Distribution of capsular serotypes and virulence markers of *Streptococcus suis* isolated from pigs with polyserositis in Korea

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Abstract

The objective of this study was to determine the capsular serotypes and potential virulence factors of *Streptococcus suis* isolated from pigs with polyserositis. Among the 24 isolates evaluated, serotype 3 [7 (29%) of the isolates] and serotype 4 [5 (21%)] were the most common. The isolates were also studied for the presence of the genes *mrp, epf,* and *sly,* which encode muramidase-released protein (MRP), extracellular factor (EF), and suilysin (SLY), respectively. Of the 24 isolates, 8 carried *mrp:* 4 of serotype 3, 2 of serotype 2, and 2 of serotype 4. One *mrp*$^+$ isolate (serotype 2) also carried the *epf* gene. All 24 isolates carried the *sly* gene. The serotype and genotype distribution greatly differed from that reported for isolates from pigs with other clinical manifestations of *S. suis* infection in other countries.

Résumé

L'objectif de la présente étude était de déterminer les sérotypes capsulaires et les facteurs de virulence potentiels d'isolats de Streptococcus suis *provenant de porcs avec une polysérosite. Parmi les 24 isolats évalués, le sérotype 3 [7 (29 %) des isolats] et le sérotype 4 [5 (21 %)] étaient les plus fréquents. Les isolats ont également été examinés pour la présence des gènes* mrp, epf, *et* sly, *qui codent respectivement pour la protéine relâchée par la muramidase (MRP), le facteur extra-cellulaire (EF) et la suilysine (SLY). Parmi les 24 isolats, 8 étaient porteurs de* mrp : *4 du sérotype 3, 2 du sérotype 2 et 2 du sérotype 4. Un isolat* mrp$^+$ *(sérotype 2) était également porteur du gène* epf. *Tous les isolats étaient porteurs du gène* sly. *La distribution des sérotypes et génotypes diffèrent grandement de celle rapportée pour les isolats provenant de porcs avec des manifestations cliniques différentes et de pays différents.*

(Traduit par Docteur Serge Messier)

Streptococcus suis is an important pathogen in intensive swine production throughout the world and is the cause of polyserositis as well as other conditions, such as bronchopneumonia, meningitis, arthritis, pericarditis, myocarditis, endocarditis, fibrinous polyserositis, septicemia, rhinitis, and abortion (1). To date, at least 35 serotypes of *S. suis* have been identified (2–4), but serotype 2 seems to be most commonly isolated (5–9). Although little is known about potential virulence factors, muramidase-released protein (MRP), extracellular factor (EF), and suilysin (SLY), encoded by the genes *mrp, epf,* and *sly,* respectively, are considered markers of virulence for Eurasian strains (10–12).

Polyserositis is a general inflammation of serous membranes such as pleura, pericardium, and peritoneum (13). It is the one of the most common necropsy findings in growth-retarded and culled pigs, especially those 4 to 12 wk old, in Korea (personal observations). Although polyserositis is caused by several pathogens (13), *S. suis* is considered the main etiologic agent of meningitis but not polyserositis among Korean swine practitioners and producers. Therefore, little is known about the prevalence of *S. suis* infection among pigs with polyserositis in Korea. The objective of this study was to determine the capsular serotypes and potential virulence factors of *S. suis* isolated from pigs with polyserositis.

In the 2-y period 2006–2008, tissues from 432 pigs aged 4 to 12 wk were examined diagnostically at the Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea. Cases of polyserositis were selected on the basis of the following criteria: (a) age between 30 and 70 d, (b) presence of clinical signs of polyserositis such as growth retardation and rough hair coat, (c) presence of a combination of pleuritis, pericarditis, and peritonitis, (d) submission of live pig, (e) isolation of *S. suis* at necropsy from lesions of polyserositis. A total of 78 cases met these criteria. The *S. suis* was isolated as previous described (14). Capsular serotyping was carried out by the coagglutination technique, as previously described (3).

Polymerase chain reaction was used to test for the presence of MRP and *mrp,* EF and *epf,* and SLY and *sly* (10–12). For *mrp* (GenBank accession number X64450) the forward and reverse primers were 5′-ATTGCTCCACAAGAGGATGG-3′ (nucleotides 3478 to 3497) and 5′-TGAGCTTTACCTGAAGCGGT-3′ (nucleotides 3665 to 3646), respectively. For *epf* (GenBank accession

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numbers X71881 and X71880) the forward and reverse primers were 5′-GCTACGACGGCCTCAGAAATC-3′ (nucleotides 2767 to 2787) and 5′-TGGATCAACCACTGGTGTTAC-3′ (nucleotides 3392 to 3372 or 5760 to 5740), respectively. For *sly* (GenBank accession number AJ416310) the forward and reverse primers were 5′-ATGAGAAAAAGTTCGCACTTG-3′ (nucleotides 338 to 358) and 5′-CCTGCTTCTTGGGCAGCCTT-3′ (nucleotides 2789 to 2770), respectively.

A total of 35 isolates visualized as α-hemolytic streptococci were taken from the primary plating for the 78 pigs with polyserositis. Three isolates were eliminated with the Gram or catalase test or were lost. The remaining 32 isolates were submitted to 10 biochemical tests; 24 were considered biochemically compatible with *S. suis*. Thus, in 24 of the 78 cases (31%) isolation of *S. suis* was confirmed.

Of the 24 confirmed isolates, 4 were autoagglutinating (2) or untypable (2) with all known antiserum and therefore were not examined further. Of the remaining 20 isolates, serotype 3 (7 isolates) and serotype 4 (5 isolates) were the most common (Table I). Of the 24 isolates, all carried the *sly* gene; in addition, 8 isolates carried the *mrp* gene, and 1 isolate carried the *epf* gene (and also the *mrp* gene).

Of the 24 isolates, 8 were from lesions of pericarditis; 3 of the isolates were serotype 3, and 1 isolate each was serotype 2, 4, or 22, was autoagglutinating, or was untypable. Of the 10 isolates from lesions of peritonitis, 4 isolates were of serotype 4, 3 isolates were of serotype 3, and 2 isolates were of serotype 8; 1 isolate was of serotype 2. Of the 6 isolates from lesions of pleuritis, 2 isolates were of serotype 33, and 1 isolate each was of serotype 3 or 16, was autoagglutinating, or was untypable. Serotype 2 with *epf* was isolated from lesions of pericarditis. Among the 8 isolates carrying *mrp*, 5 isolates were from lesions of peritonitis (2 isolates each of serotypes 3 and 4 and 1 isolate of serotype 2), 2 isolates were from lesions of pericarditis (1 isolate each of serotypes 2 and 3), and 1 isolate (serotype 3) was from lesions of pleuritis.

Histopathologically, the lesions in the serosal membranes throughout the body were similar in the 24 cases of polyserositis selected and used in this study. Of the 24 pigs, 5 had pericarditis and peritonitis, 9 had pericarditis and pleuritis, 6 had pleuritis and peritonitis, and 4 had pleuritis, pericarditis, and peritonitis. The pleural, pericardial, and peritoneal cavities were lined by greyish to yellow gelatinous and fibrinous exudates. Histopathologically, diffuse fibrinopurulent material was seen in the pleural, pericardial and peritoneal cavities.

The distribution of serotypes of *S. suis* isolated in these cases of polyserositis differs from that reported for isolates from pigs with other clinical manifestations of *S. suis* infection, such as meningitis, septicemia, and pneumonia. In the present study, the most prevalent capsular serotypes identified were 3 (29%) and 4 (21%), whereas worldwide *S. suis* serotype 2 is most frequently isolated from pigs with other clinical manifestations (6–9). In North America, serotypes 3, 2, and 1/2 have been the 3 most prevalent serotypes (7,8). In addition, serotype 2 was the common serotype isolated from slaughter pig in Korea (14). These results indicate that the distribution of capsular serotypes of *S. suis* varies with different clinical manifestations.

Besides capsular serotype, the distribution of virulence factors in the *S. suis* isolates from the cases of polyserositis in this study differs from that in cases of *S. suis* infection with other clinical manifestations, such as meningitis, septicemia, and pneumonia. In the present study, all the *S. suis* isolates carried *sly*, but only 33% and 4% carried *mrp* and *epf*, respectively. In contrast, only 65% to 70% of *S. suis* isolates from pigs with other clinical manifestations carried *sly* (8,12), and 92% and 31% carried *mrp* and *epf*, respectively (8), in North American and European countries. In addition, the phenotypes $MRP^+EF^-SLY^-$ and $MRP^-EF^-SLY^-$ were the most prevalent in the United States and France (6,8), whereas the genotypes $mrp^+epf^+sly^+$ and $mrp^-epf^+sly^+$ were the most prevalent in China (9). Thus, the distribution of virulence factors of *S. suis* isolated from cases of polyserositis appears to differ greatly from that reported for *S. suis* infection with other clinical manifestation in different countries.

Table I. Distribution of capsular serotype and virulence-associated genes of *Streptococcus suis* isolated from pigs with polyserositis in Korea

	Number of isolates			
		Virulence-associated genes		
Serotype	Total	*mrp*	*epf*	*sly*
2	2	2	1	2
3	7	4	0	7
4	5	2	0	5
8	2	0	0	2
16	1	0	0	1
22	1	0	0	1
33	2	0	0	2
Untypable[a]	2	0	0	2
Autoagglutinating	2	0	0	2
Total	24	8	1	24

[a] One isolate was recovered in co-culture with *Haemophilus parasuis*. All other *S. suis* isolates were recovered in pure culture.

There is a correlation between serotype and virulence factors for *S. suis* isolated in cases of polyserositis. Although in this study 9 serotypes, including autoagglutinating and untypable, were identified, both of the isolates of serotype 2, 4 (57%) of the 7 isolates of serotype 3, and 2 (40%) of the 5 isolates of serotype 4 carried only *mrp*. This finding suggests that isolates of these 3 serotypes carrying *mrp* are likely to be virulent in cases of polyserositis.

Serotyping is performed by the coagglutination test. An isolate that does not present any agglutination with all 35 known capsular types is considered "untypable." Some isolates, often unencapsulated ones, will autoagglutinate. This means that serotyping is impossible. Some of these isolates could belong to a known serotype, but since they autoagglutinate a positive reaction is not possible. Hence, it is important to differentiate "untypable" from "autoagglutinating" isolates.

In conclusion, isolates of serotypes 3 and 4 with *mrp* and *sly* may be the important pathogens associated with polyserositis in Korea. The prevalence of these serotypes is different from that in other countries. Although the development of effective vaccines is hindered by the various serotypes, serotype identification in pigs with different clinical manifestations is important in the control of *S. suis* infection because current bacterins provide only serotype-specific protection. The results of this study should have a significant impact

on the design of efficient vaccines for the prevention of polyserositis caused by *S. suis* in Korea.

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