

# Transmission study of Salmonella in pigs with 3 intervention strategies

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## Abstract

In this study, the effect of 3 different intervention strategies on the transmission of Salmonella in pigs was evaluated: feed supplementation with coated calcium-butyrate salt, vaccination and acidified drinking water. Strategies were evaluated serologically and bacteriologically using an experimental in vivo seeder setup. Significantly higher antibody titers were detected in the groups with acidified drinking water and vaccination. In the group with calcium-butyrate supplemented feed and in the group with oral vaccination, significantly less infected animals were observed during the transmission experiment. The overall level of Salmonella-specific antibodies in meat juice was significantly lower compared to serum. Our results indicate that vaccination with Salmoporc® and feed supplemented with coated calcium-butyrate limit Salmonella Typhimurium transmission in swine. The detection of Salmonella-specific antibodies in vaccinated pigs however, does not allow differentiation of vaccinated and infected animals and can therefore interfere with the Belgian monitoring and surveillance programme. Salmonella-specific antibody levels in meat juice are lower than those in serum, which might have consequences in surveillance programmes based on meat juice analysis.

## Introduction

Despite current control measures, Salmonella in pigs still remains a major public health problem and causes – even the more common subclinical infections in swine - large economic losses (Kranker et al., 2003). The exact proportion of human Salmonella cases in Belgium that are due to the consumption of pork/meat products is unknown. However, it is widely assumed that pork/meat products are an important source of Salmonella infections in humans (Griffith et al., 2006); the serovar Salmonella enterica subspecies enterica serovar Typhimurium representing 64% of the porcine Salmonella isolates in the period between 2005 and 2009 in Belgium (CODA-CERVA, 2009). Because a reduction in preslaughter infection rates should result in increased pork safety (Hurd et al., 2002) and better pig health, we investigated 3 control strategies against S. Typhimurium. The detection of Salmonella in persistently infected pigs continues to be problematic as well. Hence, we compared the suitability of serology on diaphragm fluid, easy to sample at slaughter, with bacteriological isolation and serum serology to determine the Salmonella-status at animal level.

## Material and Methods

Sixty-nine Salmonella-negative, 20-21 day old weaned piglets of mixed sexes were moved to the animal facility of the VAR and randomly assigned into 5 groups: The first group (n=8) received feed supplemented with (0.3%) coated calcium-butyrate salt (Green-Cab-70®, Sanluc International), the second group (n=8) was orally vaccinated at 3 and 6 weeks of age with a commercial Salmonella Typhimurium vaccine (Salmoporc®, IDT), and a third group (n=8) received drinking water adjusted to a pH 3.5-3.8 using a mixture of formic acid (50%), propionic acid (10%), acetic acid (10%) and lactic acid (5%) (Agrocid Super®, AgroLogic). A positive control group (infected/untreated; n=8) and a negative control group (uninfected/untreated; n=5) were included as well. Treatments were applied from weaning (3 weeks of age) until the end of the experiment (14 weeks of age). Each intervention group was duplicated.

At 8 weeks of age, 2 pigs of every pen - except the negative control group - were moved to a separate pen and were orally challenged (Day -1) with 108 cfu of the nalidixin-resistant Salmonella serovar Typhimurium strain 112910a, phage type 120/ad (Boyen et al., 2008). After 24h, these 'seeder' pigs were placed back in their original pens (Day 0).

From 3 weeks of age until the euthanasia at 14 weeks of age, blood samples were collected from all 69 pigs once a week and Salmonella-specific antibodies were detected with a commercial ELISA test kit (HerdChek Swine Salmonella®, IDEXX Laboratories) following the manufacturer's instructions. Before the challenge at 8 weeks of age, slurry samples were taken once a week and after challenge individual fecal samples were collected twice a week; Salmonella was isolated using the ISO 6579 annex D method. Salmonella counts were made on 3, 7 and 24 days post inoculation (DPI) using

standard enumeration protocols. At necropsy, samples from ileocecal lymph nodes, ileum, ileum contents, caecum, caecum contents, rectal feces and tonsils were analyzed for the presence of Salmonella. The diaphragm samples were frozen at  $-20^{\circ}\text{C}$ , and then thawed again to collect exudates for detection of Salmonella-specific antibodies using the above mentioned ELISA.

The transmission of Salmonella Typhimurium in every group was estimated on the basis of the stochastic 'SI' infection model (Susceptible-Infectious) using an adjusted reproduction ratio 'RT' ('Transmission') that expresses the mean number of secondary infected animals caused by 2 infectious animals ( $I_0=2$ ) in a population of 6 susceptibles ( $S_0=6$ ) during a period of 6 weeks. This RT value was estimated via the Maximum Likelihood Estimation (MLE), and this for all groups (except the negative control) and for the following parameters: i) total amount of positive feces samples, ii) number of positive samples of ileum and/or ileum contents and/or caecum and/or caecum contents, iii) number of positive lymph nodes and/or tonsils, iv) the number of all positive organs and/or feces samples (table 1). Differences in the serologic response between the intervention groups were analyzed through the module PROC MIXED in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

## Results

All pigs were seronegative before the start of the study. The evolution of serum titers of the different treatment groups is shown in figure 1. The group receiving acidified water had a significantly higher mean antibody level compared to the positive control group and the Ca-butyrate supplemented feed group.

Results on Salmonella isolation from feces are shown in figure 2; significantly fewer pigs of the vaccination and the Ca-butyrate supplemented feed group excreted the challenge strain, compared to the acidified water and the positive control group (fewest in vaccination group, most in acidified water group). The quantitative determination of Salmonella spp. in feces 3, 7 and 24 DPI confirmed these isolation results. The same significant differences were found in the organs. A significantly lower antibody level was detected in the diaphragm fluid compared to serum.

The calculated adjusted reproduction ratio RT indicated that transmission was less successful in the vaccination group ( $RT=2.6$ ) and in the group with coated butyric acid in the feed ( $RT=1.76$ ) compared to the positive control and the group with acidified water ( $RT=+$  with lower limit 1.88), looking at all organs and/or all collected faeces samples (table 1).

## Discussion

In this study we evaluated and compared 3 intervention strategies on the transmission of Salmonella Typhimurium in pigs. Previous studies have demonstrated the possible utility of organic acids in feed or drinking water, which may reduce the number of Salmonella-positive pigs in a farm, especially coated butyric acid (Boyen et al., 2008). In the present study we demonstrated that coated calcium-butyrate (Green-Cab-70<sup>®</sup>, Sanluc International NV) was also capable of reducing Salmonella transmission between animals: in this group the fewest animals got infected and the lowest number of infected organs was seen. However, the antibody level of this group did not differ significantly from the positive control.

Vaccination with the commercial vaccine Salmoporc<sup>®</sup> (IDT) has also been shown to reduce both fecal shedding and colonization of the porcine intestinal tract (Springer et al., 2001). Though in the present study, this group had the lowest number of shedding pigs, it was the group with the highest S/P ratio and the number of colonized organs was comparable with that of the positive control group. Thus, given that neither the antibody level nor the organ contamination matched the infectiousness of the concerned vaccination group, this might compromise the ambition to change the current surveillance site in Belgium from farm to slaughterhouse.

Interestingly, the third intervention, the addition of a mixture of formic acid, propionic acid, acetic acid and lactic acid (Agrocid Super<sup>®</sup>, AgroLogic) to the drinking water, increased the transmission rate: in comparison with the positive control group, more pigs got infected, the S/P ratio was higher and more organ samples turned out positive for the infected strain. The higher S/P ratio we found in serum than in meat juice, shows that it is essential to recalculate the percent optical density (OD%) data obtained from meat juice by the ELISA (IDEXX HerdChek swine Salmonella), although the instructions to the kit for the detection of Salmonella antibodies state that its product is directly suitable for antibody detection in meat juice and sera (Wilhelm et al., 2007).

## Conclusion

The results obtained demonstrate that vaccination with Salmoporc<sup>®</sup> and coated Ca-butyrate supplemented feed decrease the transmission of Salmonella Typhimurium in swine.

The detection of Salmonella-specific antibodies against the vaccine strain however, poses a new challenge for the assignment of Salmonella positive herds in the Belgian Salmonella monitoring and surveillance programme. Further investigations on new interventions that do not interfere with interpretation of serological data, or investigations on new diagnostics that are not influenced by vaccination, are needed.

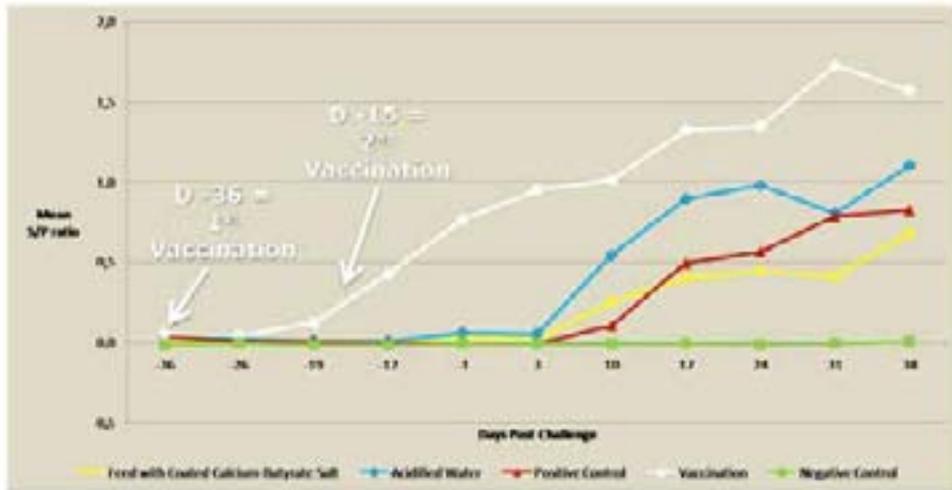


Figure 1: Serology results: Salmonella-specific antibody detection in serum

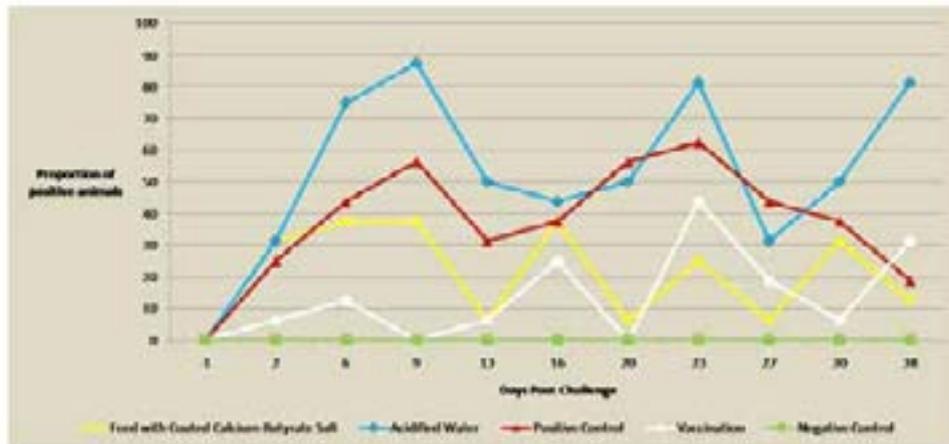


Figure 2: Bacteriology results: Isolation of inoculated Salmonella Typhimurium strain in feces.

Table 1: Calculated RT-values with the 95% confidence intervals

R <sub>T</sub>	All feces	Ileum/contents/ cecum/contents	Lymph node/ tonsil	All organs/feces
Acidified feed	1.6 [0.85; 8.43]	0.8 [0.35; 3.80]	0.3 [0.12; 2.94]	1.76 [1.02; 9.01]
Acidified Water	3.5 [1.88; 11.70]	2.5 [1.67; 24.87]	3.5 [1.88; 11.65]	+∞ [1.88; +∞]
Vaccination	1.2 [0.56; 3.95]	1.0 [0.56; 3.95]	1.2 [0.70; 7.18]	2.6 [1.21; 9.45]
Pos control	3.5 [1.88; 11.70]	1.9 [1.22; 24.78]	0.9 [0.47; 4.23]	+∞ [1.88; +∞]

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