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for the degree of Master of Veterinary Medicine

Distribution of Exfoliative Toxin Genes of Staphylococcus intermedius Group (SIG) Isolated from Dogs

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

Department of Veterinary Medicine

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Veterinary Medicine

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Staphylococcal exfoliative toxins (ETs) are known to digest desmoglein-1, a desmosomal cell‑cell adhesion molecule, thus causing intraepidermal splitting in human bullous impetigo, staphylococcal scalded skin syndrome(SSSS) and swine exudative epidermitis. *Staphylococcus pseudintermedius*, one of members of *Staphylococcus intermedius* group (SIG), has a few ETs, such as *siet*, *exp*A (formerly *exi*) and *exp*B. The aim of this study was to determine the distribution of ET genes in SIG isolated from diseased, and healthy dogs.

Total 135 and 73 isolates of SIGs were identified in diseased dogs and healthy dogs. The isolates of diseased dogs were taken from lesions related to eyes, nose, ears, skin, interdigit and urine, and those of healthy dogs

were isolated from nose, mouth and skin. The *siet* gene not related to skin exfoliation was most common in SIGs isolated from both groups, but the isolates from diseased dogs (89.6%) had much higher *siet* gene then those from healthy dogs (37.0%). In diseased dogs and helathy dogs, *exp*A and *exp*B genes were found in 29 (21.5%) and 7 (9.6%), and 8 (5.9%) and 6 (8.2%) isolates, respectively. In diseased dog group, *siet* gene was found in 100% of nose and urine samples and the prevalence rate was high in most other samples. *exp*A gene was detected in 21.3% of skin, 37.5% of interdigit, 19.4% of ear, 23.1% of nose, 12.5% of eyes and 33.3% of urine. *exp*B gene was present in 23.1% of nose, 6.6% of skin, 2.8% of ears. In healthy dogs, *siet* gene was found 40.0% of nose, 33.3% of mouth and 37.1% of skin samples. *exp*A gene was found in mouth and skin samples, and *exp*B gene was present in nose, mouth and skin samples. SIGs (65.2%) with only the *siet* gene were the most common, while in healthy dogs, those without any toxins (53.4%) were prominent. In particular, 19.3% of the isolates from the diseased dog showed *siet*-*exp*A combination, and *exp*A-*exp*B combination was found 1 strain of healthy dog-derived SIG. The *siet*-*exp*A-*exp*B combination was found in 2 and 1 isolates from the diseased dogs and healthy dogs, respectively.

In conclusion, our results showed that *exp*A and *exp*B genes were found in SIGs from various leisons of both groups and there were *exp*A-*exp*B combination in some organisms. Since *S. pseudintermedius* is well-known normal flora of dog and could cause opportunistic infection in dogs and human, especially in immune suppressed patients. This subject can be effecti ve for help of diagnosis of SIGs induced disease in veterinary medicine and risk control for dog owners who have underlying medical problems like immunosuppression.

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INTRODUCTION

Staphylococcus species are known as normal flora which could opportunistically cause various disease in human and animals [32]. Besides, coagulase-positive *Staphylococcus* species including *S. aureus, S. hycuis* and *S. pseudintermedius* are known to cause more serious disease than negative species [1,14]. Especially, *Staphylococcus intermedius* group (SIG), which comprises the three closely related species *S. intermedius, S. pseudintermedius,* and *S. delphini,* has been identified as a bacterial pathogen of concerned in canine [16,34]. *S. pseudintermedius,* the most common SIG species, is more frequently associated with dog. It was first isolated in 1976 and then identified as *S. intermedius.* Later, *S. intermedius* isolates collected from animals, was revealed as a novel species, *S. pseudintermedius,* in 2005 through DNA-DNA hybridization method [12]. *S. pseudintermedius* causes various disease in dogs including canine pyoderma, otitis externa, urinary tract and respiratory tract infections, reproductive tract infections [26]. Recently, *S. pseudintermedius* has received attention from researchers as a pathogen of zoonosis [9,31,39] and Methicillin-Resistance *S. pseudintermedius* (MRSP) [5].

Pathogenic staphylococci produce a wide variety of virulence factors, initially described in *S. aureus.* These factors include surface proteins, such as Protein A, clumping factor, fibronectin binding proteins and iron regulated surface determinants, capsular polysaccharides involved in biofilm formation, toxins related with pore forming and superantigens, or some enzymes including coagulase, staphylokinase and proteases [19]. Among the toxins staphylococcal enterotoxins and toxic shock syndrome toxin acts as superantigens triggering T-cell activation and proliferation [19], and exfoliative toxins (ETs) first reported in *S. aureus* cause staphylococcal

scaled skin syndrome (SSSS) characterized by destruction of desmoglein 1 (desmosonal cell attachments) resulting in detachment of the epidermis.

Most researches on virulence factors of other pathogenic staphylococci are based on those of *S. aureus*. Several researchers have described virulence factors in isolates of SIG including adhesion and tissue invasion [3,29], protein A [2], biofilm formation [16,39], pore-forming toxins [16,18]. ETs reported initially in *S. aureus* cause human bullous impetigo [11], SSSS as well, and include four different types, *ETA, ETB, ETD,* and *ETE.* Swine exudative epidermitis lesions caused by exfoliative toxins of *S. hyicus* [1], and six types of ETs, *ExhA, ExhB, ExhC, ExhD, SHETA,* and *SHETB,* cause blister formation of porcine skin by digesting porcine desmoglein 1 in a similar fashion to ETs from *S. aureus* [15]. Similarly, *S. pseudintermedius* and/or *S. intermedius*, which causes dog skin pyoderma, are also known to produce ETs that specifically act on desmoglein 1 in dogs [23]. ETs of those pathogens include *siet*, *exp*A (formerly *exi*), and *exp*B. *siet* have been first reported as a exfoliative toxin of *S. intermedius* [38], however Iyori *et al.* [23] raised the question of whether all *S. pseudintermedius* possesses *siet* rather than its potential as a toxin considering that *siet* is also found in strains isolated from healthy dogs, and no evident changes were reported when recombinant *siet* protein was injected to canine skin [24]. Futagawa-Saito *et al.* (2009) reported a new ET gene (*exi*) coding the first exfoliative toxin in S. pseudintermedius (*exi*) [17]. Iyori *et al.* found a novel ET gene with 70.4% homology of *SHETB* and 56.9% homology of *exi*, and proposed that *exi* be renamed *exp*A and the novel ET is named *exp*B [23]. The aim of this study is to investigate the distribution of *siet*, *exp*A, *exp*B genes from SIGs isolated from diseased, and healthy dogs in order to compare the toxin genes distribution between two group.

MATERIALS AND METHODS

Sample preparation and bacterial identification.

Samples were collected during from diseased dogs who visited Veterinary teaching hospital of Jeju National University College of Veterinary Medicine and from healthy dogs. Swabs were collected from 135 dogs suffering bacterial infection and 73 of healthy dogs. Most dogs were housekeeping dogs as companion animal. Samples were cultured at blood agar and incubated for 24 h. Isolation of *Staphylococcus* was based on Gram staining, hemolysis on blood agar, catalase test, coagulase test. A single colony was selected and subcultured for another 24 h and tested on API test (ID 32 STAPH; bioMeriux, France).

DNA extraction from *Staphylococcus* spp.

In order to extract DNA, a loopful of fresh staphylococcal colonies was suspended in 90 µl sterilized distilled water (DW) and 10 µl lysostaphin and vortex-mixed for 10 seconds. The suspension was incubated at 37℃ for 10 min and then heated at 100℃ for 10 min before adding 400 µl of DW [21].

PCR amplification

Final SIGs were identified by PCR using *Sinuc* primers previously published [4] and the presence of exfoliative genes including *siet*, *exp*A and *exp*B was also detected by PCR using published primers, *siet* [14], *exp*A [35], *exp*B [6] and *exp*Am designed in this study for *exp*A gene (Table 1). Maxime PCR PreMix Kit (I-Startaq) were used for amplification and the

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reaction mixture for the PCR consisted of 1 ㎕ of DNA extract, 1 ㎕ of 10 pmol of each primer and 17 ㎕ of distilled water (total volumed 20 ㎕). Reaction mixtures were thermally cycled as described in Table 2.

Table1. Primers used in this study

Primer	Sequence	Size of PCR products (bp)	References
*Sinuc*1	CAA TGG AGA TGG CCC TTT TA		[4]
*Sinuc*2	AGC GTA CAC GTT CAT CTT G	125	
*siet*1	ATG GAA AAT TTA GCG GCA TCT GG	359	[38]
*siet*2	CCA TTA CTT TTC GCT TGT TGT GC		
*exp*Am-F	TCA ATA GAC CTT CAC ATG CTG A	432	This study
*exp*Am-R	CTG GTA TTT TTG CAG GCT GGA		
*exp*A-F	GCGCGTCCTTCTGATCCAGAACT	574	[35]
*exp*A-R	AACGTCCCCCTTTACCTACGTG AAT		
*exp*B-F	GGGCATGCACATATGATGAAGC C	843	[6]
*exp*B-R	CCAGATCTATCTTCTGATTCAG C		

The presence of PCR products was determined by electrophoresis of 5 ㎕ of reaction product in an 1.0% agarose gel (SeaKem® GTG® agarose) with Tris-borate electrophoresis buffer and visualized under UV Transilumainator (Virber Lourmat ETX-20M).

Table 2. PCR conditions for species identification

Primer	Denaturation	Annealing	Elongation	Cycles
Sinuc	95℃, 30s	55℃, 30s	72℃, 30 s	30
siet	94℃, 30s	56℃, 30s	72℃, 1 m	30
*exp*Am	95℃, 30s	57℃, 30s	72℃, 30 s	30
*exp*A	94℃, 40s	58℃, 1m	72℃, 1 m	30
*exp*B	94℃, 40s	55℃, 50s	72℃, 1 m	30

RESULTS

Total 135 and 73 isolates of SIGs were identified by *Sinuc* PCR (125 bp, Fig. 1 A) in diseased, and healthy dogs (Table 3). The isolates of SIGs were taken from lesions related to eyes (8 strains, 5.9%), nose (13 strains, 9.6%), ears (36 strains, 26.7%), skin (61 strains, 45.2%), interdigit (8 strains, 5.9%) and urine (3 strains, 2.2%), and no informaation was found on the 6 strains (4.4%) (Table 3). Total 73 SIGs were identified by *Sinuc* PCR in healthy dogs isolated from 20 (27.4%), 18 (24.7%) and 35 (47.9%) samples of nose, mouth and skin, respectively.

Table 3. Sources of *Staphylococcus intermedius* groups identified by *Sinuc* PCR in diseased dogs and healthy dogs

Sources	No. of isolates from diseased dogs (%)	No. of isolates from healthy dogs (%)
Eyes	8 (5.9)	–
Nose	13 (9.6)	20 (27.4)
Ears	36 (26.7)	–
Skin	61 (45.2)	35 (47.9)
Interdigit	8 (5.9)	–
Urine	3 (2.2)	–
Mouth	–	18 (24.7)
No Recording	6 (4.4)	–
Total	135 (100)	73 (100)

* "–" means "not done"

Exfoliative toxin genes were amplified in expected sizes by PCR using *siet* (359 bp, Fig. 1 B), *exp*A (574 bp, Fig. 2 A) and *exp*B (843 bp, Fig. 3) primers. All positive strains for *exp*A were also amplified by *exp*Am primers designed in this study (432 bp, Fig 2. B).
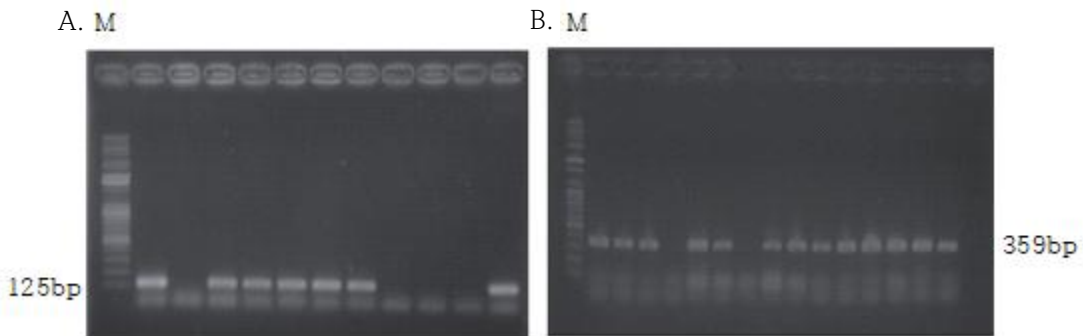

Figure 1. PCR products for *Sinuc* (A) and *siet* (B) genes of representative strains of *Staphylococcus intermedius* groups. Isolated from healthy and diseased dogs. Marker 1kb DNA ladder.
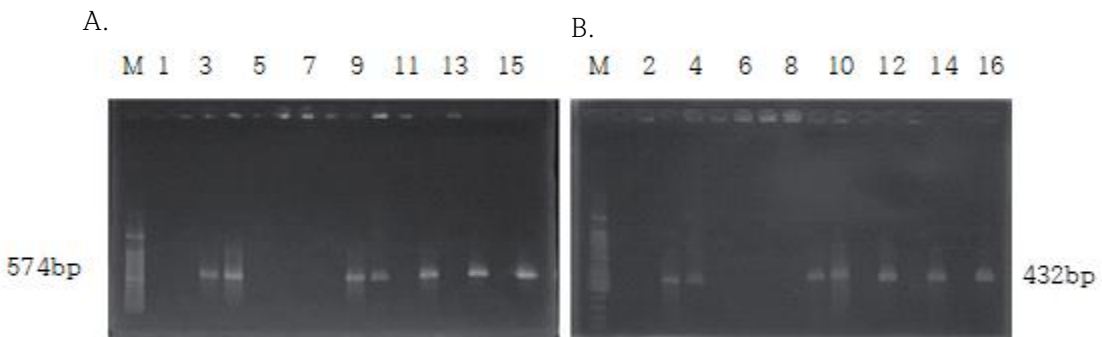

Figure 2. PCR products for *exp*A (A) and *exp*Am (B) genes of representative strains of *Staphylococcus intermedius* groups. Isolated from diseased and healthy dogs. Lanes M, 100bp DNA ladder; lanes 1-16, *Staphylococcus* spp. Strains B12A1, B12A3, B12A6, B12A7, B12B1, B12B2, B12B3, B12C8, B12D3, B12D5, B12D6, B12D8, B12E2, B12E3, B12E6 and B12E8, respectively.
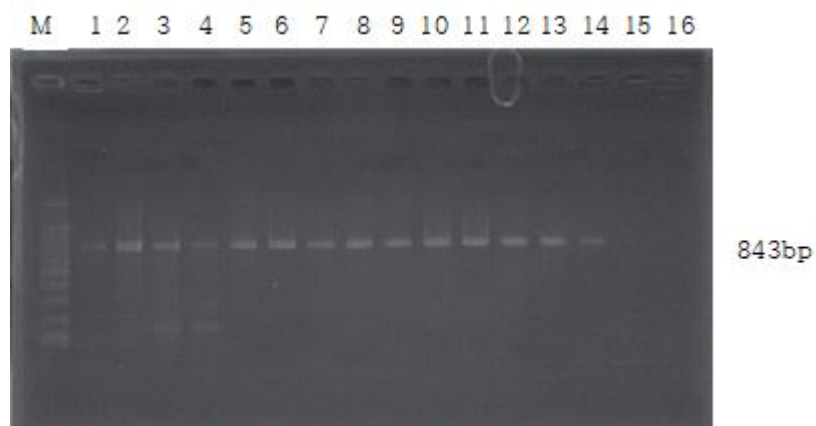
Figure 3. PCR products for *exp*B genes of representative strains of *Staphylococcus intermedius* groups. Isolated from diseased and healthy dogs. Lanes M, 100bp DNA ladder;

Following PCR amplification, the *siet* gene was mostly common, followed by *exp*A, and *exp*B genes in both groups. In detail, 71.2% (n=148) of *siet*, 17.3% (n=36) of *exp*A, and 6.7% (n=14) of *exp*B were detected in 208 SIG isolates. In diseased dogs and healthy dogs, *siet*, *exp*A and *exp*B genes were found in 121 (89.6%) and 27 (37.0%), 29 (21.5%) and 7 (9.6%), and 8 (5.9%) and 6 (8.2%) isolates, respectively (Table 4).

Table 4. Distribution of genes related to exfoliation in 208 *Staphylococcus intermedius* groups.

Primer	No. of Positive in diseased dogs (%)	No. of Positive in healthy dogs (%)	Total (%)
siet	121(89.6)	27(37.0)	148 (71.2)
*exp*A	29 (21.5)	7 (9.6)	36 (17.3)
*exp*B	8(5.9)	6(8.2)	14 (6.7)
Total	135 (100)	73 (100)	208 (100)

The distribution of both *siet* and *exp*A genes was higher in diseased group than in healthy group and *exp*B-positive rate was similar in both groups (Table 4).

In diseased dog group, *siet* gene was found in 100% of nose (n=13), urine(n=3) and no recording (n=6) samples, 91.8% (n=56) of skin, 87.5% (n=7) of interdigit, 86.1% (n=31) of ears, and 62.5% (n=5) of eyes. The *exp*A gene was detected in 13 (21.3%) of skin, 3 (37.5%) of interdigit, 7 (19.4%) of ear, 3 (23.1%) of nose, 1 (12.5%) of eyes, 1 (33.3%) of urine, and 1 (16.7%) of no recording samples. *exp*B gene was present in 23.1% (n=3) of nose, 6.6% (n=4) of skin, 2.8% (n=1) of ears. In most cases of diseased group except nose, *exp*A were more frequently detected than *exp*B (Table 5).

Table 5. Distribution of exfoliative toxin genes in 135 *Staphylococcus intermedius* groups isolated from dogs with different disease conditions.

Sites of Lesion	*siet* (%)	*exp*A (%)	*exp*B (%)	Total (%) n=135
Eyes	5 (62.5)	1 (12.5)	0 (0)	8 (5.9)
Nose	13 (100)	3 (23.1)	3 (23.1)	13 (9.6)
Ears	31 (86.1)	7 (19.4)	1 (2.8)	36 (26.7)
Skin	56 (91.8)	13 (21.3)	4 (6.6)	61 (45.2)
Interdigit	7 (87.5)	3 (37.5)	0 (0)	8 (5.9)
Urine	3 (100)	1 (33.3)	0 (0)	3 (2.2)
No Recording	6 (100)	1 (16.7)	0 (0)	6 (4.4)

Among SIGs isolates from healthy dogs, the *siet* gene was found 40.0% (n=8) of nose, 33.3% (n=6) of mouth and 37.1% (n=13) of skin samples. The *exp*A gene was found in mouth (16.7%, n=3) and in skin (11.4%, n=4), and *exp*B gene was present in nose (5.0%, n=1), mouth (16.7%, n=3) and skin (8.6%, n=1) samples (Table 6).

Table 6. Distribution of exfoliative toxin genes in 73 *Staphylococcus intermedius* groups isolated from different sampling sites of healthy dogs.

Sampling sites	*siet* (%)	*exp*A (%)	*exp*B (%)	Total (%) n=73
Nose	8 (40.0)	0 (0)	1 (5.0)	20 (100)
Mouth	6 (33.3)	3 (16.7)	3 (16.7)	18 (100)
Skin	13 (37.1)	4 (11.4)	3 (8.6)	35 (100)

In diseased dogs, SIGs (65.2%) with only the *siet* gene were the most common, while in healthy dogs, those without any toxins (53.4%) were prominent. In particular, 19.3% of the isolates from the diseased dog had both the *siet* gene and the *exp*A gene, and 1 strain with both *exp*A and *exp*B gene were found in healthy dog-derived SIG. The *siet*-*exp*A-*exp*B genotype was found in 2 and 1 isolates from the diseased and healthy dogs, respectively (Table 7).

Table 7. Distribution of toxin genes of *Staphylococcus intermedius* group isolated from healthy and diseased dogs

Genes amplified by PCR using primer				No. of staphylococci from		Total
Sinuc	*siet*	*exp*A	*exp*B	Diseased dogs	Healthy dogs	
+	–	–	–	12 (8.9)	39 (53.4)	51
+	+	–	–	88 (65.2)	22 (30.1)	110
+	–	+	–	1 (0.7)	3 (4.1)	4
+	–	–	+	1 (0.7)	3 (4.1)	4
+	+	+	–	26 (19.3)	2 (2.7)	28
+	+	–	+	5 (3.7)	2 (2.7)	7
+	–	+	+	0	1 (1.4)	1
+	+	+	+	2 (1.4)	1 (1.4)	3
–	+	–	–	0	1 (1.4)	1*
Total				135	73	208

*not included in total SIGs

DISCUSSION

Staphylococcus pseudintermedius, which belongs to *Staphylococcus intermedius* group (SIG), is recently the most prominent, so considering as a representative bacteria of SIG in most laboratories. *S. pseudintermedius* is known as the main pathogen of various diseases such as pyoderma, otitis externa, respiratory tract infections, urinary tract infections, and reproductive tract infections in dogs [27]. Especially, *S. pseudintermedius* was isolated as the predominant pathogen up to 92% in canine pyoderma, one of the most common bacterial skin disease in small animal medicine [10,20,22,30]. Moreover, it is evident that *S. pseudintermedius,* even including MRSP is not only related to dogs but also to cats in Germany, Poland, USA and Thailand [8,25,33,36]. The prevalence of *S. pseudintermedius* in healthy and sick cats was 2.49% and 7.61% according to Bierowiec K *et al.* in Poland [8]. Though humans are not the natural host, reseachers have also reported that *S. pseduintermedius* originated from companion animals caused human skin infections [37], Thus, as a pathogen of potential zoonotic disease caused by companion animals, *S. pseudintermedius* is receiving a lot of attention from the veterinary perspective as well as medical perspective these days [13,37].

Pathogenic staphylococci produce many different kinds of virulence factors and severe skin lesions were due to exfoliative toxins (ETs). The ETs are produced by some portions of *S. aureus, S. hyicus* and *S. pseudintermedius* isolates and digest desmoglein-1 of human, of swine and of canine, respectively [15]. In current study, the *exp* (ET from *S. pseudintermedius*) genes both and alone in various combinations were detected in *S. pseudintermedius* from diseased and healthy dogs by using the PCR method. A majority of the isolates were originated from skin (45.2%) and ears (26.7%) samples. The PCR outcomes demonstrated high outbreak of *siet* (89.6%)

genes in the *S. pseudintermedius* isolated from diseased dogs. This prevalence rate in diseased dogs is slightly lower than the previous studies that all isolates were carried the gene [19,21,28,37,40], however higher than those found by Ruzauskas *et al.* Lithuania(69%, 35/51) [34]. Though the isolates of healthy dogs also had a relatively high rate of *siet* (37.0%) genes in this study, it was difficult to find other previous data. The *siet* gene was highly prevalent and even was detected in 1 isolate of *Sinuc*-negative. This finding could support that the possibility that *siet* may not be the SIG's exfoliative toxin gene as formerly suggested by Futagawa *et al* [17].

This study was found *exp*A gene in 21.5% and 9.6%, and the *exp*B gene, in 5.9% and 8.2% of the diseased dogs and the healthy dogs, respectively. In the mentioned studies, *exp*A and *exp*B genes were observed in vary in the ranges of 4.0%-73.7% and 7%-23.2%), respectively. Meroni *et al.* in Italy [28] and Hritcu *et al.* in Romania and UK samples [21] found the *exp*A gene in 4%(n=73) and 9.64%(n=49), respectively, while Tabatabaei *et al.* in Iran found the *exp*A gene in 78.9% (n=19) [37] and other studies showed 23.3% (n=43) in Japan (Futagawa-Saito) [16], 30% (n=10) in USA (Banovic) and 38% (n=58) in Brazil (Ptchenin) [6,30]. Tabatabaei *et al.* [37] and Hritcu *et al.* [21] also found *exp*B gene in 5.3% and 6.25%, respectively, while Iyori *et al.* in Japan reported that *exp*B gene was found in 23.2% (n=99) of the first report on *exp*B [23]. Such various prevalence rates may be regional differences, however, it was difficult to compare the differences due to small sample sizes in the previous studies.

S. pseudintermedius isolated form diseased dogs had *exp*A gene at a relatively high rate (12.5%-37.5%) regardless of the lesion site collected, and *exp*B gene was highest (23.1%) in the nose-derived bacteria. In healthy dogs, *exp*A was found in 16.7% and 11.4%, respectively, only in the pathogens isolated from mouth and skin, and *exp*B gene was detected in all samples, both of which were highest in mouth samples. There are a few previous

studies on prevalence of *exp*A and *exp*B genes, however Pitchenin *et al* reported that *exi* (now *exp*A) gene was found in various lesion sites [30], such as keratitis, oseomyelitis, lymphadenitis, pneumonia, diarrhea, and cystitis, and the prevalence rates were high in otitis (44%) and dermatitis (29%).

The most common gene combination was *siet*-*exp*A (14.3%) and *exp*A-*exp*B combination was also in 4 isolates of both diseased and healthy group. There are many reports of gene combinations in *S. aureus* [41], but not in *S. pseudintermedius*. In a study conducted by Tabatabaei *et al.* in Iran, *exp*A and *exp*B gene was found in 1 (5.3%) and 15 (78.9%), respectively, of the 19 *S. pseudintermedius* isolates [37]. According to this study, it can be inferred that the strain with *exp*B gene has *exp*A gene, so there is a possibility that more *exp*A-*exp*B combination *S. pseudintermedius* already exist. All of the staphylococcal enterotoxins are typically encoded by the genes located on mobile genetic elements (MGEs) [41] and complete genome sequence of *S. pseudintermedius* has shown numerous MGEs encoding an array of putative virulence factors [7]. This findings of these toxin gene combinations may imply new variant of *S. pseudintermedius* but additional considerations and studies are needed on the transfer elements or the existence of MGEs carrying *exp* genes.

CONCLUSION

The aim of this study was to determine the distribution of exfoliative toxin genes among clinical isolates from cases of dogs suffering various diseases and from healthy dogs.

Total 135 and 73 isolates of SIGs were identified in diseased dogs and healthy dogs. The isolates of diseased dogs were taken from lesions related to eyes, nose, ears, skin, interdigit and urine. Healthy dogs isolates from nose, mouth and skin. The *siet* gene not related to skin exfoliation was most common in SIGs isolated from both groups, but the isolates from diseased dogs (89.6%) had much higher *siet* gene then those from healthy dogs (37.0%). In diseased dogs and helathy dogs, *exp*A and *exp*B genes were found in 29 (21.5%) and 7 (9.6%), and 8 (5.9%) and 6 (8.2% isolates, respectively. In diseased dog group, *siet* gene was found in 100% of nose and urine samples and the prevalence rate was high in most other samples. *exp*A gene was detected in 21.3% of skin, 37.5% of interdigit, 19.4% of ear, 23.1% of nose, 12.5% of eyes and 33.3% of urine. *exp*B gene was present in 23.1% of nose, 6.7% of skin, 2.8% of ears. In healthy dogs, *siet* gene was found 40.0% of nose, 33.3% of mouth and 37.1% of skin samples. *exp*A gene was found in mouth and skin samples, and *exp*B gene was present in nose, mouth and skin samples. SIGs (65.2%) with only the *siet* gene were the most common, while in healthy dogs, those without any toxins (53.4%) were prominent. In particular, 19.3% of the isolates from the diseased dog showed *siet-exp*A combination, and *exp*A-*exp*B combination was found 1 strain of healthy dog-derived SIG. The *siet-exp*A-*exp*B combination was found in 2 and 1 isolates from the diseased dogs and healthy dogs, respectively.

In conclusion, our results showed that *exp*A and *exp*B genes were found in SIGs from various lesions of both groups and there were *exp*A-*exp*B

combination in some organisms. Since *S. pseudintermedius* is well-known normal flora of dog and could cause opportunistic infection in dogs and human, especially in immune suppressed patients. This subject can be effective for help of diagnosis of SIGs induced disease in veterinary medicine and risk control for dog owners who have underlying medical problems like immunosuppression.

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국문초록

KOREAN ABSTRACT

개에서 분리한 *Staphylococcus intermedius* group의 exfoliative toxin 유전자의 분포

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*Staphylococcus*의 exofolative toxin은 데스모좀 세포 접착 분자인 desmoglein-1을 선택적으로 소화하여 사람에서 수포성 농가진, SSSS 및 돼지 삼출성 표피염을 유발하는 것으로 알려져 있다. 그 중 수의학, 특히 개에서 자주 분리되고 있는 기회감염균인 *Staphylococcus pseudintermedius* 는 SIG(*Staphylococcus intermedius*) 그룹에 속하는데, *siet, exp*A(구 *exi*), *exp*

B와 같은 몇 가지 exfoliative toxin(ET)를 보유하고 있다. 본 연구는 다양한 질병을 앓고 있는 개와 건강한 개에서 임상적으로 분리된 SIG에서의 exfoliative toxin 유전자의 분포를 확인하였다.

먼저, 질병에 걸린 개에서 총 135개, 건강한 개에서 73개의 SIG 분리주를 확보하였다. 샘플은 질병에 걸린 개의 눈, 코, 귀, 피부, 지간 및 소변과 관련된 병변에서, 건강한 개의 코, 입 및 피부에서 채취하였다. 피부 박리와 관련이 없는 *siet* 유전자가 두 그룹 모두에서 흔했지만 질병에 걸린 개에서 분리한 확률 (89.6%)이 건강한 개에서 분리한 확률(37.0%)보다 훨씬 더 높았다. 질병에 걸린 개와 건강한 개에서 expA 및 expB 유전자는 각각 29개(21.5%), 7개(9.6%) 및

8개(5.9%), 6개(8.2%)주에서 발견되었다. 질병군에서 *siet* 유전자는 코와 소변 검체에서 100% 발견되었으며 대부분의 다른 검체에서도 높은 확률로 발견되었다. *exp*A 유전자는 피부 21.3%, 손가락 37.5%, 귀 19.4%, 코 23.1%, 눈 12.5%, 소변 33.3%에서 검출되었으며 *exp*B 유전자는 코 23.1%, 피부 6.6%, 귀 2.8%에 존재했다. 건강한 개에서 *siet* 유전자는 코 40.0%, 입 33.3%, 피부 37.1%에서 발견되었다. *exp*A 유전자는 입과 피부 샘플에서, *exp*B 유전자는 코, 입 및 피부 샘플에서 발견되었다. *siet* 유전자만 있는 SIG(65.2%)가 가장 많았고 건강한 개에서는 독소가 없는 SIG(53.4%)가 두드러졌다. 특히, 질병에 걸린 개의 분리주 중 19.3%가 *siet-exp*A 조합을 보였고, *exp*A-*exp*B 조합은 건강한 개 유래 SIG 1개주를 발견했다. *siet-exp*A-*exp*B 조합은 질병군에서 2개 건강군에서 1개주를 발견했다.

 결론적으로, *exp*A 및 *exp*B 유전자가 두 그룹 모두의 다양한 해부학적 부위에서 검출된 SIG에서 발견되었으며 일부는 *exp*A-*exp*B 조합이 있음을 알게 되었다. *Staphylococcus pseudintermedius*는 개의 정상 세균총 중 하나로 개에게 기회 감염을 일으킬 수 있고, 더욱이 개를 반려동물로 키우는 사람들이 증가하고 있으므로 이런 분리주는 사람에게 전염되어 질병을 유발할 수도 있다. 임상 샘플에서 이러한 분리주를 확보하여 모니터링하면 수의학적 측면에서는 SIG 유발 질병의 진단에 도움이 될 수 있으며, 의학적으로는 면역 억제와 같은 근본적인 문제가 있는 반려견 보호자의 위험 관리에 효과적일 수 있겠다.

감사의 글

둥지를 떠난 지 8년 만에 못다한 공부를 끝내려 학교로 돌아오는 길에는 생각보다 많은 용기가 필요했습니다. 긴 시간 동안 제 자신과 주변의 상황이 예측하지 못한 방향으로 변한 차였습니다. 기초부터 많이 헤매었고, 그 때마다 용기를 잃지 않고 최대한 제 힘으로 해낼 수 있도록 다정하고 명확하게 길을 제시해주신 손원근 지도교수님께 가장 먼저 감사 인사를 드리고 싶습니다. 얼마나 답답하셨을까 생각하면 송구한 마음이 앞섭니다. 마치 며칠 전에 본 것처럼 "우리 애기 왔네!" 하며 꼬옥 안아주신 사모님, 사랑합니다. 오랜만에 전화하여 소식을 전하였더니 "원래 잘 하잖아. 그러니까 잘 할 거야. 다시 한다는 게 대단한 거야" 라는 격려로 반겨주셨던 윤영민 교수님, 그 말씀은 제 마음에 뿌리를 내려 공개발표 때 떨지 않을 수 있었습니다. 함께 공부했던 고은주 교수님, 학창시절 남다른 빛이 나던 교수님을 기억하고 있어, 교수님이 된 모습으로 다시 만나 따뜻한 이야기를 나누고 더욱이 다른 사람이 아닌 교수님의 가르침을 받을 수 있어 감사했고, 영광이었습니다. 인생 멘토로서 제 선택에 응원을 보내며 힘을 주시는 박현정 교수님, 감사합니다. 먼저 손 내밀고 챙겨주신 김주아 선배님, 여쭐 때마다 친절히 알려주신 김보라 조교선생님께도 감사를 전합니다.

끝내지 못하여 가슴에 사무치지 말라고 마무리 할 수 있도록 물심양면으로 지원을 아끼지 않으신 친정 부모님, 그리고 눈치 보지 않고 아이들을 맡기고 공부할 수 있도록 도와주신 시부모님과 도련님, 새벽까지 공부하느라 함께 자지 못하는 시간에 입이 댓발 나왔다가 슬펐다가 떼도 썼다가 그래도 엄마를 눈에 담겠다고 공부하는 모습을 보며 혼자 잠이 들던 첫째 주한이, 어린 나이에 어쩔 수 없이 어린이집에서 낮잠까지 자게 된 귀여운 둘째 하린이, 직장 생활하며 가장으로, 아빠로, 남편으로, 때로는 든든한 아들로, 사위로 힘들 법도 하건만 묵묵히 아내의 내조와 외조까지 해내는 세상에서 제일 믿고 사랑하는 인생 동지 남편께 감히 글로 표현할 수조차 없는 깊은 감사와 사랑을 전합니다.

지나고 보니 삶은 제가 예측하지 못한 방향으로 저를 이끌었습니다. 오만할 때는 겸손토록, 지쳐 있을 때는 힘을 낼 수 있도록 말입니다. 귀인을 만나 도움을 받고 소중한 인연을 귀히 여길 수 있도록 모든 감사한 분들을 제게 보내주

신 주님께 감사합니다.

 학교에서 만난 교수님들과 동료 수의사들, 소중한 가족들, 그리고 애정 어린 눈으로 저를 지켜 봐 주시는 지인들이 제 삶의 에너지이자 원동력이었음을 깨닫습니다. 저는 여러분들의 따뜻함과 격려 속에 자신감을 가지고 다시 출발하고자 합니다. 언젠가 저와 같은 후배를 만나게 되거든 제가 받은 격려와 응원, 그리고 사랑을 돌려주는 사람이 되겠습니다.

2022년 6월 실험실에서

김 정 희 올림