

Allergens Causing Atopic Diseases in Canine

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Abstract

Canine atopic skin disease is seasonal or sometimes non-seasonal immune-mediated skin disease which occurs commonly in Korea. The definite clinical sign is systemic pruritus, especially on periocular parts, external ear, interdigit spaces and lateral flank. For diagnosis of this dermatitis, complete history taking followed by intradermal skin test and serum in vitro IgE test needs to be performed. Allergen selection for the diagnosis and treatment of atopic dermatitis should be varied geographically. In this study, with intradermal skin test (IDST) the prevalence of atopic disease and what allergens are involved in are researched. Allergens used for IDST included 26 allergen extracts from six allergen groups: grasses, trees, weeds, molds, epidermal allergens and environmental allergens. The number of allergens was 42 in which the positive and negative controls are included. The most common positive allergen reaction was the house dust mites on IDST (22/35, 63%). The other positive allergen reactions were to flea (3/35, 9%), molds (1/35, 3%), house dusts (2/35, 6%), feathers (1/35, 3%), cedar/juniper (1/35, 3%), timothy grass (1/35, 3%) and dandelion (1/35, 3%). In this study, the most prevalent allergen causing atopic dermatitis in dogs in Korea was the house dust mites followed by the flea.

Key words : canine atopic disease, intradermal skin test (IDST), allergens, house dust mites.

Introduction

Allergy is an altered state of immune reactivity and atopy

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is one type of the allergy. In man, the term atopy is used to describe a triad of asthma, hay fever, and atopic dermatitis (AD). In pets, atopy historically described a pruritic dermatitis associated with the inhalation of pollen, fungal, or environmental allergens. However, in dog, the respiratory route of exposure is the subject of investigation although the exposure through the skin in man has reliable evidences [8, 11]. Canine atopic dermatitis (AD) is a common skin disease in dogs [14, 17]. In dogs, atopy is considered to be an hereditary clinical hypersensitivity state or an hereditary, reagent mediated hypersensitivity to inhalant allergens [26]. But, no genetic markers have been found for the disease [10].

Among the four types of hypersensitivity, anaphylactic or immediate type hypersensitivity reactions are of importance in relation to atopy [17]. In animals with atopy, exposure to an allergen causes production of Immunoglobulin E (IgE) and directed against that allergen; on subsequent exposure, an allergic reaction occurs. Allergen specific IgE attaches to the surface of mast cell, thus causing it to degranulate and release mediators of inflammation [1].

The breed predisposition for atopic disease has been mentioned in Lhasa apso, miniature schnauzer, pug, Sealyham terrier, Scottish terrier, West Highland white terrier, the wire haired fox terrier, cairn terriers and the golden retriever [22, 23, 26] and Cocker spaniel, setters, Labrador retrievers, and German shepherd dogs which are common breeds in Korea have also been described [12, 25].

The most common clinical signs include a history of seasonal or nonseasonal pruritus, otitis externa, recurrent and chronic inflammatory dermatitis especially in the axillary, inguinal, and flexor skin surfaces, recurrent bacterial infections, face rubbing and/or foot licking and chewing. But the signs of canine atopy are usually seasonal in the beginning, often become non-seasonal with time, and occasionally are non-seasonal from the start [17]. The allergens involved in the pathogenesis of canine atopy include house dust mites (HDM), house dust, molds, weeds, trees, grasses, epithelia and arthropods [15, 19]. Some allergens are ubiquitous in the environment (e.g. house dust); however, many other clinically important allergens vary with respect to season, climate, and/or geographic region. What is important in one geographic

region or country, may not be important in another[14, 24].

In Korea, house dust mite is known to be a common and important nonseasonal allergen in humans. This allergen is also the most common in dogs in most countries. But to date, there are no informations on the prevalence of atopic dermatitis and what allergen is most common in dogs in Korea.

For the diagnosis and management of an atopic cases, a detailed history is the most important factor. For identifying the cause of canine atopic dermatitis, two major diagnostic methods have been developed[14, 15]. Both in vivo and in vitro methods of allergy testing are available. In vitro testing involves immunoreactant measurement in serum in the allergic reaction. In vivo allergy testing involves the induction of a small scale allergic reaction by the intentional exposure of the patient to a minute amount of allergen. Intradermal skin test (IDST), as an in vivo test, is still the gold standard most commonly used and the most reliable methods. But several factors must be considered with false-positive reactions to the IDST due to improper technique, irritant test allergens, irritable skin (dermographism), cross-reactivity among allergens and possible contamination with histamine-like substance in the extracts[15, 16]. False-negative reaction to the IDST are more problematic and can occur for the following reasons; poor injection technique, degradation of allergen solutions, immune status of the dog, drug interference, inherent host factors, incorrect antigen selection and test done at the wrong time[14]. Therefore, the IDST combined with in vitro testing for the diagnosis and management of dogs with AD is recommended[4]. This study was performed to investigate what kinds of allergens involved in canine AD determined by IDST in Korea.

Materials and Methods

Patient Selection

A total of 35 dogs were admitted to Veterinary Medical Teaching Hospital, Seoul National University, Seoul, Korea between 1999 and 2001. Diagnosis of AD was made by a combination of compatible history and clinical signs along with the presence of one more positive IDST reactions that correlated well with the patients' history of exposure and the seasonality of clinical signs.

Diagnostic Evaluation

In complete cases history and physical examination were done in all 35 dogs. All of the pruritic dermatitis that could mimic AD were excluded through multiple skin scrapings for the detection of ectoparasites, skin smear for deep bacterial infection, culture and antibiotics susceptibility test. Food allergic dermatitis was ruled out on the base of dietary restriction trial, of at least 4-week duration followed by provocative testing in all cases. In most of the cases, the owners exhibited non-seasonal pruritus.

Intradermal Skin Testing

All of steroids, sedatives, immunosuppressants, antihistamines and tranquilizers were discontinued for at least 21 days before IDST[17]. A total of 40 aqueous allergenic extracts were selected for IDST that subsequently were allocated into 6 groups (grass, trees, weeds, molds, epidermal allergens, environmental allergens). All the extracts were obtained from Greer Lab (Lenoir, North Carolina, USA) and all allergens are aqueous solutions. All these allergens were diluted at the concentration shown to be non-irritant when tested in normal dogs (Table 1) following the indication of Greer Lab. All extracts of the grass, trees, weeds were diluted at a strength of 1000PNU/ml. House dust and house dust mite extracts are known irritants and so were used at a strength of 100PNU/ml and 1:5000w/v, respectively. And molds, questionable irritant allergens, were diluted at a strength of 250PNU/ml. Histamine phosphate(0.0275mg/ml) was used as the positive control and 0.9% normal saline with 0.1% phenol added was used as the negative control.

All dogs were sedated with medetomidine which did not seem to block skin test reactivity in dogs. The skin test was performed on the lateral flank after shaving or clipping and then gentle cleaning with water-soaked towel. No other chemicals or soothing agent which can affect the IDST were applied to the part. The injection sites are marked with a water based naming pen, leaving 3 cm between each injection, and 0.05ml of each allergen or control solution was injected intradermally using a 1ml syringe with a 26 gauge needle. The diameters of wheal were measured 20 min after injection[17]. The test sites were graded as follows: +++,

Table 1. Allergens used in the intradermal skin test in 35 atopic dogs in Korea and allergen dilutions of testing strength extracts

Number	Aeroallergen	Dilution	Source
<i>Grass</i>			
1	Bermuda	1000PNU/mla	Greer
2	Fescue	1000PNU/ml	Greer
3	Kentucky	1000PNU/ml	Greer
4	Orchard	1000PNU/ml	Greer
5	Rye	1000PNU/ml	Greer
6	Timothy	1000PNU/ml	Greer

Table 1. (continued)

Number	Aeroallergen	Dilution	Source
<i>Trees</i>			
1	Acacia	1000PNU/ml	Greer
2	Beech	1000PNU/ml	Greer
3	Cedar	1000PNU/ml	Greer
4	Juniper	1000PNU/ml	Greer
5	Mulberry	1000PNU/ml	Greer
6	Sycamore	1000PNU/ml	Greer
7	Willow	1000PNU/ml	Greer
8	Birch	1000PNU/ml	Greer
9	Elm	1000PNU/ml	Greer
10	Eastern oak mix	1000PNU/ml	Greer
11	Pine mix	1000PNU/ml	Greer
12	Juniper mix	1000PNU/ml	Greer
<i>Weeds</i>			
1	Ragweed	1000PNU/ml	Greer
2	Pigweed	1000PNU/ml	Greer
3	Lamb's quarter	1000PNU/ml	Greer
4	Cockle bur	1000PNU/ml	Greer
5	Dandelion	1000PNU/ml	Greer
6	Mugwort	1000PNU/ml	Greer
7	Sheep's sorrel	1000PNU/ml	Greer
8	English plantain	1000PNU/ml	Greer
<i>Moulds</i>			
1	<i>Altenaria</i>	250PNU/ml	Greer
2	<i>Aspergillus</i>	250PNU/ml	Greer
3	<i>Penicillium</i>	250PNU/ml	Greer
4	<i>Mucor</i>	250PNU/ml	Greer
5	<i>Rhizopus</i>	250PNU/ml	Greer
<i>Epidermal allergens</i>			
1	Cat epithelia	1000PNU/ml	Greer
2	Mixed feathers	1000PNU/ml	Greer
<i>Environmentals allergens</i>			
1	House dust	100PNU/ml	Greer
2	<i>D. farinae</i>	1:5000w/vb	Greer
3	<i>D. pteronyssinus</i>	1:5000w/v	Greer
4	Daisy	1000PNU/ml	Greer

µPNU= protein nitrogen unit.

bw/v= weight per volume.

equal to or greater than the diameter of the positive control; ++, equal to or greater than the mean diameter of the positive and negative controls; +, larger than the diameter

of the negative control but small than the mean diameter of the positive and negative control; -, equal to or smaller than the diameter of the negative control[4].

Results

Historical and clinical data

In this study, 32 dogs from the 35 tested were purebred and 3 dogs were mixed dogs. The presented breeds were Yorkshire terrier(7/35), Cocker spaniel(6/35), maltese(4/32), shih-tzu(5/32), mini-pin(2/35), poodle(1/35), pekinese(1/35), pug(1/35), bulldog(1/35), schunauzer(2/35), beagle(1/35), Labrador retriever(1/35) and mongrels(3/35). Twenty dogs out of the 35 were intact females, 7 dogs were intact males, 3 were spayed females and 5 were castrated males. The onset of clinical signs were ranged from 3 months to 5 years with a median age of 1.52 year. In this study, the more than 90% dogs showed clinical symptom at less than 3 years old age.

Intradermal skin test

Twenty-five out of the 35 represented cases showed positive reactions against the allergens tested (Tables 2 and 3). The other 10 dogs had no skin test reaction on the second test. The results of IDST reactions is shown in Figure 1. Eleven dogs reacted for only one antigen and 14 dogs for two or more antigens. There were large number of cases that had positive IDST reactions to house dust mites. Of the 35 dogs, 11 cases(31.5%) had positive reactions to *Dermatophagoides farinae* alone and 11 cases(31.5%) both *D. farinae* and *D. pteronyssinus*. Three dogs had positive reactions to flea and all these dogs also had positive reactions to HDM. One dog was positive to moulds, one was to house dust, one to feathers, one to timothy grass, one to cedar/juniper and one to dandelion.

Discussion

Based on the findings in this study, house dust mite is an important allergen in atopic dogs in Korea (Fig. 1). This result is similar to those of other studies in most countries including Japan. Although *D. farinae* is found to be the most common allergen causing atopic dermatitis in many regions, the prevalence of *D. pteronyssinus* is higher in some regions[13, 24]. So each individual HDM allergen was tested in this study. And in other respect, because mixed type HDM allergen have higher false positive reaction[3], the two HDM allergens were separated and the result showed *D. farinae* is more prevalent in Korea.

No information has been reported the predisposition of breeds in Korea. In this study, Cocker spaniel, Yorkshire terrier had predisposition to atopic skin disease. This result is matching with the previous reports [3, 23, 26]. There are no Korean breeds involved.

Why had the HDM much more portion of the causes of AD and why were pollens relatively rare causes? The test regions and bred-breeds-tendency seem to have a key to find the answer. Commonly, pollens are said they can move 640 Km far from the regions they produced. With this aspect, we may be able to guess all the pollens produced in Korea can affect any dogs in any regions because the diagonal distance

from Seoul to Pusan (about 450 Km) is shorter than 640 Km. But this theory may not be applied to Korea since the climate and humidity is not the same as other country. And most of the breeds tested in this study were small breeds which are kept indoors almost all the time, which aspect can make the pollens rare causes of AD.

Atopy is an hereditary clinical hypersensitivity state. In the course of diagnosing the AD, the investigation of the family line can be the one of the important sources. All the dogs tested had atopic dermatitis and/or food allergic dermatitis based on the result of case history, lesion and IDST. But since the line could not be obtained in most cases, a genetical or inherited prevalence in these cases could not be proposed. The reason why the family line couldn't be gotten in most cases is that many portions of Korean owners usually don't think it's important, especially in the case of small breeds.

As expected, pruritus was the most common conditions seen in conjunction with AD in this series. All the dogs tested in this study had pruritus especially on the periocular region, ear pinna, lateral flank and most of the dogs had the signs of licking and chewing the interdigit area. But surprisingly, the prevalence of *Malassezia* dermatitis was very low(1 of 32 dogs), although thorough clinical skin tests were performed. The probability that some atopic dogs have had *Malassezia* hypersensitivity without increased yeast counts on their skin seems unlikely, since these cases show only a partial response to glucocorticoids [20, 21]. Moreover, quite a few generalized *Malassezia* dermatitis cases have been diagnosed in our non-apotic dogs. Therefore, it is clear that this generalized fungal skin disease is rare in Korea. In contrast, otitis externa and bacterial pyoderma were the most common secondary skin diseases of AD.

The results of this study demonstrated some deviation on the prevalence according to sex. Usually, there's no predilection for atopy [13, 24] although females seem to be predisposed in some regions and estrogens may have a important role in consideration of the factors other than genetics [23]. The number of females were more than 2 times that of males in this study. But we couldn't assume that females are predisposed to AD owing to insufficient quantity of cases.

In the majority of dogs in this study, the onset of clinical signs occurred before the dog was 3 years of age. These findings were similar to those of other investigators[4]. The genetic predisposition to AD is most commonly proven by exposure to allergens in very young age. So the age of onset of clinical signs is a consistently good historical information of diagnosing canine atopic dermatitis.

A negative IDST reaction on several dogs were yielded. In these cases, the test was performed two or three times and there were no positive results. These seem to be because the causing allergens of those dogs were not chosen in the test or the seasonal incidence couldn't be met the test date.

Table 2. Intradermal skin test results of 35 dogs

	Breed(No.)	Age	Sex(No.)	Age of onset (No.)
House dust mite	Y.T(4)	4.45Y(Mean)	Fa(9) Fn(2) M(7) Mn(4)	<3Y(20) >3Y(2)
	Shih-Tzu(4)			
	C.Spaniel(3)			
	Mini pin(2)			
	L.retriever(1)			
	Mongrel(2)			
	Beagle(1)			
	Pekingese(1)			
	Pug(1)			
	Maltese(1)			
Schunauzer(2)				
Flea	Mongrel	9Y	Mn	<1Y
	Y.T	5Y	F	1Y
	Y.T	3Y	Mn	1Y
House dust	C. spaniel	5Y	M	1Y
	Shih tzu	6Y	F	2Y
Cat epithelia	Schunauzer	4Y	F	1Y
Mucor	Pug	9Y	F	6M
Feathers				
	Y.T	2Y	F	8M
Timothy grass	Maltese	2.5Y	F	6M
Dandelion	Bull dog	3Y	F	6M
Cedar/juniper	Y.T	5Y	F	1Y

aF: female

bFn: female neutered

cM: male

dMn: male neutered

eY.T: Yorkshire terrier

Table 3. Causative allergens in canine AD detected by IDST

Allergen	Number of cases (total=35, %)	Allergen	Number of cases (total=35, %)
<i>D. farinae</i>	22 (63%)	Feathers	1 (3%)
<i>D. pteronyssinus</i>	11 (31%)	Cedar/juniper	1 (3%)
Flea	3 (9%)	Cat epithelia	1 (3%)
House dust	2 (6%)	Dandelion	1 (3%)
Mucor	1 (3%)		

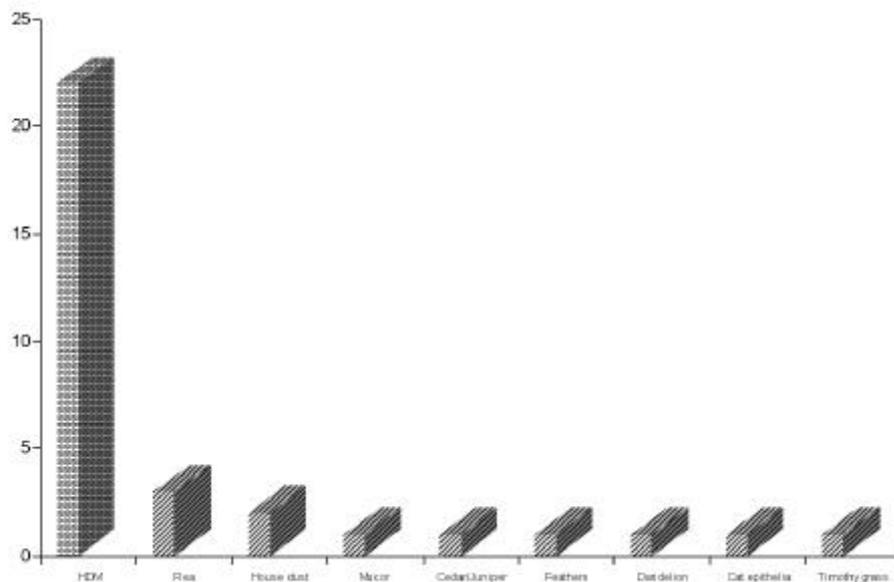


Fig. 1. Prevalent allergens in Korea House dust mites are most frequently detected causative allergens shown in left side.

Food allergic dermatitis (FAD) is the most common form of canine allergic dermatitis in most countries and the second one is AD. Both allergic dermatitis occurs together in 35~70% of allergic dermatitis. In this study, FAD coincides with AD in 5 cases (14%) although the exact cause of FAD is not investigated very well because most of the owners complained the procedure of provocative testing in the course of diagnosing AD.

There are advantages and disadvantages of the currently used diagnostic tests for canine atopy. In IDST, the major diagnostic problems are false positive and false negative reactions and/or problems with cross-reactivity among allergens.

Actually, the major diagnostic problem with IDST is that the test procedure has not been standardized by veterinary dermatologists and results are based on subjective evaluation.

This study describes the prevalence of positive reactions to selected allergens in atopic dogs in Korea. In this study, HDM is the most frequent and important pathogenesis of canine atopy in dogs from Korea although other allergens which were not included in this study must be considered to be included in later IDST.

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