MATERNAL VACCINATION AS AN EFFECTIVE SALMONELLA REDUCTION STRATEGY

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Introduction

Salmonella is a widespread pathogen that can infect a variety of animals, including man. Pork is considered, after eggs, the major source of infection in humans in the EU, with S. Typhimurium (ST), including monophasic strains (mST; S. 1,4,[5],12:i- and S. 1,4,12:i-) being frequently implicated [1]. Reducing the prevalence of infected pigs on-farm contributes to minimising contamination of pig meat and edible offal for human consumption, as the slaughter process alone cannot cope with high levels of contamination. The persistent and frequently asymptomatic nature of porcine Salmonella infection, the organism’s colonisation of farm pests, such as rodents and wild birds, and ability to survive in the environment, means that effective control generally requires multiple measures [2]. It is generally accepted that vaccination can play a role in reducing the prevalence of Salmonella in pigs and could assist other on-farm control measures by helping to prevent Salmonella colonizing the gut and reducing the subsequent shedding and development of a carrier state [3]. Several vaccines for Salmonella have been developed; from inactivated bacterins to elicit a humoral response, to live or adjuvanted vaccines that can stimulate cell-mediated immunity. A recent study examined the vaccination of sows in three farms with follow-up of the breeding and rearing animals for up to two years after the initial pre-vaccination visit [4]. The study provided evidence for sustained reductions of ST and mST-shedding among pigs up to slaughter age, although it was based on an uncontrolled observational field study. This project was tasked with investigating the effectiveness of sow vaccination with a live Salmonella Typhimurium vaccine by comparing results from eight indoor farrow-to-finish herds that were vaccinated and eight similar control herds.

Material and methods

Farms were selected based on the following inclusion criteria: (i) indoor breeder-finisher enterprise, (ii) Herd size of 100-600 sows, (iii) a recent occurrence of ST or mST, (iv) presence of ST or mST in finishing pigs, (v) sows free of significant clinical disease which may have affected the efficacy of the vaccine. Herds were randomised into vaccinated (n=8) and non-vaccinated control groups (n=8). Herds were followed for approximately 69 weeks after the start of the trial, with four sampling visits. Sows were vaccinated with a live attenuated vaccine by subcutaneous injection (Salmoporc STM, IDT Biologika GmbH, Dessau-Rosslau, Germany). Vaccine was administered to pre-partum sows (6 weeks and 3 weeks ante-partum) with a single booster dose three weeks before each subsequent farrowing. The first dose was given to the first batch of
sows in week 1, with sampling visits taking place prior to vaccination (week 0); at a point when half of the progeny were estimated to originate from vaccinated sows (week 21); when all of the finishers were from vaccinated sows (week 55); and a final sampling visit three to four months after visit 3 (week 69).

A target of sixty individual floor faeces samples were collected at each visit from each of the following pig stages: weaners, growers, and finishers, providing a 95% probability of detection per group, assuming a 5% prevalence. In addition, pooled pen faeces samples (one or two pools per pen according to the number of pigs in the pen) were taken from the following pig stages: gestation, farrowing, weaners, growers, finishers and a combination of dry sows, gilts and boars. For each pig stage, up to a maximum of 20 pooled samples were collected per building and 60 per pig stage to ensure effective detection of *Salmonella* prevalence and diversity of serovars across the farm. In addition, wildlife and environmental samples (wildlife faeces, pooled water, etc) were collected at each visit. Material was cultured for *Salmonella*, using a modification of the ISO 6579:2002 (Annex D) method, as described previously [5]. Briefly, all pooled faeces samples (approximately 25 g) and swabs were pre-enriched in 225 ml BPW at 37°C for 18 h followed by enrichment in Modified Semi-Solid Rappaport-Vassiliadis medium (MRSV) for 24h and 48h at 41.5°C then plating on Rambach agar which was incubated for 24h at 37°C. Sub-samples (2 g) of individual pig faeces samples were pre-enriched in 20 ml BPW and cultured as above. For *Salmonella*-positive individual faeces samples, a subset from each farm, building and epidemiological group sampled was subjected to a semi-quantitative enumeration procedure by creating a decimal dilution series in BPW immediately before pre-enrichment. A selection (all isolates from pooled samples and individual samples that was cultured semi-quantitatively) of *Salmonella* isolates were serotyped using standard methodology.

A mixed-effects logistic regression model was used to assess the effect of vaccination, to examine the association between time from the start of vaccination and the odds of a sample being *Salmonella*-positive. The *a priori* variables were pig stage from which the sample was collected (named pig type), sample type (individual or pooled) and sampling season (winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) and autumn (Sep-Nov)). The farm study identifier was added as a random effect to account for the non-independence of sample results from the same farm. An interaction term, including visit number and experimental group (vaccine or control), was added. Two outcomes were tested in the model: presence of all *Salmonella* or presence of only serovars of public health concern (ST/mST). All analyses were performed in Stata 12 (StataCorp, College Station, Texas, USA).

**Results**

A total of 22,246 samples (9,747 pooled faeces samples, 10,905 individual faeces samples and 1,594 environmental samples) were collected from farm visits conducted between April 2014 and May 2016. The initial visit (visit 1) results demonstrated a similar high prevalence of *Salmonella* from faeces samples in both vaccine and control groups; 30.8% vs 36.2% of pooled samples, 19.1% vs 21.9% of individual samples, and 34.6% vs 53.0% of environmental samples, for vaccine and control groups respectively (Table 1). Clinical problems associated with *Salmonella* infections were reported from six vaccine and three control farms respectively at visit 1.
Table 1. Results from the pooled and individual faecal samples and environmental samples collected for the evaluation of the protection against Salmonella Typhimurium and its monophasic variants conferred by a vaccine administered to sows on eight pig herds and compared to eight control farms. Salmonella vaccination commenced between the first and second visit (N: total number of samples.)

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<tr>
<th>Visit</th>
<th>Pooled samples</th>
<th>Individual samples</th>
<th>Environmental samples</th>
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<tbody>
<tr>
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<td>Vaccine</td>
<td>Control</td>
<td>Vaccine</td>
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<tr>
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<td>1,279</td>
<td>26.1</td>
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<td>4</td>
<td>1,288</td>
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S. Typhimurium and monophasic variants -positive

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At the second and third visits, following the start of the vaccination programme, reduction in prevalence of Salmonella and ST/mST was not apparent in control farms. However, vaccine farms showed sustained reduction of Salmonella prevalence up to the final visit. In addition, a higher proportion of vaccine farms (5 farms out of 6) resolved clinical salmonellosis than control farms (2 of 3). The effect of vaccination was not consistent on all farms; in one farm prevalence increased at visit 2 and this rise was sustained up to the final visit for both pooled samples and individual samples. Another vaccine farm showed only a slight reduction after vaccination, with a similar sample prevalence observed at visits 2 and 3 to that at visit 1. The results of Salmonella enumeration in positive faecal samples showed no apparent significant effect of vaccination.

The mixed-effects models showed a significantly decreased odds ratio of Salmonella-positive (Odds Ratio (OR) = 0.73, p<0.001) and ST/mST-positive samples (OR = 0.71, P<0.001) for vaccine farms in comparison to control farms and that samples collected at visit 2 where at significantly lower odds for both outcomes than at the first visit. Examining the interaction between the experimental groups and visit number showed there was a significantly decreased odds of Salmonella-positive (OR = 0.51, p<0.001) or ST/mST-positive (OR = 0.61, p<0.001) at visit 4 for vaccine farms only. The inclusion of the a priori variables accounted for a significantly increased odds of isolation of Salmonella and ST/mST in pooled samples. There was a significantly increased odds of isolation of summer of Salmonella and ST/mST and an increase in spring and autumn for ST/mST-positive when compared with winter. Finally, the model showed significantly increased odds of Salmonella-positive and ST/mST-positive samples for all pig group types (except boars) and significantly reduced odds for farrowing groups, when compared against the gestation group.
Conclusion

The significant results of the mixed-effects model have demonstrated that the strategy of maternal vaccination against ST is able to reduce, in a substantial proportion of treated farms, both faecal and environmental prevalence of *Salmonella* in farrow-to-finish pig herds. Although a beneficial association between vaccination and *Salmonella* reduction was observed, vaccination strategies alone were not sufficient to eliminate infection and vaccines should preferably be applied to uninfected animals on a preventative basis rather than in the face in infection [6].

Vaccinal protection of sows is particularly relevant in farrow-to-finish pig herds where breeders and finishers are housed in the same environment and weaned pigs present a continuous source of environmental contamination with ST or mST. Once all sows were vaccinated a reduction in *Salmonella* prevalence was observed in all stages of pig production, and mainly in finishers, hence reducing the *Salmonella* burden before slaughter. Previous findings have also shown that pigs born from vaccinated sows have reduced *Salmonella* faecal shedding and that the effect on environmental contamination and re-cycling of infection is also important [4].

However, the *Salmonella* prevalence reduction observed in the vaccinated farms was not observed in all herds, and this is consistent with other studies. De Ridder et al [7] observed response variability after oral vaccinated of piglets with the same product on three farrow-to-finish pig herds. In our study, vaccination did not have a marked effect on two herds which had clinical salmonellosis reported before the start of vaccination, which may have represented a recent outbreak caused by a new strain and presenting an overwhelming challenge for the vaccine within the timescale of the study. Our results provide evidence that maternal vaccination on a farrow-to-finish pig herd was a suitable ST/ mST reduction strategy and helped to control clinical salmonellosis. *Salmonella* vaccines therefore have the potential to reduce prevalence of *Salmonella* in pigs and result in a reduction of human cases.

Acknowledgements

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References


