Experimental colonization of piglets and gilts with systemic strains of *Haemophilus parasuis* and *Streptococcus suis* to prevent disease

Simone Oliveira, Laura Batista, Montserrat Torremorell, Carlos Pijoan

Abstract

Haemophilus parasuis and *Streptococcus suis* are both major causes of losses during the nursery period, especially in herds using the segregated early weaning system. In this system, only a few piglets may be colonized with the herd's prevalent systemic strain, which results in infection of naïve penmates late in the nursery. In view of these factors, the objectives of this study were: (1) to evaluate the early colonization of piglets with the farm's prevalent systemic strain of *H. parasuis* and *S. suis* as an alternative method for disease prevention; and (2) to evaluate 2 different protocols for experimental colonization: direct colonization of piglets and colonization of piglets through nose-to-nose contact with inoculated sows. *Haemophilus parasuis* and *S. suis* isolates recovered from diseased nursery pigs were characterized by the rep-PCR technique and the herd's prevalent strains were used for colonization. Piglets in the experimentally colonized groups were inoculated at 5 days of age by the oral route using a spray pump. Sows were colonized at 2 weeks prior to farrowing using a similar protocol. Although both colonization protocols were successful in getting the piglets colonized, direct inoculation of 5-day-old piglets with the herd's systemic strains of *H. parasuis* and *S. suis* tended to be more effective in reducing the morbidity and the mortality than the colonization of piglets by nose-to-nose contact with inoculated sows.

Résumé

Haemophilus parasuis *et* Streptococcus suis *sont deux causes importantes de perte durant la période en pouponnière, plus spécialement dans les élevages où le sevrage hâtif est pratiqué. Dans ce système, seulement quelques porcelets peuvent être colonisés par la souche qui prévaut dans l'élevage, ce qui entraînera une infection des porcelets naïfs lors de la mise en pouponnière. Les objectifs de l'étude étaient : 1) évaluer chez des porcelets une colonisation hâtive par les souches d'*H. parasuis *et de* S. suis *qui prévalent dans l'élevage comme une méthode alternative de prévention de la maladie; 2) évaluer deux protocoles différents de colonisation expérimentale : une colonisation directe des porcelets et une colonisation des porcelets via un contact nez-à-nez avec des truies inoculées. Les isolats d'*H. parasuis *et de* S. suis *provenant des porcelets en pouponnières malades furent caractérisés par la technique de rep-PCR et les souches prévalentes dans l'élevage utilisées pour la colonisation. Les porcelets des groupes colonisés de manière expérimentale furent inoculés à 5 jours d'âge par voie orale à l'aide d'une pompe à vaporiser. Les truies furent colonisées 2 semaines précédant la mise-bas en utilisant un protocole similaire. Bien que les deux protocoles de colonisation permirent d'obtenir des porcelets colonisés, une inoculation directe d'un porcelet de 6 j à l'aide des souches d'*H. parasuis *et de* S. suis *prévalentes dans l'élevage semblait plus efficace à réduire la morbidité et la mortalité que la colonisation des porcelets par le contact nez-à-nez avec des truies contaminées.*

(Traduit par docteur Serge Messier)

Introduction

Haemophilus parasuis and *Streptococcus suis* are both early colonizers of the upper respiratory tract of healthy pigs (1,2). They are also 2 of the most common causes of death in herds where the segregated early weaning (SEW) method is used (3). Control of *H. parasuis* and *S. suis* infections has been reported as difficult and not always successful, especially when commercial vaccines are used (4,5). Conversely, the use of autogenous vaccines has proven to be more effective in controlling disease, particularly when the herd's prevalent systemic strains are used (6).

Epidemiological studies have shown that strains of *H. parasuis* and *S. suis* present in the upper respiratory tract tend to be highly heterogeneous, representing various serotypes and genotypes (7,8). Conversely, pathogenic strains recovered from systemic sites of diseased pigs tend to be highly homogeneous, and generally only a few strains are simultaneously responsible for disease in a herd (9). The herd's prevalent systemic strains of *H. parasuis* and *S. suis* are rarely found colonizing the upper respiratory tract of sows and gilts, especially in specific pathogen-free herds (3,10,8). Consequently, only a small number of piglets reach the nursery colonized with the herd's systemic strains. When piglets are mixed in the nursery,

Department of Clinical and Population Sciences, College of Veterinary Medicine, University of Minnesota, Animal Science Building, Room 385, 1988 Fitch Avenue, St. Paul, Minnesota 55108 USA (Oliveira, Batista, Pijoan); Pig Improvement Company, Franklin, Kentucky 42134 USA (Torremorell).

Address correspondence and reprint requests to Dr. Carlos Pijoan, telephone: 612-624-3237, fax: 612-625-1210.

Received March 21, 2001. Accepted May 28, 2001.

the carrier piglets can act as sources of infection for non-colonized animals. Disease caused by *H. parasuis* and *S. suis* is commonly observed between the 4th and the 6th weeks in the nursery, presumably when maternal immunity starts to decrease (11).

Recently, an alternative method has been proposed to prevent *S. suis* infections during the nursery and finishing periods: the early colonization of piglets with the herd's prevalent systemic strain of *S. suis* while they are protected by maternal immunity. Exposure of 5-day-old piglets with the herd's prevalent strain of *S. suis* significantly reduced the number of animals showing clinical signs and the number of antibiotic treatments due to *S. suis* infections (12). However, in some farms, both *H. parasuis* and *S. suis* are involved in most of the nursery mortality (3). No studies have been conducted to evaluate the effect of early colonization by the herd's prevalent systemic strain of *H. parasuis* as means to prevent disease later in the nursery.

The objectives of this study were 1) to evaluate the early colonization of young pigs with systemic strains of *H. parasuis* and *S. suis* as a method for disease prevention; and 2) to compare 2 methods of colonization: direct colonization of piglets through tonsillar inoculation and natural colonization of piglets through nose-to-nose contact with inoculated sows.

Materials and methods

Farms

Pigs used in this experiment were obtained from 2 sow farms with 2400 (A) and 1800 (B) Landrace x Yorkshire sows, respectively. These farms were selected based on a previous history of clinical disease caused by *H. parasuis* and *S. suis* in pigs in the late nursery and early finishing stages of production. Pigs were weaned at 15 d of age and moved to 2 off-site nurseries, where they remained for 8 wk. This experiment was approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Assurance of Compliance # A3456-01).

Isolation and characterization of *H. parasuis* and *S. suis* systemic strains

Pigs weaned from the 2 sow farms were sent to 19 different off-site nurseries. Thirty diseased pigs were selected from 10 of these nurseries for isolation of *H. parasuis* and *S. suis* systemic strains. The selected pigs were in the nursery for 4 to 6 wk and were showing clinical sings characteristic of *H. parasuis* and/or *S. suis* infection, such as lameness, swollen joints, and central nervous system (CNS) signs. Pigs were necropsied and samples from non-respiratory organs with obvious lesions of polyserositis were collected for bacterial isolation. Collected samples were inoculated onto blood agar (BA) and incubated at 37°C for 18 h. A *Staphylococcus aureus* streak was placed on every BA plate to allow *H. parasuis* growth and isolation (13). Colonies resembling *S. suis* and *H. parasuis* were subcultured in BA and chocolate agar, respectively. *Streptococcus suis* colonies were further identified using a strip test (API 20; bioMerieux, Marcy l'Étoile, France). *Haemophilus parasuis* identification was confirmed by polymerase chain reaction (PCR) (14). *Haemophilus parasuis* and *S. suis* obtained from systemic body sites were genotyped and compared using the repetitive element-based PCR (Rep-PCR) technique (15). Briefly, primers targeting the enterobacterial repetitive intergenic consensus (ERIC) were used. Rep-PCR was performed in a 50 μL reaction mixture containing 100 ng of template DNA, 1.5 μM of each primer, 50 mM of KCl, 10 mM of Tris-HCL, 3 mM of $MgCl_2$, 0.24 mM of each deoxynucleoside triphosphate, and 2 U of *Taq* DNA polymerase (Roche Diagnostics, Indianapolis, Indiana, USA). The PCR was carried out for 30 cycles consisting of denaturation for 30 s at 94°C, annealing for 1.5 min at 40°C and extension for 2 min at 72°C, using a thermal cycler (GeneAmp model 2400; Perkin-Elmer Applied Biosystems, Foster City, California, USA). Products from PCR were separated by electrophoresis in 2% agarose gel for 3.5 h at 70 V. Gels were stained with an ethidium bromide solution (Continental Lab Products, San Diego, California, USA) and photographed (Eagle Eye; Stratagene, La Jolla, California, USA).

Table I. Experimental design for colonization of sows and piglets using systemic strains of *Haemophilus parasuis* and *Streptococcus suis*

Group #	Number of animals	Experimental colonization[a]
1	50	Sow colonization with *H. parasuis*
2	50	Sow colonization with *S. suis*
3	50	Sow colonization with *H. parasuis* + *S. suis*
4	50	Piglet colonization with *H. parasuis*
5	50	Piglet colonization with *S. suis*
6	50	Piglet colonization with *H. parasuis* + *S. suis*
7	50	Control group (no colonization)
Total	350	

[a] Similar protocol was used in farms A and B

Table II. *Haemophilus parasuis* and *Streptococcus suis* isolates recovered from diseased pigs

Isolation	Animal #	Site of isolation	Genotype
S. suis	1	Lung	A[a]
	2	Pericardial fluid	A[a,b]
	2	Pleura	A
	2	Pericardium	A
	3	Abdominal fluid	A
	3	Abdominal fluid	A
	3	Abdominal fluid	A
	3	Abdominal fluid	A[a]
	3	Abdominal fluid	A
	6	Abdominal fluid	A[a]
	7	Lung	A
	7	Abdominal fluid	A
H. parasuis	10	Lung	B
	11	Lung	B
	12	Lung	B[a,b]
	12	Lung	B[a]

[a] Clonally related; slight differences in fingerprint patterns
[b] Strains selected for colonization

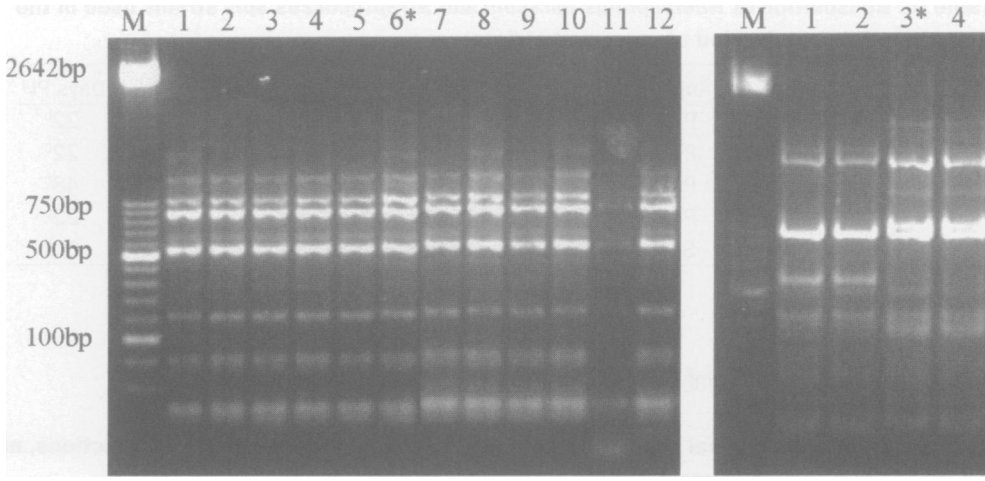

Figure 1. Genomic fingerprints of *Streptococcus suis* (A) and *Haemophilus parasuis* (B) isolates recovered from diseased pigs. (*) Strains selected for colonization. (M) Base pair marker.

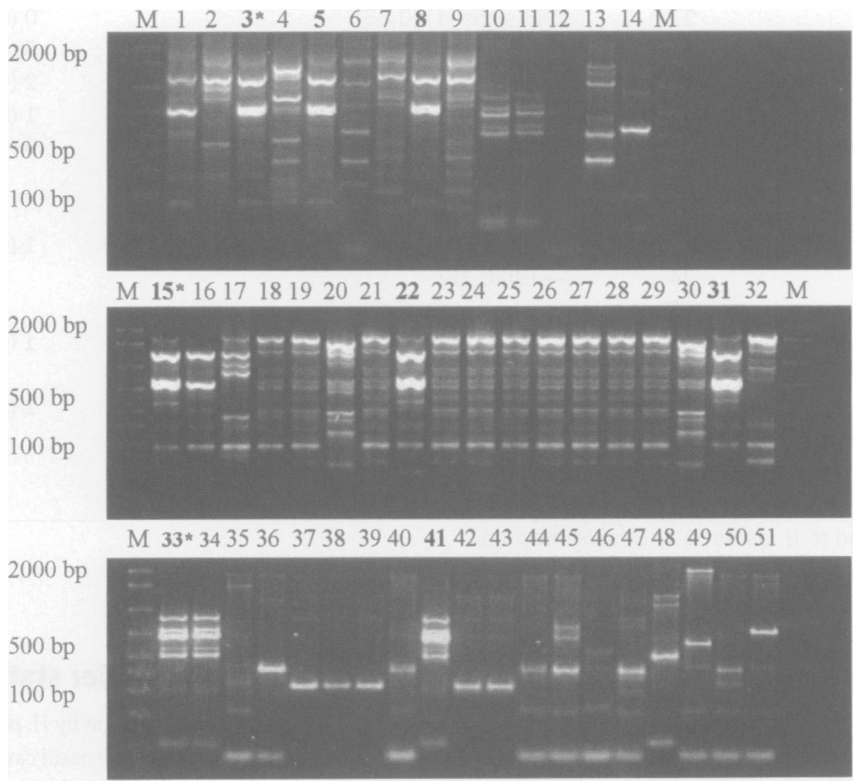

Figure 2. Comparison of the genomic fingerprints from *Haemophilus parasuis* and *Streptococcus suis* systemic strains used for colonization (*) and strains isolated from nasal and tonsillar swabs. Lanes 1 to 32 *H. parasuis* isolates. Lanes 33 to 51 *S. suis* isolates. Four *H. parasuis* isolates (lanes 5, 8, 22 and 31) and 1 *S. suis* isolate (lane 41) were genetically similar to the strains used for colonization. Lanes 1, 16 and 34 are positive controls with known fingerprints by rep-PCR. M — Base pair marker.

Experimental colonization

Based on the genotyping results, the herd's prevalent strains of *H. parasuis* and *S. suis* were selected to be used in the experimental colonization of piglets and sows. Selected strains were grown separately on PPLO medium for 18 h at 37°C. Bacterial cultures were frozen in 10-mL aliquots at −80°C. One tube from each culture was thawed and a standard bacterial count was performed. Original cultures were diluted to produce an inoculum containing 1×10^4 cfu/mL. Delivery of the inoculum was performed using sterile spray pumps. Each animal received one spray of the inoculum by the oral route, which corresponded to a 0.7-mL dose or 7×10^3 cfu/pig. Piglets and sows that were colonized with both *S. suis* and *H. parasuis* received one spray of each inoculum. Antibiotic treatments were avoided for 1 wk following inoculation.

Three hundred fifty piglets from each sow farm were divided into 7 experimental groups, according to the colonization scheme adopted (Table I). The same protocol was applied for farms A and B, with a total of 700 animals involved in the experiment. Two

Table III. Re-isolation of *Haemophilus parasuis* and *Streptococcus suis* strains used in the experimental colonization of sows and piglets

Isolation	Swab	Animal	Group	Days PI[a]
H. parasuis	Tonsil	Pig	Sow colonization with *H. parasuis* + *S. suis*	22[b]
	Tonsil	Pig	Sow colonization with *H. parasuis*	22[b]
	Tonsil	Pig	Piglet colonization with *H. parasuis*	48[b]
	Tonsil	Pig	Piglet colonization with *S. suis*	22[c]
S. suis	Nasal	Sow	Piglet colonization with *H. parasuis*	0[b,d]

[a] Post-infection
[b] Farm A
[c] Farm B
[d] Two weeks prior to farrowing, before inoculation with *H. parasuis*

Table IV. Number of animals presenting clinical signs related to *H. parasuis* (*Hps*) and/or *S. suis* infections, number of antibiotic treatments and mortality of piglets from farms A and B

Farm	Group identification	*n*	Clinical signs	Antibiotic treatments[a]	Death
A	*Hps* — Sow	37	Swollen joint (1)[b] (0.84)[c]	2 (0.40)[c]	1 (0.84)[c]
	S. suis — Sow	27	CNS signs (1) (0.66)	0 (0.45)	1 (0.66)
	Hps + *S. suis* — Sow	31	Swollen joint (3) (0.13)	5 (0.02)	0 (0.42)
	Hps — Piglet	42	Swollen joint (2) (0.47)	2 (0.47)	0 (0.35)
	S. suis — Piglet	42	Swollen joint (1) (0.91)	1 (0.91)	0 (0.35)
	Hps + *S. suis* — Piglet	39	— (0) (0.37)	0 (0.37)	1 (0.87)
	Negative control	49	Swollen joint (1)	1	1
	Total	267	9	11	4
B	*Hps* — Sow	38	Reddish extremities, prostration (1) (0.32)	1 (0.31)	2 (0.66)
	S. suis — Sow	24	— (0) (0.16)	0	0 (0.16)
	Hps + *S. suis* — Sow	43	Prostration (3) (0.90)	0	3 (0.90)
	Hps — Piglet	37	— (0) (0,08)	1 (0.34)	1 (0.33)
	S. suis — Piglet	37	Swollen joint (1) (0.69)		
			Prostration (1)	1 (0.66)	2 (0.69)
	Hps + *S. suis* — Piglet	44	— (0) (0.06)	0	0 (0.06)
	Negative control	39	Prostration (3)	0	3
	Total	262	9	3	11

[a] Due to clinical signs related to *H. parasuis* or *S. suis* infection
[b] Number of pigs affected
[c] *P* values for the difference between experimental groups and the control group

colonization protocols were tested: sow colonization and piglet colonization. On each farm, the sow colonization protocol consisted of inoculating 15 sows with *H. parasuis* (5 sows), *S. suis* (5 sows), or both *H. parasuis* and *S. suis* (5 sows) 2 wk prior to farrowing. Piglets that were born from these sows were ear-tagged with 1 of 3 different colored ear tags and maintained with their original sow for 15 d. Piglets from colonized sows were expected to be naturally colonized by nose-to-nose contact with the sow. The second protocol consisted in the direct colonization of 5-day-old piglets. One hundred fifty piglets were divided into 3 groups and colonized with *H. parasuis* (50 animals), *S. suis* (50 animals), or both *H. parasuis* and *S. suis* (50 animals). Piglets in each group were assigned 1 of 3 different ear tag colors. Gloves were used every time pigs were manipulated and were changed between different experimental groups. All groups were maintained separated by solid partitions in both the sow farms and the nurseries. Cross-fostering was allowed only between groups with the same ear tag color.

Evaluation of the carrier state

To evaluate if the colonization by *H. parasuis* and *S. suis* strains had been achieved, swabs from the nasal cavity (sows) and tonsillar area (piglets) were taken immediately before inoculation and on days 6, 14, 22, 36, and 48 post inoculation. Ten animals were sampled in each group, totalling 70 swabs per visit. Swabs were transported under refrigeration, streaked onto BA plates and incubated at 37°C for 18 h. Isolated *H. parasuis* and *S. suis* colonies were genotyped (15) and compared with the *H. parasuis* and *S. suis* strains used in the colonization procedures.

Evaluation of the early colonization procedure for disease prevention

The endpoints used for evaluation were: morbidity, number of antibiotic treatments and mortality. The morbidity was evaluated as the number of pigs that showed clinical signs, including lameness, swollen joints, CNS signs, and/or recumbency. Antibiotic treatments

Table V. Morbidity, antibiotic treatments and mortality in piglets directly inoculated or colonized by inoculated sows from farms A and B

Farm	Colonization method	*n*	Clinical signs	Antibiotic treatments	Deaths
A + B	Sow	200	9 (4.5%) (0.97)[a]	8 (4%) (0.20)	7 (3.5%) (0.67)
	Piglet	241	5 (2.07%) (0.22)	5 (2.07%) (0.57)	4 (1.66%) (0.13)
	Control	88	4 (4.54%)	1 (1.14%)	4 (4.54%)

[a] *P* values

were administered according to the farms' protocols. The farm's owners were instructed to notify all deaths of animals involved in the experiment. Most of these animals were subsequently necropsied and isolation of *H. parasuis* and *S. suis* was then attempted.

Statistical analysis

Percent morbidity and mortality, as well as number of antibiotic treatments were compared between individual experimental groups and the control group using multiple chi-squared tests. The pig was considered to be the experimental unit since the animals were individually identified and, therefore, they could be individually monitored. Results were considered to be statistically significant at $P < 0.05$.

Results

Isolation and identification of *H. parasuis* and *S. suis* systemic strains

Twelve *S. suis* isolates were recovered from 6 animals and 4 *H. parasuis* isolates were recovered from 3 animals (Table II). Evaluation of rep-PCR profiles showed that all *S. suis* isolates had very similar genomic fingerprints (Figure 1). Therefore, it was concluded that a single predominant *S. suis* pathogenic strain was responsible for the mortality present on the farm. *Haemophilus parasuis* isolates were also very similar among themselves; however, 2 distinct genotypes could be identified. Based on the rep-PCR results, an *S. suis* isolate recovered from a pig with severe purulent pericarditis and an *H. parasuis* isolate recovered from the pleura of a pig with severe fibrinous polyserositis were selected to be used as the inoculum.

Experimental colonization and evaluation of the carrier state

Results from the *H. parasuis* and *S. suis* isolation, genotyping and comparison with the systemic strains used for colonization are summarized in Table III. Contamination of samples by the bacterial flora of the upper respiratory tract made isolation of both *S. suis* and *H. parasuis* difficult. Several BA plates were overgrown by motile bacteria (*Proteus* sp.), which compromised the recovery of isolated colonies. A total of 29 *H. parasuis* (15 from herd A and 14 from herd B) and 53 *S. suis* isolates (26 from herd A and 27 from herd B) were recovered from nasal and tonsillar swabs. Four *H. parasuis* isolates recovered from tonsillar swabs and one *S. suis* isolate recovered from a nasal swab had a genotype identical to the systemic strains used for inoculation. The remaining isolates had highly variable genomic patterns and were not similar to the inoculum strains (Figure 2).

Evaluation of the early colonization procedure for disease prevention

Not all pigs that were ear-tagged on the sow farms reached the nursery. Table 4 shows the changes in the number of pigs in each experimental group per farm for the 8-week period in the nursery. The relatively high pre-weaning mortality (23.7% in herd A and 25% in herd B) was partially related to the restrictions regarding cross-fostering and antibiotic treatments during the first week of life. Observed clinical signs resembling *H. parasuis* and/or *S. suis* infections included swollen joints, recumbency with reddish extremities (septicemia), prostration/non-responsiveness, and paddling (CNS signs). Antibiotic treatments were administered mostly due to swollen joints, especially in farm A. A total of 15 pigs died during the experiment, 4 from farm A and 11 from farm B. Differences between experimental groups and the control group were not statistically significant in both farms, except in the number of antibiotic treatments used in animals naturally colonized by inoculated sows in farm A ($P = 0.02$) (Table IV). However, the comparison between the 2 colonization methods showed that piglets directly inoculated with the herd's systemic strains of *H. parasuis* and *S. suis* tended to have less morbidity and mortality than piglets colonized by nose-to-nose contact with inoculated sows (Table V). It was not possible to necropsy all pigs that died during the experiment. A total of 6 pigs were necropsied, including 4 from farm A and 2 from farm B. One of the necropsied pigs from farm A (control group) was a low viability pig with purulent lesions on the face and abscesses in the lung. The 2nd pig (sow inoculation with *H. parasuis* group) died after antibiotic treatment and application of an elastic band to correct a rectal prolapse. This pig had a bladder rupture and fibrinous peritonitis. The 3rd pig from farm A (sow inoculation with *S. suis* group) presented with CNS signs and was euthanized. No lesions were found in this pig. The 4th pig from farm A (piglet inoculation with *H. parasuis* + *S. suis*) was also a low viability pig and no lesions were found at necropsy. One of the pigs necropsied from farm B (piglet inoculation with *H. parasuis*) had a hepatic rupture and a consequent hemoperitoneum. The 2nd pig necropsied from farm B (sow inoculation with *H. parasuis* + *S. suis*) was a runt pig with no evident lesions. Neither *H. parasuis* nor *S. suis* was isolated from these pigs. Results of morbidity, antibiotic treatments and mortality in each group are summarized in Table IV.

Discussion

The use of the segregated early weaning technique interferes with one of the most important features related to the natural control of *H. parasuis* and *S. suis* infections: the colonization of piglets with the herd's potentially pathogenic strains while they still are protected by maternal immunity. Previous studies (12) have shown that the direct colonization of 5-day-old piglets with the herd's prevalent systemic strain of *S. suis* significantly reduced the morbidity and the number of antibiotic treatments in the experimentally exposed group. *Haemophilus parasuis* and *S. suis* are both early colonizers of the upper respiratory tract of swine (3), and, in several herds both organisms are involved in clinical disease during the late nursery period. The present study evaluated the early colonization of piglets with systemic strains of *H. parasuis* and *S. suis* as an alternative method to control disease in herds historically affected by both agents. Considering that the individual colonization of piglets is time consuming and laborious, we decided to compare 2 protocols for piglet colonization: direct colonization through tonsillar inoculation and natural colonization through nose-to-nose contact with inoculated sows. In addition, instead of colonizing piglets and sows using tonsillar swabs, we used a spray pump that allowed the inoculation of a higher amount of inoculum in a lower and safer dosage: 7×10^3 bacteria/pig.

In order to perform the colonization of young piglets with the herd's pathogenic strains of *H. parasuis* and *S. suis*, we selected isolates recovered from systemic sites of diseased pigs to be used as inoculum. *Haemophilus parasuis* and *S. suis* isolates obtained from sick nursery pigs were highly homogeneous among themselves and were also genetically different from isolates recovered from the nasal cavity and tonsillar area of healthy sows and piglets from the same farm. These findings are in agreement with previous studies, which have shown that systemic strains of *H. parasuis* (8) and *S. suis* (10,12) are generally different from those found in the upper respiratory tract of swine. Evaluation of the rep-PCR profiles of isolates recovered from diseased pigs also revealed that only one *S. suis* and 2 closely related *H. parasuis* strains were causing disease in the herd. These results also support previous findings, where it was shown that only a few *H. parasuis* (9) and *S. suis* (12) strains were causing clinical disease in the studied herds.

Recovery of the *H. parasuis* strain used for colonization from inoculated piglets, as well as from those exposed to inoculated sows, demonstrated that both protocols were successful in getting piglets colonized. The *S. suis* strain used for colonization was not recovered from experimentally colonized piglets. Torremorell et al (12) also could not re-isolate the *S. suis* strains used in the colonization of baby pigs. The isolation of specific organisms from nasal or tonsillar swabs is generally impaired by overgrowth of other bacteria from the normal flora of the upper respiratory tract (16). The *H. parasuis* strain used for colonization was isolated from a piglet directly colonized with the herd's systemic strain of *S. suis* and the *S. suis* strain used in the inoculum was isolated from a sow before the experimental colonization. Based on these results, we assume that the farm's pathogenic strains of *H. parasuis* and *S. suis* can be present in the sow farm, but that only a small number of animals are colonized with these strains.

Torremorell et al (12) found a significant reduction of morbidity after colonizing 5-day-old piglets with a systemic strain of *S. suis*. In our experiment, we could not find significant differences between the experimental and control groups. However, comparison of the results obtained for morbidity and mortality between piglets directly inoculated or colonized by inoculated sows revealed that the piglet inoculation was more effective than the sow inoculation in preventing disease in the nursery. The number of animals showing clinical signs and the number of deaths in the group of directly inoculated piglets was half that seen in piglets colonized by inoculated sows (Table V). Results from this experiment also confirmed the inherent safety of the early colonization of piglets with the herd's pathogenic strains of *H. parasuis* and *S. suis*. Similar to previous studies (12), we did not find any clinical sign or mortality that could be directly attributed to the inoculation, even though the strains of *H. parasuis* and *S. suis* that were used were presumed to be fully virulent.

In our experiment, the level of infection of the control group was very low, especially on farm A. This partly explains why no statistical differences were found between control and treated groups. Farm A and B presented similar morbidity throughout the experiment. In both farms, a total of 9 animals showed clinical signs related to *H. parasuis* or *S. suis* infections. The major differences between the 2 farms were the number of antibiotic treatments and deaths. Most of the antibiotic treatments used in animals from farm A were due to swollen joints. This farm used a full-slatted cement floor in the nursery, which had large openings between the slats. This resulted in leg injury in several pigs.

In conclusion, early colonization of piglets with the herd's systemic strains of *H. parasuis* and *S. suis* can be used safely in an attempt to prevent and control infections by both organisms during the nursery period. Evaluation of colonization protocols (piglet colonization and sow colonization) demonstrated that direct inoculation of 5-day-old piglets with the herd's pathogenic strains of *H. parasuis* and *S. suis* tended to be more effective in preventing disease when compared with sow colonization, although these results were not statistically significant. Further studies are necessary in order to fully characterize the influence of piglet colonization in disease prevention.

Acknowledgment

We would like to thank Dr. Tim Klein for providing the farms where this experiment was developed and the National Pork Producers Association for funding this project.

References

1. Amass SF, Clark LK, Wu CC. Source and timing of *Streptococcus suis* infection in neonatal pigs: Implications for early weaning procedures. J Swine Health Prod 1995;3:189–193.
2. Smart NL, Miniats OP, MacInnes JI. Analysis of *Haemophilus parasuis* isolates from southern Ontario swine by restriction endonuclease fingerprinting. Can J Vet Res 1988;52:319–324.
3. Pijoan C, Torremorell M, Solano G. Colonization patterns by the bacterial flora of young pigs. Proc Am Assoc Swine Pract 1997: 463–464.

4. Rapp-Gabrielson VJ, Kocur GJ, Clark JT, Muir, SK. *Haemophilus parasuis*: immunity in swine after vaccination. Vet Med 1997;92: 83–90.
5. Torremorell M, Pijoan C, Trigo E. Vaccination against *Streptococcus suis*: effect on nursery mortality. J Swine Health Prod 1997;4:139–143.
6. Miniats OP, Smart NL, Ewert E. Vaccination of gnotobiotic primary specific pathogen-free pigs against *Haemophilus parasuis*. Can J Vet Res 1991;55:33–36.
7. Mogollon JD, Pijoan C, Murtaugh MP, Collins JE, Cleary PP. Identification of epidemic strains of *Streptococcus suis* by genomic fingerprinting. J Clin Microbiol 1991;29:782–787.
8. Smart NL, Hurnik D, MacInnes JI. An investigation of enzootic Glasser's disease in a specific-pathogen-free grower-finisher facility using restriction endonuclease analysis. Can Vet J 1993;34: 487–490.
9. Ruiz A, Oliveira S, Torremorell M, Pijoan C. Outer membrane proteins and DNA profiles in strains of *Haemophilus parasuis* recovered from systemic and respiratory sites. J Clin Microbiol 2001;39:1757–1762.
10. Torremorell M, Calsamiglia M, Pijoan C. Colonization of suckling pigs by *Streptococcus suis* with particular reference to systemic serotype 2 strains. Can J Vet Res 1998;62:21–26.
11. Solano-Aguilar GI, Pijoan C, Rapp-Gabrielson V, Collins J, Carvalho LF, Winkelman N. Protective role of maternal antibodies against *Haemophilus parasuis* infection. Am J Vet Res 1999;60:81–7.
12. Torremorell M, Pijoan C, Dee S. Experimental exposure of young pigs using a pathogenic strain of *Streptococcus suis* serotype 2 and evaluation of this method for disease prevention. Can J Vet Res 1999;63:269–275.
13. Segales J, Domingo M, Solano GI, Pijoan C. Immunohistochemical detection of *Haemophilus parasuis* serovar 5 in formalin-fixed, paraffin-embedded tissues of experimentally infected swine. J Vet Diagn Invest 1997;9:237–243.
14. Oliveira SR, Galina L, Pijoan C. Development of a PCR test to detect *Haemophilus parasuis* infections. Proc 81st Conf Res Work Anim Dis, 2000:149.
15. Versalovic J, Koeuth T, Lupski JR. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Meth Mol Cel Biol 1994;5:25–40.
16. Little TWA, Harding JDJ. The interaction of *Haemophilus parahaemolyticus* and *Pasteurella multocida* in the respiratory tract of the pig. Br Vet J 1980;136:371–383.