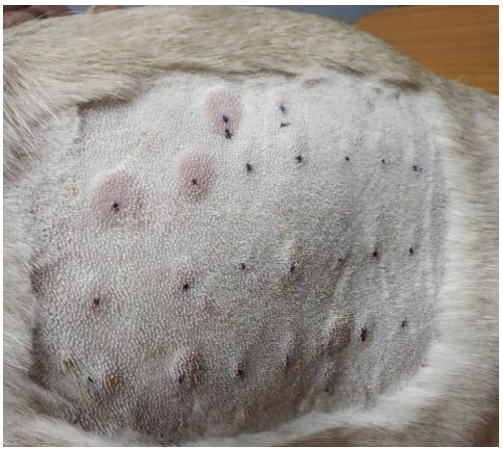

Plate 3: Positive intradermal test showing reactions to three allergens

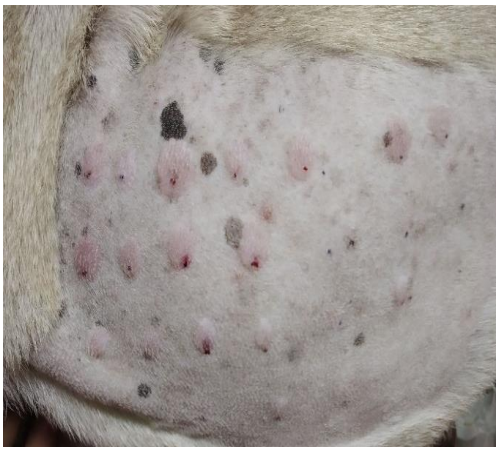

Plate 4: Positive intradermal test showing reactions to more than six allergens

In the present study majority of the dogs showed positive reactions to house dust mites and similar finding were reported by Saridomichelakis *et al.* (1999), Masuda *et al*. (2000), Mueller *et al.* (2000), Tarpataki *et al*. (2006), Chanthick *et al*. (2008), Vaseem (2008). Zur *et al*. (2012), Subarevic *et al*. (2014). Dust mites are the free-living arachnids of the family Pyroglyphidae.

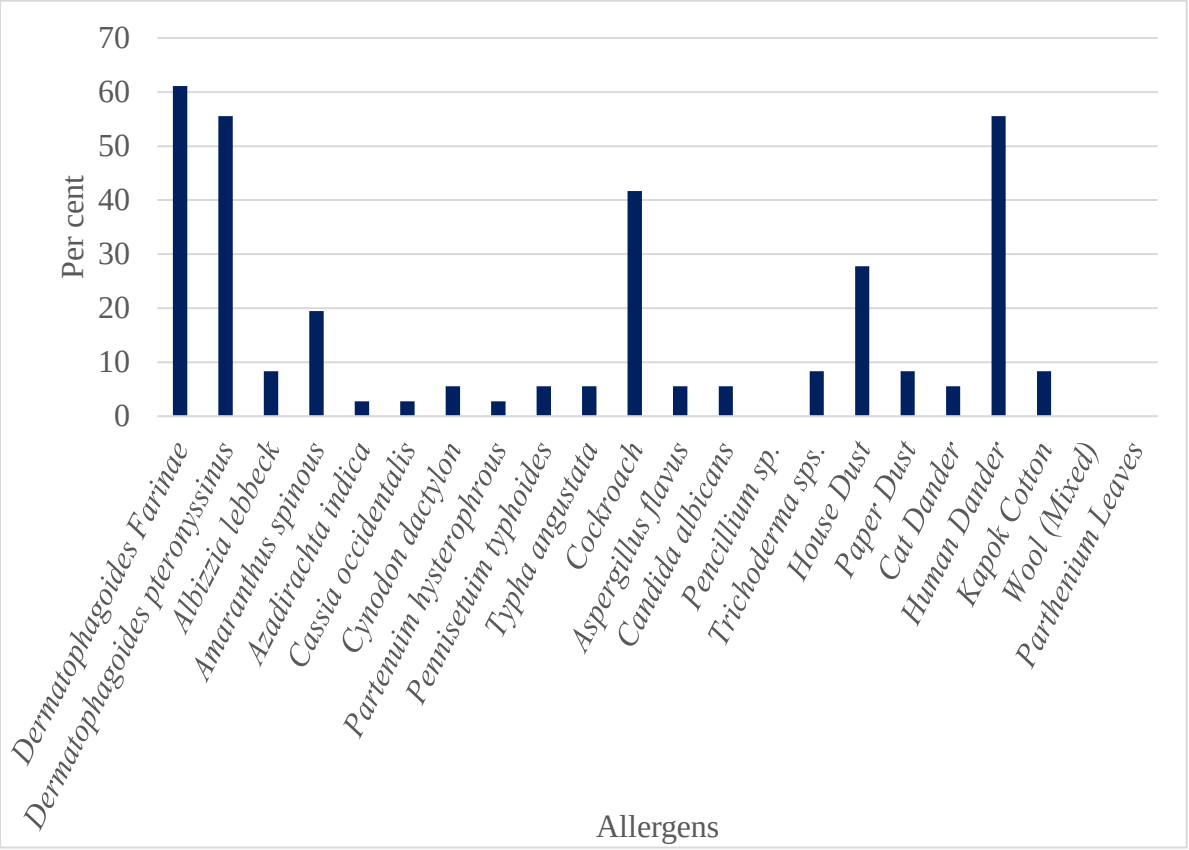

Figure 1: Number of animals showed positive reactions to various allergens in intradermal test (n=36)

The major source of dust mites in household are mattresses, fabric- upholstered furniture and carpet. In a survey of homes with dust mites, 60% of the dust mite population is reported from the bed, mattresses and pillows, 30% in upholstery and 10% in carpet (Sharma *et al.,* 2011). Der f 15 and Der f 18 can be considered major dust mite allergens in the dog (Nuttall *et al.,* 2001).

Followed by dust mites, human dander, cockroach allergen, house dust and different fungal species were the other allergens associated with atopic dermatitis in Pugs and this is in accordance with findings of Vaseem (2008) and Kim *et al.* (2011). House dust is a heterogeneous mixture of animal and human danders, molds, house dust mites, insect debris, bacteria, food particles, breakdown products of clothing and inorganic substances, each one can vary in its antigenicity and irritability (Miller *et al*., 2013). Insect allergies other than flea allergies are relatively rare in dogs. Allergy to insects is prevailing in areas with poor sanitation systems. In India, cockroach allergens have been considered as one of the triggering factors for the development of atopic asthma. The most commonly found species of cockroach is the American cockroach (*Periplaneta americana*) (Bhattacharya *et al*., 2018). Cockroach allergens are derived principally from fecal material and saliva.

Fungal allergens are found primarily in the spores but also occur in other structures, such as mycelia. Fungal allergens may be derived from the structural components of the organism or from the material excreted into the environment. The mycotoxins and other excreted materials may be allergens or act as modifiers to amplify or accentuate the allergic response (Ledford, 1994). Sensitization to mould allergens occurs in dogs with atopic dermatitis. However, percentages of dogs with IgE against fungal allergens vary considerably among studies. These discrepancies may reflect lack of standardization in allergen extracts used in these studies or low specificity of available assays. Higher sensitization in North American studies, than in Europe, Australia or Asia suggested geographical influences (Mueller *et al*., 2016).

In India, the humid climate is considered as ideal for the growth of fungi. Hence, allergic disorders elicited by moulds are quite frequent in almost every part of India. Different species of *Aspergillus* are highly predominant in the ambient air of India and are major contributing factors for the development of asthma as they discharge a huge number of allergen-containing spores (Bhattacharya *et al*., 2018). *Amaranthus spinosus* is an important aeroallergen in India. Grows in the different parts of the country and causes hypersensitivity reactions in humans in India. This

is also associated with atopic dermatitis in Pugs (19.44%) and this correlates with the findings of Vaseem (2008). In the present study nine (25%) Pugs showed positive reactions to single allergen out of 22 allergens tested on IDT. Remaining 27 Pugs were multisensitive and showed positive reactions to more than one allergen (Table 2).

Table 2: Allergic pattern in CAD to various allergens based on IDT results

S. No.	No. of Allergens	No. of animals showed positive reaction	Per cent
1	1	9	25
2	2	7	19.4
3	3	8	22.2
4	4	4	11.11
5	5	4	11.11
6	6	1	2.7
7	> 6	3	8.3

False negative results could be due to improper technique, too low-test concentration of allergens (Hensel *et al*., 2004; Bauer *et al*., 2010), drug interference (Olivry and Saridomichelakis, 2013), intrinsic host factors, incorrect selection of allergens, IDT performed too long after (>60 days) or during the peak allergy season and presence of a condition called atopic-like dermatitis (Hillier and DeBoer, 2001). Fourteen dogs which did not show any reaction to allergen tested, although they were showing clinical signs suggestive of CAD can be categorised as atopic-like dermatitis, which is described as "an inflammatory and pruritic skin disease with clinical features identical to those seen in canine atopic dermatitis in which an IgE response to environmental or other allergens cannot be documented" (Halliwell, 2006).

Conclusion

The common allergens associated with atopic dermatitis in Pugs were house dust mites, human dander, cockroach, house dust, fungal allergens and *Amaranthus spinosus* pollen. Majority of the dogs showed positive reactions to indoor allergens as compared to outdoor allergengs this could be due to the fact that Pugs are commonly housed indoor, rather than outdoor and could be because of limited exposure to the outdoor allergens.

Conflict of Interests

There is no conflict of interest.

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