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# Identification of Allergens of *Dermatophagoides farinae* on Canine Atopic Dermatitis in Korea

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**Abstract :** *Dermatophagoides farinae* plays important role in the pathogenesis of canine atopic dermatitis as environmental allergens. Also, many studies revealed that *D. farinae* was the main causative allergen for Korean dogs with atopic dermatitis. To identify major allergens of *D. farinae* in Korean atopic dogs allergic to *D. farinae* by immunoblot using commercial allergenic extracts, 26 dogs from two groups were enrolled in the study. Control group consists of 10 dogs with no clinical signs of disease and atopic group consists of 16 dogs diagnosed as atopic dermatitis. Sera from Korean dogs with atopic dermatitis showed six allergens of *D. farinae* extract by procedure of immunoblot. The molecular weights of identifying protein bands were 177, 109, 75, 44, 27, 15 kDa. The major allergens showing reactivity with greater than 50% of atopic dogs were detected at approximately 44, 109 and 177 kDa. Subsequent investigations will be carried out to verify the identity of the allergens detected in this study.

***Key words*** **:** canine atopic dermatitis, *Dermatophagoides farinae,* allergen, immunoblot.

## Introduction

Canine atopic dermatitis is genetically predisposed, inflammatory and pruritic dermatitis of dog with characteristic clinical manifestations, associated with specific IgE antibodies against most commonly environmental allergen (6).

House dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, play important role in the pathogenesis of canine atopic dermatitis as environmental allergens (11). Also, many studies revealed that house dust mites were the main causative allergen for Korean dogs with atopic dermatitis (9,18,23). In spite of geographic difference in distribution of these two mite species, positive results of intradermal test of *D. farinae* were more dominant than those of *D. pteronyssinus* (7,12,19). Therefore, *D. farinae* is important indoor allergen in canine atopic dermatitis.

Fifteen allergenic protein groups (Der f 1-3, 6, 7, 10, 11, 13-18, and 22, 24) of *D. farinae* are identified in human, and biochemical name and molecular weight of each group are listed in the database of the World Health Organization and International Union of Immunological Societies.

The major allergens were considered these was identified by more than 50% of sera from atopic dogs conventionally, whereas minor allergens are recognized by less than 50% of sera from atopic dogs (17). Investigations about the allergenic proteins of *D. farinae* in dogs were reported. In Netherlands, they were identified that Der 1 and Der 2 were minor allergens and reaction to 90 kDa allergen found at high frequency by immunoblot (14). Sensitivity to purified Der 1 and Der 2 allergens in half of Japanese dogs was appeared at the immunodot method and ELISA (12). There were found that the majority of sensitized American dogs had IgE antibodies specific to two proteins of apparent molecular weights of 98 and 109 kDa, Der f 15 (13). One study confirmed that high molecular weight allergens were the most important *Dermatophagoides* allergens, rather than the low molecular weight group 1 and 2 proteins (15). But other study verified high frequency of positive response to Der f 2 (22). One study described 60 kDa allergen of *D. farinae* was a major allergen for atopic dogs (21). There was demonstrated that high molecular weight allergens are important for Brazilian dogs with atopic dermatitis (2)

It is important to identify which groups of *D. farinae* give rise to atopic dermatitis for the development of more effective diagnosis or immunotherapeutic protocols and reagents. In human, major allergens are regarded as low molecular allergens such as group 1 and 2, which are a 25 kDa cysteine protease and a 14 kDa epididymal protein (20). Besides, considerable studies have been conducted to understand the biological, chemical, and structural properties of several allergens of *D. farinae*. In comparison with human, investigations about major allergens of *D. farinae* in dogs are deficient, and it is controversial that which allergens are major in dogs. In addition, there have been no studies characterizing *D. farinae* using sera from atopic dogs in Korea.

Therefore, the purpose of this study was to identify major allergens of *D. farinae* in Korean atopic dogs allergic to *D. farinae* by immunoblot using commercial allergenic extracts.

## Materials and Methods

### Study groups

Twenty-six dogs from two groups were enrolled in the

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study. The first group is called control group and consists of ten dogs with no clinical signs of disease. The second group is called atopic group and consists of sixteen other dogs diagnosed as atopic dermatitis at the Veterinary Medical Teaching Hospital of Chungnam National University, based on Favrot's criteria (3). All dogs were intra dermally tested using the standard protocol (8) with *D. farinae* extract (Greer Laboratories, Lenoir, USA). None of control dogs had positive reaction to *D. farinae*, and all of atopic dogs had positive reaction to *D. farinae*.

**Sera**

Blood was obtained by venipuncture from all dogs. After collection, the blood samples were kept at room temperature for 10 minutes to allow clotting, and centrifuged at 3,000 rpm. Sera were stored at −20ºC until used.

**Allergen**

The *Dermatophagoides farinae* extract was purchased from Greer Laboratories (Lenoir, USA). The extract was centrifuged by using a centrifugal filter device (Millipore, Carrigtwohill, Ireland) according to manufacturer's protocol. This device consists of regenerated cellulose membrane that separates proteins with a molecular weight > 10 kDa. Then, protein concentration was determined by the Bradford protein assay, using bovine serum albumin (Bio-Rad, Herculus, USA) as standard (1).

**SDS-polyacrylamide gel electrophoresis (PAGE)**

Ten microgram of concentrated *Dermatophagoides farinae* extract was diluted 1:1 in sample buffer containing 62.5 mM Tris-HCL, 25% glycerol, 2% SDS, 0.01% bromophenol blue, 5% 2-mercaptoethanol. The sample was boiled for 10 minutes and then applied to the 4-20% gradient gel (Bio-Rad, Hercules, USA). Also, the molecular marker (Precision Plus Protein Dual Color Standards; Bio-Rad, Hercules, USA) was applied to the gel for identification of molecular weight. Electrophoresis was carried out at 100 V for approximately 90 minutes in a Mini-PROTEAN® Tetra Cell (Bio-Rad, Hercules, USA). Then, the gel was stained with 0.1% Coomassie brilliant blue to confirm the protein bands of the *D. farinae* extract and photographed by Universal Hood II Image Analyzer (Bio-Rad, Hercules, USA).

**Immunoblot**

Reconstituted *D. farinae* extract (10 μg per lane) was separated by SDS-PAGE as described above. The protein was transferred to a 0.45 μm pore size PVDF membrane (Millipore, Billerica, USA) at 100 V for 1 hour in a chamber containing transfer buffer (25 mM Tris, 120 mM glycine, and 20% methanol). The quality of transfer was evaluated by staining the membrane with Ponceau S solution (Sigma Aldrich, St. Louis, USA) to demonstrate the absence of proteins in the gel. The membrane was cut into strips and each strip was washed with tris-buffered saline (TBS)/Tween 20 (0.05%).

The membrane was blocked with 3% bovine serum albumin in TBS/Tween 20 (0.05%) at room temperature for 3 hours. Next, the membrane was incubated with the 1/50 dilution of sera at 4ºC overnight. After washing with TBS/Tween 20 (0.05%) 3 times for 10 minutes, the membrane was incubated with the 1/8,000 dilution of horseradish peroxidase-conjugated goat anti-canine IgE for 1 hour at room temperature. The membrane was again washed with TBS/Tween 20 (0.05%) and exposed to the luminol substrate (West-Q Chemiluminescent Substrate Kit plus; GenDEPOT, Baker, USA). The bands were visualized with Universal Hood II Image Analyzer (Bio-Rad, Hercules, USA).

## Results

**SDS-polyacrylamide gel electrophoresis (PAGE)**

The result of Coomassie-stained SDS-PAGE of the *D. farinae* extract is shown in Fig 1. Analysis of the *D. farinae* extract by SDS-PAGE under reducing condition revealed protein bands at approximately 15 kDa, 20 kDa, 27 kDa, 32 kDa, 44 kDa, 60 kDa, 70 kDa, 109 kDa, 177 kDa, 220 kDa.

**Immunoblot**

The results of immunoblot using sera from sixteen atopic dogs with positive responses in intradermal test for *D. farinae* are shown in Fig 2. The molecular weights of identifying protein bands were 177, 109, 75, 44, 27, 15 kDa. Fourteen of the sixteen sera showed protein band of molecular weights of about 109 kDa, thirteen appeared as band of molecular weights of 44 kDa, eleven appeared as band of approximately 177 kDa, seven showed band of about 27 kDa, five appeared as band of approximately 75 kDa, three showed band of about 15 kDa. The 177, 109 and 44 kDa allergens were identified by greater than 50% of atopic group

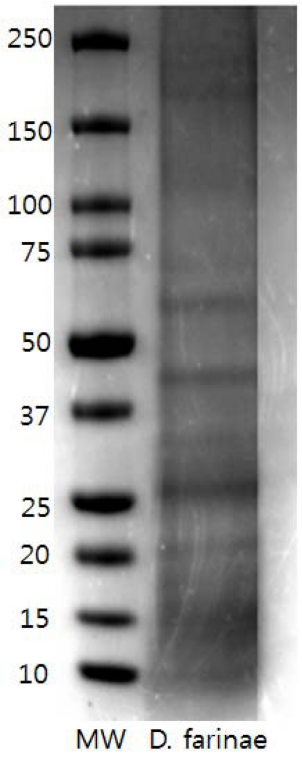


**Fig 1.** Coomassie-stained SDS-PAGE of the *Dermatophagoides farinae* (*D. farinae*) extract under reducing condition. MW: molecular weight marker (kDa).

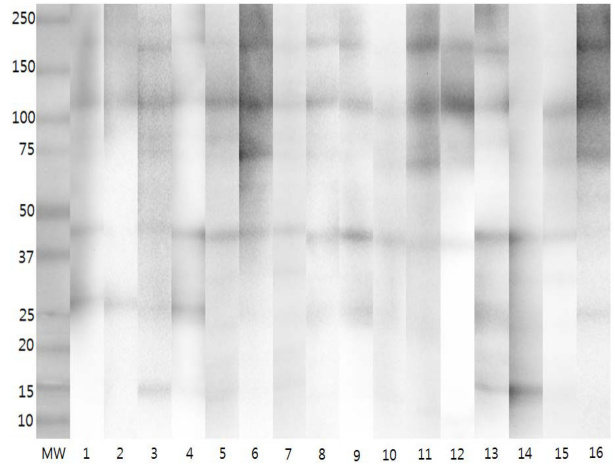


**Fig 2.** Immunoblot of *D. farinae* extract separated by SDS-PAGE plus incubated canine sera. MW: molecular weight marker (kDa).

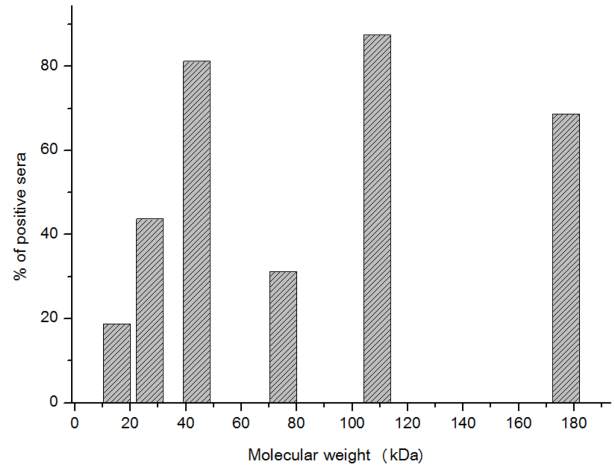


**Fig 3.** Percentage of positive sera of individual allergens in *D. farinae* extract. Bars greater than 50 percent indicate major allergens.

and considered as major allergens as shown in Fig 3. Sera from control group contained no IgE binding to any components of the *D. farinae* extract used in the immunoblot.

The positive sera showed variable binding patterns. IgE in four sera (#s 2, 5, 10 and 14) bound to two protein bands, IgE in six sera (#s 1, 6, 8, 9, 12 and 15) bound to three protein bands, IgE in five sera (#s 4, 7, 11, 13 and 16) bound to four protein bands, IgE in one serum (# 3) bound to five protein bands. No serum was identified to have only one protein band.

## Discussion

In this study, the results of immunoblot assay using the *D. farinae* extract showed bands with different molecular weights ranging from 15 to 177 kDa.

The protein band of approximately 109 kDa in fourteen dogs with atopic dermatitis was identified, being presumably Der f 15. Der f 15 usually appears as double bands at 98 and 109 kDa on both reducing and non-reducing blots (15). Der f 15 is heavily glycosylated protein with molecular weights of approximately 62 kDa and homologous N-terminal sequences with insect chitinase (13). In our study, only one band of molecular weights of about 109 kDa was observed without a 98 kDa band. It is assumed that this result is associated with allergen extract or laboratory conditions, because the absence of a protein band of 98 kDa allergen appeared on the SDS-PAGE in this study.

In previous study, fragment or denatured forms of glycosylated allergens with high molecular weight were identified at 44-45, 65-69, 84-85 kDa on reducing blot (10). This fact supports that 44 kDa and 75 kDa allergens in thirteen and five atopic dogs were also fragment or denatured forms of glycosylated allergens.

The 177 kDa of allergens recognized by eleven dogs sensitized with *D. farinae* might be considered as Der f 14. Der f 14 is apolipophorin susceptible to protease, termed Mag 3 (4). In human, the breakdown of this allergen induced more allergic reaction than did the intact form of allergen (5). However, reactivity to this allergen has not been studied in dogs. Because of sensitivity to protease, the heterogeneity was seen in the antigenic constitution of crude extract (5). So, this band of 177 kDa will be more investigated.

The 44, 109 and 177 kDa allergens appeared at high frequency, so these allergens will be helpful for diagnosis and therapy of canine atopic dermatitis. Further studies as cDNA and/or N-terminal amino acid sequencing are needed to understand the biological, chemical and structural properties of these allergens observed in this investigation.

Der f 18, glycosylated chitinase with molecular weights of 60 kDa, identified as major allergen in dogs suffering from atopic dermatitis in previous studies (21). In this study, none of sera showed a band at 60 kDa.

Seven sera from atopic dogs showed band at 27 kDa, three sera showed band at 15 kDa. These bands correspond to Der f 1 and Der f 2, major allergens for humans. In veterinary medicine, it is reported that these low molecular weight allergens is considered rather minor allergen in America and Europe (2,13,14,15). In Japan, one study reported half of the atopic dogs had sensitivity to Der 1 and Der 2 (12). Also, another report identified high IgE-reactivity to Der f 2 from Japanese dogs (22). In this investigation, Der f 1 and Der f 2 were recognized by fewer than 50% of *D. farinae*-sensitized dogs, so it is determined that Der f 1 and Der f 2 are not major allergens in Korea.

In both humans and dogs, house dust mites are considered as most important allergens, whereas major allergens of house dust mites are recognized differently. Previous studies speculated that canine antigen presenting cells having a different array of peptides or T-cell receptor and MHC II repertoires very distinct from humans caused the difference of allergen profiles (13,16,21).

Because of this dissimilarity in recognition of major allergens of house dust mite, separated diagnosis and treatment are needed for dogs. Containing insufficiency quantities of allergens, Commercial materials used for the immunotherapy in humans could be inappropriate for dog. Therefore, the identification and production of major allergens for dogs could contribute to make more effective treatment of atopic

dermatitis.

Sera from Korean dogs with atopic dermatitis showed six allergens of *D. farinae* extract by procedure of immunoblot. The major allergens showing reactivity with greater than 50% of atopic dogs were detected at approximately 44, 109 and 177 kDa. Subsequent investigations will be carried out to verify the identity of the allergens detected in this study.

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# 국내 개에서 발생한 아토피성 피부염에서 *Dermatophagoides farinae*의 항원 확인

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**요 약** : 개의 아토피성 피부염에서 *Dermatophagoides farinae*의 항원을 immunoblot을 통해 확인하였다. 실험은 *D. farinae*에 의해 아토피성 피부염이 유발된 16마리와 임상적으로 건강한 10마리 개의 혈청으로 수행하였으며, SDS-PAGE를 실시하여 *D. farinae* 추출물의 항원들이 15에서 220 kDa 범위 내에 존재하는 것을 관찰하였다. Immunoblot 결과, 임상적으로 건강한 10마리 개의 혈청에서는 단백띠가 보이지 않았으며, *D. farinae*에 의해 아토피성 피부염이 유발된 16마리 개의 혈청을 이용한 실험에서는 14마리에서 109 kDa, 13마리에서 44 kDa, 11마리에서 177 kDa, 7마리에서 27 kDa, 5마리에서 75 kDa, 3마리에서 15 kDa의 단백띠가 관찰되었다. 즉, *D. farinae*에 의해 아토피성 피부염이 유발된 개체 중 50% 이상에서 관찰되는 주요 항원은 177 kDa, 109 kDa, 44 kDa 항원이었다. 본 실험과 같이 주요 항원들을 확인하는 이러한 연구는 아토피성 피부염에 대한 새로운 면역 치료의 전략과 제제의 개발에 기여할 수 있을 것으로 생각된다.

**주요어** : 개 아토피성 피부염, *Dermatophagoides farina,* 항원, immunoblot