Effects of attenuated vaccine protocols against *Salmonella Typhimurium* on *Salmonella* serology in subclinically infected pig herds

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**A B S T R A C T**

Vaccination of pigs against *Salmonella Typhimurium* (S. Typhimurium) can be effective for the control of *Salmonella* infections at the farm level and reduce the risk of *Salmonella* contamination in the food chain. However, vaccination may interfere with herd serological status in serology-based *Salmonella* monitoring programs. The present study investigated the effects of an attenuated S. Typhimurium vaccine (Salmoporc, IDT Biologika) on *Salmonella* serology in sows, neonatal piglets and slaughter pigs from three subclinically infected herds. Within each herd, five different vaccination protocols were tested as follows: group 1, vaccination of sows; group 2, vaccination of sows and piglets; group 3, vaccination of sows and fattening pigs; group 4, vaccination of piglets; and group 5 vaccination of fattening pigs. Each group was compared to a non-vaccinated control group (group 6). Sera were analyzed by ELISA (HerdChek Swine *Salmonella*, IDEXX Laboratories) and sample-to-positive (S/P) ratios were calculated.

At day 3 after farrowing, but not before vaccination, S/P ratios in vaccinated sows (mean: 2.21) were significantly higher than S/P ratios in non-vaccinated sows (mean: 0.87, \(P < 0.001\)). S/P ratios in 3-day old piglets from vaccinated sows (mean: 2.46) were significantly higher than S/P ratios in similar piglets from non-vaccinated sows (mean: 0.73, \(P < 0.001\)). At slaughter, S/P ratios in pigs from groups 2, 3, 4 and 5 were significantly higher than those in the non-vaccinated control group (\(P < 0.001\)). Therefore, vaccination of piglets and fattening pigs could have implications for current serology-based *Salmonella* monitoring programs in slaughter pigs.

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**Introduction**

Pig herds are frequently colonized with *Salmonella enterica* serovar *Typhimurium* (S. Typhimurium). Infections with S. Typhimurium in pigs constitute a major risk for human salmonellosis, which is the second most reported foodborne zoonosis in Europe [EFSA, 2015a,b, 2016, 2017]. Most S. Typhimurium infections in pigs are subclinical (presence of S. Typhimurium, without clinical signs of *Salmonella* infection, e.g. diarrhea), which, together with the intermittent shedding pattern of infected pigs and the common occurrence of carriers, make it difficult to detect and control subclinical infections at the farm level [Funk et al., 2001; Pires et al., 2013].

Vaccination is useful for the control of *Salmonella* infections in pig herds (Haesebrouck et al., 2004; Denagamage et al., 2007; Boyen et al., 2008; de la Cruz et al., 2017; Wales and Davies, 2017). The main goals of vaccination are to prevent clinical salmonellosis, to decrease shedding and colonization, and to decrease the probability of contamination of carcasses (Haesebrouck et al., 2004; Meeussen et al., 2007; Boyen et al., 2008; Arguello et al., 2012; Wales and Davies, 2017). Several papers report the effects of different vaccines against *Salmonella* infection in pigs, either under experimental or field conditions (Denagamage et al., 2007; de la Cruz et al., 2017; Wales and Davies, 2017). Most studies measured vaccine efficacy by comparing the presence of *Salmonella* in faeces and/or organs in vaccinated and control groups (de la Cruz et al., 2017).

Evaluation of the serological response after vaccination is important because it may interfere with the currently widely used (compulsory) serology-based *Salmonella* monitoring programs (e.g. in Belgium, Denmark, The Netherlands and Germany).
(Pearson et al., 2017; Wales and Davies, 2017). Most serology-based Salmonella monitoring programs classify farms into risk categories by probability of contamination of carcasses based on the presence of Salmonella-specific antibodies in blood samples collected from fattening pigs. The classification system, cut-off values, and possible consequences (e.g. logistic/separated slaughter or penalties) vary between countries.

The aim of this study was to investigate the effects of five different vaccination protocols against S. Typhimurium on Salmonella serology in subclinically infected pig herds; specifically, vaccination of sows, sows and piglets, sows and fattening pigs, piglets only, or fattening pigs only. The specific objectives were to evaluate the serological response of sows after vaccination, the effect of sow-vaccination on maternally derived antibodies in 3-day old piglets, and the effect of different vaccination protocols on Salmonella serology in slaughter pigs.

Materials and methods

Farm selection and description

Three Belgian pig farms (A, B, C) were selected for the study based on the presence of S. Typhimurium, high Salmonella-specific antibody concentrations in fattening pigs (sample-to-positive [S/P] ratios > 0.6), and the absence of clinical salmonellosis outbreaks. A description of the selected farms is shown in Table 1.

Experimental design

In each farm, one batch of sows was selected, from which 72 sows of different parities were allocated to six experimental groups using a convenience method, each group consisting of 12 sows and their offspring. Five different vaccination protocols were used as follows: group 1, vaccination of sows; group 2, vaccination of sows and piglets; group 3, vaccination of sows and fattening pigs; group 4, vaccination of piglets; group 5, vaccination of fattening pigs. These five experimental groups were compared to a non-vaccinated control group (group 6). The sows were monitored during two consecutive production cycles and their piglets were followed from birth until slaughter.

An attenuated histidine-adenine auxotrophic vaccine (Salmoporc, IDT Biologika) was used for all vaccinations. Details of the vaccination protocols are shown in Table 2. The administration of antimicrobials was prohibited from 5 days before vaccination until 5 days after vaccination. In the farrowing house, transfer of piglets to another sow was only allowed within the same experimental group. During the nursery and fattening period, the pigs from each experimental group were housed in separate pens within the same room. Nose-to-nose-contact with pigs from different experimental groups was possible in all fattening houses, and in the nurseries of farm C.

Sampling design

In the first production cycle, blood samples from three sows/group/farm were collected before the first vaccination. Blood samples from the same sows were collected 3 days after farrowing. From every sampled sow, 3 or 4 piglets were selected using a convenience sampling method (total: 10 piglets/group/farm) and a blood sample was collected 3 days after farrowing. In the slaughterhouse, blood samples of 10 slaughter pigs/group/farm were collected.

In the second production cycle, blood samples of 10 slaughter pigs/group/farm were collected in the slaughterhouse.

The study was approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University (Approval number: EC 2015/06; Approval date: 9 May, 2015).

Serological analysis

After coagulation, blood samples were centrifuged for 5 min at 3000 × g to collect serum. All serum samples were analyzed for the presence of Salmonella-specific antibodies with a commercial ELISA kit based on lipopolysaccharide (LPS) O-antigens of serogroup B, C1 and D (Herdevet Swine Salmonella, IDEXX Laboratories) and the results were calculated according to the manufacturer's guidelines. The results were expressed as sample to positive (S/P) ratios, which is common in Belgium. In other countries, optical density percentage (OD%) is used to express results. S/P ratios can be converted to OD% using the following formula: (S/P ratio/2.5) × 100%.

Statistical analysis

Prior to the statistical analysis, all S/P ratios were transformed using natural logarithms to create normally distributed data. For the analysis of the S/P ratios in sows and piglets at day 3 after farrowing, linear regression models were used. Farm and group were included as fixed effects. To compare the S/P ratios in sows and piglets per farm, independent sample t tests were used. Pearson-correlations between the S/P ratios in sows and their piglets were determined using non-transformed S/P ratios. For the analysis of S/P ratios in slaughter pigs in each production cycle, linear regression models, in which farm and group were included as fixed effects, were used. For the analysis of the combined results from production cycles 1 and 2, a linear regression model, in which farm, production cycle and group were included as fixed effects, was used. ANOVA-tests were used to compare the S/P ratios in slaughter pigs by farm. The least significant difference (LSD) procedure was used for post-hoc-testing to determine differences between vaccinated groups and the non-vaccinated control group. To determine differences between groups mutually, the Scheffe’s post-hoc test was used. All statistical analyses were performed in SPSS Statistics (version 25). P values < 0.05 were considered statistically significant.

Results

Serology of sows and 3-day old piglets

The mean S/P ratios in sows and 3-day old piglets are shown in Table 3. In the combined analysis of all farms at day 3 after farrowing, the S/P ratios in vaccinated sows (mean: 2.21) were significantly higher than those in non-vaccinated sows (mean: 0.87), but they were not significantly different before vaccination (P > 0.05). The S/P ratios in 3-day old piglets from vaccinated sows (mean: 2.46) were significantly higher than those in piglets from non-vaccinated sows (mean: 0.73).

In Fig. 1, the S/P ratios in piglets are plotted against the S/P ratios in sows. The Pearson-correlation between S/P ratios in sows and piglets was 0.923 (P < 0.001).

Serology at slaughter

The mean S/P ratios from the slaughter pigs on farms A, B and C in production cycles 1 and 2 are shown in Table 4. The mean S/P ratios from the slaughter pigs in production cycles 1 and 2 combined are presented in Fig. 2. In all linear regression models, there were significant differences between farms (P < 0.05). In the analysis of combined results from production cycles 1 and 2, the differences between groups were related to farm and cycle, but

<table>
<thead>
<tr>
<th>Farm</th>
<th>Farm A</th>
<th>Farm B</th>
<th>Farm C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm type</td>
<td>Farrow-to-finish</td>
<td>Multiplier</td>
<td>Farrow-to-finish</td>
</tr>
<tr>
<td>Number of sows</td>
<td>400</td>
<td>550</td>
<td>450</td>
</tr>
<tr>
<td>Batch production system for the sows (weeks)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Weaning age (days)</td>
<td>20</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Nursery: floor type</td>
<td>Fully slatted</td>
<td>Fully slatted</td>
<td>Fully slatted</td>
</tr>
<tr>
<td>Nursery: feed</td>
<td>Crumbled</td>
<td>Meal</td>
<td>Meal</td>
</tr>
<tr>
<td>Fattening stable: floor type</td>
<td>Fully slatted</td>
<td>Fully slatted</td>
<td>Partially slatted</td>
</tr>
<tr>
<td>Fattening stable: feed</td>
<td>Pellets</td>
<td>Meal</td>
<td>Meal/crumbled</td>
</tr>
<tr>
<td>Start study</td>
<td>Spring 2015</td>
<td>Spring 2015</td>
<td>Spring 2016</td>
</tr>
</tbody>
</table>
Table 2
Vaccination schedule for different groups\(^a\) on farms A, B and C.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sows (subcutaneously)</th>
<th>Piglets (orally)</th>
<th>Fattening pigs (subcutaneously)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 and 3 weeks before farrowing</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>6 and 3 weeks before farrowing</td>
<td>Day 3 and day 24</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>6 and 3 weeks before farrowing</td>
<td>NA</td>
<td>11–12 weeks and 14–15 weeks of age</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>Day 3 and day 24</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>11–12 weeks and 14–15 weeks of age</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, Not applicable.
\(^a\) Group 1, vaccination of sows; group 2, vaccination of sows and piglets; group 3, vaccination of sows and fattening pigs; group 4, vaccination of piglets; group 5, vaccination of fattening pigs; group 6, non-vaccinated control group. Sows, piglets and fattening pigs were vaccinated against S. Typhimurium with an attenuated histidine-adenine auxotrophic vaccine (SalmoporC, IDT Biologika). In the second production cycle, sows were vaccinated at 3 weeks before farrowing and replacement gilts in groups 1, 2 and 3 (n = 40; 2–7)group/farm were vaccinated 6 and 3 weeks before farrowing.

Table 3
Mean S/P ratios and standard deviations (SD) of sows\(^a\) and 3-day old piglets from vaccinated and non-vaccinated sows in farms A, B and C in production cycle 1.

<table>
<thead>
<tr>
<th>Farm A</th>
<th>Farm B</th>
<th>Farm C</th>
<th>Farm A+B+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean S/P-ratio (n)</td>
<td>SD</td>
<td>P</td>
<td>Mean S/P-ratio (n)</td>
</tr>
<tr>
<td>Before vaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated sows</td>
<td>1.45 (9)</td>
<td>0.80</td>
<td>0.556</td>
</tr>
<tr>
<td>Non-vaccinated sows</td>
<td>1.40 (9)</td>
<td>0.53</td>
<td>0.67 (7)</td>
</tr>
<tr>
<td>Day 3 after farrowing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated sows</td>
<td>2.53 (9)</td>
<td>0.40</td>
<td>0.001</td>
</tr>
<tr>
<td>Non-vaccinated sows</td>
<td>1.11 (9)</td>
<td>0.58</td>
<td>0.45 (7)</td>
</tr>
<tr>
<td>Piglets from vaccinated sows</td>
<td>2.67 (30)</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Piglets from non-vaccinated sows</td>
<td>1.33 (30)</td>
<td>0.79</td>
<td>0.40 (30)</td>
</tr>
</tbody>
</table>

\(^a\) Blood samples from the same sows were collected before vaccination and 3 days after farrowing. At day 3 after farrowing, the S/P ratios in vaccinated sows were significantly higher than those in non-vaccinated sows, but they were not significantly different before vaccination. The S/P ratios in 3-day old piglets from vaccinated sows were significantly higher than those in piglets from non-vaccinated sows.

Fig. 1. Correlation between the sample-to-positive (S/P) ratios in sows and 3-day old piglets on farms A, B and C in production cycle 1. Blood samples from three sows/group/ farm and three or four piglets from each sampled sow (total: 10 piglets/group/farm) were collected 3 days after farrowing. Serum samples were analyzed for Salmonella-specific antibodies with a commercial ELISA kit (HerdChek Swine Salmonella, IDEXX Laboratories). Pearson-correlation coefficient for S/P ratios in sows and piglets was 0.923 (P < 0.001).

There were no significant differences between production cycles. Results from farm A and C were mostly consistent with the results of the combined analysis of all farms together, but results from Farm B did not follow the same pattern.

In the combined analysis of all farms in both production cycles, S/P ratios in slaughter pigs from group 2 (vaccination of sows and piglets, mean: 1.50), group 3 (vaccination of sows and fattening pigs, mean: 1.76), group 4 (vaccination of piglets, mean: 1.75) and group 5 (vaccination of fattening pigs, mean: 1.88) were significantly higher than those in slaughter pigs from the non-vaccinated control group (mean: 1.03). There were no significant differences between the S/P ratios in slaughter pigs from group 1 (vaccination of sows, mean: 0.80) and the non-vaccinated control group (P > 0.05).

Using the Scheffe’s post-hoc test, there were two homogeneous subsets: subset 1, which consisted of group 1 (vaccination
of sows) and the non-vaccinated control group; and subset 2, which consisted of group 2 (vaccination of sows and piglets), group 3 (vaccination of sows and fattening pigs), group 4 (vaccination of piglets) and group 5 (vaccination of fattening pigs).

**Discussion**

Vaccination of sows induced a serological response and increased the concentration of maternally derived antibodies in 3-day old piglets. There was also a significant correlation between the serological results in sows and their offspring. These results are consistent with transfer of maternal antibodies, since sow colostrum is mainly a transudate from sow serum (Bourne, 1973), and piglets completely depend on colostrum as a source of antibodies (Bourne, 1976).

Slaughter pigs that had been vaccinated either as piglets or fattening pigs had significantly higher S/P ratios than non-vaccinated pigs. The S/P ratios in unvaccinated pigs from vaccinated sows (group 1) were not significantly different from S/P ratios in non-vaccinated pigs at slaughter. Although results varied between farms, this suggests that vaccination of piglets and fattening pigs could interfere with currently used serology-based Salmonella monitoring programs in slaughter pigs.

Previous studies using the same attenuated S. Typhimurium vaccine in sows and/or piglets of 3–42 days of age (Lindner et al., 2007; Roesler et al., 2010), did not demonstrate measurable effects on Salmonella serology at slaughter. However, it is important to consider the cut-off value that was applied. When the cut-off value used in the formerly compulsory serology-based Salmonella monitoring program in Belgium was used (S/P ratio 0.6),
vaccination did not result in a change in herd serological status. All groups, including the non-vaccinated control group, would be classified as ‘at risk’ in the first and second production cycle. When an S/P ratio of 1.00 was used as the cut-off value (as it is in The Netherlands and Germany), vaccination of piglets and fattening pigs resulted in a change in herd serological status, as homogeneous subset 1 (group 1: vaccination of sows and the non-vaccinated control group) would be classified as ‘no risk/ borderline risk’, while homogeneous subset 2 (group 2, vaccination of sows and piglets; group 3, vaccination of sows and fattening pigs; group 4, vaccination of piglets; and group 5, vaccination of fattening pigs) would be classified as ‘at risk’.

The serological test used in this study detects antibodies against LPS from Salmonella serogroups B, C1 and D. Therefore, antibodies against S. Typhimurium and other serotypes in these serogroups are detected. In addition, when vaccination against S. Typhimurium is administered, antibodies against S. Typhimurium can be produced in response to vaccination, and/or to field infection. Consequently, the S/P ratios in our study might have resulted from the combined effect of vaccination and field infections. In endemically infected farms, vaccination might prime the immune system and result in more pronounced antibody responses upon subsequent field infections (Wales and Davies, 2017). In farm B, in contrast with farms A and C, none of the faecal and overshoe samples collected in the fattening unit were Salmonella positive (data not shown). Therefore, the infection pressure in the fattening unit of farm B was assumed to be low, which might explain the less pronounced antibody response in the slaughter pigs from this farm.

Since it takes at least 7–14 days for pigs to seroconvert after an experimental Salmonella infection (Nielsen et al., 1995; van Winsen et al., 2001), and possibly even longer after field infection (Kranker et al., 2003), serology-based Salmonella monitoring programs in slaughter pigs might underestimate the number of pigs infected with Salmonella. The agreement between serology results and bacteriological evaluation of samples collected at slaughter is weak, especially at the individual pig level (Botteldoorn et al., 2003; Nollet et al., 2005; Van Parys et al., 2013). Therefore, to correctly classify farms according to the risk they pose for (cross-) contamination at the slaughterhouse, Salmonella monitoring programs should ideally be based on bacteriological evaluation of samples collected at slaughter.

The development of efficacious DIVA (differentiating infected from vaccinated animals) vaccines (Leyman et al., 2011; De Ridder et al., 2013; Pearson et al., 2017) could be helpful to overcome the issue of vaccination interfering with the currently used serology-based Salmonella monitoring programs in slaughter pigs. Alternatively, although several practical issues need to be overcome first (e.g. correct and confirmed categorization of farms/animals), implementing a special category for farms which use vaccination is worthy of further investigation.

Conclusions

Vaccination of sows induced a serological response and increased the concentration of maternally derived antibodies in 3-day-old piglets. Vaccination of piglets and fattening pigs resulted in higher S/P ratios at slaughter, which could interfere with widely used serology-based Salmonella monitoring programs in slaughter pigs.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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References


