Identification of Epidemic Strains of *Streptococcus suis* by Genomic Fingerprinting

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A natural outbreak of *Streptococcus suis* meningitis in two closed swine herds was studied. DNA fingerprinting, serotyping, and biochemical profiles were assessed. Multiple serotypes were recovered from these herds. In farm A, 50 *S. suis* strains were isolated from 330 swabs collected. Eighteen strains belonged to serotype 2, and 32 strains belonged to serotypes 3, 5, 6, 8, 9, and 11. In farm B, 16 *S. suis* strains were recovered from a total of 70 samples. Eight strains belonged to serotype 7, and eight belonged to serotypes 2, 3, 5, and 8. In each epidemiological situation, a single strain characterized by a distinctive restriction fragment pattern predominated among affected penmates. The epidemic serotype 2 strain was detected in farm A in weaned pigs between the ages of 5 and 7 weeks. In contrast, the pathogenic strain in farm B belonged to serotype 7 and was isolated from pigs up to 3 weeks of age. The results from both farms strongly suggest a lateral spread of these organisms. No vertical transmission could be shown in either herd. It was concluded that genomic fingerprinting is an appropriate method to distinguish outbreak isolates of *S. suis* from nonoutbreak strains, within the same serotype or from epidemiologically unrelated clusters of strains.

*Streptococcus suis* infections have risen steadily to become one of the major disease problems in the pork industry. *S. suis* was originally recognized as a cause of meningitis, septicemia, and purulent arthritis in young pigs (15). Recently, however, strains showing features of this species have been commonly isolated from pigs with pneumonia (23). In addition, concern over this form of streptococcal meningitis has increased because of reports of cases occurring in humans (1, 20).

Both the tonsils and the nose have been shown to be carrier sites of *S. suis* in pigs, and the throat has been suggested as a first site of colonization for these bacteria (16, 26). Nevertheless, colonization alone is not a good criterion for virulence because these events may be independent of the development of clinical disease in susceptible piglets. A carrier state in convalescent animals and apparent infection of pigs are both important in an epidemiological context, since they may create reservoirs of *S. suis* (6). However, because of differences in the virulence of isolates, a correlation has not been detected between the carrier state and actual disease prevalence (5, 6).

Because *S. suis* is widespread in pig herds, epidemiological studies of this disease have been infrequent. It has proven impossible to identify either the source of infection in outbreaks or carrier animals harboring the virulent strains. The ability to further characterize individual strains of *S. suis* would be valuable in studies of the epidemiology of this disease.

Recently, Mogollon et al. (14) found that chromosomal restriction endonuclease analysis of *S. suis* may provide a set of epidemiological markers to study the transmission patterns of this organism and the relationship between epidemic strains and other strains present in a swine herd.

This technique has been used successfully as an epidemiologic tool for the identification of subspecies and for the tracking of group A, C, and G streptococci, as well as a variety of other pathogenic microorganisms (4, 13, 18, 19, 22).

The purpose of this study was to assess the applicability of this approach for the identification of outbreak-specific strains of *S. suis* and for tracking the organisms in a given population. The results presented here suggest that genomic fingerprinting of *S. suis* is an important method to distinguish strains of the natural flora from pathogenic strains of epidemiological importance. This information may help to elucidate the natural history of this infectious disease.

**MATERIALS AND METHODS**

**Herds.** A natural outbreak of *S. suis* meningitis in two closed farrow-to-finish herds from Minnesota was traced by DNA fingerprinting, serotyping, and biochemical profiles. The affected herd in farm A comprised 200 crossbred sows. The highest incidence of disease was among weaners between 5 and 7 weeks old, which had a 5% mortality rate. Piglets were weaned at 3 weeks of age into two nursery rooms with coated expanded metal as flooring material, each room containing between 150 and 200 pigs. The outbreak had lasted almost 2 years despite the use of an autogenous vaccine and antibiotics. Because the results obtained in farm A indicated that DNA fingerprinting was useful in answering questions about the epidemiology of *S. suis* infection in a swine population, it was decided to validate some of the observations by studying another outbreak.

In farm B, the disease occurred in piglets in the farrowing room, and the mortality rate was about 14%. Piglets were weaned at 16 days of age into hot prenurseries, and the herd comprised 400 F1 sows. This outbreak had lasted 4 months at the time of sampling. The affected animals were receiving

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high doses (25,000 IU/kg of body weight) of injectable penicillin.

**Bacteriological sampling.** In both farms, pigs of different ages were screened at random by swabbing the tonsils and noses of apparently healthy pigs or by doing necropsies on the affected animals.

Nasal swabs were collected from 20 farrowing sows and 10 gilts belonging to farm A. Samples from the tonsils of 12 of these 20 sows were also taken. To sample the tonsils, the mouth was held open with a mouth speculum. Double-guarded swabs were rubbed over the surface of one tonsil. Nasopharyngeal swab samples were also taken from three piglets of each litter. Samples were also taken from the noses and tonsils of 54 apparently healthy nursery piglets, 5 to 7 weeks old, as previously described (16).

Twenty piglets that were showing signs of meningitis or were recumbent were necropsied, and swab samples were collected from the brains and in some cases from the lungs. Tonsils were removed and cut deeply with sterile scissors, and samples were taken by rubbing the exposed tissue with a bacteriological loop. A total of 330 samples were collected from farm A for bacteriological analysis. Representative samples of some of the brains were also taken and fixed in phosphate-buffered 10% Formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin by routine procedures.

Sampling from herd B was carried out as described above. Nasal and tonsil swabs were taken from six farrowing sows and from six piglets per litter. Thirteen prenursery piglets were also sampled. Necropsy and brain sampling were performed on 10 piglets that died with signs of central nervous system (CNS) disturbance. A total of 70 samples were collected from this herd for bacteriological studies.

**Bacteriology.** *S. suis* isolates were identified biochemically by routine procedures. Primary isolation was done by plating onto Columbia blood agar. Colonies of alpha-hemolytic, catalase-negative cocci resembling *S. suis* were subcultured onto brain heart infusion blood agar. Biochemical reactions and growth tolerance were examined by the methods of Cowan (7) and Hommez et al. (9). Briefly, isolates were tested for the following: growth in Todd-Hewitt broth, growth on bile esculin agar, growth in 6.5% NaCl, and production of acid from inulin and raffinose. All suspected *S. suis* strains were tested with the API 20 Strep system according to the directions of the manufacturer (API, Montalieu, Vercier, France).

**Serotyping.** Colonies of *S. suis* strains were subcultured in Todd-Hewitt broth. Cells were pelleted by centrifugation and tested by slide agglutination and coagglutination by using antisera prepared in rabbits against reference strains of serotypes 1/2 through 16.

**DNA extraction and analysis.** Thirty milliliters of Todd-Hewitt broth with 1% yeast extract was inoculated with 1 ml of Todd-Hewitt broth culture and incubated overnight at 37°C. Cells were recovered by centrifugation at 10,000 × g and washed twice with phosphate-buffered saline. Protothecae were produced by digesting *S. suis* cells with 10 mg of lysozyme per ml in 5 ml of TE sucrose buffer (25% sucrose in 50 mM Tris hydrochloride [pH 8.0], 50 mM EDTA, and 10 mM NaCl) for 1 h at room temperature with frequent mixing.

DNA was extracted from protoplasts as described by Gebhart et al. (8). Streptococcal DNA was digested for 3 h at 37°C with the restriction endonuclease *Hae*III. Digested DNA was displayed by electrophoresis in 0.55% agarose as previously described (14).

**RESULTS**

**Clinical signs, postmortem, and histopathological examinations.** In both farms, the affected animals showed classical signs of CNS disturbance. Signs of meningitis such as opisthotonos, nygmus, and paddling in lateral recumbency were seen in farm A only in the nursery piglets between 5 and 7 weeks of age. In contrast, in farm B the affected piglets were younger, less than 3 weeks of age.

Gross lesions were observed in only 2 of 20 diseased piglets from farm A. A fibrinopurulent pericarditis and vegetative valvular endocarditis were noted in one pig, and in another a multifocal lobular bronchopneumonia was observed. The only change present in the CNS of affected pigs from both farms was a mild congestion of the leptomeningeal blood vessels. The common histological lesion was fibrinopurulent leptomenigitis and choroiditis.

**Bacteriological analysis.** Fifty *S. suis* strains were isolated from 330 swabs collected from farm A. Multiple serotypes were recovered from this herd. As shown in Tables 1 and 2, 18 strains belonged to serotype 2 and 32 strains belonged to serotypes 3, 5, 6, 8, 9, and 11. On farm A, the disease outbreak was confined to nursery piglets. Serotype 2 strains were associated with meningitis, pneumonia, and pericarditis. However, two isolates of serotype 2 were recovered from apparently healthy weaned pigs, one from the nasal cavity and the other from the tonsils. Some animals yielded multiple isolates; two strains of serotype 2 were isolated from the brain and tonsils of a weaner pig and three more were isolated from the brain, heart, and lung of another. In addition, one *S. suis* serotype 2 strain was isolated from the tonsils of a farrowing sow. The distribution of *S. suis* isolates according to serotyping results is shown in Tables 1 and 2. It was also found that one sick nursery piglet was positive for two different serotypes. It harbored one strain of serotype 2 in the meninges and tonsils and another of serotype 11 in the tonsils.

**TABLE 1.** *S. suis* strains recovered from pigs in the farrowing room of farm A

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<tr>
<th>Serotype</th>
<th>Piglets</th>
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<td>Nose</td>
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**TABLE 2.** *S. suis* strains recovered from nursery pigs of farm A

<table>
<thead>
<tr>
<th>Serotype</th>
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Sixteen *S. suis* strains were recovered from 70 samples collected from farm B. Eight belonged to serotype 7 and another seven belonged to serotypes 2, 3, 5, and 8 (Table 3). On farm B, the outbreak occurred in the farrowing room. Seven of the serotype 7 strains were isolated from piglets affected with signs of CNS disturbance, and one tonsil isolate was recovered from a sow that was adjacent to a sow nursing several affected piglets.

**Restriction endonuclease patterns of *S. suis* isolates.** All *S. suis* strains isolated from both farms were analyzed for their restriction enzyme electrophoretic patterns in order to establish a clonal relationship among strains within a given serotype. Five additional serotype 7 strains collected from farm B by a private laboratory were included in the analysis.

Strains of the same genotype are usually expected to have the same restriction pattern. In farm A, it was found that all 14 invasive strains of *S. suis* serotype 2 (associated with disease in nursery piglets within the same room) possessed identical DNA fingerprints, strongly suggesting that these isolates belonged to the same clone (Fig. 1). Conversely, two isolates from healthy carriers, one sow and one weaner pig which had serotype 2 strains in the tonsils and nose, respectively, had clearly different restriction patterns (Fig. 2, lanes 6 and 7). They differed from the outbreak strain (Fig. 2, lanes 2 and 3) and also from the prototype strain of serotype 2 (Fig. 2, lane 1). Two other apparently healthy animals yielded *S. suis* serotype 2 from the nose or tonsils. These strains showed DNA fingerprints identical to those observed in the affected animals, suggesting a carrier state (Fig. 2, lanes 4 and 5).

The ethidium bromide staining intensity of the bands in Fig. 1 and 2 appeared to be relatively constant. This observation suggests that all fragments are present in equimolar proportions, most likely at one copy per cell. Plasmids present at more than one copy per cell would be observed as more intensely stained bands relative to their immediate neighbors in the agarose gel. Thus, the similarities and differences seen in Fig. 1 and 2 are due to restriction fragment length polymorphisms in genomic DNA and are not due to the presence or absence of nonchromosomal plasmids.

A meningeal strain of serotype 2 isolated the year before from the same herd A (Fig. 3, lane 2) had a DNA fingerprint clearly different from that of the clone detected in this outbreak (Fig. 3, lanes 3 through 6). The findings in the present study also show that *S. suis* isolated from different sites (systemic isolates) in the same animal possessed identical DNA fingerprints. Lanes 3, 4, and 5 in Fig. 3 show the restriction profiles of *S. suis* strains cultured from the brain, heart, and lung of the same affected nursery piglet.

*S. suis* isolates from healthy carriers that harbored serotypes 3, 6, 8, 9, and 11 showed a wide variety of banding patterns. The DNA fingerprints of six strains of serotype 6 are displayed in Fig. 4 (lanes 3 through 8) to illustrate this
VOL. 29, undigested DNA.

The prototype heterogeneity. Figure 4 also shows the DNA fingerprints of the prototype 2 strain (lane 1) and the strain involved in disease in herd A (lane 2).

In contrast to farm A, the epidemic strain in farm B belonged to serotype 7. A total of 13 strains of \textit{S. suis} serotype 7 were analyzed for their restriction enzyme electrophoresis patterns. All isolates tested had identical DNA fingerprints and may represent a single clone. Figure 5 shows the restriction profiles of six strains of serotype 7 isolated from pigs showing signs of CNS disturbance (lanes 2 through 7). They were different from the prototype strain 7 (lane 1) and also different from a serotype 7 strain isolated from the tonsils of a healthy carrier piglet (lane 8). One nasal isolate of serotype 8 from a piglet in the hot nursery is also shown in Fig. 5 (lane 9) for comparative purposes. It should be noted that the strain in Fig. 5 (lane 7) which had a pattern identical to that of the meningal isolate was recovered from the tonsils of an apparently healthy sow. A nontypeable \textit{S. suis} strain was isolated from the nose of one piglet of her litter (Table 3). It had a restriction pattern different from that of the epidemic strain in farm B (data not shown).

**FIG. 3.** Comparison of restriction fragment patterns of \textit{S. suis} serotype 2 cultured from different tissues. \(\lambda\), Lambda DNA size markers. The origins of strains are as follows: lane 1, prototype 2; lane 2, brain (isolated the previous year); lane 3, brain; lane 4, heart; lane 5, lung; lane 6, brain. The upper band in lane 2 represents undigested DNA.

**FIG. 4.** Restriction fragment patterns of \textit{S. suis} strains unrelated to the outbreak in farm A. Lanes: \(\lambda\), lambda DNA size markers; 1, prototype 2; 2, epidemic strain from farm A. Lanes 3 through 8 contain nondisease strains. Lanes: 3, 11-3V; 4, strain 22-3V; 5, strain 105-3V; 6, strain P3T1; 7, strain 71.2; 8, strain 75.

**FIG. 5.** Restriction fragment patterns of \textit{S. suis} strains collected from farm B. Lanes: \(\lambda\), lambda DNA size markers; 1, prototype strain 7; 2, strain D2M; 3, strain 14; 4, strain 15; 5, strain 16; 6, strain 17; 7, strain 10; 8, strain 15-2T; 9, strain 7.

**DISCUSSION**

This study demonstrated the practical usefulness of the DNA fingerprinting method, since it allowed the identification of two \textit{S. suis} clones causing natural outbreaks of meningeal disease in suckling and nursery piglets. In farm A, the epidemic clone was detected only in weaned pigs 5 to 7 weeks old, and in farm B, it was detected in piglets up to 3 weeks old. The organism is apparently transmitted horizontally, since affected animals were penmates and all the outbreak \textit{S. suis} strains of serotype 2 or 7 isolated from them had identical restriction profiles. No vertical transmission could be shown in either herd, since restriction patterns of \textit{S. suis} strains isolated from sows did not match the restriction patterns of \textit{S. suis} strains isolated from their litters. It was found that in farm B, one farrowing sow had the epidemic strain (serotype 7) in her tonsils (Fig. 5, lane 7). This sow was adjacent to the pen containing the sick piglets. An \textit{S. suis} strain was also recovered from the nose of one piglet of her litter. However, this piglet was colonized by a nontypeable strain which had a restriction pattern different from that of the epidemic strain (data not shown).

The restriction enzyme cleavage patterns of \textit{S. suis} strains of serotype 2 isolated from two asymptomatic nursery pig-
lets (farm A) were identical to the DNA fingerprints of invasive strains isolated from the brains, lungs, or hearts of pigs affected with meningitis (Fig. 1, 2, and 3). This observation appears to implicate healthy carriers in the transmission of disease. These findings may support those of Clifton-Hadley et al. (6) and Van Leengoed et al. (24), who detected carrier rates of between 0 and 80% and of 45%, respectively, in endemic herds. Alternatively, finding potentially virulent strains in asymptomatic pigs may only be a chance occurrence. It could be that these animals were in the incubation period and would develop clinical signs of CNS disturbance later. Since the animals were not monitored individually, it was not possible to ascertain whether a true asymptomatic carrier state existed.

It was shown that a herd may harbor a heterogeneous population of S. suis, as six and five different serotypes were recognized in farms A and B, respectively. Multiple genotypes were found, indicating genetic diversity. This genetic diversity may explain why S. suis is a ubiquitous microorganism in the pig microbiota (10, 14) and indicates that care must be taken when interpreting isolations of these organisms from animals with no signs of CNS disturbance. This serotype diversity among S. suis isolated from a given herd has been reported before. Serological typing has revealed the presence of various serotypes cultured from clinically healthy piglets belonging to herds with or without histories of streptococcal meningitis (3, 16, 25).

One sick piglet from farm A harbored in the tonsils the epidemic strain of S. suis type 2 and another strain of serotype 11. Brisebois et al. (3) and Sihvonen et al. (21) also found carrier piglets harboring two or more different serotypes. Multiple colonization may explain the apparent ease with which S. suis can spread after its introduction into a susceptible herd (6, 24). However, as stated above, the existence of a carrier state has not been totally proven yet.

It is noteworthy that the meningitis outbreak in farm B was due to serotype 7. This serotype is more frequently associated with cases of meningitis, septicemia, and arthritis in piglets less than 3 weeks of age in Denmark and in other countries (2).

It appears that despite the wide variety of S. suis strains within a swine population, a defined cluster of one genomic variety tends to be responsible for an outbreak of disease. This homogenous cluster shows the same serotype and tends to have very similar DNA fingerprints. On the other hand, some strains of the same serotype may not be related with the outbreak, as demonstrated in this study (Fig. 2 and 5). A similar epidemiological pattern has been described in epidemics of nosocomial infections in hospitals and in closed communities in humans (17, 12). The epidemiology of S. suis may also be comparable to that of type B15 meningococci and group G streptococci (11, 13).

Comparing isolates from an outbreak of S. suis by DNA fingerprinting may yield clinically relevant information for the entire herd. The demonstration that the same strain is present in many brain cultures would indicate that this is the virulent strain, and it could then be included in autogenous vaccines to control that outbreak. In this study, an S. suis serotype 2 strain which was being used in the autogenous vaccine in farm A had a DNA pattern different from those of meningococci strains isolated from the present outbreak (Fig. 3, lane 2). This observation may explain why that vaccine was not effective in farm A.

In conclusion, genomic fingerprinting of S. suis isolates proved to be a suitable technique to monitor the epidemiology of this infectious disease.

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