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# Profile of the Staphylococcal Exotoxin Gene and its Relation with Canine Atopic Dermatitis

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**Abstract :** *Staphylococcus* spp. is one of the most common bacteria isolated from the lesions of atopic dermatitis (AD) in humans, and their colonization is known to be a possible trigger factor of clinical signs. The aim of this study was to determine the prevalence of *Staphylococcus* spp. in canine AD (CAD), the types of exotoxins present, and their relation with the clinical severity of CAD. From 79 dogs with AD, 72 samples of *Staphylococcus* spp. were isolated (91.1%), and 65 (90.3%) were confirmed as *Staphylococcus pseudintermedius*. Concerning the profile of the exotoxin gene, 50 isolates (69.4%) contained at least one exotoxin gene, and 28 isolates (56%) were found to contain more than 2 different exotoxins. There was a significant difference in clinical severity with the presence of staphylococcal exotoxins ($P = 0.028$), whereas no correlation was found with the presence of *Staphylococcus* spp. ($P = 0.598$). The clinical severity of CAD increased only in relation to staphylococcal enterotoxin D (SED) and exfoliative toxins ($P < 0.05$). Some clinical evaluation criteria (erythema, papule/pustule) were correlated with the presence of the exotoxin gene ($P < 0.05$). This study showed that the high prevalence of *Staphylococcus* spp. and staphylococcal exotoxins in lesions from dogs with AD may be regarded as an important trigger factor for exacerbation of the clinical signs of CAD.

***Key words*** : Atopic dermatitis, dog, exotoxin, *Staphylococcus*.

## Introduction

Atopic dermatitis (AD) is a complex chronic inflammatory skin disorder with a recently increasing incidence. AD is associated with cutaneous erythema, induration, severe pruritus, eosinophilia, and elevated serum IgE and has a hereditary disposition (14,21). AD is often associated with skin colonization by *Staphylococcus* spp. *Staphylococcus* spp. is one of the most common bacteria isolated from humans and a wide range of animal species, and it is associated with many virulence factors, such as coagulase, hemolysins, leucocidin, and enterotoxins. *Staphylococcus* spp. colonization contributes to allergic sensitization and inflammation (26). Scratching increases the binding of *Staphylococcus* spp. to the skin, and an increase in *Staphylococcus* spp. produces seramidase, which can aggravate defects in skin barrier function. Staphylococcal exotoxins increase inflammation and provoke the generation of exotoxin-specific IgE, levels of which correlate with the severity of the disease (25,34).

The role of staphylococcal exotoxin secretion on lesion severity in the pathogenesis of AD has become more evident (7,8,36). Staphylococcal exotoxins include staphylococcal enterotoxin A (SEA), B (SEB), C (SEC), D (SED), and E (SEE); exfoliative toxin A (ETA) and B (ETB); and toxic shock syndrome toxin-1 (TSST-1) (9,15,19,39). These exotoxins can contribute to the inflammation of AD by causing continued T cell activation and the release of pro-inflammatory mediators. Secreted exotoxins play a role as superantigens, which are characterized by their ability to bind to major histocompatibility complex (MHC) class II molecules and subsequently activate a large fraction of T lymphocytes in an antigen non-specific manner (10,40,41). Superantigens penetrate the injured skin barrier more easily in patients with AD than in persons with an intact skin barrier; therefore, they appear to play a role in the induction, prolongation, and exacerbation of AD in the majority of AD patients (7,27).

Recently, some studies of the relation between the presence of staphylococcal exotoxins and the severity of clinical signs in humans with AD were conducted (31,36,42). However, little is known about the role of staphylococcal exotoxins in canine AD (CAD) (5,9,17). The aim of this study was to determine the prevalence of staphylococcal infection, the gene patterns of staphylococcal exotoxins, and the relation of staphylococcal exotoxins with the clinical severity of CAD.

## Materials and Methods

### Study population

A total 79 dogs with CAD were involved in this study,

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which was performed from August 2007 to April 2009. CAD was diagnosed on the basis of history, clinical findings, a positive result from a serum allergen-specific IgE test and/or an intradermal skin test, and the absence of other pruritic disease (e.g. sarcoptic acariasis) using standard diagnostic methods (12,14). All dogs underwent a 6-week trial with an appropriate home-cooked or commercial single protein diet, or a commercial hydrolyzed hypoallergenic diet to rule out adverse food reactions. No dog in this study had received topical or systemic antimicrobial therapies for at least 3 weeks prior to sampling. The study population consisted of 13 intact males, 33 castrated males, 22 intact females and 11 spayed females. The mean age of the animals was 6.3 years (range: 1-12 years). The dog breeds included Shih Tzu (n = 33), Cocker Spaniel (n = 18), Maltese (n = 8), mixed breed (n = 5), Yorkshire Terrier (n = 7), Pekingese (n = 4), Schnauzer (n = 2), Miniature Pinscher (n = 1) and Bichon Frise (n = 1). Forty-five healthy dogs with no history or current clinical evidence of skin disease were studied as controls.

**Evaluation of clinical severity**

Clinical severity at the sampling site was assessed using a lesional scoring system modified from the third version of the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) and Scoring of Atopic Dermatitis (SCORAD) to estimate the severity of the clinical signs of dogs and humans with AD, which consists of 6 clinical criteria (erythema, lichenification, excoriation, oozing, scale, and papule/pustule) (23,32). For each combination, a score from 0 to 3 was given (0, none; 1, mild; 2, moderate; and 3, severe). The total score was defined as the sum of these 6 clinical criteria for each dog; thus, a maximum score of 18 was possible.

**Bacterial isolation and identification**

Samples were taken from the skin that had no *Malassezia* populations by cytologic evaluation in the dogs with CAD. So, swabs from atopic dogs with pyoderma (n = 52) were obtained from skin lesions (papules, pustules and underside of crusts) consistent with superficial pyoderma; swabs from atopic dogs without pyoderma (n = 27) were obtained from CAD-affected skin; and swabs from healthy dogs (n = 55) were obtained from mucosal sites. All specimens were inoculated in trypticase soy broth (TSB) and subcultured in blood agar at 37°C for 24 h. Staphylococcal isolates were identified on the basis of colony characteristics, gram staining, and the production of coagulase (tube coagulase test) (35). Identification was confirmed based on results of API STAPH (bioMerieux, France) and the VITEK II system (bio Merieux Vitek, Hazelwood, MO, USA). Strains identified as *S. intermedius* were classified as the *S. intermedius* group (SIG) and included *S. intermedius, S. delphini*, and *S. pseudintermedius*. It was difficult to differentiate these 3 species based on biochemical characteristics. All isolates were suspended in 50% glycerol and stored at −70°C until analyzed further.

The following toxigenic *S. aureus* reference strains were used as a positive control: FRI 472 (SED), FRI913 (SEA, SEC, SEE,

**Table 1.** PCR primers for the detection of Staphylococcal exotoxin genes used in this study

| Gene | Primer | Oligonucleotide sequence (5'-3') | Location within gene | Size of amplified product (bp) | Multiplex PCR set |
|---|---|---|---|---|---|
| *sea* | GSEAR-1 | GGTTATCAATGTGCGGGTGG | 349-368 | 102 | A |
| | GSEAR-2 | CGGCACTTTTTTCTCTTCGG | 431-450 | | |
| *seb* | GSEBR-1 | GTATGGTGGTGTAACTGAGC | 666-685 | 164 | A |
| | GSEBR-2 | CCAAATAGTGACGAGTTAGG | 810-829 | | |
| *sec* | GSECR-1 | AGATGAAGTAGTTGATGTGTATGG | 432-455 | 451 | A |
| | GSECR-2 | CACACTTTTAGAATCAACCG | 863-882 | | |
| *sed* | GSEDR-1 | CCAATAATAGGAGAAAATAAAAG | 492-514 | 278 | A |
| | GSEDR-2 | ATTGGTATTTTTTTTCGTTC | 750-769 | | |
| *see* | GSEER-1 | AGGTTTTTTCACAGGTCATCC | 237-257 | 209 | A |
| | GSEER-2 | CTTTTTTTTCTTCGGTCAATC | 425-445 | | |
| *femA* | GFEMAR-1 | AAAAAAGCACATAACAAGCG | 1444-1463 | 132 | A and B |
| | GFEMAR-2 | GATAAAGAAGAAACCAGCAG | 1556-1575 | | |
| *eta* | GETAR-1 | GCAGGTGTTGATTTAGCATT | 775-794 | 93 | B |
| | GETAR-2 | AGATGTCCCTATTTTTGCTG | 848-867 | | |
| *etb* | GETBR-1 | ACAAGCAAAAGAATACAGCG | 509-528 | 226 | B |
| | GETBR-2 | GTTTTTGGCTGCTTCTCTTG | 715-734 | | |
| *tst* | GTSSTR-1 | ACCCCTGTTCCCTTATCATC | 88-107 | 326 | B |
| | GTSSTR-2 | TTTTCAGTATTTGTAACGCC | 394-113 | | |

and TST-1), MNHOCH (SEB), UT0003 (ETA), and UT0007 (ETB) (20,30). These strains were provided by the Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University.

### DNA extraction and identification of *S. pseudintermedius*

Genomic DNA from isolated *Staphylococcus* was extracted using a DNeasy tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions with a modification of the cell lysis step performed with 50 U/ml lysostaphin (Sigma, St. Louis, MO, USA). Staphylococcal cultures were grown overnight in 2 ml TSB with shaking.

Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was conducted to differentiate *S. pseudintermedius* from the other SIG species (4). PCR amplification of the 320-bp *pta* gene fragment (primer 1, AAAGACAAACTTTCAGGTAA; primer 2, GCATAAACAAGCATTGTACCG) was performed, and *pta* PCR products were digested with MboI restriction enzyme, which resulted in 2 restriction fragments of 213 bp and 107 bp, respectively, as reported previously.

### Multiplex PCR reaction for detecting exotoxin gene

Primers specific to 8 kinds of staphylococcal exotoxin genes (*sea* to *see*, *eta*, *etb*, and *tst*) in 2 sets of the reactions were selected from the published DNA sequences of the *S. aureus* genes (Table 1) (29). For multiplex PCRs, 2 primers sets were prepared: set A was designed to amplify *sea*, *seb*, *sec*, *sed*, *see*, and *femA*, whereas set B was designed to amplify *eta*, *etb*, *tst*, and *femA*. The *femA* gene involved in the synthesis of the staphylococcal cell wall was used as an internal positive control target and confirmed the fidelity of the multiplex PCR. The *femA* gene primer was included in every set. The primer sequences, their amplified PCR product sizes, and the type of PCR set used in the multiplex PCRs are described in Table 1.

Multiplex PCR reactions were performed with a reaction mixture that contained 5 μl of prepared template DNA, 2.5 mM dNTPs, 1× reaction buffer, 1× gel loading buffer, 2.5 U i-StarTaq™ DNA Polymerase (iNtRON, Seoul, Korea), and each of a pair of primers (primer set A: 40 pmol of *sed* primer and 20 pmol each of *sea*, *seb*, *sec*, *see*, and *femA* primers; primer set B: 50 pmol of *eta* and 20 pmol each of *etb*, *tst*, and *femA*). The final volume was adjusted to 20 μl with DEPC. The PCR amplification reaction was carried out using the following procedure: initial denaturation at 94°C for 5 min was followed by 37 cycles of amplification (denaturation at 94°C for 2 min, annealing at 57°C for 2 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 7 min. The PCR amplification products were analyzed by electrophoresis on 3% agarose gel (Sigma) containing 1 μg/ml ethidium bromide for 50 min at 100 V in 0.5× TBE buffer and visualized with UV light. The PCR products were purified using the MEGAquick-spin™ kit (iNtRon, Korea) according to the manufacturer's instructions, and then directly sequenced using an automatic sequencer (ABI 3730XL DNA analyser, Macrogen Inc.). The sequence data were analyzed using the Blast program from the National Center for Biotechnology Information.

### Statistical analysis

A statistical analysis was conducted to evaluate the relation between the production of staphylococcal exotoxin gene and clinical severity of CAD. All results were performed using chi-square test, Fisher's exact test and 2-sample t-tests with the computer software SAS program. $P$ values $< 0.05$ were considered statistically significant.

## Results

### *Staphylococcus* isolation and exotoxin gene profile

*Staphylococcus* spp. was isolated from 72 of the 79 dogs (91.1%) with CAD. Of these 72 isolates, SIG strains were isolated from 65 dogs (90.3%), all of which were confirmed as *S. pseudintermedius*. Two *S. epidermis* strains and one each of *S. aureus*, *S. haemolyticus*, *S. lugdunensis*, *S. caprae*, and *S. warneri* were also identified. *Staphylococcus* spp. strains isolated from 50 of the 72 CAD dogs (69.4%) were found to produce more than 1 different exotoxins, and 28 isolates (56%) were found to contain more than 2 different exotoxins (Table 2). The multiplex PCR reactions were performed on reference strains without nonspecific or additional bands (Fig 1). SEA was the exotoxin most frequently detected (28 isolates, 56%), either alone or in combination with other toxins, followed by ETA (16 isolates, 32%), TSST-1 (16 isolates, 32%), and ETB (15 isolates, 30%). In 45 healthy control dogs, *Staphylococcus*

**Table 2.** Exotoxin profile of *Staphylococcus* isolates

| Single | Isolate(n) | | |
|---|---|---|---|
| SEA only | 8 | | |
| SEB only | 3 | | |
| SEC only | 1 | | |
| ETA only | 3 | | |
| ETB only | 1 | | |
| TSST-1 only | 6 | | |
| Multiple | Isolate(n) | Multiple | Isolate(n) |
| SEA/SEB | 1 | SEA/SEB/TSST-1 | 1 |
| SEA/ETA | 1 | SEA/SED/ETA | 2 |
| SEA/ETB | 6 | SEA/ETA/ETB | 1 |
| SEA/TSST-1 | 2 | SEA/ETA/TSST-1 | 1 |
| SEB/SEE | 1 | SEB/SEC/TSST-1 | 1 |
| SEB/ETB | 1 | SED/ETA/ETB | 1 |
| SEB/TSST-1 | 1 | SEA/SEB/ETA/TSST-1 | 1 |
| SED/ETA | 1 | SEB/ETA/ETB/TSST-1 | 1 |
| ETA/ETB | 1 | SEA/SED/ETA/ETB/TSST-1 | 1 |
| SEA/SEB/ETA | 1 | SEA/SEB/SEE/ETA/ETB/TSST-1 | 1 |
| SEA/SEB/ETB | 1 | | |

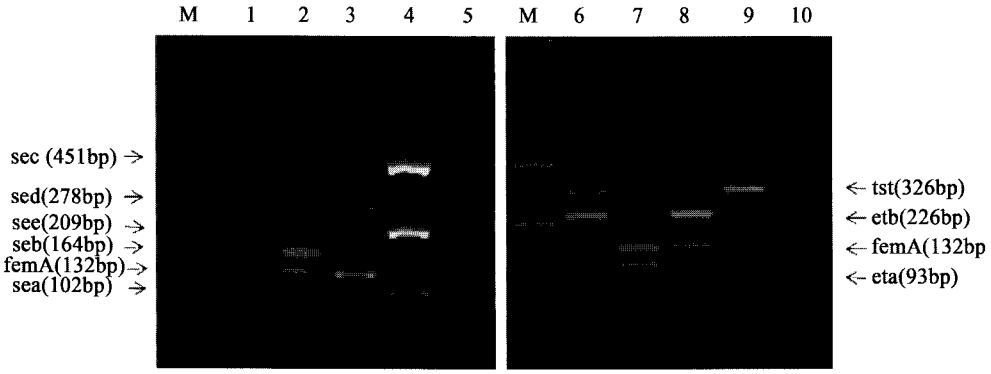


**Fig 1.** Agarose gel electrophoresis patterns showing multiplex polymerase chain reaction (PCR) results on *Staphylococcus aureus* reference strains. Lane M: 20 bp marker; lanes 1 to 5, PCR amplifications from primer set A; lanes 6 to 10, PCR amplification from primer set B. Lane 1: *sea*, *seb*, *sec*, *sed*, *see*, and *femA* simultaneously; Lane 2: MNHOCH (*seb* and *femA*), Lane 3: FRI472 (*sed* and *femA*), Lane 4: FRI913 (*sea*, *sec*, *see*, and *femA*), Lane 5: negative control, Lane 6: *eta*, *etb*, *tst*, and *femA*; Lane 7: *eta* and *femA*; Lane 8: *etb* and *femA*; Lane 9: FRI 913 (*tst* and *femA*), and Lane 10: negative control.

spp. strains were isolated from 21 dogs (46.7%), and *S. pseudintermedius* was identified in 16 isolated strains (76.2%). None of these isolated strains were detected exotoxins.

The detection rate of *Staphylococcus* spp. and staphylococcal exotoxins was significantly higher in the CAD group than in the normal control dogs ($P < 0.0001$ and $P < 0.0001$, respectively) (Tables 3 and 4).

### Correlation between staphylococcal colonization with exotoxin genes and clinical severity of CAD

This study assessed the influence of staphylococcal colonization and exotoxins on CAD by comparing the clinical severity scores of the dogs. No significant difference in clinical severity was observed based on staphylococcal colonization in lesions from dogs with CAD ($8.14 \pm 6.01$ vs. $9.19 \pm 4.92$, $P = 0.598$), but the number of exotoxin genes detected was related to clinical severity ($9.30 \pm 4.58$ vs. $11.37 \pm 4.81$, $P = 0.028$) (Fig 2). Clinical severity was not related to the number of exotoxin genes, but an increasing tendency was detected with an increasing number of exotoxin genes.

In comparison with clinical severity according to the type of staphylococcal exotoxins, SED, ETA and ETB gene contaning isolates showed a statistically significant difference in the dogs with higher clinical severity score ($P = 0.017$, $P = 0.01$ and $P = 0.007$, respectively) (Table 5). The correlation between each criteria of the clinical severity of CAD and staphylococcal exotoxin gene detection was shown in Table 6. Only erythema and papule/pustule criteria were significantly correlated with staphylococcal exotoxin gene detection ($P < 0.05$).

**Table 3.** Comparison of detection rates of *Staphylococcus* spp. between skin from the canine atopic dermatitis group and that from the control group

| | Canine atopic dermatitis (%) | Normal control (%) |
|---|---|---|
| *Staphylococcus* spp. (+) | 72 (91.1) | 21 (46.7) |
| *Staphylococcus* spp. (-) | 7 (8.9) | 24 (53.3) |
| Total | 79 (100) | 45 (100) |

$P < 0.0001$, chi-square test

**Table 4.** Comparison of detection rates of Staphylococcal exotoxins between skin from the canine atopic dermatitis group and that from the control group

| | Canine atopic dermatitis (%) | Normal control (%) |
|---|---|---|
| Exotoxin (+) | 50 (69.4) | 0 (0) |
| Exotoxin (-) | 22 (30.6) | 21 (100) |
| Total | 72 (100) | 21 (100) |

$P < 0.0001$, Fisher's exact test

## Discussion

Dogs with atopic dermatitis frequently exhibit concurrent skin infections with a variety of microorganisms, including *Staphylococcus* spp. bacteria or *Malassezia* yeast (13). Most studies revealed that the prevalence of the skin colonization by *Staphylococcus* spp. and degree of colonization density on the skin in dogs with AD are higher than in normal healthy dogs like in human AD (3,16). And it was reported in human that positive correlation between the clinical severity of AD and the colonization density of *Staphylococcus* spp. on skin lesions (43). Several possible mechanisms responsible for the high prevalence of skin colonization and worsening AD by *Staphylococcus* spp. have been suggested, including skin barrier dysfunction, decreased production of endogenous antimicrobial peptides and increased synthesis of extracellular matrix proteins (11,28,33). Several studies investigated that the skin with AD by the combination therapy of corticosteroid and antibiotics compared to a corticosteroid alone was able to reduce the colonization of *Staphylococcus* spp. and to lead to an improve-

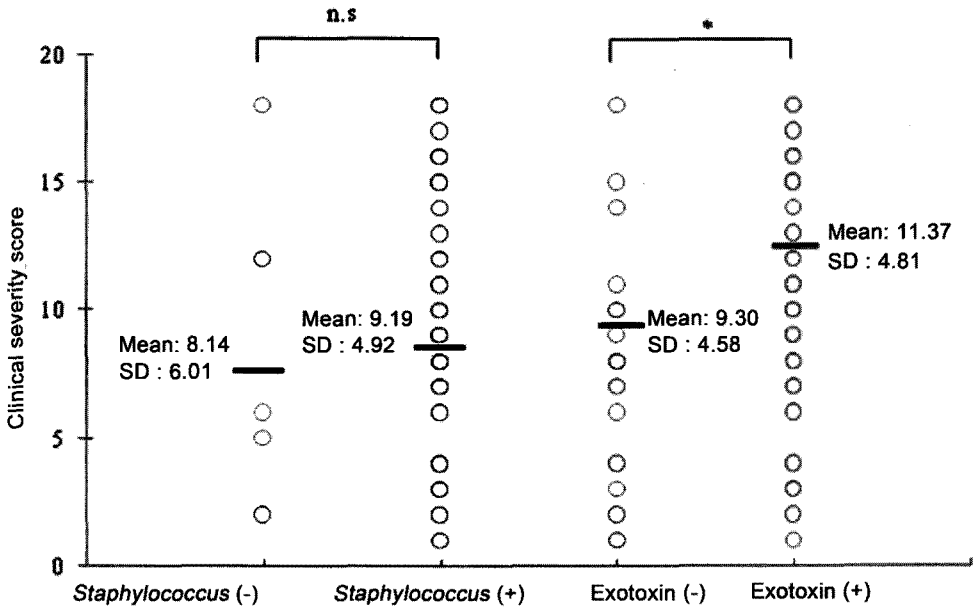


**Fig 2.** Relation of clinical severity score with *Staphylococcus* spp. colonization and with the number of staphylococcal exotoxins. No significant difference in clinical severity was observed based on staphylococcal colonization in lesions from dogs with CAD ($P = 0.598$), but the number of exotoxin genes detected was significantly related to clinical severity ($P = 0.028$).

**Table 5.** Comparison of clinical severity scores according to exotoxin type. There was a statistically significant difference ($P < 0.05$) in the correlation between clinical severity of CAD and the type of exotoxin, such as SED, ETA, and ETB

| | Mean ± SD | *P* value |
|---|---|---|
| SEA | 9.71 ± 5.08 | 0.641 |
| SEB | 8.62 ± 4.03 | 0.232 |
| SEC | 8.67 ± 0.58 | 0.839 |
| SED | 14.80 ± 1.79 | 0.017 |
| SEE | 9.00 ± 2.83 | 0.768 |
| ETA | 12.50 ± 4.86 | 0.010 |
| ETB | 12.73 ± 4.13 | 0.007 |
| TSST-1 | 9.06 ± 3.86 | 0.350 |

**Table 6.** Comparison of exotoxin production according to multiple parameters of disease severity. There was a significant difference ($P < 0.05$) in the correlation between exotoxin production and parameters of disease severity, such as erythema and papule/pustule

| Parameter | Mean ± SD | *P* value |
|---|---|---|
| Excoriation | 1.72 ± 1.03 | 0.183 |
| Lichenification | 1.42 ± 1.16 | 0.139 |
| Oozing | 1.44 ± 1.03 | 0.061 |
| Erythema | 2.54 ± 0.65 | 0.025 |
| Scale | 1.52 ± 1.02 | 0.082 |
| Papule/Pustule | 1.36 ± 0.96 | 0.026 |

ment of AD skin lesions, which suggest the contribution of immunological factors that support colonization (1,6,22,37,38).

Little evidence supports the role of staphylococcal exotoxin production in the pathogenesis of CAD (24). This study showed that the prevalence of staphylococcal colonization and staphylococcal exotoxins was higher in dogs with CAD than in dogs without CAD, as in previous studies (2,18). This study investigated the prevalence of staphylococcal infection, its exotoxin gene patterns, and the relation of exotoxin gene patterns with the clinical severity of CAD. No significant difference in clinical severity was observed in relation to the isolation of *Staphylococcus* from the lesions of dogs with CAD, but the exotoxin gene pattern was related to clinical severity. In addition, the clinical severity of CAD was not related to the number of exotoxin genes detected, but an increased incidence of CAD was related to an increased number of exotoxin genes detected.

This result indicates that the presence of staphylococcal exotoxins, rather than *Staphylococcus* spp., is associated with an exacerbation of clinical signs of CAD. Some exotoxin genes, such as SED, ETA, and ETB, were more frequently detected in *Staphylococcus* from lesions of dogs with CAD that had higher clinical severity scores. This finding suggests that SED, ETA, and ETB may play an important role in the clinical severity of CAD. There also appeared to be a difference between indicators of the clinical severity of CAD, especially erythema and papule/pustule. These findings suggest that exotoxin-producing *Staphylococcus* spp. act as a trigger in the pathogenesis of CAD. Patients with CAD colonized with *Staphylococcus* spp. continuously release exotoxins into the skin, which contributes to the persistence and exacerbation of skin inflammation

associated with CAD. Thus, staphylococcal exotoxins seem to be an exacerbating rather than an induction factor of CAD.

However, this study was unable to document whether or not superantigen activity is involved in the pathogenesis of CAD (44). Additional studies are required to elucidate whether staphylococcal exotoxins play a role as superantigens or as a new group of allergens through an IgE-mediated immune response resulting in the exacerbation of skin inflammation in CAD. Detection of a serum IgE titer against staphylococcal exotoxins, peripheral blood T cell activation, and homing receptor expression may provide important information relevant to the severity of CAD. Also, this study found that SEA was the most common exotoxin gene unlike the one reported in the recent study conducted in Korea (45). The reason for the difference in exotoxin gene profile of atopic dogs is unclear. Thus, additional studies would be necessary to investigate prevalence of staphylococcal exotoxin gene patterns observed in atopic dogs with pyoderma versus dogs with a single episode of pyoderma. As shown in this study, staphylococcal exotoxins may contribute to the clinical severity of CAD, and new strategies for the treatment of CAD may include ones that interfere with the production of staphylococcal exotoxins.

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# 포도알구균의 외독소 유전자 분석과 그 외독소가 개 아토피 피부염에 미치는 영향

**남의화 · 정태호 · 김지현 · 박설희 · 김효은 · 윤화영 · 채준석 · 박용호 · 황철용**[1]

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**요 약** : 포도알구균은 아토피 피부염 환자의 피부병변에서 가장 많이 발견되는 세균으로, 이들 균집락의 정도는 사람 아토피 피부염의 임상증상 악화요인으로 알려져있다. 이에 본 연구에서는 개 아토피 피부염 환자의 피부에서 포도알구균의 존재를 확인하고, 이들 균주가 생산하는 외독소 유형을 분석하여 개 아토피 피부염 환자의 임상증상과의 연관성을 알아보았다. 79마리의 개 아토피 피부염 환자 중 91.1%인 72마리에서 포도알구균이 검출되었으며, 이 중 62마리에서 *Staphylococcus pseudintermedius*가 가장 높은 빈도로 확인되었다. 외독소 유전자 분석에서는 69.4%인 50마리에서 1가지 이상의 외독소 유전자를 포함하였고, 이들 중 56%인 28마리에서 2가지 이상의 다른 외독소 유전자를 가지고 있는 것으로 나타났다. 개 아토피 피부염 환자를 포도알구균의 존재 유무에 따라 분류하였을 때, 임상증상 점수의 차이에 통계적인 의미는 없었지만 ($P = 0.598$), 외독소 유무에 따라 임상증상 점수를 비교하였을 때는 의미있는 차이를 보였다 ($P = 0.028$). 또한 외독소 유형에 따라 분류하였을 때 외독소 중 SED와 exfoliative toxins에서 임상증상에 의미있는 차이를 보였으며 ($P < 0.05$), 외독소 유무에 따라 분류하였을 때는 임상증상 점수 중 특히 발적과 구진/농포에서 의미있는 차이를 보였다 ($P < 0.05$). 이와 같은 결과를 통해 개 아토피 피부염에서 포도알구균이 생산하는 외독소가 개 아토피 피부염의 증상악화와 관련이 있는 것으로 사료된다.

**주요어** : 아토피 피부염, 개, 외독소, 포도알구균