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# Allergen Sensitization Patterns of Allergic Dogs: IgE-microarray Analysis

Yun-Hsia Hsiao<sup>1\*</sup> Charles Chen<sup>1</sup> Ton Willemse<sup>2</sup>

## Abstract

It was the aim of this study to determine sensitization rates to environmental allergens in 50 dogs with allergic dermatitis. Protein microarray of the dogs' sera was used to identify allergen-specific IgE against 25 most common allergens. Increased IgE levels were most frequently observed for house dust mites (*D. pteronyssinus* 42.0%, *D. farinae* 38.0%) and storage mites (22-50%). In particular, the dogs with non-food-induced allergic dermatitis showed higher sensitization rates to mites compared to the dogs with food-induced allergic dermatitis, or a combination thereof. Reactions to insects were, with the exception of one dog sensitized to cockroach, entirely negative, and variable for epithelia (wool, 2-10%; cow, 36%; mixed feathers, 2%). Clinicians should consider testing these allergens only if there is a clear history of exposure. In this study 18 out of 50 dogs with AD had *Malassezia* infection based on cytology, but only 2 dogs showed elevated levels of *Malassezia*-specific IgE. Although their clinical significance was unclear, reactions to *Alternaria* and *Aspergillus* sp. were more common (10.0 and 18.0%, respectively). With the exception of *Eucalyptus* sp. allergen, sensitization was noticed to all tested pollen, but most commonly observed to grasses: *Poa pratensis* (22%) and *Cynodon dactylon* (20%). It is concluded that the fluorescence-linked immunosorbent protein array can be successfully used to identify sensitization. Careful selection of dogs with allergic dermatitis by means of Favrot's diagnostic criteria is important, as the results may be the basis for subsequent immunotherapy.

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**Keywords:** allergens, canine allergic dermatitis, Favrot's criteria, protein microarray, sensitization

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## Introduction

Atopic dermatitis (AD) is the most common allergic skin disease in dogs. The true incidence of the disease in the dog population is unknown, but estimates vary from 3 to 15 per cent (Hillier and Griffin, 2001). Although atopic disease can be recognized in any dog, the disorder is recognized more frequently in certain breeds such as poodle, West Highland white terrier, French bulldog, Shih Tzu, Jack Russell terrier, and Labrador retriever (Favrot et al., 2010; Wilhelm et al., 2010).

AD in dogs is defined as a genetically-predisposed, inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with IgE antibodies to environmental allergens such as house dust mites, insects, pollens and epithelia (Pucheu-Haston et al., 2015). A diagnosis of AD is based on history, physical examination and clinical manifestations (Hensel et al., 2015). The latter involve the presence of pruritus in

particular on the muzzle, the lips, the pinnae and/or the feet. Otitis externa is also a common finding in atopic dogs (up to 80 per cent) and even in 45 per cent of dogs as the initial problem (Favrot et al., 2010). The occurrence in a predisposed breed and first signs before the age of 3 years enhance the possibility for AD (Griffin and DeBoer, 2001; Hensel et al., 2015). In more than 70 per cent of dogs a secondary staphylococcal infection due to *Staphylococcus pseudintermedius* is observed, whereas concurrent *Malassezia pachydermatis* infection is seen in approximately half of the atopic dogs (Griffin and DeBoer, 2001). Recently, a validation study was performed regarding the characteristic symptoms and signs related to canine AD (Favrot et al., 2010). This study led to the adaptation of the existing list of criteria for the clinical diagnosis of cAD (Willemsse, 1986). These so-called Favrot's criteria are currently used to identify atopic dogs, both food-induced based and/or related to environmental allergens (Favrot et al., 2010; Table 1).

**Table 1** Favrot's 2010 criteria for canine atopic dermatitis

1.	Onset of signs under 3 years of age
2.	Dog living mostly indoors
3.	Glucocorticoid-responsive pruritus
4.	Pruritus sine materia at onset (i.e. aleisional pruritus)
5.	Affected front feet
6.	Affected ear pinnae
7.	Non-affected ear margins
8.	Non-affected dorso-lumbar area

A combination of five satisfied criteria has a sensitivity of 85% and a specificity of 79% to differentiate dogs with AD from dogs with chronic or recurrent pruritus without AD.

In order to identify offending allergens for immunotherapy, the intradermal allergy test or *in vitro* tests measuring allergen-specific IgE in serum, are the most common procedures of choice. However, the intradermal test is time-consuming, needs withdrawal of most antipruritic medication prior to testing (Olivry et al., 2013), and acquires specific skills of the clinician. Protein (allergen) microarrays have recently been introduced for the determination of allergen-specific IgE, not only for research purpose, but also for routine diagnostic settings. Promising results have been obtained for the diagnosis of insect bite hypersensitivity in horses and pollen allergy in humans (Gadermaier et al., 2008; Marti et al., 2015). Intradermal tests (IDT) and allergen-specific IgE serology (ASIS) still lack standardization and a poor correlation exists between the two tests (Foster et al., 2003; Pucheu-Haston et al., 2015). In addition, the success rate of allergen-specific immunotherapy (ASIT) based on IDT versus ASIS is not significantly different (Park et al., 2000), and justifies limiting the study to the use of the IgE microarray.

Advantages of the protein microarray technology are the simultaneous measurement of many allergen samples and the use of minute amounts of serum. Computer-based evaluation of the gathered data not only facilitates diagnosis of allergic diseases, but also provides much more comprehensive information on patients' sensitization patterns. Furthermore, spotted protein microarray slides can be stored up to at least 6 months at -20°C, which makes

them ideal for large-scale production and use in clinical routine diagnostics.

Hence, it was the aim of this blinded study to identify the sensitization pattern of dogs with atopic dermatitis for environmental allergens using a fluorescence-linked immunosorbent protein array.

## Materials and Methods

**Animal selection:** A total number of 50 dogs, of which 30 dogs were female, were included in the study. Their median age at presentation was 5.5 yrs (ranging from 0.5-12.0 yrs) and at onset of atopic symptoms was 3.0 yrs (ranging from 0.5-9.0 yrs). There were a large number of breeds included in the study, ranging from Maltese, West Highland white terrier, Shih Tzu, beagle, to poodle. Four groups of dogs were created on the basis of their preliminary diagnosis, with comparable age ranges and sex ratio (Table 2): dogs with non-food-induced AD (NFIAD), dogs with unidentified AD (UAD), dogs with food-induced AD (FIAD), and dogs with both NFIAD and FIAD (NFIAD/FIAD). NFIAD was observed in 52 per cent of the dogs, whereas FIAD was diagnosed in 9 out of the 50 dogs. In addition, 20 dogs had UAD, but were comparable to the other groups regarding their demographic data. In the NFIAD group and the NFIAD/FIAD group, the dogs were mostly dachshunds and poodles.

Client-owned dogs randomly presented to the clinic for pruritic dermatological problems underwent a general physical and dermatological

examination. To rule out primary bacterial, parasitic and fungal diseases, in all dogs Woods' light examination, microscopy of multiple skin scrapings, hair plucking, and impression smears were carried out. Dermatophyte Test Medium was used to culture potential dermatophytes (BBL™ Mycosel agar, Becton, Dickinson Co., USA), and impression smears were stained with Diff-Quick stain (Diff-Quik®, Laboklin, Germany) for microscopy. Dogs with a primary parasitic, bacterial or fungal skin disease were not included.

If the dogs subsequently fulfilled Favrot's diagnostic criteria for atopic dermatitis (AD) (Favrot et

al., 2010), a clinical diagnosis of non-food-induced and/or food-induced AD was made. If allowed, dogs underwent a food trial of at least 8 weeks using a home-cooked novel protein diet (ostrich and rice, potatoes or yams) or a commercially-available, hydrolysed protein diet (Hill's Z/d or Royal Canin DR21). NFIAD/FIAD dogs are dogs that fulfilled Favrot's criteria and had an incomplete response (60-90%) to the dietary trial. For dogs that fulfilled Favrot's criteria but were not allowed by their owners to take part in the dietary trial, a so-called diagnosis of undetermined AD (UAD) was made (Favrot et al., 2010).

**Table 2** Demographic data of 50 dogs with atopic dermatitis

	Age at presentation (yr)	Age at onset (yr)	Duration of diseases (yr)	Sex
Total (n = 50)	5.5 (0.5-12) <sup>1</sup>	3 (0.5-9.5)	3 (0.5-11)	M20, F30 <sup>2</sup>
NFIAD <sup>3</sup> (n = 21)	6 (2-11.5)	3.5 (0.5-7.5)	3 (0.5-9.5)	M11, F10
UAD <sup>4</sup> (n = 20)	5 (0.5-12)	2.5 (0.5-6.5)	2.5 (0.5-11)	M6, F14
FIAD <sup>5</sup> (n = 4)	7 (2.5-10)	4 (1-9.5)	2.5 (0.5-7.5)	M1, F3
NFIAD/FIAD <sup>6</sup> (n = 5)	5.5 (2.5-8)	2.5 (1-5)	3 (0.5-6)	M2, F3

<sup>1</sup>median age (range), <sup>2</sup>M: male, F: female, <sup>3</sup>non-food-induced atopic dermatitis, <sup>4</sup>unidentified atopic dermatitis, <sup>5</sup>food-induced atopic dermatitis, <sup>6</sup>dogs with both NFIAD and FIAD

**Fluorescence-linked immunosorbent protein array:** In all dogs with the diagnosis of AD, 1 ml of blood was collected by jugular vein puncture and serum was obtained after centrifugation. The serum samples were stored at -70°C until use, and sent blinded to the laboratory without any information of the dogs. Allergen-specific IgE was determined by means of a commercially-available fluorescence-linked immunosorbent protein array (AllerSpot®, Excelsior Bio-System Incorporation, Taiwan).

Briefly, allergen extracts were spotted in triplicate onto epoxy-coated glass slides (superepoxy 3 microarray substrate slides, Arrayit Corporation, CA, USA) by a spotting system (BioDot AD3200, BioDot, CA, USA). Stable coupling of proteins to the chip surface was achieved by incubating the slides at 37°C for 30 min (JorFai Corporation, Taiwan). The chips were then immersed with blocking buffer (Abcam, Cambridge, UK) at 37°C for 1 hr and washed with deionized water three times for 1 min. Hereafter, the chips were incubated with the sample serum (15 microliter diluted 1:10 with blocking buffer) at 37°C for 1 hr and rinsed three times for 30 sec. After adding mouse anti-canine IgE-Cy3 (LTK Biolaboratories, Taiwan) the chips were incubated for 1 hr at 37°C in the dark. Finally, the slides were rinsed twice with PBS (UniRegion Bio-Tech, Taiwan) and deionized water, each for 1 min, dried with nitrogen gas, and subsequently scanned on a InnoScan 710-AL microarray scanner (Innopsys, Carbonne, France). Slide images were analyzed for feature intensity extraction by Mapix 6.5 software (Innopsys). In this study, PBS was used as a negative control. Sample signals from the microarray images were scored on the basis of fluorescence intensity by deduction of the negative control value from the mean of the triplicate serum values. Response levels were scored from 0 (fluorescence intensity: 0-500) to 6 (> 16,000). According to the manufacturer's instructions only

responses at level 3 (intensity 2,000-4,000) or higher were considered positive (<http://www.ebs.com.tw>). Tested allergens included: house dust mites - *Dermatophagoides pteronyssinus* and *D. farinae*; storage mites - *Acarus siro*, *Blomia tropicalis* and *Tyrophagus putrescentiae*; insects - flea mixture (*Ctenocephalides felis* and *C. canis*), *Aedes communis* (common snow mosquito), *Culex pipiens* (common house mosquito) and *Blattella germanica* (German cockroach); epithelia - mixed feathers, wool, cow, and cat; molds - *Cladosporium herbarum*, *Alternaria alternata*, *Penicillium chrysogenum*, *Aspergillus fumigatus* and *Malassezia pachydermatis*; and pollens - *Eucalyptus globulus* (blue gum tree), *Poa pratensis* (common meadow grass), *Amaranthus retroflexus* (redroot pigweed), *Rumex acetosella* (sheep sorrel), *Acacia Baileyana* (Cootemundra wattle), *Cynodon dactylon* (Bermuda grass), and *Ambrosia artemisiifolia* (common ragweed). The tested allergens were chosen based on them being the most common in the area.

## Results

Table 3 presents the sensitization rates of the dogs in this study. In the total group of 50 dogs, increased allergen-specific IgE was most commonly observed for the regular house dust mites (*D. pteronyssinus* 42.0%, *D. farinae* 38.0%) and the storage mites (22-50%). In particular, the NFIAD group showed higher sensitization rates to the various mites compared to the other groups of dogs as shown in Table 3. In contrast, reactions to insects were, with the exception of one dog sensitized to cockroach, entirely negative, and rare for the wool (2-10%) and mixed feathers (2%). However, a high sensitization rate was seen for cow allergen (36%).

Reactions to molds were observed in every group but at a low percentage, as were those to pollens. With the exception of *Eucalyptus* sp. allergen, sensitization was noticed to all kinds of pollen, but

mostly to the grasses *Poa pratensis* (22%) and *Cynodon dactylon* (20%) among the 50 AD dogs. Those pollens were also the only allergens to which sensitization occurred in the four dogs with FIAD.

### Discussion

Atopic dermatitis in dogs is associated with sensitization to environmental allergens of which house dust mites, pollens, epithelia, and molds are the most well-known. Interestingly, evidence suggests that dogs also have a predisposition to develop clinical signs compatible with AD triggered by food antigens

(Olivry et al., 2007; Pucheu-Haston et al., 2015). Therefore, certain dogs with food-induced AD and non-food-induced AD cannot be differentiated on a clinical basis.

Tests to identify sensitization are conducted by measuring allergen-specific IgE, as being strongly associated with the presence of allergic skin disease in dogs (Pucheu-Haston et al., 2015). In this study the fluorescence-linked immunosorbent protein array was used to identify sensitization, which implies exposure of a predisposed animal, to the most common allergens in Taiwan in dogs fulfilling the diagnostic criteria of AD (Favrot et al., 2010).

**Table 3** Environmental allergens sensitization rate of 50 dogs with atopic dermatitis

Allergens	NFIAD <sup>1</sup> (n = 21)	FIAD <sup>2</sup> (n = 4)	NFIAD/FIAD <sup>3</sup> (n = 5)	UAD <sup>4</sup> (n = 20)	
Mites	<i>D. pteronyssinus</i>	10 (20%) <sup>5</sup>	3 (6%)	2 (4%)	6 (12%)
	<i>D. farinae</i>	8 (16%)	1 (2%)	3 (6%)	7 (14%)
	<i>Acarus siro</i>	5 (10%)	1 (2%)	1 (2%)	4 (8%)
	<i>T. putrescentiae</i>	11 (22%)	3 (6%)	3 (6%)	8 (16%)
	<i>Blomia tropicalis</i>	6 (12%)	1 (2%)	2 (4%)	5 (10%)
Insects	<i>Ctenocephalides</i> spp.	0	0	0	0
	<i>Culex pipiens</i>	0	0	0	0
	<i>Aedes communis</i>	0	0	0	0
	<i>Blatella germanica</i>	1 (2%)	0	0	0
Epithelia	mixed feathers	1 (2%)	0	0	0
	wool	3 (6%)	0	0	2 (4%)
	cat	0	0	0	0
	cow	6 (12%)	1 (2%)	7 (14%)	4 (8%)
Molds	<i>C. herbarum</i>	2 (4%)	0	1 (2%)	0
	<i>A. alternate</i>	4 (8%)	0	1 (2%)	2 (2%)
	<i>P. chrysogenum</i>	0	0	1 (2%)	0
	<i>A. fumigatus</i>	1 (2%)	2 (4%)	1 (2%)	5 (10%)
	<i>M. pachydermatis</i>	2 (4%)	1 (2%)	0	0
Pollens	<i>Eucalyptus globulus</i>	0	0	0	0
	<i>Poa pratensis</i>	3 (6%)	3 (6%)	3 (6%)	2 (4%)
	<i>A. retroflexus</i>	0	0	0	3 (6%)
	<i>Rumex acetosella</i>	2 (4%)	0	2 (4%)	2 (4%)
	<i>A. artemisiifolia</i>	1 (2%)	0	1 (2%)	3 (6%)
	<i>Acacia Baileyana</i>	0	0	0	1 (2%)
	<i>Cynodon dactylon</i>	2 (4%)	1 (2%)	4 (8%)	3 (6%)

<sup>1</sup>non-food-induced atopic dermatitis, <sup>2</sup>food-induced atopic dermatitis, <sup>3</sup>dogs with both NFIAD and FIAD, <sup>4</sup>unidentified atopic dermatitis, <sup>5</sup> number of dogs reacting to allergen (percentage among total study group)

In all groups in this study the age at onset was around 3 years, which is slightly higher than those in other studies, in which at least 75 per cent of dogs had the first symptoms before the age of 3 years (Youn et al., 2002; Favrot et al., 2010; Kang et al., 2014). Moreover, poodles and dachshunds were more frequently affected in this study, which is different from the report of Tsai et al. (2012), indicating that golden retrievers and Maltese were the predisposed breeds in Taiwan. It is unknown whether this is due to the change in popularly owned breeds or only regional differences.

In this study allergen-specific IgE was most commonly detected for the house dust mites *D. pteronyssinus* (42.0%) and *D. farinae* (38.0%). From the total group of dogs 62 per cent showed elevated IgE levels to one or more mites. Although it depends on the geographical area, most studies in western countries

showed *D. farinae* to be the most important house dust mite related to the development of AD in dogs (Nuttall et al., 2006). Not only these are present in the environment and on coat (Randall et al., 2003; Jackson et al., 2004), but upon cutaneous exposure AD-like lesions are elicited (Marsella et al., 2005). In another study using the same microarray test, slightly lower sensitization rates were found to *D. pteronyssinus* (29.5%) and *D. farinae* (17.0%) in a group of 505 household dogs with suspected AD (Tsai et al., 2012). However, in both studies the sensitization to *D. pteronyssinus* was found to be more common. Interestingly, Macan et al. (2003) found *D. pteronyssinus* to be more common than *D. farinae* along the Mediterranean coast (with a maritime climate and higher temperatures), and the reverse to be true inland, supporting our findings. From the study of Tsai et al. (2012) it also became apparent that sensitization to

mites is the highest in the summer and fall season. Although most of the dogs participated in our study between May and October, it is not clear whether this is an explanation for the higher rates. Studies of sensitization in AD dogs in other Asian countries are relatively rare. In 61.4 per cent of dogs with NFIAD in Korea increased house dust mite-specific IgE levels were observed (Kang et al., 2014), whereas in Thailand rates between 53.5 and 74.6 per cent were reported based on skin test results (Chanthick et al., 2008).

Sensitization to storage mites is commonly observed in dogs with AD both by means of skin testing and in IgE serology tests (Masuda et al., 2000; Bensignor and Carlotti, 2002; Arlian et al., 2003; Nuttall et al., 2006). However, the relevance of these species for the development of canine AD has not yet been established. In our study half of the dogs showed increased IgE levels to *T. putrescentiae* and 20-30 per cent to *A. siro* and *B. tropicalis*. Although most dogs are exposed to low concentrations of these mites in regular house dust (Mueller et al., 2005), coming into contact with stored foodstuffs including dry pet food is also a suggested route of exposure (Brazis et al., 2008; Gill et al., 2011; Hibberson and Vogelneust, 2014). However, in a house dust sample study in Thailand, large amounts of *B. tropicalis* and *T. putrescentiae* were found together with *Dermatophagoides* mites (Insung and Homchan, 2004), indicating a high likelihood for direct exposure from the environment. In the study of Tsai et al. (2012) lower rates (ranging between 14.9 and 24.6%) were found in 505 Taiwan household dogs with symptoms suggesting AD. As speculated before, the selection of dogs, variable housing conditions and seasonal differences may be the underlying factors for this difference.

Sensitization of dogs to epithelia such as mixed feathers, cow, sheep/wool, and cat by means of the detection of allergen-specific IgE has been reported in Korea and Taiwan (Tsai et al., 2012; Kang et al., 2014). Whereas the sensitization rates are high in Korea (feathers 25.7%, cat 9.9%, mouse 22.9%), the reported values in Taiwan are much lower (cow 5.4%, cat 3.2%, sheep 0%, pigeon 1.6%). The latter are much in line with our observations regarding the sensitization to mixed feathers and cat. In contrast, our study found a much higher sensitization rate to cow epithelia (36.0%). However, especially with respect to sensitization to epithelia, it is important to realize that clinical history should provide evidence of exposure before these data can be translated to a clinical setting. In our study group there was only a positive history for the dog with feather sensitization, but as far as could be ascertained no such relationship was found for exposure to cows. Therefore, the significance of these data is unknown. Sensitization to sheep/wool is also more difficult to correlate, as also rugs and carpets can be the source of exposure. Hence, it is recommended to test for sensitization to epithelia only if there is a positive history of exposure, and not routinely.

*Malassezia pachydermatis* infection is a common secondary complication in canine AD, but is occasionally also observed in non-atopic dogs. Moreover, these yeasts are constituents of the normal skin flora (Bond et al., 1996; Kennis et al., 1996; Carfachia et al., 2006). In our study 18 dogs with some

forms of AD had *Malassezia* infection or overgrowth based on the cytology, but only 2 dogs with NFIAD showed elevated levels of *Malassezia*-specific IgE. This finding implies that although these 18 dogs had been colonized with yeasts, only a limited number of sensitization occurred. This observation supports the results of Nuttall and Halliwell (2001), indicating that the presence of *Malassezia*-specific IgE is not dependent on the number of yeasts on the skin surface. In addition, the percentage of dogs sensitized in our study, is lower than those reported by others (Nuttall and Halliwell, 2001; Oldenhoff et al., 2014), including observations in Asia (21.9%) (Kang et al., 2014). According to Nuttall and Halliwell (2001), cross-reactivity with environmental fungi is unlikely, as the finding of *Malassezia*-specific IgE in serum is not paralleled by skin test reactivity to *Alternaria* or *Penicillium* sp.

Geographic and climate conditions such as temperature and humidity are closely related to the presence and exposure to molds (Beggs, 2004). Therefore, it is not surprising that 14 of the 50 dogs in our study were sensitized to one or more mold species despite the AD group to which they belonged. However, reactions to *Alternaria* and *Aspergillus* sp. were most common (10.0 and 18.0%, respectively), which is slightly lower for *Alternaria* and similar for *Aspergillus* sp. compared to another study concerning Taiwan dogs with suspected AD (15.8 and 18.0%, respectively) (Tsai et al., 2012). In addition, different sensitization rates were noticed in NFIAD dogs in Korea for *Alternaria* (39.6%), *Penicillium* (36.6%) and *Aspergillus* (9.8%) (Kang et al., 2014), whereas 14.2% of the NFIAD dogs in our study showed increased allergen-specific IgE to these molds. In contrast, much lower rates of 3.5 and 2.6% were observed in a study in Thailand, but were established by means of skin test reactivity (Chanthick et al., 2008). The clinical significance of molds in relation to canine AD is unclear. However, it should be realized that in humans molds are only associated with asthmatic and allergic rhinitis patients (Sritipsukho, 2004). It is the authors' opinion that also in dogs it is unlikely that these molds are associated with the development of AD.

Although sensitization to black ants (20.2%), German cockroach (6.7-15.2%) and mosquitos (1.4-15.8%) has been reported in Thailand, Taiwan and Korea (Chanthick et al., 2008; Tsai et al., 2012; Kang et al., 2014), their clinical relevance is unlikely in dogs with clinical symptoms of AD and has never been described before. This is in contrast to cats, where mosquito bite hypersensitivity is observed in particular on non-hairy parts of the body such as pinnae, feet and temporal area (Miller et al., 2013). The results of our study indicate a low exposure to these insects and they are in line with a previous study in NFIAD dogs in Taiwan (Tsai et al., 2012). Finally, our study did not observe sensitization to the mixed flea extract. Based on the clinical appearance of the dogs in this study, and the absence of skin involvement on the dorsal back, hind limbs and tail base (Hensel et al., 2015), these results were to be expected.

The sensitization rates to various pollen was lower than 10 per cent in the dogs of our study and independent of their preliminary diagnosis. These

rates are more or less comparable to the observations of Tsai et al. in 505 dogs with allergic dermatitis (ranging from 5.4-14.5%) (Tsai et al., 2012). Although there are differences regarding the specific grass and tree pollen between the two studies, it should be realized that pollination of the various plants may differ over the seasons and the geographic area. Consequently, it depends on the time of collection of the serum samples whether increased sensitization will be detected, and air pollen concentrations may be different in various parts of a country, which makes it difficult to compare results of various studies. In our study the dogs with only food-induced AD showed increased sensitization rates to pollen. In humans, up to 60 per cent of food allergies are linked with an inhalant allergy, most likely due to cross-reactivity between inhalants such as tree pollen and mugwort, and food allergens (Werfel et al., 2015). Whether this is a possible explanation of our findings, or that these dogs will develop classical AD at a later stage, is unclear.

In summary, the fluorescence-linked immunosorbent protein array was successfully used to identify sensitization to various allergens in dogs fulfilling Favrot's criteria of canine atopic dermatitis. The sensitization to various mites and pollen was most relevant. In particular, the increased levels of allergen-specific IgE for *D. pteronyssinus* were more common than for the other mite species. With respect to epithelia, clinicians should consider testing these allergens only if there is a clear history of exposure. As the significance of sensitization rates to molds is unclear and is unlikely the reason for the development of AD, test results for these allergens should be interpreted reluctantly. Finally, it is important to carefully select dogs prior to *in vitro* testing for allergens, as the results may be the basis for subsequent allergen-specific immunotherapy (ASIT). It may be of interest to evaluate the results of ASIT based on the IgE-microarray analysis in a subsequent double blind, placebo-controlled study.

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## บทคัดย่อ

### Allergen Sensitization Patterns of Allergic Dogs: IgE-microarray Analysis

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วัตถุประสงค์ของการศึกษานี้เพื่อรายงานอัตราการแพ้สารจากสิ่งแวดล้อมในสุนัขจำนวน 50 ตัว โดยสุนัขในการศึกษานี้ได้รับการวินิจฉัยว่าเป็นโรคผิวหนังที่เกิดจากภาวะภูมิแพ้สารจากสิ่งแวดล้อม ทำการตรวจหาอัตราการแพ้สารจากสิ่งแวดล้อมโดยการวัดระดับ allergen-specific IgE ต่อสารจากสิ่งแวดล้อมทั้งหมด 25 สารด้วยวิธี protein microarray จากซีรัมของสุนัข พบว่าสารจากสิ่งแวดล้อมที่สุนัขแพ้มากที่สุด โดยวัดจากการเพิ่มขึ้นของ IgE คือ house dust mites (*D. pteronyssinus* 42.0%, *D. farinae* 38.0%) และ storage mites (22-50%) สุนัขที่มีภาวะภูมิแพ้สารจากสิ่งแวดล้อมแต่ไม่มีภาวะภูมิแพ้โปรตีนในอาหารร่วมด้วย (non-food-induced allergic dermatitis) มีอัตราการแพ้สารจากไรฝุ่นมากกว่าสุนัขที่เป็นโรคผิวหนังที่เกิดจากภาวะภูมิแพ้โปรตีนในอาหาร (food-induced allergic dermatitis) จากการจัดระดับอัตราการแพ้ พบว่าสุนัขมีอัตราการแพ้สารจากสิ่งแวดล้อมพวกแมลง ยกเว้นพวกแมลงสาบ น้อยมาก และมีอัตราการแพ้สารจากสิ่งแวดล้อมพวกขนสัตว์แตกต่างกันไป เช่น wool: 2-10%, cow: 36%, mixed feathers: 2% ดังนั้น การเลือกสารจากสิ่งแวดล้อมมาตรวจควรจะขึ้นอยู่กับประวัติและที่อยู่อาศัยของสุนัขแต่ละตัว ในการศึกษานี้พบเชื้อ *Malassezia* ในสุนัข 18 จาก 50 ตัวจากการทำ cytology แต่กลับพบสุนัขเพียง 2 ตัวที่วัดระดับ IgE ต่อ *Malassezia* allergen ได้ สำหรับสารจากสิ่งแวดล้อมประเภท mold พบว่าสุนัขมีอัตราการแพ้ต่อ *Alternaria* 10% และ *Aspergillus* sp. 18% สำหรับสารจากสิ่งแวดล้อมที่มาจากละอองเกสร พบว่าสุนัขมีอัตราการแพ้หญ้ากลุ่ม *Poa pratensis* และ *Cynodon dactylon* 22% และ 20% ตามลำดับ จากการศึกษาครั้งนี้สรุปได้ว่า fluorescence-linked immunosorbent protein array สามารถใช้วัดระดับการแพ้ต่อสารจากสิ่งแวดล้อม การเลือกสุนัขที่มาตรวจควรจะผ่านการคัดเลือกว่าเป็นโรคผิวหนังที่เกิดจากภาวะภูมิแพ้สารจากสิ่งแวดล้อมโดยใช้ Favrot's diagnostic criteria และสารจากสิ่งแวดล้อมที่ตรวจพบระดับการแพ้จากการตรวจด้วยวิธีนี้สามารถนำไปเป็นข้อมูลในการรักษาด้วยวิธี immunotherapy ต่อไป

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**คำสำคัญ:** allergens canine allergic dermatitis Favrot's criteria protein microarray sensitization

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