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Short communication

Relatedness of *Streptococcus equi* subsp. *zooepidemicus* strains isolated from harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) of various origins of the North Sea during 1988–2005

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Abstract

The present study was designed to identify 15 beta-hemolytic streptococci isolated during a period between 1988 and 2005 from nine harbour seals and six grey seals from various origins of the North Sea. All isolates were identified as *Streptococcus equi* subsp. *zooepidemicus*. The bacteria were additionally investigated for relatedness by restriction fragment length polymorphism analysis of PCR amplified 16S-23S rDNA intergenic spacer region and gene *szp* and by macrorestriction analysis of chromosomal DNA of the strains by pulsed field gel electrophoresis. The molecular analysis yielded identical or closely related patterns within the strains of the present study and with the *S. equi* subsp. *zooepidemicus* strains isolated from harbour seals of German North Sea which were investigated previously [Akineden, Ö., Hassan, A.A., Alber, J., El-Sayed, A., Estoepangestie, A.T.S., Lämmler, C., Weiss, R., Siebert, U., 2005. Phenotypic and genotypic properties of *S. equi* subsp. *zooepidemicus* isolated from harbor seals (*Phoca vitulina*) from the German North Sea during the phocine distemper outbreak in 2002. Vet. Microbiol. 110, 147–152]. This indicates that this single or closely related bacterial clone existed during both phocine distemper virus epidemics in 1988 and 2002 and that a direct transmission of the

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strains has occurred between two seal species and between seal populations of far distant regions possibly with grey seals as a vector.

Keywords: *S. equi* subsp. *zooepidemicus*; Harbour seal; Grey seal; Phocine Distemper Virus

1. Introduction

In 1988 and 2002 two phocine distemper virus (PDV) epidemics in the European harbour seal (*Phoca vitulina*) population resulted in the death of approximately 23,000 harbour seals in 1988 and 30,000 in 2002 (Härkönen et al., 2006). According to these authors the major clinical symptoms caused by PDV included respiratory problems, fever, diarrhoea and abortion with *Bordetella bronchiseptica* as a frequent secondary bacterial infection. However, some PDV infected harbour seals were also found to be coinfected with various other bacterial species namely *Arcanobacterium phocae*, *Brucella* spp., *Erysipelothrix rhusiopathiae* and beta-hemolytic streptococci (Akineden et al., 2005). Beta-haemolytic streptococci isolated from seals could generally be classified as *Streptococcus phocae* (Skaar et al., 1994; Vossen et al., 2004). However, according to the findings of Akineden et al. (2005) the beta-hemolytic streptococci of the latter could be identified as *Streptococcus equi* subsp. *zooepidemicus*. Additionally performed molecular analysis revealed that these *S. equi* subsp. *zooepidemicus* strains isolated from harbour seals of various origins of the German North Sea represented a single bacterial clone indicating that, comparable to PDV, a direct transfer from animal to animal seems to be a plausible mean of transmission.

Following these results it was of interest to investigate *S. equi* subsp. *zooepidemicus* strains isolated during a period from 1988 to 2005 from harbour and grey seals (*Halichoerus grypus*) found in various far distant regions within Northern Europe for relatedness and for relationship to this previously described bacterial clone.

2. Materials and methods

2.1. Bacterial strains

The bacterial strains included 15 strains recovered from specimens obtained from harbour seals ($n = 9$) and grey seals ($n = 6$) of the North Sea in the period

Table 1
Origin of the 15 *S. equi* subsp. *zooepidemicus* strains obtained from harbour seals and grey seals used in this study

Animal number	Animal designation	Status and date of animal when found[a]		Tissue	Location/country	*S. equi* subsp. *zooepidemicus* strain designation
1	MA907/88/1 Harbour seal	S	23.09.88	Lung	North Kessock/Northeast-Scotland	88
2	M 186/93/2 Grey seal	S	27.01.93	Lung	Balmedie/Northeast-Scotland	P2469
3	M 2475/96/1 Grey seal	S	22.12.96	Lung	Shetland Islands/Scotland	P2471
4	M 517/02/2 Grey seal	S	25.11.02	Bronchii	Fraserburgh/Northeast-Scotland	P2468
5	M 615/02/2 Grey seal	S	17.12.02	Lung	Fife/East-Scotland	P2461
6	NH 14 Harbour seal	S	22.08.02	Lung	North-Holland/Netherlands	P419
7	Tx 143 Harbor seal	S	03.09.02	Lung	Texel/Netherlands	P422
8	Tx148 Harbour seal	S	04.09.02	Lung	Texel/Netherlands	P423
9	Tx 147 Harbour seal	S	04.09.02	Lung	Texel/Netherlands	P420
10	2147 Harbour seal	S	18.09.02	Lung	Westerhever/Germany	P6810-03
11	2258 Harbour seal	C	09.04.03	Anus	Lorenzenplate/Germany	P3101-03
12	2435 Harbour seal	K	24.10.03	Intestine	Sylt/Germany	1388-04
13	2510 Harbour seal	C	13.04.04	Anus	Rømø/Denmark	P2826-04
14	2936 Grey seal	S	17.04.05	Lung	Sylt/Germany	1816
15	2938 Grey seal	S	23.04.05	Lung	Sylt/Germany	1817

[a] K: killed because of poor condition; S: stranded; C: captured alive.

from 1988 to 2005 in Scotland, The Netherlands, Denmark and Germany. Strain details including the animal species, the animal designation, the status and date of the animal when found, the location of the seals and the tissues from which the beta-hemolytic streptococci were isolated are shown in Table 1. For comparative purposes the previously characterized *S. equi* subsp. *zooepidemicus* strain 354 isolated from a harbour seal of the German North Sea was used (Akineden et al., 2005). All controls were performed as described by Akineden et al. (2005).

2.2. *Identification and molecular characterization*

The streptococci were identified biochemically (Lämmler and Hahn, 1994), serologically by the use of a commercial Streptococcal grouping kit (Oxoid, Wesel, Germany) and by molecular methods using the previously described *sod*A–*see*I multiplex PCR (Alber et al., 2004). The strains were additionally characterized by PCR-amplification and restriction endonuclease digestion of 16S-23S rDNA intergenic spacer region (ISR) and the Szp encoding gene *szp* using the restriction enzymes *Hae*III (ISR) and *Hin*fI (*szp*), respectively. The extraction of bacterial DNA, the primer sequences, thermalcycler programmes and running conditions have been described previously (Akineden et al., 2005). The strains were finally analyzed by macrorestriction analysis of chromosomal DNA (PFGE) using the restriction enzyme *Sma*I (Soedarmanto et al., 1996).

3. Results and discussion

According to the data reviewed by Härkönen et al. (2006) both PDV epidemics started at the Danish island of Anholt in the central Kattegat and subsequently spread to adjacent colonies. However, some infections appeared far away from the initially infected population. This suggests a jump of the virus to populations in, e.g. the Irish Sea and the Dutch Wadden Sea (Reijnders et al., 2003). Because the harbour seal is a rather sedentary species another vector species might have been involved in the spread of the disease among the colonies. According to Härkönen et al. (2006) it seems likely that grey seals, a species exhibiting long distance movements, contributed to the spread into the various

Table 2
Genotypic properties of the 16 *S. equi* subsp. *zooepidemicus* strains investigated in the present study

S. equi subsp. *zooepidemicus* strains	ISR			*szp* gene			PFGE pattern[a]
	Size of amplicon (bp)	*Hae*III		Size of amplicon (bp)	*Hin*fI		
		No. of bands	Size of RLFP-fragments (bp)		No. of bands	Size of RLFP-fragments (bp)	
88	950	2	620, 400	1200	3	650, 400, 120	A
P2469	950	2	620, 400	1100	3	380, 250, 120	B
P2471	950	2	620, 400	1200	3	650, 400, 120	B
P2468	800	2	600, 220	1200	3	650, 400, 120	B
P2461	950	2	620, 400	1100	3	380, 250, 120	A
P419	950	2	620, 400	1200	3	650, 400, 120	B
P422	950	2	620, 400	1200	3	650, 400, 120	B
P423	950	2	620, 400	1200	3	650, 400, 120	B
P420	950	2	620, 400	1200	3	650, 400, 120	B
P6810-03	950	2	620, 400	1100	3	380, 250, 120	C
P3101-03	950	2	620, 400	1100	3	380, 250, 120	C
1388-04	950	2	620, 400	1100	3	380, 250, 120	C
P2826-04	950	2	620, 400	1100	3	380, 250, 120	C
1816	950	2	620, 400	1100	3	380, 250, 120	B
1817	950	2	620, 400	1100	3	380, 250, 120	B
354[b]	950	2	620, 400	1100	3	380, 250, 120	C

[a] Differences between PFGE pattern A to B one fragment, A to C three fragments, B to C two fragments.
[b] This strain had been characterized previously (Akineden et al., 2005).

regions. In addition grey seals appear to be less sensitive to PDV infection possibly causing a continued circulation of PDV after 1988 (Härkönen et al., 2006).

Grey seals might also represent a vector contributing to the dissemination of beta-haemolytic streptococci between grey and harbour seals and between seal colonies in various regions of the North Sea. The beta-hemolytic streptococci of the present study, which were identified as *S. equi* subsp. *zooepidemicus* by biochemical, serological and molecular analysis (data not shown) and characterized by RFLP and PFGE analysis yielded generally identical RFLP patterns and identical or, according to the principles of Tenover et al. (1995), closely related PFGE patterns of the 15 *S. equi* subsp. *zooepidemicus* strains obtained from harbour seals and grey seals. Comparing the patterns with the RFLP and PFGE pattern of the previously investigated *S. equi* subsp. *zooepidemicus* strains obtained from harbour seals of the German North Sea (Akineden et al., 2005) revealed that they were identical or closely related. The only difference in ISR pattern of the 15 strains of the present study was observed for a single strain P2468 which was cultured from a harbour seal from north-east-Scotland in 2002. The *szp* patterns separated the *S. equi* subsp. *zooepidemicus* isolates into two groups. However, the differences in RFLP pattern seemed to be neither related to the seal species from which the isolates were obtained nor to the PFGE pattern of the strains. These differences in RFLP and PFGE pattern of closely related bacterial clones could possibly be explained by ongoing evolutionary processes of a single bacterial clone during the isolation time between 1988 and 2005 or in the various regions. The results are summarized in Table 2. Typical RFLP fragment patterns and PFGE restriction patterns are shown in Figs. 1 and 2.

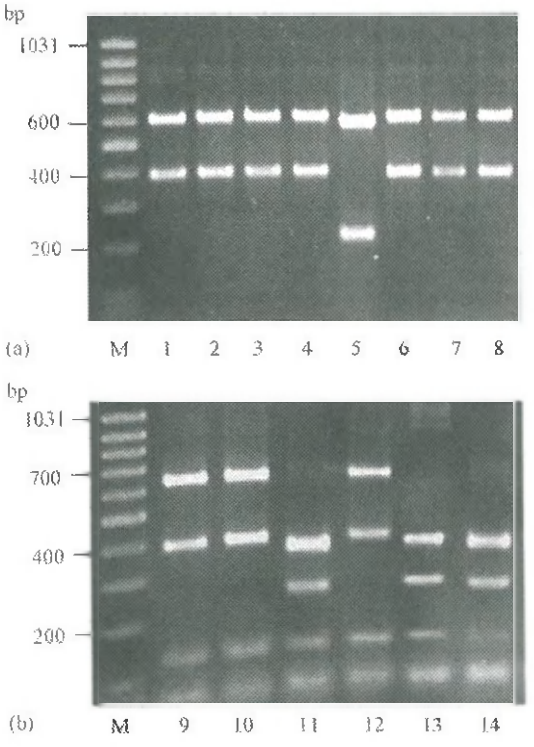

Fig. 1. Typical RFLP restriction patterns of ISR (a) and *szp* (b) of *S. equi* subsp. *zooepidemicus* strains isolated from seals after digestion with *Hae*III [1 (88), 2 (P2469), 3 (P2471), 4 (P419), 5 (P2468), 6 (P423), 7 (P420), 8 (1388-04)] and *Hin*fI [9 (88), 10 (P423), 11 (P2469), 12 (P2471), 13 (P3101-03), 14 (P2461)]. M: GeneRuler™ 100 bp DNA Ladder (MBI Fermentas, St. Leon-Rot, Germany).

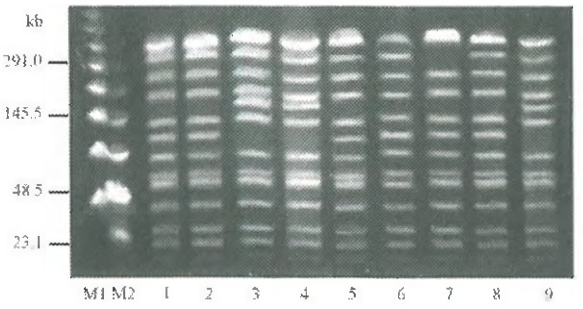

Fig. 2. PFGE pattern of nine *S. equi* subsp. *zooepidemicus* strains with pattern A: 7 (P2461); pattern B: 1 (P422), 2 (P423), 5 (P2469), 6 (P2468), 8 (P2471); and pattern C: 3 (P2826-04), 4 (P3101-03) and 9 (354). M1: Lambda Ladder PFGE Marker (50–1000 kb) and M2: "low range" PFGE Marker (0.1–200 kb) (both Sigma–Aldrich Chemie GmbH, Steinheim, Germany).

As concluded by Härkönen et al. (2006) for the PDV infection, the area of Anholt in Denmark might possess specific conditions also promoting a cross-species infection of harbour seals and grey seals with *S. equi* subsp. *zooepidemicus* and subsequently allow a spread of this bacterial species to more distant regions by the highly migratory grey seal. However, at present nothing is known about the pathogenic importance these bacteria might have in seal infections.

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