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Demonstration of Vertical Transmission of *Streptococcus suis* in Swine by Genomic Fingerprinting

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Isolates of *Streptococcus suis* serotype 5 collected from three sows and nine of their pigs at birth were analyzed by genomic DNA fingerprinting. The cleavage patterns of DNA from *S. suis* isolated from the sows matched the cleavage patterns of DNA from *S. suis* isolated from their respective pigs.

Streptococcus suis infection is a worldwide cause of meningitis in nursery pigs (11). Most clinically healthy pigs are carriers of multiple serotypes of *S. suis* (5). The piglets become colonized during parturition when they contact and/or swallow *S. suis* from sow vaginal secretions (1). Colonized piglets carry *S. suis* into the nursery, where it may be transmitted among other pigs and cause clinical disease as maternal immunity declines.

Thirty-five serotypes of *S. suis*, and subtypes defined as different genotypes within these serotypes, have been identified (9, 10). Vertical transmission of *S. suis* has been demonstrated based on serotypes, but not subtypes, by the coagglutination method (1). Mogollon et al. have described genomic fingerprinting as a technique to study *S. suis* epidemiology (10). Use of this technique with the coagglutination method allows subtypes of *S. suis* to be identified (3, 10). The purpose of this study was to demonstrate vertical transmission of *S. suis* subtypes in swine.

All *S. suis* isolates used in this study originated in healthy sows and pigs from a single herd. Samples were collected with sterile swabs (S/P brand culturette system; Baxter Diagnostics Inc., Deerfield, Ill.). Oral and vaginal swab samples were collected from each sow prior to farrowing. Piglets were removed from the vagina with sterile obstetrical sleeves into which the piglets were delivered. Sterile gauze was used to remove the fetal membranes covering each piglet's rostral surface. Swab samples from the oropharyngeal region and surface of each piglet were collected.

Sow oral swab samples were plated onto sheep blood agar and incubated within 24 h of collection. These samples were not enriched, because *S. suis* was isolated from unenriched cultures of saliva from five of seven sows studied previously (2). Sow vaginal samples, piglet oropharyngeal samples, and piglet surface swab samples were plated onto sheep blood agar within 11 h of collection. These swab samples were then enriched in Todd-Hewitt broth (THB) for 12 h, and a sample of the THB was plated onto phenylethyl alcohol agar with 5% sheep blood and incubated for 18 h. Sow vaginal samples were enriched, because *S. suis* was isolated from unenriched vaginal fluids of only three of seven sows studied previously (2). After 18 to 24 h of incubation at 37°C with 5% CO_2, up to three- 0.5- to 1-mm, flat, alpha-hemolytic colonies per site for each animal sampled were selected and subcultured onto sheep blood agar. These isolates were then Gram stained and biochemically tested. Gram-positive, catalase-negative, acetoin-negative, amylase-positive diplobacilli which did not grow in 6.5% NaCl solution were suspected to be *S. suis* (6, 8).

All organisms suspected of being *S. suis* were serotyped by a polyvalent coagglutination method followed by a monovalent coagglutination method (7) with antisera to *S. suis* types 1/2 to 34 (antisera provided by R. Higgins, University of Montreal, Saint-Hyacinthe, Canada). Isolates were considered *S. suis* if their biochemical test results were consistent with *S. suis* and they were agglutinated by the antiserum.

Fifteen isolates of *S. suis* serotype 5 collected from three sows and nine of their pigs were chosen from the 54 original isolates (1) for genomic DNA fingerprinting. An isolate of *S. suis* serotype 4 was used as a negative control. DNA was isolated by a modification of the procedure of Bickley and Owen (4). Ten milliliters of THB was inoculated with a loopful of *S. suis* culture in THB and incubated for 18 h at 37°C with 5% CO_2. Cells were harvested by centrifugation, resuspended in 100 μl of TE-glucose (25% [wt/vol] glucose, 100 mM Tris HCl, 10 mM EDTA [pH 7.0]) containing 20 μg of mutanolysin per ml, and incubated in a 37°C shaker water bath for 1 h. Cells were resuspended in 0.5 ml of GES reagent (5 M guanidinium isothiocyanate, 0.1 M EDTA, 0.5% [vol/vol] Sarkosyl) and incubated for 50 min at room temperature with frequent mixing. Two hundred fifty microliters of cold 7.5 M ammonium acetate was added, and the mixture was incubated on ice for 10 min. Five hundred microliters of chloroform reagent (24:1 ratio of chloroform to isoamyl alcohol) was added. The mixture was incubated on ice for 10 min and then centrifuged in a microcentrifuge. The DNA was precipitated from the supernatant with 0.54 volume of cold isopropanol and washed three times with cold 70% ethanol. The DNA was dried under vacuum for 10 min and redissolved in 100 μl of 1× TE (10 mM Tris HCl, 1 mM EDTA [pH 8.0]) buffer overnight at 4°C.

DNA purity was estimated visually after electrophoresis in a horizontal gel containing 0.6% agarose, 44.6 mM Tris base, 44.5 mM boric acid, 1 mM EDTA, and 0.01 mg of ethidium bromide at 100 V for 1 h in 0.5× TBE (44.6 mM Tris base, 44.5 mM boric acid, 1 mM EDTA, 0.0002 mg of ethidium bromide/ml [pH 8.0]) buffer.

Streptococcal DNA samples were digested for 2 h in a 37°C water bath in 20-μl volumes containing *Hae*III in the appropriate buffer (New England Biolabs, Inc., Beverly, Mass.).

Digested DNA was electrophoresed in a horizontal gel containing 0.6% agarose, 89.2 mM Tris base, 89 mM boric acid, and 2 mM EDTA at 60 V for 20 h in 1× TBE (89.2 mM Tris base, 89 mM boric acid, 2 mM EDTA [pH 8.0]) buffer. DNA fragments were stained with 0.5 mg of ethidium bromide in distilled water for 20 min. DNA was visualized with shortwave

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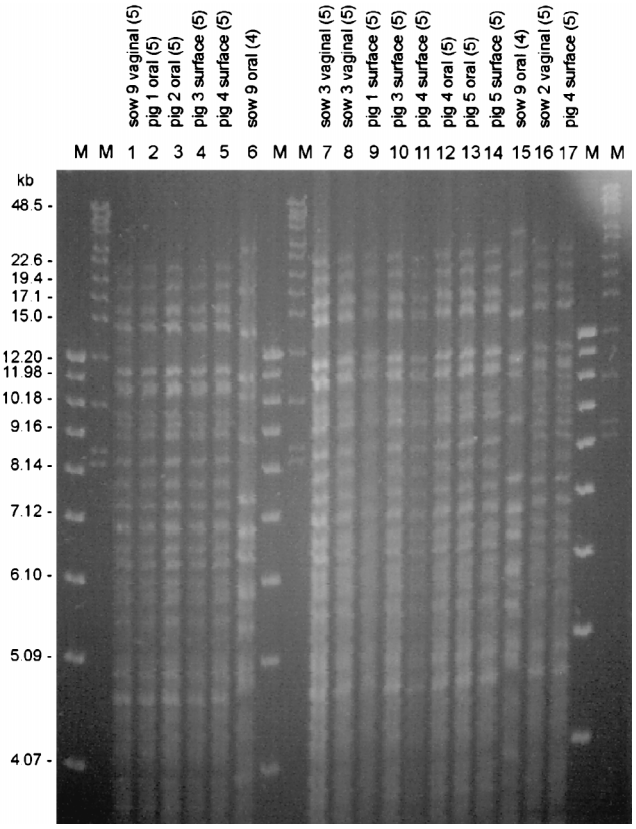

FIG. 1. Genomic fingerprints of *Hae*III-digested DNA of *S. suis* serotype 5 isolated from sows and their pigs. *S. suis* serotype 4 was used as a negative control (lanes 6 and 15). Lanes M contain DNA size markers. DNA was visualized as described in the text (Gel print 2000i [BioPhotonics Corporation]). Text was added to the figure by using CorelDRAW (Salinas, Calif.).

UV light, and the image was preserved by computer (Gel print 2000i; BioPhotonics Corporation, Ann Arbor, Mich.).

The cleavage pattern of DNA from *S. suis* serotype 5 isolated from a vaginal swab from sow 9 matched those of DNA from *S. suis* serotype 5 isolated from oral or surface swabs from each of her four pigs (Fig. 1, lanes 1 through 5). The cleavage patterns of DNA from two different isolates of *S. suis* serotype 5 isolated from a vaginal swab from sow 3 matched those of DNA from *S. suis* serotype 5 isolated from oral and/or surface swabs from four of her pigs (Fig. 1, lanes 7 through 14). Moreover, the cleavage patterns of the DNA from *S. suis* serotype 5 isolated from sow 9, sow 3, and their piglets were identical. The cleavage pattern of DNA from an isolate of *S. suis* serotype 5 isolated from a vaginal swab from sow 2 matched that of DNA from *S. suis* serotype 5 isolated from a surface swab from one of her pigs (Fig. 1, lanes 16 and 17). However, this cleavage pattern did not match those of *S. suis* serotype 5 isolated from the other two sows and their piglets. In each of the three litters, all *S. suis* subtype 5 isolates from the pigs matched the *S. suis* subtype 5 isolate carried by their dam. None of the cleavage patterns of the DNA from the strains of *S. suis* serotype 5 isolated in this study matched that of the negative-control DNA from *S. suis* serotype 4 (Fig. 1, lanes 6 and 15).

Comparisons of cleavage patterns coupled with the results of coagglutination tests support the findings of others working with *S. suis* by identifying subtypes within serotype 5 in this herd (3, 10). The DNA cleavage patterns for sows and their respective pigs were identical for the serotype and subtype carried. This study provides definitive evidence that pigs become colonized with *S. suis* of the same subtype as carried by the sow during parturition when they contact and/or swallow *S. suis* from sow vaginal secretions.

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