**Streptococcus suis** Serotype 2 Capsule In Vivo

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Many *Streptococcus suis* isolates from porcine endocarditis in slaughterhouses have lost their capsule and are considered avirulent. However, we retrieved capsule- and virulence-recovered *S. suis* after in vivo passages of a nonencapsulated strain in mice, suggesting that nonencapsulated *S. suis* are still potentially hazardous for persons in the swine industry.

*Streptococcus suis* is a gram-positive bacterium that infects pigs and causes severe economic losses to the swine industry. Moreover, it causes severe disease in persons in close contact with diseased pigs or their products (1). In Japan, *S. suis* has been frequently isolated from pigs with endocarditis in slaughterhouses; most of the isolates were expected to be sequence types (STs) that are potentially hazardous to humans (2). Many isolates from porcine endocarditis lost their capsule, and all the nonencapsulated isolates analyzed had mutations in the capsular polysaccharide synthesis (*cps*) genes (3,4). The capsule of *S. suis* is a major virulence factor (1). Although loss of the capsule gives *S. suis* some benefit in causing endocarditis by enhancing the ability of bacterial cells to adhere to porcine and human platelets, a major virulence determinant for infective endocarditis (3), nonencapsulated *S. suis* are generally considered avirulent (5). However, whether nonencapsulated *S. suis* lurking in porcine endocarditis poses a threat to persons working in the swine industry is unknown. To investigate whether nonencapsulated *S. suis* can restore the ability to express the capsule and become virulent again, we repeated in vitro or in vivo passages of nonencapsulated *S. suis* and attempted to retrieve capsule-recovered strains.

The Study

For the in vitro passages, we used 29 *S. suis* strains isolated from pigs with endocarditis. These isolates had the *cps* gene cluster of serotype 2 but had lost their capsule. We subcultured them twice in liquid media and separated the cells according to the buoyant density by Percoll density gradient centrifugation (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/15-1640-Techapp1.pdf). Because encapsulated cells show lower density than nonencapsulated cells (6,7), we investigated capsular expression of *S. suis* cells with low density by coagglutination tests using serotype 2 antiserum (online Technical Appendix). The retrieved *S. suis* was also used for the next subcultures. We repeated 4 cycles of this experiment (in total 8 subcultures) but obtained no encapsulated *S. suis* from any of the strains tested.

Although these results suggested that mutations in *cps* genes are not repaired easily, the conditions faced by *S. suis* in vivo could influence capsular expression. To investigate this possibility, we selected strain NL119 as a representative. NL119 is an ST1 strain, one of the types hazardous to humans, but one that has lost the capsule because of a point mutation that occurred at nt 490 (T490C, Cys164Arg) of a glycosyltransferase gene (*cps2F*) (Table; Figure 1, panel A) (4). We inoculated groups of 5 mice with 5 × 10⁶ CFU of NL119 (online Technical Appendix). Bacteria persistent in mice were retrieved 36 h after infection from the blood, in which capsular expression works favorably for survival. We investigated capsular expression of the retrieved NL119 by coagglutination tests and used the colony giving the strongest reaction within 30 s for the subsequent in vivo passage.

As expected, the coagglutination test of the parental strain NL119 showed a negative result. Similarly, NL119 after the first and second passages (NL119 P1 and P2, respectively) reacted weakly, comparable to those of the parental strain, suggesting poor encapsulation. Meanwhile, NL119 after the third and fourth passages (NL119 P3 and P4, respectively) reacted strongly, suggesting recovery of the capsule. To confirm this finding, we further analyzed formalin-killed bacteria by dot-ELISA using monoclonal antibody Z3, which reacts with the sialic acid moiety of the serotype 2 capsule (8), and an anti–*S. suis* serotype 2 serum adsorbed with parental strain NL119 to select the capsule-specific antibodies (online Technical Appendix). In accordance with the coagglutination test, NL119 P1 and P2 gave weak reactions similar to those of NL119, whereas strong signals were detected in NL119 P3 and P4 with both the monoclonal antibody and serum (Figure 1, panels B, C). Because NL119 P1–P4 were also ST1 as determined by multilocus sequence

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infected with NL119 had blood bacterial titers <10^4 CFU/mL. In contrast, except for 1 mouse, all mice in either NL119 or NL119 P4 (online Technical Appendix). Rates of death differed significantly (p<0.05): 50% death occurred in the NL119 P4-infected mice 14 days after infection, compared with 0% for the nonencapsulated NL119 (Figure 2, panel A). Recovery of the capsule also significantly increased its survival in blood 24 h after infection (p<0.05). All but 1 surviving NL119 P4-infected mice had significant blood bacterial titers (≥5 × 10^3 CFU/mL; geometric mean 10^4 CFU/mL). In contrast, except for 1 mouse, all mice infected with NL119 had blood bacterial titers <10^4 CFU/mL (geometric mean 10^2 CFU/mL) (Figure 2, panel B).

**Conclusions**

Although capsule loss might contribute to *S. suis* infection by enhancing bacterial adherence to host cells and
biofilm formation (3,10–12), capsule loss makes *S. suis* cells susceptible to phagocytosis; therefore, the virulence of nonencapsulated mutants was attenuated when evaluated in animal models (5). In accordance with previous studies, nonencapsulated NL119 was avirulent. However, NL119 P4, which recovered its capsule in vivo, also recovered virulence. Because various mutations in *cps* genes, including large deletions and insertions, cause capsule loss in *S. suis* (3,4), not all mutations will be repaired like NL119. However, our results demonstrated the presence of a nonencapsulated mutant, which can recover the capsule and virulence in vivo. Hence, nonencapsulated *S. suis* strains can cause severe diseases to the next hosts by recovering the capsule, which indicates that some nonencapsulated *S. suis* lurking in pigs with endocarditis are still potentially hazardous to
persons handling such pigs and their products. Further investigations using a variety of naturally occurring and laboratory-derived mutants are needed for a comprehensive understanding of the biological significance and mechanisms of this phenomenon.

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References

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EID Podcast: *Shigella Sonnei* and Shiga Toxin

Shiga toxins (Stx) are primarily associated with Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* serotype 1. Stx production by other shigellae is uncommon, but in 2014, Stx1-producing *S. sonnei* infections were detected in California. During June 2014–April 2015, 56 cases of Stx1-producing *S. sonnei* were identified, in 2 clusters. Continued surveillance of Stx1-producing *S. sonnei* in California is necessary to characterize its features and plan for reduction of its spread in the United States.