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Controlling *H. parasuis* mortality through exposure of suckling pigs

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Haemophilus parasuis can be normally isolated from the nasal cavity, tonsils, and trachea of healthy pigs. This organism can potentially invade the host and cause severe systemic infection characterized by fibrinous polyserositis, arthritis, and meningitis. The factors involved in systemic invasion by *H. parasuis* have not been clearly defined. The relationship between *H. parasuis* serovar, genotype, whole-cell protein profiles, and virulence has also been evaluated. Although virulent strains share similar genotype and protein profiles, no specific virulence factors have been described for *H. parasuis* so far.

Control of *H. parasuis* can be performed by means of antibiotic treatments or vaccination, using either commercial or autogenous products. The effectiveness of experimental vaccines against homologous challenge has been demonstrated. However, a lack of cross-protection between different *H. parasuis* serovars and strains has also been reported. Commercial vaccines have been shown to provide satisfactory homologous protection against *H. parasuis* strains from the same serovar group. However, vaccination failures using commercially available products have also been demonstrated, especially when the *H. parasuis* strains prevalent in the herd differ from those included in the vaccine. In this case, autogenous vaccines can be used to reduce nursery mortality.

Controlled exposure of five-day old pigs to a low dose of live, virulent *H. parasuis* has been recently proposed as an alternative method to control nursery mortality. This method is based on the hypothesis that early colonization of piglets with the prevalent strains of *H. parasuis* in the presence of maternal immunity reduces the risks of systemic infection after weaning. This control measure has been shown to successfully reduce morbidity and mortality due to *Streptococcus suis* and *H. parasuis* systemic infection in affected nurseries.

Materials and methods

Multi-farm system

A multi-farm system (30,000 sows) experiencing high nursery mortality due to *H. parasuis* infections was selected for the evaluation of vaccination and controlled exposure as control measures. Nursery pigs from this system were positive for porcine respiratory and reproductive syndrome virus (PRRSV), Circovirus, swine influenza virus (SIV), and negative for *Mycoplasma hyopneumoniae*. Pigs were weaned at 21 days and transferred to off-site nurseries. Each nursery contained eight rooms which were filled with approximately 1,000 pigs each.

Experiment I (commercial vaccine)

Historical nonvaccinated groups had 3.25% average mortality (sd 1.1%). Sample size calculation was performed with 80% power of detecting 1% decrease in mortality with alpha of 0.05. Five barns with eight rooms per barn were used for the trial. A randomized complete block design was used with barn as the blocking factor. At the start of the trial, each barn was emptied, cleaned, and disinfected. One barn was filled each week for five consecutive weeks. The producer requested a minimum of control, nonvaccinated groups, so that a 3:1 ratio of treatment:control was selected. Treatment was allocated to rooms within barns by randomly selecting the first room of the first barn to be control or vaccinated group and consecutive rooms were systematically allocated thereafter assuring a 3:1 ratio of vaccinated:nonvaccinated rooms in every barn. Pigs in the treatment group were vaccinated intramuscularly at weaning (21 days) using a single-dose of a commercially available *H. parasuis* bacterin (Ingelvac(r) HP One dose, Boehringer Ingelheim).

Experiment II (autogenous vaccine)

In order to select the *H. parasuis* strains that would be included in the autogenous vaccine, 32 nursery pigs, including 15 clinically affected and 17 found dead pigs, were necropsied and swabs from pleura, pericardium, peritoneum, joints and meninges, plus lung tissue, were collected for *H. parasuis* isolation. Collected swabs were also tested by PCR to assess the prevalence of *H. parasuis* systemic infection in the nursery. *Haemophilus parasuis* isolates were genotyped by ERIC-PCR. Genomic fingerprints were analyzed using the BioNumerics software and one representative isolate from each prevalent group of *H. parasuis* strains was used to produce an autogenous vaccine. The experimental design was similar to that described for Experiment I, with the exception of the room allocation and the ratio of vaccinated:nonvaccinated rooms (see **Table 1**). Control pigs were always allocated

to the first four rooms in each barn, whereas vaccinated pigs were allocated to the remaining rooms. Pigs in the autogenous treatment group were vaccinated intramuscularly at processing (4-5 days) and at weaning (21 days).

Experiment III (controlled exposure)

Forty-three animals, including 41 clinically affected and two found dead pigs, were necropsied. Sample collection and characterization of the obtained *H. parasuis* isolates were performed as described in Experiment II. One representative isolate of each group of prevalent strains was included in the inoculum used for controlled exposure. *Haemophilus parasuis* isolates were cultured on chocolate agar during 24 h at 37° C using a candle jar. A seed culture containing the prevalent *H. parasuis* strains isolated from affected animals was prepared by harvesting the bacterial growth from chocolate agar plates and suspending it in 10% skim milk. The bacterial suspension was distributed in 1 mL aliquots and stored at -20° C until use. The final inoculum was prepared by diluting one 1 mL aliquot in 1 liter of sterile PBS. The inoculum was then transferred to plastic spray pumps and maintained at 4° C until use.

The experimental design was similar to those used in Experiment I and II, with the exception of the number and allocation of treatment and control rooms per barn (see **Table 1**). Four rooms per barn were used to allocate treatment (two rooms) and control (two rooms) groups. The same rooms were used to allocate these groups in all the repetitions. Due to the reduction in the number of experimental groups, this experiment was repeated nine times (nine consecutive weeks of production). Each piglet in the treatment group received one spray (1 mL dose) of the inoculum at processing. When the first group of inoculated pigs reached four weeks in the nursery, 2-3 pigs from each treated and control rooms were selected for euthanasia, based on clinical signs characteristic of *H. parasuis* systemic infection. Samples collected from these pigs were submitted to isolation and PCR, and isolates were genotyped and compared with the prevalent strains previously identified in the herd.

Statistical analysis

The room (air space) was used as the experimental unit. Main effects ANOVA was performed in Statistica(r) with percent mortality at closeout as the dependent variable and room and treatment as the independent variables.

Results

H. parasuis isolation, PCR, and genotyping

Haemophilus parasuis isolation and PCR results are summarized in **Table 2**. Prevalence of *H. parasuis* systemic infection in the nursery varied from 50% in the first visit

Table 1: Experimental design used to assess the effectiveness of vaccination and controlled exposure to control nursery mortality due to *H. parasuis* infection.

Exp.	Control measure	Timing	Control:treated rooms	Reps
I	Commercial vaccine	At weaning[A]	2:6	5
II	Autogenous vaccine	At processing[B] and at weaning	4:4	5
III	Controlled exposure	At processing	2:2	9

AWeaning at 21 days
BProcessing at 3-5 days of age

Table 2: *Haemophilus parasuis* isolation and PCR results for swabs collected from euthanized (E) and found dead (FD) pigs.

Farm visit	Number of pigs necropsied	Weeks in the nursery	Pigs positive by isolation	Pigs positive by PCR[A]	Prevalent genotypes
1 (Nov 2001)	32 15 (E) + 17 (FD)	2-6	7 (21.88%)	16 (50%)	A and B
2 (Oct 2002)	43 41 (E) + 2 (FD)	2-5	20 (46.51%)	23 (53.48%)	A, B, and C
3 (Mar 2003)	17 (E)	3-4	5 (29.41%)	6 (35.29%)	A and B

APigs positive by PCR were also positive by isolation.

to 53.48% in the second visit and 35.29% in the third visit, based on PCR results. Two prevalent genotypes (A and B) were identified among the isolates recovered from seven pigs in the first farm visit and a third group of prevalent strains (genotype C) was identified among the isolates recovered from the 20 pigs necropsied in the second farm visit. Isolates A and B were found to be still prevalent one year after the first isolation. In the third visit, when the first group of inoculated pigs had four weeks placed in the nursery, only strains A and B were recovered from clinically affected animals. *Haemophilus parasuis* was mainly isolated from non-treated pigs (four out of five isolates), compared to one isolate recovered from the joint of an inoculated pig.

Experiment I (commercial vaccine)

The commercial vaccine used in the present study contained a non-typable *H. parasuis* isolate, which was reported to be cross-protective against heterologous *H. parasuis* serovars and to protect susceptible animals against homologous challenge. Percent mortality appeared to be normally distributed across all rooms, with an average 5.09% (sd 1.74%). Nonvaccinated groups (n = 30) had average mortality of 4.81% (sd 1.17%) and vaccinated groups (n = 10) had 5.18% (sd 1.22%) (see **Table 3**). Room was significantly associated with mortality while treatment was not. The average mortality rate observed in the treated group, including the five repetitions, was 1.08 times higher than that observed in the control group (see **Figure 1**).

Experiment II (autogenous vaccine)

The autogenous vaccine was prepared using a representative isolate of each prevalent genotype group (A and B) identified after the first farm visit (November 2001). Similar to Experiment I, percent mortality appeared to be normally distributed across all rooms, with average 7.38% (sd 3.28%). Nonvaccinated groups (n = 20) had average mortality of 7.66% (sd 2.02%) and vaccinated groups (n = 20) had 7.10% (sd 2.47%). Treatment was not significantly associated with mortality. The average mortality rate observed in the control group, including the five repetitions, was 1.08 times higher than that observed in the treated group (see **Figure 1**).

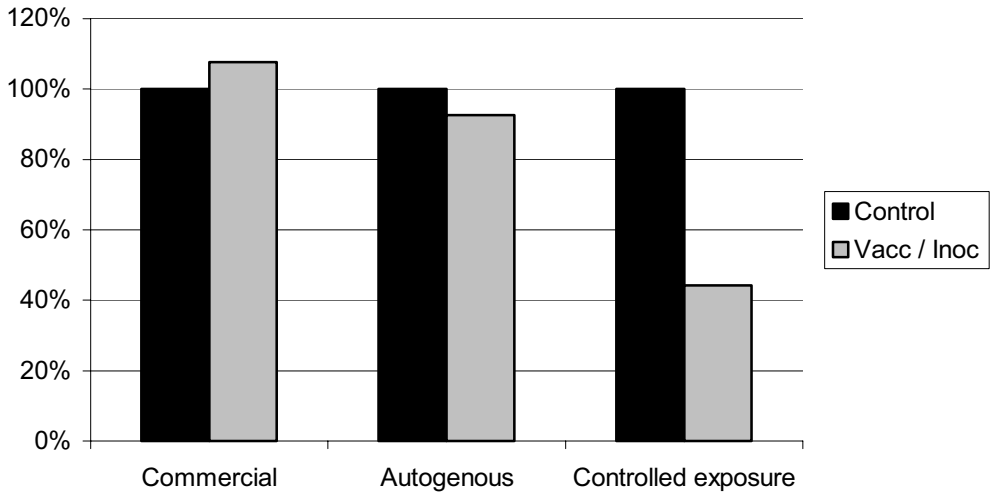
Experiment III (controlled exposure)

The inoculum used for controlled exposure was prepared using a mixture of representative isolates from each prevalent genotype group (A, B, and C) identified after the second farm visit (October 2002). Pigs were inoculated with 105-6 CFU/mL. Across all rooms, percent mortality appeared to be normally distributed, with an average 10.34% (sd 5.99%). Nonvaccinated groups (n = 18) had an average mortality of 14.34% (sd 4.98%) and vaccinated groups (n = 18) had 6.35% (sd 1.79%) . Room and treatment were significantly associated with mortality. The average

Table 3: Mortality rates observed in each nursery at closeout in Experiments I (commercial vaccine), II (autogenous vaccine) and III (controlled exposure).

Experiment	Nursery	Average Mortality Rate (%)	
		Control (SD)	Treated (SD)
I	1A (304)	4.81 (1.72)	6.75 (1.9)
Commercial	1B (201)	5.75 (1.07)	5.54 (1.27)
vaccine	1C (202)	5.64 (1.69)	5.45 (1.76)
	1D (401)	2.84 (0.5)	3.41 (1.38)
	1E (402)	5.02 (1.18)	4.77 (1.44)
Average 5 weeks	5 repetitions	4.81 (1.17)	5.18 (1.22)
II	2A (303)	5.6 (1.52)	5.36 (2.48)
Autogenous	2B (304)	6.61 (2.45)	4.66 (0.78)
vaccine	2C (201)	6.48 (1.42)	6.35 (2.72)
	2D (202)	9.31 (2.95)	8.43 (3.80)
	2E (401)	10.27 (4.72)	10.71 (3.79)
Average 5 weeks	5 repetitions	7.66 (2.02)	7.10 (2.47)
III	3A (402)	21.72 (6.7)	8.07 (2.28)
Controlled	3B (301)	17.25 (6.46)	7.2 (2.95)
exposure	3C (302)	19.86 (5.41)	7.65 (1.06)
	3D (303)	14.5 (1.51)	3.96 (1.72)
	3E (304)	13 (4.57)	5.03 (0.7)
	3F (201)	7.89 (2.98)	4 (0.74)
	3G (202)	9.05 (1.94)	5.37 (0.74)
	3H (401)	9.03 (2.45)	7.13 (3.06)
	3I (402)	16.72 (5.63)	8.77 (3.72)
Average 9 weeks	9 repetitions	14.34 (4.98)	6.35 (1.79)

Figure 1: Mortality rates in treated groups expressed as the percentage of the mortality observed in the control groups for Experiments I (commercial vaccine), II (autogenous vaccine) and III (controlled exposure).



mortality observed in the inoculated groups was significantly lower than that observed in the control groups, which indicates that controlled exposure of young pigs to a low dose of live *H. parasuis* was effective in reducing nursery mortality in the treated group. The average mortality rate observed in the control group, including the nine repetitions, was 2.26 times higher than that observed in the control group (see **Figure 1**).

Discussion

In the present study, the effectiveness of vaccination and controlled exposure to reduce nursery mortality due to *H. parasuis* systemic infection was evaluated. Results showed that neither the commercial nor the autogenous vaccines tested reduced nursery mortality, whereas controlled exposure of piglets to the virulent *H. parasuis* strains prevalent in the herd reduced mortality by 55.72% compared to the control group (see **Figure 1**).

A commercially available one-shot *H. parasuis* vaccine, which contains a non-typable strain, was selected to be used in the first trial. Although the one-shot vaccine considerably reduced the workload involved in the implementation of the vaccination program, mortality in the treated group exceeded the mortality observed in the control group, indicating that there was no effect of vaccination in nursery mortality. Several factors could have influenced this outcome. The lack of cross-protection between the vaccine strain and the prevalent *H. parasuis* strains in the herd is one of the potential factors that could have influenced the effectiveness of the selected vaccine. Other factors to be considered include the incorrect timing of vaccination, the interference of maternal immunity, the dose used, and even the need for a booster vaccination.

In the second trial, an autogenous vaccine was tested to reduce nursery mortality. This vaccine contained two isolates representative of the prevalent strains identified in the herd after the first visit (A and B). Results showed that there was a non-significant reduction in mortality in the vaccinated group compared with the control group. The failure in vaccination using the autogenous product was unexpected, since homologous protection has been reported to be highly effective between *H. parasuis* serovars and strains.

Results obtained in these field trails suggest that autogenous vaccines can be a viable alternative to control *H. parasuis* when commercial products are not effective. However, in the present study, it was demonstrated that this may not be true for all swine herds. Again, several factors could have influenced the effectiveness of the autogenous vaccine, especially timing of vaccination and maternal immunity interference. In the present study, pigs were vaccinated with the autogenous product at processing and at weaning, due to the early onset of *H. parasuis* systemic infection in the nursery (3-4 weeks after weaning). When pigs are vaccinated at such an early age, there is always a concern regarding interference of maternal immunity in the development of active immune response induced by the vaccine.

In the third trial, the effectiveness of an alternative control measure to reduce nursery mortality due to *H. parasuis* systemic infection was evaluated. Piglets were inoculated by the oral route with a low dose of live, virulent *H.*

parasuis cells. Strains A, B, and C were included in the inoculum used for controlled exposure.

We had previously hypothesized that the early colonization of piglets with the herd's prevalent strains of *H. parasuis* while they are still protected by maternal immunity could reduce the risk of systemic infection after weaning. Following this hypothesis, it would be desirable that a large number of pigs be exposed to these potential virulent strains before they become susceptible, which coincides with the decrease in maternal antibody levels.

In the present study, the use of controlled exposure significantly reduced nursery mortality compared with both the control and with the groups vaccinated with the commercial or autogenous vaccine.

The sample size and number of repetitions (8,000 pigs per group, 5-9 repetitions) used in the present study were considerably larger compared with our previous report (50 pigs per group, 2 repetitions). The herd used in the present study had a greater prevalence of systemic infection and mortality due to *H. parasuis* compared with the one used in our previous study. Also, the allocation of treated and control groups in the nursery, as well as the experimental unit used for statistical analysis, differed between studies. In the present study, treated and control groups were allocated to different rooms in a single barn and room, or air space, was considered the experimental unit. In our previous study, all groups were allocated to different pens in the same nursery barn (same air space), and the pig was considered the experimental unit. Therefore, there is a possibility that the *H. parasuis* and *S. suis* strains used for colonization could have spread from treated to control groups, even though we did not isolate these strains from the control pigs.

Results obtained in the present study demonstrated that early exposure of pigs to a low dose of live, virulent *H. parasuis* can significantly reduce nursery mortality compared with autogenous and commercial vaccines. This technique has several advantages compared with traditional vaccination, including lower cost and reduction of workload. Furthermore, timing does not seem to be an issue with controlled exposure, whereas maternal immunity may interfere with pig vaccination. Although controlled exposure was successful in reducing nursery mortality, there are some concerns regarding the safety of this method. Although the interaction between PRRS virus and *H. parasuis* has not been scientifically demonstrated, field experiences suggest that these organisms may co-infect nursery pigs, resulting in increased mortality. It would appear to be counter-indicated to inoculate pigs with *H. parasuis* if PRRS virus infection is active in the sow farm.