NOTE *Bacteriology*

Characteristics of *Staphylococcus intermedius* Isolates from Diseased and Healthy Dogs

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ABSTRACT. *Staphylococcus intermedius* isolates from diseased and healthy dogs were examined for production of extracellular enzymes and toxins, and phage patterns. There were no significant differences between the two groups of isolates in the production rates of DNase, protease, lipase, gelatinase, hyaluronidase, hemolysins, protein A, and TSST-1, or in phage patterns. But the production rate of enterotoxins in isolates from diseased dogs was significantly higher than that in isolates from healthy dogs. PFGE analysis was performed with isolates from different body sites in individual dogs. In 3 of 6 healthy dogs, identical PFGE patterns were seen in isolates from the nares, external auditory meatus or skin. The remaining 3 dogs yielded isolates of different patterns. In 4 of 6 diseased dogs, identical patterns were seen in isolates from lesions as well as from the other normal sites.

KEY WORDS: diseased and healthy dog, PFGE, *Staphylococcus intermedius*.

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Staphylococcus intermedius has been considered a primary pathogen of skin infections in dogs, such as otitis externa [5, 7] and pyoderma [1, 22, 23]. Some strains of *S. intermedius* produce a variety of extracellular enzymes and toxins and virulence-associated factors [1, 13], which are thought to contribute to the pathogenicity of the organism.

S. intermedius is also present in the nasal vestibulum, external auditory canal, anal mucosa, and on the skin surface and hair coat of healthy dogs. Knowledge of the characteristics of the normal *S. intermedius* flora on these sites in healthy dogs is an important factor in understanding the epidemiology of staphylococcal skin infections in dogs. There are several reports on the population sizes and frequency of the organism at these carrier sites in healthy dogs [2, 3, 9, 22]. Nevertheless, comparison of the characteristics of isolates from different sites in individual healthy dogs has not been studied sufficiently [4]. The organism has frequently been cultured from other body sites besides lesion sites in diseased dogs, but information on the clonal relationship between the organisms in the lesions and those in the nose or skin is lacking.

The purpose of this study was to compare characteristics of *S. intermedius* isolates from diseased dogs with those from healthy dogs by using biological properties and phage typing. In addition, the present study was to characterize isolates from different sites in individual healthy and diseased dogs, by means of pulsed-field gel electrophoresis (PFGE).

A total of 96 *S. intermedius* isolates were subjected to biological characterization and phage typing. Forty-seven isolates were from dogs affected with otitis externa (n=8), dermatitis (n=11), pyoderma (n=7), impedigo (n=4), eczema (n=8), and folliculitis (n=9). Forty-nine isolates were from the mouth (n=3), nares (n=17), external auditory meatus (n=18), and skin (n=11) of apparently healthy dogs. Ninety of the 96 isolates had been used in a previous antimicrobial susceptibility study [26].

Isolation of *S. intermedius* from different sites in individual healthy and diseased dogs was performed. Four of 6 healthy dogs examined carried *S. intermedius* in both the nares and external auditory meatus. One dog carried the organism in both the nares and skin. In the remaining one dog, the organism was recovered from the nares, external auditory meatus, and skin. In 5 of 6 diseased dogs, *S. intermedius* was recovered from other healthy sites (i.e. the nares, external auditory meatus and skin) besides the lesion sites. One dog suffered from both otitis externa and pyoderma.

Detection of extracellular enzymes and toxins was performed by the method of Hájek [14] and Devriese *et al.* [8,10]. Staphylococcal enterotoxins (SEs: SEA, SEB, SEC, and SED) and toxic shock syndrome toxin-1 (TSST-1) were detected by the reversed-passive latex agglutination (RPLA) method with SET-RPLA and TSST-RPLA (Denka Seiken). Phage typing was performed as described previously [20] with 14 phages of the typing set for *S. intermedius*. Isolates found untypable at a routine test dilution (RTD) were retested at 100 × RTD. Preparation of chromosomal DNA of *S. intermedius* isolates and fragmentation of DNA with *Sma*I (New England BioLabs, Beverly, Mass.) were performed as described previously [25]. PFGE was performed with a 1% agarose slab gel (Seakem GTG, FMC Bioproducts, Rockland, Me.) in a CHEF-DR II system (Bio-Rad Laboratories Inc, Hercules, Calif.) in a 0.5 × Tris-borate-EDTA maintained at 14°C. The running parameters used were as follows: initial pulse time, 5 sec; final pulse time, 40 sec; voltage, 6V/cm; running time, 22 hr.

All 47 isolates from diseased dogs produced coagulase,

Table 1. Extracellular enzyme and toxin production by *S. intermedius* isolates from diseased and healthy dogs

Characteristic	Diseased dogs (n=47)	Healthy dogs (n=49)
Coagulation of rabbit plasma	47[a)]	49
DNase	46	48
Protease	45	47
Lipase	46	46
Gelatinase	37	37
Hyaluronidase	0	0
Hemolysin		
α	0	0
β	45	46
non	2	3
Protein A	0	0
Enterotoxin		
A	0	2
C	9	6
AC	8	0
non	30	41
TSST-1	0	0

a) No. of positive isolates.

and almost all of them produced DNase, protease, lipase, gelatinase and *β*-hemolysin. None produced hyaluronidase, protein A or TSST-1 (Table 1). Forty-nine healthy dog isolates showed similar characteristics. Allaker *et al.* [1] were also unable to find differences between strains of *S. intermedius* isolated from canine pyoderma and healthy carriers with respect to the production of various extracellular enzymes and toxins of putative virulence factors.

Some strains of *S. intermedius* isolated from dogs produce SEs; SEC is the most common [1, 11, 18, 19]. In the present study, the production rate of SEs in isolates from diseased dogs (17/47) was significantly higher than that in isolates from healthy dogs (8/49) ($P<0.05$). Twenty-three (92.0%) of the 25 SEs-producing isolates yielded SEC alone or in combination with SEA (Table 1). All of the 8 SEs-producing isolates from healthy dogs were single SE type, whereas 9 and 8 of the 17 SEs-producing isolates from diseased dogs were single and mixed SE types, respectively. The reasons why the mixed SE type are found only in diseased dogs are not known. In humans with atopic dermatitis, SEs or TSST-1 secreted by *S. aureus* may play an important role in the severity of skin lesions and pruritus associated with pyoderma [21]. In contrast, production of SEs or TSST-1 by *S. intermedius* may not play a role in the recurrent nature of pyoderma in atopic dogs or in determining the type of lesion or severity of pruritus associated with pyoderma [6]. Further studies are needed to determine whether SEC-producing *S. intermedius* contributes significantly to various skin infections in dogs.

In the present study, *S. intermedius* isolates were examined for their production of 4 classical SEs (SEA, SEB, SEC, and SED) by SET-RPLA assay. Recently, 18 types of SEs in *S. aureus* have been reported comprising the five classical (SEA through SEE) and the 13 newly described (SEG through SER and SEU). Also, the existence of the 2 new types ($SEC_{-canine}$ and SE_{-int}) in *S. intermedius* has been reported [11,12]. Further work is needed to confirm the existence of these new SE genes in order to clarify the roles of SEs in staphylococcal skin infections in dogs.

Table 2. Phage patterns of *S. intermedius* isolates from diseased and healthy dogs

Phage pattern	Diseased dogs (n=47)	Healthy dogs (n=49)
06	6[a)]	7
40	3	
93		2
177	1	4
248		1
06/40	1	5
06/58	1	1
06/93		1
06/248	1	
40/58	2	2
58/93	1	
58/248	1	
93/177		1
06/40/58	8	7
06/40/248		2
06/58/248		1
06/58/E42	1	
06/40/58/93	6	5
06/40/58/248	1	
06/40/58/H35		1
06/40/58/D11		1
06/40/58/E41	1	
06/40/58/93/177	1	
06/40/58/93/248	1	
06/40/58/93/D11	1	
06/40/58/177/D11	1	
06/40/58/248/H35	1	
06/40/58/93/177/248	1	
06/40/58/93/177/P13	1	
06/40/58/93/248/D11	1	
Untypable	5	8

a) No. of isolates.

On the whole, 83 (86.5%) of the 96 isolates were phage typable, including 51 isolates at RTD and 32 at 100 × RTD. A total of 29 different phage patterns were detected (Table 2). Isolates from dogs affected with skin disease and from healthy dogs were indistinguishable by phage typing. Likewise, Hesselbarth *et al.* [17] and Shimizu *et al.* [25] were unable to separate lesion strains from healthy carrier strains by PFGE analysis.

PFGE analyses of isolates from different sites on the same dog are shown in Table 3 and Fig. 1. In 3 (Nos. 1–3) of 6 healthy dogs examined, identical PFGE patterns were seen in the isolates from the nares and external auditory meatus or the skin of individual dogs. The remaining 3 dogs (Nos. 4–6) yielded isolates with different PFGE patterns. In 4 of 6 diseased dogs (Nos. 7, 9–11), isolates from the lesion sites and other normal sites such as the nares, external auditory meatus, and skin, had identical PFGE patterns. An isolate from the normal skin of one dog (No. 12) differed from

Table 3. Sources of *S. intermedius* and lane number in PFGE

Dog No.	Lane No.[a)]	Site of isolation	PFGE pattern	Phage pattern	SE type
Healthy dogs					
①	1	nares	A	06/40/58	
	2	external auditory meatus	A	06/40/58	
	3	skin	A	06/40/58	
②	4	nares	B	06	C
	5	external auditory meatus	B	06	C
③	6	nares	C	untypable	C
	7	external auditory meatus	C	untypable	C
④	8	nares	D	untypable	
	9	external auditory meatus	E	untypable	
⑤	10	nares	G	06/93	
	11	external auditory meatus	H	40/58	
⑥	12	nares	I	06	C
	13	skin	J	06	C
Diseased dogs					
⑦	14	otitis externa	K	06/40/58	
	15	nares	K	06/40/58	
	16	skin	K	06/40/58	
⑧	17	otitis externa	L	untypable	AC
	18	pyoderma	L	untypable	AC
⑨	19	pyoderma	M	06/40/58/93/248/D11	AC
	20	nares	M	06/40/58/93/248/D11	AC
⑩	21	dermatitis	N	40	C
	22	nares	N	40	C
⑪	23	dermatitis	O	untypable	
	24	nares	O	untypable	
	25	external auditory meatus	O	untypable	
⑫	26	otitis externa	P	06/40/58	
	27	skin	Q	06/40/58	

a) Lane Nos. correspond to those of lane Nos. in Fig. 1.

the lesion isolate. Interestingly, isolates from one dog (No. 8) with both otitis externa and pyoderma had an identical PFGE pattern. Phage typing is often unsatisfactory in differentiating *S. intermedius* strains, since its typability is less than that of the PFGE typing method. In the present study, isolates with the same PFGE pattern had the same phage pattern (Table 3).

Barrs *et al.* [4] reported that *S. intermedius* isolates from different sites on the same healthy dog were not all identical in terms of electrophoretic type. Partially similar results were obtained in the present study. On the other hand, the present study indicated clonal spread of the same strain within individual healthy dogs. In most of the healthy subjects in chicken studies, similar or identical strains of *S. aureus* were shown to be present on the skin of the underwing and in the nasal sinuses of individual chickens [16, 27].

In most cases of diseased dogs examined in the present study, isolates of *S. intermedius* present in the nares and external auditory meatus or on the skin were found to have identical genotypes to those of isolates from lesions. This finding is comparable to the case of chickens with staphylococcal arthritis [15]. In humans, phage-typing studies have revealed that *S. aureus* strains isolated from sites of infection are often of the same phage type as those present in patients' noses [28].

Devriese and De Pelsmaeker [8] suggested that elimination of the mucosal populations of *S. intermedius* by disinfection or some other means might contribute to the successful treatment of canine recurrent staphylococcal infections in susceptible patients. Subsequently, Saijonmaa-Kolumies *et al.* [24] reported that elimination of *S. intermedius* in cutaneous and mucosal carriage in healthy dogs by treatment with fusidic acid significantly decreased the cutaneous populations and frequency of *S. intermedius*, and that this form of therapy may be useful as an additional tool against canine recurrent pyoderma. Also, elimination of nasal carriage of *S. aureus* has decreased the rate of staphylococcal infection in human patients undergoing haemodialysis, providing further evidence of the clinical

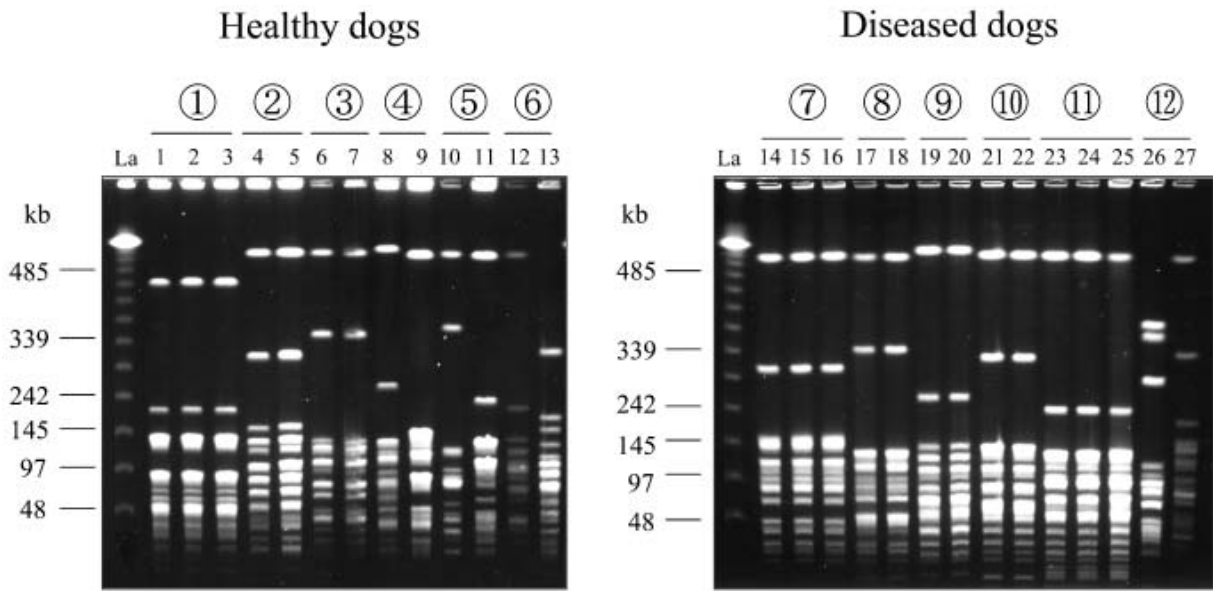

Fig. 1. PFGE of *Sma*I-digested genomic fragments of *S. intermedius* from healthy and diseased dogs. Dog No. and lane No. correspond to those in Table 3. M indicates the lambda ladder DNA concatemers used as molecular size markers (kb).

importance of mucosal populations [28].

In this study, we could not differentiate between isolates from diseased and healthy dogs with respect to the production of some extracellular substances and phage patterns. These results are consistent with the known role of *S. intermedius* as an opportunistic pathogen [4, 17]. The epidemiologic analysis of the isolates from different sites on the same healthy and diseased dogs suggested that elimination of *S. intermedius* from healthy carrier sites by disinfection in addition to elimination from lesions would be worthwhile in the treatment of staphylococcal skin disease including otitis externa in dogs.

REFERENCES

1. Allaker, R.P., Lamport, A.I., Lloyd, D.H. and Noble, W.C. 1991. *Microbial. Ecol. Health. Dis.* **4**:169–173.
2. Allaker, R.P., Lloyd, D.H. and Bailey, R.M. 1992. *Vet. Rec.* **130**: 303–304.
3. Allaker, R.P., Lloyd, D.H. and Simpson, A.I. 1992. *Res. Vet. Sci.* **52**: 174–176.
4. Barrs, V.R., Briscoe, D., Malik, R. and Love, D.N. 2000. *Aust. Vet. J.* **78**: 267–272.
5. Biberstein, E.L., Jang, S.S. and Hirsh, D. 1984. *J. Clin. Microbiol.* **19**: 610–615.
6. Burkett, G. and Frank, L.A. 1998. *J. Am. Vet. Med. Assoc.* **213**: 232–234.
7. Cole, L.K., Kwochka, K.W., Kowalski, J.J.and Hillier, A. 1998. *J. Am. Vet. Med. Assoc.* **212**: 534–538.
8. Devriese, L.A. 1984. *J. Appl. Bacteriol.* **56**: 215–220.
9. Devriese, L.A. and De Pelsmaecker, K. 1987. *Vet. Rec.* **121**: 302–303.
10. Devriese, L.A. and Oeding, P. 1975. *J. Appl. Bacteriol.* **39**: 197–297.
11. Edwards, V.M., Deringer, J.R., Callantine, S.D., Deobald, C.F., Berger, P.H., Kapur, V., Stauffacher, C.V. and Bohach, G.A. 1997. *Infect. Immun.* **65**: 2346–2352.
12. Futagawa-Saito, K., Suzuki, M., Ohsawa, M., Ohshima, S., Sakurai, N., Ba-Thein, W. and Fukuyasu, T. 2004. *J. Appl. Microbiol.* **96**: 1361–1366.
13. Greene, R.T. and Lämmler, C.H. 1993. *J. Vet. Med. B* **40**: 206–214.
14. Hájek, V. 1976. *Int. J. Syst. Bacteriol.* **26**: 401–408.
15. Harry, E.G. 1967. *Res. Vet. Sci.* **8**: 479–489.
16. Harry, E.G. 1967. *Res. Vet. Sci.* **8**: 490–499.
17. Hesselbarth, J., Witte, W., Cuny, C., Rohde, R. and Amtsberg, G. 1994. *Vet. Microbiol.* **41**: 259–266.
18. Kato, E., Kaji, Y. and Kaneko, K. 1978. *Am. J. Vet. Res.* **39**: 1771–1773.
19. Kaji, Y. and Kato, E. 1980. *Jpn. J. Vet. Res.* **28**: 86–94.
20. Kawano, J., Shimizu, A., Kimura, S. and Blouse, L. 1982. *Zbl. Bakt. Hyg., I. Abt. Orig. A* **253**: 321–330.
21. Leung, D.Y.M., Harbeck, R., Bina, P., Reiser, R.F., Yang, E., Norris, D.A., Hanifin, J.M. and Sampson, H.A. 1993. *J. Clin. Invest.* **92**: 1374–1380
22. Lloyd, D.H., Allaker, R.P. and Pattinson, A. 1991. *Vet. Dermatol.* **2**: 161–164.
23. Phillips, W.E. Jr. and Kloos, W.E. 1981. *J. Clin. Microbiol.* **14**: 671–673.
24. Saijonmaa-Koulumies, L., Parsons, E. and Lloyd, D.H. 1998. *J. Small. Anim. Pract.* **39**: 341–347.
25. Shimizu, A., Berkhoff, H.A., Kloos, W.E., George, C.G. and Ballard, D.N. 1996. *Am. J. Vet. Res.* **57**: 1458–1462.
26. Shimizu, A., Wakita, Y., Nagase, S., Okabe, M., Koji, T., Hayashi, T., Nagase, N., Sasaki, A., Kawano, J., Yamashita, K. and Takagi, M. 2001. *J. Vet. Med. Sci.* **63**: 357–360.
27. Shimizu, A., Yamamoto, C., Kawano, J., Inubushi, H., Kakutani, O. and Hikami, Y. 1997. *J. Jpn. Soc. Poult. Dis.* **33**: 24–29.
28. Yu, V.L., Goetz, A., Wagener, M., Smith, P.B., Rihs, J.D., Hanchett, J. and Zuravleff, J.J. 1986. *New Engl. J. Med.* **315**: 91–96.