

Source and timing of *Streptococcus suis* infection in neonatal pigs: Implications for early weaning procedures

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Summary: To determine the possible sources of *Streptococcus suis* that colonize neonatal pigs, and the youngest age at which *S. suis* may be isolated from pigs, multiple samples were collected from 35 pigs, their dams, and the environment. *Streptococcus suis* was not isolated from environmental samples collected before sows were placed in the farrowing house. At the day of farrowing and for 6 days thereafter, five pigs were euthanized daily and tonsils and meningeal swabs were collected from each pig. Additionally, multiple samples (sow vaginal swab, placental swab, milk, sow teat skin swab, sow feces, sow saliva, sow nasal secretions, piglet water, swab of piglet mat, environmental air) were collected at the time the pig was removed. All samples were culturally examined for *S. suis*. *Streptococcus suis* isolates were serotyped and antimicrobial susceptibility tests were performed. No conclusions were drawn from the meningeal swab samples; cultural examination confirmed that they had become contaminated during sampling. Forty-five isolates of *S. suis* serotypes 1–8 were isolated. The most prevalent serotype of *S. suis* isolated was serotype 3 (33.3%), followed by 5 (22.2%), 7 (17.8%), 4 (11.1%), 6 (6.7%), 8 (6.7%), and 2 (2.2%). Often (six of seven dams), multiple serotypes of *S. suis* were isolated from samples collected from a single dam. *Streptococcus suis* was isolated from samples of sow origin beginning on day 0 (two of seven sows), and the tonsils of pigs as early as day 1 (two of five pigs). In four of seven dams, *S. suis* of the same serotype isolated from samples collected from the dam was detected in samples of the tonsil of that dam's pig. These results suggest that the source of *S. suis* was the sow, and that *S. suis* could have colonized the tonsil of the pig shortly after birth when the pig contacted sow excretions and secretions. Ninety-one percent of the *S. suis* isolates were susceptible to ampicillin. Less than 50% of these isolates were susceptible to apramycin, lincomycin, penicillin, spectinomycin, tetracycline, and tylosin. Consequently, the use of antimicrobial susceptibility testing on herd isolates of *S. suis* is recommended due to the variability in sensitivity between isolates.

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There are at least 29 serotypes of *Streptococcus suis*, with types 1, 2, 3, and 8 being the most common in North America.¹ *Streptococcus suis* may cause disease outbreaks in pigs of all ages.² Many of the serotypes have been associated with septicemia resulting in meningitis, pneumonia, arthritis, endocarditis, and polyserositis.

Mechanisms of *S. suis* transmission have been investigated for the past 10 years. Clifton-Hadley, et al.,² concluded that although *S. suis* can infect suckling pigs, the major spread was among weaned pigs within intensive production units. More recently, researchers have been unable to eliminate *S. suis* from pigs using medicated early weaning (MEW) techniques. Wiseman, et al.,³ have shown that commingled pigs from source herds affected by porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV), *Bordetella bronchiseptica*, *S. suis*, *Serpulina hyodysenteriae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, and *Haemophilus parasuis* could be weaned at less than 10 days old, medicated, reared in isolation, and remain free of all organisms except *S. suis*. Clark, et al.,⁴ examined pigs weaned at 7, 10, and 21 days old to determine whether age segregation would produce high-health-status pigs without the high cost of medication. Again, *S. suis* survived all components of these early weaning procedures.

In segregated early weaning (SEW) studies that were part of the National Pork Producers Council's genetic evaluation program, researchers treated approximately 200 of 400 pigs at different times against clinical signs consistent with *S. suis* infection during a 42-day time period. In some cases, the same pigs were treated for a second episode of disease, and approximately five pigs were treated for as many as six disease episodes. The time interval between treatments ranged from 5–9.5 days.⁵

Thus, *S. suis* infection is responsible for a large portion of the treatment and mortality costs of raising SEW pigs. Moreover, with current SEW procedures, there is considerable risk of subsequent epidemics of *S. suis* infection developing in older pigs.

From the results of previous SEW studies,⁴ we concluded that *S. suis* was transmitted from dam to pig before the pig was 7 days old, and that dam-to-pig transmission was of prime importance.

The purposes of this study were to determine the youngest age at which *S. suis* could be isolated from pigs, and possible sources of the *S. suis* that infects SEW pigs. Additionally, all *S. suis* isolates were serotyped, and antimicrobial sensitivity tests were performed for future research to develop a protocol for eliminating *S. suis* from SEW pigs.

Materials and methods

Experimental design

Newborn to 6-day-old crossbred pigs from a single herd were used. Although samples were only taken from 35 pigs, the study population of 49 pigs included 14 additional pigs to ensure an adequate sample size after preweaning mortality. The 49 pigs were from seven sows in one weekly farrowing group. A 50% herd prevalence of *S. suis* was assumed among the population. Thus, the organism should have been detected in at least one pig with 95% confidence with a sample size of five pigs (Episcope, K. Frankena and J.O. Goelema, Wageningen, the Netherlands) for the sample population of 35 pigs.

The farrowing room was pressure washed and disinfected 3 days before the start of the project. Samples of the air, piglet water, and a swab of the surface of the piglet floor mat were culturally examined to determine whether the room was free of *S. suis* before the sows entered. All sows were washed with Biosentry S-3™ before being placed in the farrowing room. Farrowing was induced with 263 mcg cloprostenol sodium injected intramuscularly (Estrumate®, Miles, Shawnee, Kansas), about 24 hours before the start of the project. Two pigs from one sow were manually delivered due to uterine inertia. Crate design prohibited physical contact between pigs from litters in adjacent crates.

Five randomly selected pigs from the study population of 49 pigs were removed from their dams on each of days 0 through 6 and euthanized. If a pig allocated to be removed from its dam on a given day had already died, one of the extra 14 pigs in the study population was used instead. All pigs, except for those collected at birth, remained in the farrowing crates with their dams and were allowed to nurse until their designated day of removal. No cross-fostering was performed in the sample litters.

Piglet sample collection

All samples were collected at the farm of origin. The allocated pig was euthanized by intracardial injection of 1 mL of pentobarbital sodium (6 grains per mL) per 4.5 kg (10 lb) bodyweight. The tonsil of the pig was removed using aseptic technique. Next, the calvarium was bisected with a knife, and manually forced open. The meninges were exposed and swabbed (S/P® Brand culturette system; Baxter Diagnostics Inc., Deerfield, Illinois). A complete necropsy examination was performed, and all gross lesions were recorded.

Dam and environmental sample collection

Multiple microbiological samples were taken at the time the allo-

cated pig was removed from the dam. Sources chosen to be sampled were those that the pig would have come in contact with during the first week of life.

Sources from the dam of the allocated piglet (all taken by sterile swab) included:

- placenta,
- vaginal fluids,
- milk,
- teat skin,
- feces,
- saliva, and
- nasal secretions.

Sources from the environment of the allocated piglet included:

- air (sampled by placing an opened sheep blood agar plate on the floor of the pig's crate for 30 seconds),
- swab of the piglet nipple waterer,
- swab of the surface of the pig mat under the heat lamp.

All swab samples were taken using sterile swabs (S/P® Brand culturette system; Baxter Diagnostics Inc., Deerfield, Illinois). If it happened that two piglets from the same litter were allocated to be removed from their dam on the same day, two sets of dam-source and environmental swabs were taken, one for each piglet.

Cultural examination

All samples were kept at 4°C and plated on to sheep blood agar within 48 hours of collection. Tonsils were macerated in 10 mL of trypticase soy broth. One tenth of one mL of the solution was then plated onto sheep blood agar. After 24 hours of aerobic incubation at 37°C, three 1- to 2-mm flat alpha-hemolytic colonies were selected and subcultured onto sheep blood agar. These isolates were then Gram stained and biochemically tested. Gram-positive catalase-negative diplobacilli were considered to be *S. suis* suspects. Each suspect isolate was inoculated into a tube containing a 6.5% NaCl solution. *Enterococcus* spp. were suspected if there was bacterial growth in the salt solution after 48 hours of incubation. The isolate was serotyped with antiserum to *S. suis* types 1-8 if there was no growth in the salt solution after 48 hours of incubation at 37°C. The culture was considered to be *S. suis* if it satisfied all of the above-mentioned criteria and agglutinated the antiserum. No conclusions were drawn from isolates that satisfied all requirements but did not agglutinate the antisera to *S. suis* 1-8, even though these isolates could potentially be *S. suis* of other serotypes. Antimicrobial susceptibility tests were performed on all *S. suis* isolates type 1-8 (Sensititre LTD®, West Sussex, England.)

Three additional suspect colonies were chosen randomly from nine of the sheep blood agar plates inoculated with the solution of macerated tonsil in trypticase soy broth to further determine whether the serotypes of the three chosen suspect colonies were representative of the serotypes of the unselected suspect colonies on that sheep blood agar plate.

Results

Necropsy revealed no gross abnormalities with the exception of one pig with unilateral hydronephrosis. The nine cases of cultural examination of tonsil where three additional suspect colonies were evaluated were all culturally negative for *S. suis* types 1–8. Samples from the air, water, and piglet mat before the sows were placed in the farrowing house were all culturally negative for *S. suis* types 1–8.

Seven hundred and four isolates of 1119 suspect colonies isolated during days 0–6 were Gram-positive diplobacilli that did not grow in 9.5% NaCl. Forty-five of these 704 isolates, some from sow sources and some from piglet tonsils, agglutinated *S. suis* antisera types 2–8 (Figure 1). The most frequently isolated serotype of *S. suis* was serotype 3 (33.3%), followed by 5, 7, 4, 6, 8, and 2.

Streptococcus suis serotypes 2–8 were isolated both from piglet tonsils and from most sow-source swabs, including:

- saliva,
- teat skin,
- nasal secretions,
- milk,
- feces, and
- vaginal fluids (Table 1).

Streptococcus suis serotypes 1–8 were not isolated from placental swabs. Environment-source *S. suis* was isolated only once, when *S. suis* serotype 5 was isolated from the piglet mat. *Streptococcus suis* types 2, 3, 5, 6, 7, and 8 were isolated from the tonsil of pigs as early as 24 hours after birth (Table 1). In four of seven dams, *S. suis* of the same serotype isolated from samples collected from the dam was detected in samples of the tonsil of that dam's pig. Meningeal swabs were found to be contaminated upon cultural examination.

Figure 1

Serotype	Number of isolates (% of total)
2	1 (2.2%)
3	15 (33.3%)
4	5 (11.1%)
5	10 (22.2%)
6	3 (6.7%)
7	8 (17.8%)
8	3 (6.7%)

Distribution of the 45 *S. suis* serotypes 2–8 isolated.

Table 1

Serotype, source, and day of collection of *S. suis* that were culturally isolated. *S. suis* type 5 was also isolated once from from the piglet mat.

Sow ID	Dam Serotype (day collected, source)	Pigs of dam serotype (day of collection; all from tonsil)
A	4 (day 3; saliva) 5 (day 0; teat skin)	
B	6 (day 2; saliva) 7 (day 2; nasal secretions)	6 (day 2)
C	4 (day 4; saliva) 8 (day 4; saliva)	2 (day 5)
D	3 (days 2, 5; teat skin, milk) 4 (day 2; saliva, nasal secretions) 7 (day 3; milk)	3 (day 1)
E	3 (days 1,5; nasal secretions, feces) 5 (days 1,5; vaginal fluids) 7 (day 4; vaginal fluids)	8 (day 6)
F	3 (days 0,2,3; nasal secretions) 4 (day 1; saliva) 5 (day 4; vaginal fluids) 6 (day 1; teat skin)	5 (day 1) 3 (day 2)
G	7 (day 2; vaginal fluids, feces)	7 (day 2)

Table 2 Percentage of *S. suis* isolates that were susceptible to commonly used antibiotics. (Based on the criteria of the National Committee for Clinical Laboratory Standards.)

Antibiotic	Serotype (number of isolates)							All(44)
	2(1)	3(15)	4(5)	5(10)	6(3)	7(7)	8(3)	
Ampicillin	100	87	100	90	67	100	100	91
Apramycin	0	53	60	40	0	57	33	45
Ceftiofur	0	87	100	80	67	100	67	84
Enrofloxacin	100	60	80	80	33	100	100	75
Gentamicin	100	60	100	60	67	71	67	68
Lincomycin	0	40	20	10	33	14	33	25
Penicillin	0	47	0	20	0	43	33	30
Spectinomycin	100	47	80	20	0	57	33	43
Tetracycline	0	0	0	20	0	14	0	7
Tiamulin	100	73	80	40	0	86	67	64
TMS (trimethoprim-sulfa)	100	87	80	90	67	100	100	86
Tylosin	100	47	60	60	67	29	67	52

Antimicrobial susceptibility was performed on 44 of the 45 isolates. The percentage of isolates susceptible to antibiotics varied among serotypes (Table 2). Ninety-one percent of *S. suis* isolates were susceptible to ampicillin. Less than 50% of the isolates were susceptible to apramycin, lincomycin, penicillin, spectinomycin, tetracycline, and tylosin.

Discussion

The relative frequency of each serotype included in our sample differed from that of recent surveys in the United States.^{6,7} The low number of isolates in our sample must be noted as a contributing factor. *Streptococcus suis* serotype 2 was the serotype least frequently isolated in our study, whereas in recent surveys this serotype was the most commonly isolated. These surveys found our most frequent isolate, *S. suis* serotype 3, to be the second-most-common isolate. It is interesting to note the reported increase in prevalence of serotype 7, which was previously considered rare in the United States, but is the most frequently isolated serotype in Scandinavian countries.¹ It is possible that breeding stock being imported into the United States from Scandinavia are carriers of the organism.

Our findings are consistent with those of others who used early-weaning protocols and were unable to eliminate *S. suis* if pigs were weaned at 7 days of age. Our results also support the findings of Robertson and Blackmore,⁸ who isolated *S. suis* from the nasal swabs of pigs derived from sows whose vaginas were colonized by *S. suis*. We reached different conclusions than Mogollon, et al.,⁹ who studied outbreaks of *S. suis* meningitis in two swine herds. They were unable to demonstrate vertical transmission, and concluded that horizontal transmission occurred. Although Mogollon, et al.,⁹ did not indicate the number of colonies chosen per sample, by extrapolation from available data it appears that only one colony was used. On the basis of this limited sampling, it is quite possible that vertical transmission was not detected.

We concluded that *S. suis* colonized the tonsil as early as 24 hours after birth, but it is possible that colonization occurred before the piglets were born (i.e., in utero or during birth.) Because we chose only three suspects from each sample we collected, our ability to detect *S. suis* was limited, and our results may not be conclusive. The suspect isolates were too numerous to count in most cases. It is possible that *S. suis* was present in the tonsil on day 0, but that suspect colony was not included in our sample. It is also possible that *S. suis* was present in the placental samples for similar reasons. Additionally, it is possible that the inability to detect identical serotypes in some sow-pig combinations was also related to sample size. We were unable to determine whether the serotypes of the three chosen suspect colonies were representative of the serotypes of the unselected suspect colonies on the sheep blood agar plate because we could not confirm additional isolates of *S. suis* types 1–8 in the nine samples where three additional suspect colonies were culturally examined.

No conclusions were drawn from the cultural examination of the meningeal swabs because they were exposed to contaminated

room air for an extended period of time while the culturette was being prepared for use. We confirmed that this sampling procedure allowed the opportunity for contamination via cultural examination. Unlike the sampling technique for meningeal swabs, the technique used for removing the tonsils was immediate and aseptic.

The possibility exists that both the sows and pigs were infected with *S. suis* from the environment. We believe this to be unlikely, because the rooms were effectively cleaned and disinfected, as confirmed by cultural examination, before sows were moved into the farrowing rooms. Supportive evidence of the effectiveness of disinfectants in inhibiting the growth of *S. suis* has been reported by others.¹⁰

Our results provide strong evidence that the sow continually sheds multiple serotypes of *S. suis*. Although we isolated *S. suis* from milk samples, the extent to which the milk was contaminated by organisms on the teat skin is unknown. Future studies using genomic fingerprinting may be useful for identifying clonal relationships among organisms shed by the sow and those isolated from her progeny.⁹ We concluded that secretions and excretions from the dam were the primary source of *S. suis* that colonized the tonsils of neonatal pigs. Thus, SEW procedures without the use of antibiotics would not be expected to be effective in eliminating this pathogen from early-weaned pigs.

The results of our antimicrobial susceptibility tests were similar to the findings of Cantin, et al.,¹¹ who reported that antimicrobial susceptibility varied among different serotypes of *S. suis*. We were unable to determine the antimicrobial susceptibility pattern of one *S. suis* serotype-7 isolate after repeated attempts, because it would not grow in the Sensititre® sensitivity testing system. We support Cantin's suggestion,¹¹ and encourage using antimicrobial susceptibility testing on herd isolates of *S. suis* if medication is to be used strategically to decrease or eliminate the *S. suis* carrier state from a herd in early-weaned pigs, or to treat pigs demonstrating clinical signs of *S. suis* infection.

Implications

- We conclude that the sow sheds multiple serotypes of *S. suis* in bodily excretions and secretions, including saliva, nasal secretions, feces, vaginal fluids, and possibly milk.
- These sources most likely contaminate the skin of the dam's underline and the environment.
- The tonsils of the pig can become colonized with *S. suis* shortly after birth, when the pig contacts these contaminated surfaces, secretions, and excretions.
- It is possible, but unproven, that the pig becomes infected *in utero* or during birth.
- If infection occurs in utero, neither Cesarean section-derived pigs nor depopulation and subsequent repopulation would be effective in eradicating *S. suis* from swine herds.

- Early weaning and segregation protocols will not prevent *S. suis* from colonizing the tonsil of the pig.
- Developing MEW protocols will be difficult because susceptibility to antimicrobials varies among the different serotypes of *S. suis*.
- We encourage antimicrobial susceptibility testing on multiple herd isolates of *S. suis* from clinically affected pigs to develop a protocol for treating *S. suis* infections in pigs from that herd.

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