Experimental Exposure of Young Pigs Using a Pathogenic Strain of *Streptococcus suis* Serotype 2 and Evaluation of this Method for Disease Prevention

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ABSTRACT

Control of *Streptococcus suis* infections and associated disease have proven to be a difficult challenge under most farm conditions. The objective of this study was to experimentally expose young pigs with a pathogenic strain of *S. suis* serotype 2 as a means of controlling the disease in a commercial swine farm. Prior to the start of the study, the pathogenic *S. suis* strain responsible for mortality in the farm was identified and used to experimentally inoculate baby piglets. Over a 3-week period, groups of pigs were selected (100 pigs/wk) and divided into 2 groups: control (50 pigs/week) and experimentally exposed (50 pigs/week). Pigs in the experimentally exposed group were inoculated at 5 d old by tonsillar swabbing with the pathogenic *S. suis* farm isolate. The effect of exposure with this pathogenic strain was evaluated during the nursery and finishing stages and was based on: morbidity (pigs with central nervous signs (CNS) and/or lameness), mortality and number of treatments required by pigs that had either CNS or lameness. The relative risk (RR) of acquiring disease due to *S. suis* infection was also calculated. Results showed that morbidity in the experimentally exposed groups was lower than in the control group and these results were statistically different ($P = 0.006$). Experimentally exposed pigs also showed a statistically significant reduction in lameness problems ($P = 0.012$), but not in CNS ($P = 0.20$) or mortality ($P = 0.59$). Pigs in the control group had an increased RR of 4.76, 8.77 and 2.7 for morbidity, to have lameness or to have CNS signs, respectively. In conclusion, experimental exposure of young pigs with the farm's pathogenic *S. suis* strain at a young age, had a positive effect in reducing clinical signs characteristics of *S. suis* infection. This method constitutes a novel approach to the control of *S. suis* infections in swine farms.

RÉSUMÉ

Le contrôle des infections à *Streptococcus suis* et des maladies associées s'avèrent un défi de taille dans la majorité des élevages. L'objectif de l'étude était d'exposer expérimentalement de jeunes porcs à un isolat pathogène de *S. suis* sérotype 2 comme moyen pour contrôler la maladie dans un élevage commercial. Avant le début de l'étude, la souche pathogène de *S. suis* responsable de mortalité dans l'élevage fut identifiée et utilisée pour inoculer expérimentalement de jeunes porcelets. Pendant 3 semaines, des groupes hebdomadaire de porcs furent sélectionnés (100 porcs/semaine) et divisés en deux groupes : des animaux témoins (50 porcs/semaine) et des animaux exposés expérimentalement (50 porcs/semaine). Les porcs dans le groupe exposé expérimentalement furent inoculés à l'âge de 5 jours par badigeonnage des amygdales avec l'isolat pathogène de *S. suis* provenant de la ferme. L'effet d'une exposition à la souche pathogène fut évalué durant le séjour des animaux en pouponnière et en période de finition en se basant sur la morbidité (porcs avec signes nerveux centraux (SNC) ou boiterie), la mortalité et le nombre de traitements requis par les porcs avec SNC ou boiterie. Le risque relatif (RR) de contracter une maladie due à une infection par *S. suis* fut aussi calculé. Les résultats ont démontré que dans le groupe exposé expérimentalement la morbidité était significativement ($P = 0,006$) plus faible que dans le groupe témoin et qu'il y avait également moins de problèmes de boiteries ($P = 0,012$). Aucune différence significative ne fut observée entre les deux groupes pour ce qui est des SNC ($P = 0,20$) et de la mortalité ($P = 0,59$). Les porcs dans le groupe témoin présentaient des RR élevés de 4,76, 8,77 et 2,7 pour la morbidité, le développement de boiterie ou l'apparition de SNC, respectivement. L'exposition expérimentale en bas âge de jeunes porcelets à une souche pathogène endogène à la ferme eu un effet positif en réduisant les signes cliniques caractéristiques d'une infection par *S. suis*. Cette méthode constitue une approche nouvelle au contrôle à la ferme des infections par *S. suis*. Toutefois, compte tenu du potentiel zoonotique de *S. suis*, des précautions doivent être prises lors de la manipulation de ce microorganisme, surtout lorsque l'on met en place des mesures de contrôle dans des élevages commerciaux de porcs.

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INTRODUCTION

Disease caused by *Streptococcus suis* is one of the most common problems observed in swine farms. The disease affects mostly nursery pigs causing meningitis, arthritis, septicemia and endocarditis (1). Pigs are colonized by *S. suis* essentially at birth (2). These strains, part of the normal flora and isolated from respiratory sites, tend to be very heterogeneous, even in closed populations, representing various serotypes and genotypes (3). In contrast, recent molecular epidemiological studies have shown that in closed swine populations, there is a tendency for very few strains to cause mortality, to be established, and to persist over time (3). Unlike commensal strains of the normal flora, pathogenic strains are found at a low prevalence at weaning (4) and it has been hypothesized that this could be a predisposing factor for the latter onset of disease (5). According to this hypothesis, the problem would be exacerbated by the adoption of segregated early weaning (SEW) techniques since pigs in these systems are weaned early and separated from the sow herd. This hypothesis states that maternal immunity levels towards these systemic strains are high at weaning towards these systemic strains, therefore interfering with the colonization of baby piglets. However, a few sows in the group do shed the organism and infect their litter. In SEW systems as maternal immunity decreases after weaning, the pathogenic strains would be transmitted to the non-colonized pigs, therefore resulting in *S. suis*-induced disease. In contrast, in conventional systems in which pigs are weaned older, and where piglets are under constant challenge from older pigs, colonization would occur rapidly in the presence of adequate maternal immunity, resulting in active immunity in the piglets. As a result, in these conventional systems, most pigs are colonized under maternal antibody protection, reducing the incidence of postweaning disease. If this hypothesis is correct, early colonization of suckling pigs with pathogenic strains in the presence of maternal immunity should prove beneficial.

The mechanisms of protective immune responses against *S. suis* infection are not well understood. Studies on its pathogenesis suggest that in the early stages of the infection, *S. suis* invades the tonsils and is phagocytosed by mononuclear cells. Infected monocytes migrate to the local lymph nodes and then to the blood in order to reach the cerebral spinal fluid after crossing the blood-brain barrier (6,7). Therefore, it appears that both cell and antibody-mediated immune responses may be necessary in order to resist the disease. Commercial bacterins, which mainly stimulate antibody production (8), do not seem to provide full protection against *S. suis* infection (9,10). Vaccines using live strains seem to give better results under experimental conditions (11). However, success in using live vaccines in commercial farms is uncertain, since in the published experimental trials, challenges were done intravenously, which may not reproduce the original form of the disease. An alternative approach would be to inoculate live *S. suis* strains onto mucosal sites in young pigs, which should simulate natural infection and a correspondingly appropriate host response. This would, therefore, result in disease control under farm conditions.

The objective of this study was to experimentally expose young piglets to a pathogenic strain of *S. suis* serotype 2 as a means of controlling the disease in a commercial swine farm.

MATERIALS AND METHODS

FARM

A 250-sow farm with Landrace × Yorkshire commercial pigs was selected for this study. The farm had a history of clinical disease caused by *S. suis* serotype 2 in the late nursery phase, as well as the early stages of the finishing period. Mortality rates ranged from 1% to 8%, with a large variability between weekly groups of pigs. Pigs in this farm were weaned at 15 d and moved to an off-site nursery where they stayed for 8 wk. After that, pigs were moved to the finisher building which was situated on the same site as the nursery, but was managed as a separate site. All weekly groups of pigs were raised independently from each other with strict all-in/all-out measures between groups, thereby avoiding mixing of pigs of different ages. Rooms were also cleaned and disinfected between groups of pigs.

Breeding stock was purchased from a single source which had remained constant since the farm was established. The study farm was free from both porcine reproductive and respiratory syndrome (PRRS) viruses and Aujeszky's disease virus. Clinical cases of *Haemophilus parasuis*, *Mycoplasma hyorhinis*, hemolytic *Escherichia coli* and *Actinobacillus suis* had not been reported.

IDENTIFICATION OF THE PATHOGENIC STRAINS

Prior to the start of the study, mortality observed in the nursery and finisher units was studied. Pigs that showed clinical signs of either meningitis or arthritis prior to death were considered to be affected by *S. suis*. Pigs with central nervous signs (CNS) characterized by recumbency, convulsions and paddling were diagnosed as having meningitis. Arthritis was clinically characterized as lame pigs with inflamed joints. Pigs with clinical signs of disease of either meningitis and/or arthritis were necropsied during the preliminary farm visits. The visits were conducted over a one-year period. Samples were taken from brain and joints as well as tonsils of healthy pigs, incubated at 37°C on blood agar plates (BAP) and identified using standard tests (12,13). *Streptococcus suis* isolates were serotyped by the coagglutination method (14). *Streptococcus suis* serotype 2 was isolated in pure culture from the necropsied pigs. This organism was considered to be responsible for the aforementioned clinical problems. By contrast, *S. suis* isolates from tonsils were not serotype 2 and were considered non-pathogenic.

In order to further characterize the *S. suis* isolates collected during the 12 mo prior to the start of the study, a comparison was done by genotyping in an attempt to determine if these isolates were all the same or if they represented several different strains responsible for the mortality (15,16). Isolates were genotyped by Rep-PCR as described elsewhere (4).

EXPERIMENTAL INOCULATION

Genetic analysis revealed that all isolates recovered from necropsies

had an identical genetic pattern. Therefore, it was assumed that a single strain of *S. suis* was responsible for the mortality and clinical signs of meningitis and arthritis observed in the farm under study. Isolates recovered from tonsils were genetically heterogeneous and different from the pathogenic strain.

The first *S. suis* isolate recovered from the meninges of a dead pig was used as the inoculum for the experimental inoculation. This isolate was grown overnight at 37°C in BAP, collected the next day in phosphate-buffered saline (PBS), and frozen in vials at −80°C in 50% skim milk. One of the vials was thawed and the colonies were counted in order to assess the number of cfu/mL and the necessary dilution for the experimental exposure. The organism concentration was adjusted to 1×10^7 cfu/mL prior to the start of the experimental exposure.

The experiment was performed on the farm of origin. Three groups of pigs were selected weekly, exposed experimentally to the pathogenic *S. suis* strain and then observed throughout the nursery and finisher stages (3 trials). About 100 pigs were included in each trial (Table I) and divided into experimentally exposed and control groups.

Pigs were randomly selected, eartagged and exposed at about 5 d old in the farrowing room. Pigs in trial 1 were experimentally exposed with 1×10^6 cfu/mL of the pathogenic strain, using a nasal swab. The swab was introduced approximately 2 cm into each nostril. Pigs in trials 2 and 3 were exposed with 1×10^7 cfu/mL of the same strain, using both nasal and tonsillar swabbing, in order to get more consistent colonization. Pigs included in the experiment were housed in pens with solid partitions at all times, in order to prevent direct contact with pigs not included in the study. Pigs from both control and experimentally exposed groups were also housed in separated pens and not mixed. Farm personnel were the same throughout the study and did not know which pens corresponded to the control or to the experimentally exposed pigs. Pigs were managed according to the standard procedures of the farm.

TABLE I. *Streptococcus suis*-related and total (in parenthesis) morbidity, mortality as well as the number of pigs that showed CNS signs or lameness and the total number of treatments administered during the duration of the experiment

		Morbidity	CNS signs	Lameness	Mortality	Treatment
1st trial	Control (n = 40)	1 (1)	1 (1)	0 (0)	0 (0)	1 (1)
	Exposed (n = 53)	1 (4)	0 (0)	1 (1)	0 (1)	2 (2)
2nd trial	Control (n = 52)	4 (4)	2 (2)	2 (2)	1 (1)	8 (8)
	Exposed (n = 54)	1 (1)	1 (1)	0 (0)	0 (0)	1 (1)
3rd trial	Control (n = 53)	8 (9)	2 (2)	6 (6)	1 (2)	15 (15)
	Exposed (n = 52)	1 (2)	1 (1)	0 (0)	1 (2)	0 (0)
Total	Control (n = 145)	13 (14)	5 (5)	8 (8)	2 (3)	24 (24)
	Exposed (n = 159)	3 (7)	2 (2)	1 (3)	1 (3)	3 (3)

Numbers in parentheses are the totals for pigs exhibiting morbidity, mortality, CNS signs, and lameness, as well as the total number of treatments administered over the duration of the experiment

EVALUATION OF THE CARRIER STATE

Tonsillar swabs were taken in order to confirm the carrier state of the pigs and whether the pigs had been successfully colonized. In each trial, 10 pigs were randomly selected from each group and sampled before inoculation and at 6, 14, 22, and 44 d post inoculation. Swabs were plated onto colistin-nalidixic acid plates and incubated at 37°C overnight. Three α-hemolytic colonies were selected from each plate in trial 1 and 8 colonies were selected and reisolated in trials 2 and 3. Isolates were further subcultured and serotyped using an immunofluorescence antibody test (IFAT) against serotype 2, which previously had been standardized in the laboratory. The optimal serum and conjugate dilutions were determined by checkerboard titration (results not shown). Briefly, about three colonies were resuspended in 20 μL of PBS on a glass slide and fixed with acetone for 10 min. Twenty-five microliters of a serotype 2 polyclonal antiserum produced in rabbits (dilution 1:75) was added to the bacterial suspension. Slides were incubated at 37°C for 30 min in a moist chamber. Slides were then washed 3 times with PBS and 25 μL of fluorescein labeled anti-rabbit IgG (Sigma Chemical Co., St. Louis, Missouri, USA) (dilution 1:100) was added and incubated for 30 min at 37°C. Slides were rinsed 3 times with PBS and observed under a fluorescence microscope. Serotype 2 isolates were viewed as cocci covered by a full, bright green, fluorescent capsule. Controls of this test included the *S. suis* isolate used as inoculum (positive control) and the reference strain for serotype 1 (negative control). Serotype 2 IFAT-positive isolates were further confirmed by the coagglutination method (14).

Following serotypification, all confirmed serotype 2 isolates were analyzed by Rep-PCR (4) and the genetic pattern was compared to the farm-specific pathogenic strain used as the inoculum for the experimental exposure.

ISOLATION OF *S. SUIS* FROM DEAD PIGS

Attempts were made to isolate *S. suis* throughout the experiment from any pigs that died during the study. Two pigs, one from the control and one from the experimentally exposed group, as well as 3 pigs from the farm but not included in the study, were necropsied and meningeal and inflamed joint swabs were collected. Isolated strains were characterized and genotyped as described above.

PARAMETERS EVALUATED

The effect of the experimental exposure of young piglets to a pathogenic *S. suis* strain was evaluated according to the following parameters: 1) mortality, 2) morbidity, and 3) number of antibiotic treatments administered during the nursery and finishing stages. Primary signs recorded in morbidity included CNS signs and/or lameness with clinically inflamed joints. The number of antibiotic treatments was calculated as total treatments per group and as number of treatments per pig with clinical signs of *S. suis* infection. Pigs were treated according to the farm protocols and pigs that showed signs of *S. suis* disease consisting of either CNS or lameness, as described above, were treated with injectable penicillin.

The relative risk (RR) was used to indicate the likelihood of developing

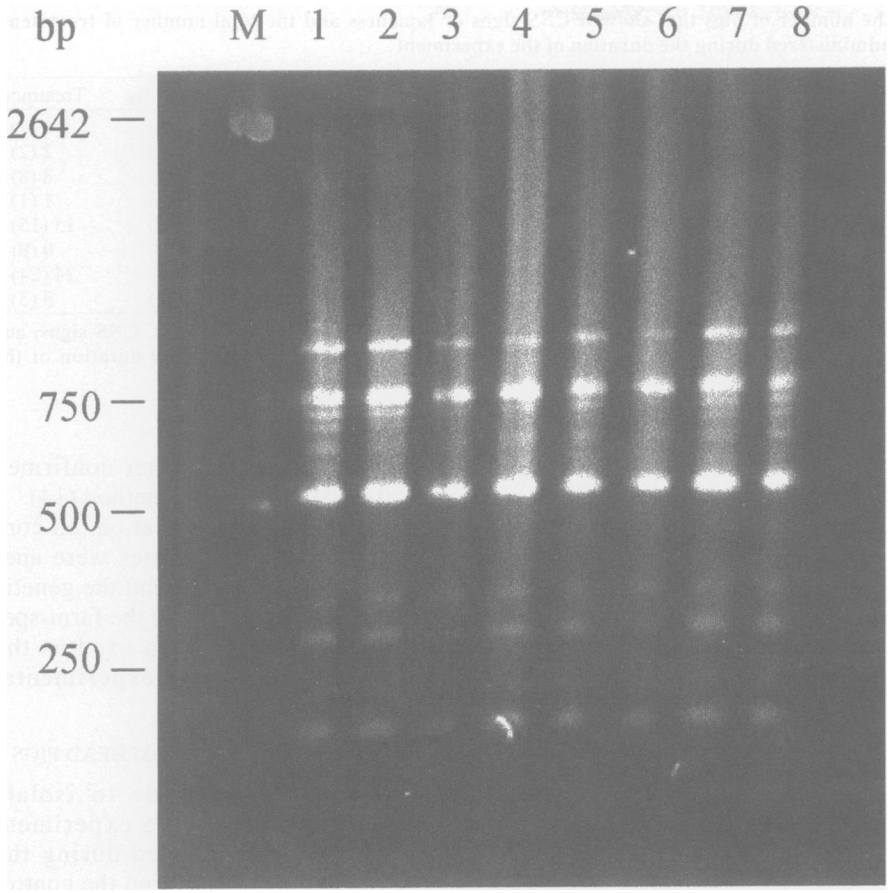

Figure 1. Genetic pattern of the *S. suis* pathogenic strains collected from the farm during the study. bp — base pair. Lane M — molecular weight marker. Lanes 1 to 4 — isolates collected from 4 pigs during a 10-month period previous to the start of the study. Lanes 5 to 7 — isolates collected from the tonsils of experimentally colonized pigs in trial 2. Lane 8 — isolate recovered from a farm pig not included in the experiment that died during the study. These patterns were obtained by Rep-PCR, using ERIC 1R and ERIC 2 primers in the reaction.

S. suis-related disease as defined by pigs that had CNS signs and/or lameness in the control group relative to the experimentally exposed group. It was calculated as the ratio of the incidence of disease in the control group (non-exposed) divided by the corresponding incidence of disease in the experimentally exposed group. The RR was determined for morbidity, number of pigs that showed CNS signs, number of pigs that showed lameness, and for mortality.

STATISTICAL ANALYSIS

Results were compared between the experimentally exposed and control groups. Data of *S. suis*-related disease on morbidity, mortality, and pigs that showed CNS signs and lameness was analyzed. Results from the 3 trials were pooled and analyzed using the chi-squared test. 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

IDENTIFICATION OF THE PATHOGENIC STRAINS

Eight pigs were necropsied during the 10 mo prior to the start of the study. Seven *S. suis* isolates were recovered from 4 pigs during that period. The primary sites of isolation were the brain or joint capsule. Four of the pigs did not yield *S. suis* at necropsy, but they had been treated with injectable penicillin upon early signs of meningitis. These animals died soon after treatment and other pathogens were not isolated. Systemic *S. suis* isolates were determined to be serotype 2. Molecular analysis demonstrated a single genetic pattern among these isolates (Figure 1). This uniformity suggested that all these isolates belonged to a single strain of *S. suis*, which was involved in the mortality. This strain was established in the farm and had persisted for at least 1 y.

EXPERIMENTAL INOCULATION

Clinical signs were not observed in the 5-day-old-piglets for at least 4 wk following the experimental inoculation. Results were recorded as total numbers of pigs that had any sign of disease and as number of pigs that had signs compatible with disease caused by *S. suis* (Table I). Results of morbidity, mortality, as well as number of pigs having CNS signs or lameness, and number of total treatments, are shown for each of the trials. Few pigs in trial 1 were affected. In contrast, control pigs in trials 2 and 3 had disease rates similar to values observed in the farm prior to the start of the experiment. A total of 14 control pigs were affected during the 3 trials. One pig died acutely without it being possible to determine the cause of death. This animal was, therefore, excluded from the study. Seven experimentally exposed pigs were affected. While clinical signs in 3 pigs were determined to be due to *S. suis*, 2 other pigs were considered to be poor-doers, another one died from castration complications, and another died acutely without any apparent cause.

Since the 3 trials were independent and the pigs studied in each of the trials were different, the results were grouped and analyzed together. The number of pigs that had signs of disease related to *S. suis* in both control and experimentally exposed groups, were statistically different ($P = 0.006$). These disease signs were further analyzed according to the main signs observed, namely CNS or lameness (Figure 2). Lameness was the predominant sign with 5.5% (8 pigs) in the control pigs, whereas < 1% (one pig) was affected in the experimentally exposed group. These results were statistically significant ($P = 0.012$). Five pigs (3.4%) in the control groups had CNS signs while only 2 pigs (1.2%) had CNS signs in the experimentally exposed group. These results, however, were not statistically significant ($P = 0.20$). Differences in the level of mortality were

not significant between the groups ($P = 0.5086$), despite a higher mortality in controls (1.3%) vs in the experimentally exposed animals (0.06%). However, mortality in the study pigs was lower than in the contemporaneous groups on the farm (2.5%). *Streptococcus suis* was isolated from joints and meninges of one dead control pig. Isolation was negative in one experimentally exposed pig that had CNS signs previous to death, probably due to the treatment received. The *S. suis* pathogenic strain was isolated in 3 pigs present in the farm that died at the time of the experiment but not included in the study (Figure 1). All isolates had identical genetic patterns to the farm pathogenic strain previously characterized, and used for the experimental inoculation.

The RR of being affected with *S. suis* for the control non-exposed pigs was 4.76 times higher than for the experimentally exposed animals. The RR for control pigs to develop CNS signs or lameness was 2.7 and 8.77, respectively.

Control pigs required more treatments than the experimentally exposed pigs. Twenty-four treatments were required in the control group, whereas only 2 treatments were required in the experimentally exposed group. The control group required 2 treatments per affected animal, whereas animals in the experimentally exposed group required only one treatment.

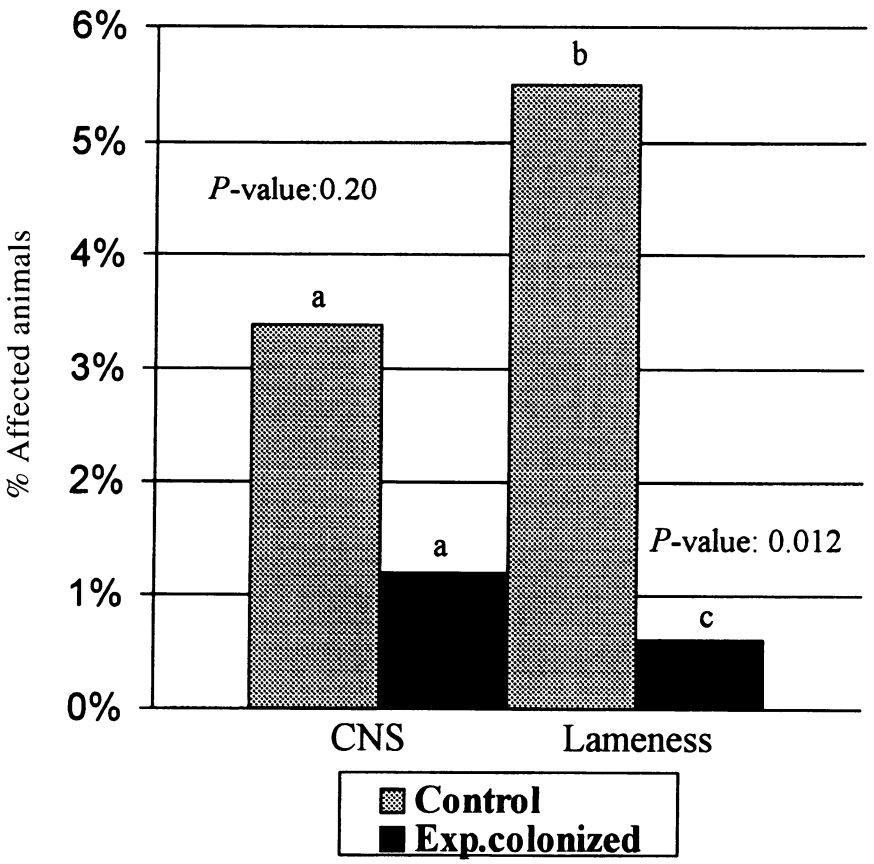

Figure 2. Percentage of pigs that showed either CNS signs or lameness with clinically inflamed joints during the duration of the study. Differences in CNS results between control and experimentally colonized pigs had a *P*-value equal to 0.20. Differences in lameness between groups had a *P*-value equal to 0.012.

EVALUATION OF THE CARRIER STATE

Evaluation of the carrier state was performed by superficial tonsillar swabbing. The farm's pathogenic strain could not be isolated from the tonsils of either the control or the experimentally exposed pigs in trials 1 and 3; however, the strain was successfully isolated in trial 2 from 3 different experimentally inoculated pigs (Figure 1). These isolates were recovered at 22, 34 and 44 d after the experimental exposure. The pigs that carried the inoculated strain in their tonsils did not show any sign of *S. suis* disease during the experiment.

DISCUSSION

Experimental exposure of healthy animals with pathogenic strains as a means of preventing disease is a new concept for the control of *S. suis* infection in pigs. In this study, 5-day-old pigs were exposed to a specific pathogenic strain of *S. suis* which was responsible for the clinical disease in the farm under study.

This study was based on 2 major points: a) identification of a pathogenic strain of *S. suis* specific for the farm and responsible for the *S. suis*-related disease; b) to expose young piglets to this strain in order to stimulate an appropriate immune response by simulating time when colonization occurs in conventional systems.

This study was conducted to investigate alternatives to the control of *S. suis* disease, since *S. suis* killed vaccines are not efficacious under field conditions (10).

Most pigs are colonized by *S. suis* very early in their lives. The *S. suis* strains that are found colonizing the upper respiratory tract tend to be different from strains causing disease (17). Field observations suggest that colonization by non-virulent strains does not provide protection against disease, since pigs colonized by non-virulent strains are still affected by these specific strains. Most of the studies that describe colonization by *S. suis* do not differentiate between common flora *S. suis* and pathogenic strains. Two studies in which this differentiation was performed showed that pathogenic strains could be found in the tonsils of pigs (17,18). However, in these studies, pigs were not monitored individually and it was, therefore, not possible to ascertain whether a true asymptomatic carrier state existed. In the present study, pigs were monitored individually and we showed that healthy animals can carry the pathogenic strain in their tonsils without being affected, therefore constituting a possible focus for disease transmission.

In an effort to evaluate the effect of the experimental exposure on disease, tonsillar swabs were taken following the experimental inoculation. Surprisingly, the isolation rate of the pathogenic strain from the tonsils of

experimentally exposed pigs was very low. However, prior studies had shown that isolation of pathogenic strains from the tonsils of healthy pigs was difficult. In this study, one explanation for this may have been that the experimental exposure did not result in colonization. This, however, is unlikely, since tonsils are the normal habitat for *S. suis* (18,19). More likely, the pathogenic strain was present in the pig, but it could not be detected by superficial tonsillar swabbing. In an attempt to improve the sensitivity of the bacteriological isolation, up to 8 colonies were studied per animal and per sampling. In the 3 pigs where the pathogenic strain could be isolated, only one of the 8 α-hemolytic colonies analyzed was the pathogenic strain and this strain could not be isolated twice from the same animal. This highlights the difficulty in successfully achieving microbiological isolation from the tonsils, which may result in misleading results. These results are perhaps not surprising as previous studies have also shown that *S. suis* is more likely to be found deep in the crypts of the tonsils, so superficial swabbing of the tonsillar surface may be inadequate (19).

The experimental exposure of young pigs with a pathogenic strain reduced the number of pigs that developed clinical signs of *S. suis*-related disease. However, pigs in trial 1 did not show signs of disease in either the control or experimentally exposed groups. The cohort group of pigs from the farm of the same age, which was not included in the study, did not show signs of disease either, suggesting that this weekly group of pigs that was selected for trial 1, was a low disease group. Control pigs in trials 2 and 3 showed signs of disease, and experimental exposure to a pathogenic *S. suis* strain had a positive effect on limiting development of disease, resulting in higher morbidity in the control group. Differences in the number of pigs that showed lameness were statistically significant, but differences in CNS signs were not statistically significant at $P < 0.05$. Differences in mortality were also not statistically significant. Mortality in the farm pigs not included in the experiment was higher than in the study pigs, suggesting that overall disease in the study pigs was lower than expected. Mortality caused by *S. suis* is due to meningitis, and since lameness with clinically inflamed joints was the main sign observed, mortality was expected to be low. Only 3 pigs that showed signs of meningitis died during the experiment, and none of the arthritic pigs died. Assessment of the RR also showed that control pigs had a higher likelihood of developing disease compared to the experimentally exposed pigs. Control pigs had almost 5 times the risk of having *S. suis*-related clinical signs, 2.7 times for showing CNS signs and 8.77 times risk for having lameness, than experimentally exposed pigs. However, relative risk for mortality was only 1.09, indicating that the mortality was similar between the control and experimentally exposed groups. Therefore, exposure with the pathogenic strain proved to be advantageous in reducing the clinical signs of the disease but not necessarily in reducing the level of mortality.

The source of the pathogenic strain in the control pigs that died remains undefined. Control pigs were affected by the same specific pathogenic strain, even though they had not been experimentally exposed. This suggests that several sows were the most likely source of infection. However, since not all piglets were exposed during lactation, they presumably did not develop an appropriate immune response, and by the time they reached the nursery, the pigs carrying the pathogenic strain may have infected the naive pigs, resulting in disease. By contrast, in the experimentally exposed group, most of the pigs were exposed to the pathogenic strain when they were with the sow, and in the presence of maternal immunity, simulating what would occur under natural conditions. After weaning, the pigs would already carry the strains and, therefore, would resist further challenge with the same strain.

An immediate disadvantage for the applicability of this procedure in commercial swine farms would be that the method for experimental inoculation is cumbersome. Additional research is necessary to create a more user-friendly device for large-scale colonization. In addition, since *S. suis* is potentially zoonotic, its handling can result in serious disease to humans. In this study, special handling instructions and management procedures were applied in order to prevent transmission to the personnel in charge of the pigs. The implementation of safety measures using live *S. suis* strains has to be carefully reviewed and planned.

In conclusion, protection against *S. suis* disease was observed in young piglets that had been exposed to the pathogenic strain responsible for the mortality in the farm under study. This study constitutes a novel approach on how to control *S. suis* infection in swine farms; however, more studies are necessary in order to further evaluate the problem and to understand the pathogenesis and the epidemiology of the disease caused by *S. suis*.

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