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Maternal vaccination as a Salmonella Typhimurium reduction strategy on pig farms

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Abstract

The control of *Salmonella enterica* in pig production is necessary for both public and animal health. Vaccination is one control measure that has potential to decrease slaughter pig prevalence. The study examined the efficacy of a licensed live *Salmonella* Typhimurium vaccine, administered to sows on eight commercial farrow-to-finish pig herds experiencing clinical salmonellosis or high prevalence of *Salmonella* carriage associated with *S*. Typhimurium or its monophasic variants (*S.* 1,4,[5],12:i- or *S*. 1,4,12:i-). Results of longitudinal *Salmonella* sampling were compared against eight similarly selected and studied control farms. One year after the start of vaccination, when all finishing stock had been born to vaccinated sows, clinical salmonellosis resolved and both faecal shedding and environmental prevalence of *S*. Typhimurium substantially declined in the majority of farms. However, *Salmonella* counts in positive faeces samples were similar between non-vaccinated and vaccinated herds. In addition, vaccination did not have a measurable impact on piglets' and sows' performance, including average daily liveweight gain for piglets. The results suggest that maternal vaccination as a *Salmonella* Typhimurium reduction strategy in farrow-to-finish pig herds seems to be a suitable option, especially for *S*. Typhimurium and its monophasic variants , although significant cross-protection against other serovars was not observed.

Keywords: Salmonella, Typhimurium, Vaccination, vaccine, Pig, Swine, Field study

1. Introduction

The 2015 EFSA summary report on zoonoses, zoonotic agents and food-borne outbreaks reported that *Salmonella* was responsible for the vast majority of food-borne outbreaks in the EU (21.8%, EFSA, 2016). It is estimated that 13% of outbreaks are associated with pig meat and products thereof (EFSA, 2016). Pork is considered, after eggs, the major source of infection in humans in the EU, with *S*. Typhimurium, including monophasic strains (*S*.1,4,[5],12:i- and *S*. 1,4,12:i-) being frequently implicated (Andres and Davies, 2015; Davies et al., 2016). Nonetheless, within the EU, there is no mandatory programme for the control of *Salmonella* at pork primary production level. The European Commission (EC) has considered the measures that could be applied in order to reduce the *Salmonella* prevalence in pigs across the member states, and it is likely that successful control will include effective pre-harvest actions in breeding herds (Andres and Davies, 2015). The EU was originally expected to introduce regulations concerning the monitoring and control of *Salmonella* in pigs after an initial focus on the control of Salmonella in poultry and its subsequent reduction, although proposals were dropped following a negative cost–benefit analysis (DG SANCO 2010). However, despite enhanced hygiene interventions at slaughter , the control of *Salmonella* carriage and shedding remains a challenge in most countries (Davies et al., 2016).

The persistent and frequently asymptomatic nature of porcine *Salmonella* infection and the organism's ability to colonize other animal species, such as rodents and wild birds on farms, and to survive in the environment means that effective control generally requires multiple measures (Wales and Davies, 2017). In summary, control measures against *Salmonella* infection can be divided into five broad interventions: biosecurity/SPF status, feed management, acidification of feed or water, manipulation of gut microbiota, and vaccination (Andres and Davies, 2015; Wilhelm et al., 2017). Wilhelm et al. (2017) suggests that biosecurity and vaccination seem to be the intervention categories showing the greatest potential to minimise *Salmonella* on an infected

58 farm, and only culling of infected pigs can totallly eliminate infection, but in most countries a
59 source of *Salmonella*-free replacement pigs is not available and the risk of re-infection is high
60 (Wales & Davies, 2017).

61 It is generally accepted that vaccination can play a role in reducing the prevalence of *Salmonella* in
62 pigs and could become an adjunct to other on-farm control measures (Denagamage et al., 2007)
63 by helping to prevent *Salmonella* colonizing the gut and reducing the subsequent shedding and
64 development of a carrier state (Haesebrouck et al., 2004). Over many years, several candidate
65 vaccines for *Salmonella* in pigs have been developed; from inactivated bacterins to elicit a humoral
66 response to live or adjuvanted vaccines that additionally stimulate cell-mediated immunity (Davies
67 et al., 2016). Live vaccines theoretically offer the best option, since they are able to stimulate cell-
68 mediated immunity (Mastroeni et al., 2001; Haesebrouck et al., 2004), but the extent of this may
69 be limited by the attenuation process necessary for licencing the vaccine as being cleared from the
70 body before slaughter of pigs and non-persistent in the environment. Vaccination strategies that
71 involve stimulating both passive immunity from the dam plus active immunity in offspring appear
72 to be most efficacious, although either approach alone can yield significant control of *Salmonella*
73 (Wales and Davies, 2017). Relatively few vaccination studies with *Salmonella* have been
74 undertaken under field conditions on pig farms and most of these have been conducted with small
75 numbers of animals (Schwarz et al., 2011; Arguello et al., 2013; De Ridder et al., 2014; Ruggeri et
76 al., 2015; Davies et al., 2016). Several studies have evaluated live vaccines for *Salmonella*
77 Choleraesuis, a serovar that is particularly pathogenic to both pigs and some humans (Schwarz et
78 al., 2011), but is now rarely reported in Europe (EFSA, 2016; Wales and Davies et al., 2017). The
79 remaining studies have been carried out with an attenuated vaccine for *S.* Typhimurium
80 (Salmoporc STM, IDT Biologika) available commercially for use in the EU. Currently, monophasic
81 variants of *S*. Typhimurium (mST) (*S*. 1,4,[5],12:i- and *S*. 1,4,12:i-) have emerged as a public health

threat, and mST is the third most frequently isolated serovar from human cases of salmonellosis in Europe, representing 8.3%, of 69,663 confirmed human cases in 2015 (EFSA, 2016). These vaccination studies found a reduction of faecal shedding by fattening pigs (Arguello et al., 2013; De Ridder et al., 2014). When sows plus piglets were vaccinated, a consistent reduction in shedding was observed, but results were more variable and lacked statistical significance (Ruggeri et al., 2015). Recently, Davies et al. (2016) examined the immunization 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. Although the study provided sustained reductions in *Salmonella* Typhimurium and mST-shedding among pigs up to slaughter age, it was based on an observational study under field conditions, which was uncontrolled. Longitudinal field studies examining natural infections are comparatively uncommon amongst reports of *Salmonella* vaccination trials in pigs (Davies et al., 2016).

In the present study, we developed a long-term longitudinal field study to evaluate the efficacy of vaccination with an inactivated *S.* Typhimurium vaccine to all breeding sows present in the herd as a strategy to reduce the prevalence of *Salmonella* infection throughout rearing in farms with a salmonellosis problem.

2. Materials and methods

2.1. Farms

A total of 35 farms were invited to participate in the study. Farms were selected based on the following inclusion criteria: (i) indoor breeder-finisher enterprise, (ii) Herd size of 100-600 sows, (iii) a significant recent occurrence of *S.* Typhimurium (ST) or mST, (iv) presence of ST or mST in

finishing pigs, (v) farmer willing to be involved for the entire study period and (vi) sows free of significant clinical disease which may affect the efficacy of the vaccine.

2.2. Sampling visits and vaccination schedule

Farms were randomised into vaccinated (n=8) and non-vaccine (n=8). Farms were followed for approximately 69 weeks after the start of the trial, with sampling intervals as follow (details of the study design are summarised in Table 1). Briefly, sows were vaccinated with a live attenuated vaccine by subcutaneous injection (Salmoporc STM, IDT Biologika, Dessau-Rosslau, Germany). Vaccine was administered to pre-partum sows (6 weeks and 3 weeks ante-partum) and one booster dose three weeks before each subsequent farrowing. The first dose was given to the first batch of sows in week 1 and the second dose in week 4. The piglets (progeny) from the first batch of vaccine sows were estimated to go to slaughter during week 33. The last batch of sows was vaccinated in weeks 23 and 26 and farrowed in week 29, with their progeny going to slaughter in week 55. Sampling visits took place prior to vaccination (week 0), at a point where half of the progeny on the farm came were estimated to come from vaccinated sows (week 21), when all of the finishers on the farm came from vaccinated sows (week 55) and a final “follow-up” sampling visit took place up to three-four months after all of the finishers on the farm came from vaccinated sows (week 69). Sows were observed closely and any sows showing signs of ill health were treated as appropriate. All veterinary treatments were recorded including identity of sow, clinical signs, medication used and dosage.

2.3. Sampling and Salmonella detection

A minimum of sixty individual faeces samples were collected at each visit per epidemiological group (gestation, farrowing, weaners, growers, finishers, gilts, dry sows and boars) where possible given the number of animals present, providing a 95% probability of detection assuming a 5% prevalence and 100% sensitivity of detection. Faeces were collected in sterile stool sample tubes using an integral spoon. In addition, pooled pen faeces samples (one or two pools per pen according to the number of pigs in the pen, including pre-weaned piglet faeces in farrowing accommodation) were taken, using a sterile gauze swab held with a clean disposable glove for each sample. In addition, wildlife and environmental samples were collected. Solid and semi-solid material was collected using sterile gauze swabs, whilst surfaces were wiped with gauze swabs that had been pre-autoclaved in buffered peptone water (BPW). Materials and areas sampled included: rodent faeces and (occasionally) carcases; wild bird faeces; flies; cleaned and empty pens and farrowing crates; pooled water water sources, feed barrows and dust from feed handling areas; piglet transporters; handling facilities; manure heaps and associated run-off fluid; vehicles, trailers, loader buckets and scrapers.

Samples were transported to the testing laboratory on the day of collection. Material was cultured for *Salmonella* either immediately upon arrival (pooled faeces and swabs in BPW) or after overnight storage at 4 °C (individual faeces samples), using a modification of the ISO 6579:2002 (Annex D) method, as described previously (Martelli et al., 2014). Briefly, all pooled faeces samples (approximately 25 g) and swabs were pre-enriched in 225 ml BPW at 37oC for 18 h followed by enrichment in Modified Semi-Solid Rappaport-Vassiliadis medium (MRSV) for 24h and 48h at 41.5oC then plating on Rambach agar which was incubated for 24h at 37oC. Sub-samples (2 g) of individual pig faeces samples, and samples of aseptically dissected rodent carcass intestines plus liver and spleen, were pre-enriched in 20 ml BPW and cultured as above. The residue of the individual pig faeces samples were stored unprocessed at 4 °C. Among individual faeces samples

that proved *Salmonella*-positive, a representative subset of the stored material was subjected to a semi-quantitative enumeration procedure by creating a decimal dilution series in BPW immediately before pre-enrichment, as described elsewhere (Wales et al., 2006).

A selection (all isolates from pooled samples and any individual sample that was cultured semi-quantitatively) of *Salmonella* isolates were fully sero- and phage-typed in the APHA *Salmonella* reference laboratory using standard methodology (Jones et al., 2000).

2.4. Herd performance

Herd performance data was collected at the start of the vaccination programme and at the end of the study. The parameters collected were piglets born, piglets weaned, pigs sold per sow per year, slaughter live weight, litters per sow, sow mortality, sow replacement rate, sow parity max, pre-weaning mortality, weaning age, weaners mortality, growers mortality, finishers mortality, daily live weight gain and feed conversion rate.

2.5. Statistical analyses

The prevalence of *Salmonella* in faecal (pooled and individual) and environmental samples at each visit was analysed in a general linear model using experimental groups (vaccine and non-vaccine) as a fixed effect, within each visit. This statistical analysis was performed using SPSS (version 21.0) software. A p value of less than 0.05 was considered to indicate a statistically significant difference.

For overall analysis of the effect of vaccination, a mixed-effects logistic regression model was used, to examine the association between time from the start of vaccination (represented by visit number, with the first visit being before the introduction of vaccination) and the odds of a sample being *Salmonella*-positive, the hypothesis being that vaccination would progressively reduce the odds of a sample being positive over time. The *a priori* variables were pig stage from which the sample was collected (named Pig type), faeces sample type (individual or pooled), season (winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) and autumn (Sep-Nov), with the visit to the farm included as a fixed effect. The Farm study identifier was added as a random effect to account for the non-independence of sample results from the same farm. The use of farm and group random effects were tested, but the addition of group did not significantly improve the fit of the model (likelihood ratio test). An interaction term was added to allow for different effects of the vaccine over visits on the different farms. Two outcomes were tested in the model: Salmonella-positive or ST/mST-positive.

A paired T-test was used to compare herd performance at the initial visit of the vaccination programme (visit 1) and at the final visit (visit 4) for vaccine and non-vaccine groups. Statistical analyses were performed in Stata 12 (StataCorp, 2011. Stata Statistical Software: Release 12. College Station, TX: Stata-Corp LP).

3. Results

From 35 farms invited to participate in the study, six farms were not eligible as no ST/mST was detected from pen faecal swab sent to confirm status. A further seven farms were rejected due to complex multi-site operations which would have limited the ability to trace the vaccine effect in finishers. Three farms had an ineligible farm type and one farm was too small. In addition, two farms had started vaccination programmes, but were discarded as it was not possible to provide a

baseline situation. Therefore, data from 16 farms are presented in this study. From the 8 farms in the vaccine group, 5 farms employed a weekly batch sow management system and the others 3 farms, employed a two, three and four weeks batch system, respectively. In the non-vaccine group, 7 farms used a weekly batch management system and 1 farm employed a three week batch system. The mean number of sow and gilts per herd was 321 (range from 150 to 550) for vaccine farms and 406 (range from 150 to 750) for non-vaccine farms. Clinical problems (diarrhoea, septicaemia, ill-thrift and increased mortality) in weaned pigs, associated with *Salmonella* infections were identified at 5 and 3 vaccine and non-vaccine farms , respectively, ST/mST serovars had been detected in weaned pigs on all farms before the start of the trial.

3.1. Bacteriological results

A total of 22,246 samples (9,747 pooled faeces samples, 10,905 individual faeces samples and 1,594 environmental samples) were collected between April 2014 and May 2016, with an intense level of sampling per visit (mean of 374 samples collected in each visit), which increases the degree of confidence in the results . Bacteriological findings from faeces samples are summarised in Table 2. 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 vs non-vaccine groups, respectively. The proportion of *Salmonella*-positive samples ranged from 3.7% to 62.2% for vaccine farms and from 11.5% to 67.0% for non-vaccine farms in pooled samples. Prevalence of ST/mST was also high at visit 1 in both experimental groups (26.6% vs 31.3% of pooled samples, 17.8% vs 21.7% of individual samples and 30.1% vs 46.3% of environmental samples, for vaccine vs non-vaccine groups, respectively). 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 significantly (p=0.000) reduced *Salmonella* prevalence at the final visit (Table 2). For pooled faecal samples, 15.5% of vaccine farms' samples were positive for *Salmonella*, while 46.5% of samples from control farms were positive (p=0.005). For individual faeces, 11.9% of the of vaccine farms samples were positive compared with 35.5% of samples from the non-vaccine farms (p=0.009). Finally, for environmental samples, 22.7% of the samples from vaccinated farms were *Salmonella*-positive compared with 48.9% of the non-vaccine farms (p=0.035). The prevalence of *Salmonella* and ST/mST was reduced around 20% and 15%, respectively for all samples types. Vaccine farms showed significantly reduced ST/mST prevalence at the final visit (Table 2). For pooled faecal samples, 14.5% of samples from the vaccine farms contained *Salmonella* compared with 38.8% for the non-vaccine farms (p=0.019). For individual faeces, 11.6% of samples from vaccine farms were positive for *Salmonella* compared with 29.7% of samples from non-vaccine farms (p=0.035). However, for environmental samples the difference in prevalence was not significant (22.0% vs 41.0% for vaccinated vs non-vaccine group, respectively, p=0.168), probably due to the more limited number of samples..

Figure 1 summarizes the effect of sow vaccination on the *Salmonella* ~~shedding~~ sample prevalence of pigs for all the rearing stages. Weaners and finishers born from vaccinated sows showed significantly reduced *Salmonella* sample positivity (p=0.006 and p=0.000, respectively. Figure 1). Samples from rowers born from vaccinated sows also showed *Salmonella* prevalence, although the difference was not significant (p=0.057, Figure 1).

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 (3.7%, 35.8%, 29.5% and 38.5% for visits 1, 2, 3 and 4, respectively) and individual samples (0.0%, 16.2%, 26.9% and 23.3%

for visits 1, 2, 3 and 4, respectively). Another vaccine farm showed a slight reduction after the start with the vaccination, however at visits 3 and 4 had a similar sample prevalence to that observed at the beginning of the experiment (20.1%, 8.6%, 17.6% and 19.3% of pooled samples for visits 1, 2, 3 and 4, respectively, and 16.5%, 12.2%, 18.9% and 12.4% of individual samples for visit 1, 2, 3 and 4, respectively). Similarly, the sample prevalence on the non-vaccine farms was not consistent over time on all units. In one farm, a marked reduction in prevalence of *Salmonella*-positive and ST/mSTs-positive pooled and individual faeces samples was observed from visit 2 (11.5%, 3.2%, 1.5% and 2.1% of pooled samples for visits 1, 2, 3 and 4, respectively, and 8.9%, 1.7%, 1.1% and 7.8% of individual samples for visits 1, 2, 3 and 4, respectively). Nevertheless, analyses excluding data from inconsistent farms showed that vaccinated farms (75%, 6/8) experienced a significant reduction in *Salmonella*-positive and ST/mST-positive samples at the final visit of around 50% for all sample types (Table 3). However, it should also be noted that although two vaccine farms retained a ST/mST prevalence of over 20.0% at the final visit in the pooled samples, no vaccinated farm had a prevalence of over 20.0% in the individual samples.

Findings from the logistic regression analyses are summarised in table 4. Examining the relationship between vaccine and non-vaccine farms, there was a significantly decreased odds ratio (OR = 0.726, P<0.001) of *Salmonella*-positive and ST/mST-positive samples (OR = 0.706, P<0.001) for vaccine farms. Examining the relationship between vaccine and non-vaccine farms and visit number, there was a significantly decreased odds ratio (OR = 0.512, P<0.001) of *Salmonella*-positive or ST/mST-positive (OR = 0.613, P<0.001) at visit 4 for vaccine farms only. The analysis of the sample type on all the farms revealed significant increases in the odds of isolation in pooled samples (OR = 2.697, P<0.001) of *Salmonella* and ST/mST- (OR = 2.558, P<0.001). There were significant increases in the odds of isolation in summer (OR = 1.214, P=0.004) of *Salmonella* or ST/mST-positive (OR = 1.198, P=0.013) and a slight increase in Spring and Autumn (OR = 1.119,

279 P=0.025 and OR = 1.130, P=0.047) compared with Winter. Finally, the model showed significantly
280 increased odds (P<0.001) of *Salmonella*-positive and ST/mST-positive samples for all main pig
281 group types compared with boars, dry sows and environmental samples.
282
283 The results of *Salmonella* enumeration in faecal samples across the trial are shown in Table 5.
284 Although a significant reduction of *Salmonella* and ST/mST prevalence at final visit was observed,
285 there was not a significant effect of the vaccination on *Salmonella* concentration in the faecal
286 samples.
287
288 Although farms were selected because of significant occurrence of ST/mST, a total of 23 different
289 *Salmonella* serovars were identified over the entire period of the study. Nevertheless, 19 serovars
290 represent less than 1% of positive samples (data not shown). Non-ST/mST isolates from pigs were
291 confined, in decreasing order, to serovars Kedougou (5.9%) and Derby (1.8%).
292
293 3.2. Herd performance
294
295 The herd performance in first and final visits is shown in Table 6. In summary, vaccination did not
296 have any influence on the evaluated variables, including average daily live-weight gain and feed
297 conversion ratio for piglets.
298
299 **4. Discussion**
300
301 This study is the first of its kind to demonstrate that the strategy of maternal vaccination against
302 *Salmonella* Typhimurium is able to reduce, in a substantial proportion of treated farms, both

303 faecal and environmental prevalence of *Salmonella* in farrow-to-finish pig herds, especially for
304 serovars *S*. Typhimurium and its monophasic variants. Nevertheless, according to previous studies,
305 although a beneficial association between vaccination and *Salmonella* reduction was observed,
306 vaccination strategies alone are not sufficientto eliminate infection that is already present on
307 breeding pig farms and all vaccines aimed at intestinal bacteria should preferably be applied to
308 uninfected animals on a preventative basis rather than in the face in infection (Wales et al., 2011;
309 Soumpasis et al., 2012). The persistent and frequently asymptomatic nature of porcine *Salmonella*
310 infection and the organism's abilities to colonize other animal species and to survive, or even
311 multiply, in the environment mean that effective control of subclinical Salmonella infection
312 generally requires multiple approaches applied simultaneously, although clinical salmonellosis can
313 usually be markedly improved by vaccination alone, as demonstrated in the current study (Wales
314 and Davies, 2017; Wilhelm et al., 2016). In conjunction with other control measures against
315 *Salmonella* infection, vaccination may assist in the protection of animal health, reduction of
316 antibiotic usage, enhancement of food safety as well as reduction of economic losses and
317 environmental contamination associated with faecal waste, run-off and dust from pig farms and
318 transmission of *Salmonella* to other food animal species, such as poultry, by wildlife vectors
319 (Bearson et al., 2016). Vaccination is the second most frequently studied on-farm intervention
320 measure for *Salmonella* control (Wilhelm et al., 2016). However, longitudinal field studies (such as
321 the present one) examining natural infections are comparatively uncommon amongst reports of
322 *Salmonella* vaccination trials in pigs (Davies et al., 2016; Wilhelm et al., 2016). This study was novel
323 in that the trial was run under field conditions, without any interference with the farming practices
324 used on the farms, used a large numbers of animals and focused a controlled and randomized
325 study on farms with an existing *Salmonella* problem (Davies et al., 2016). Although direct
326 comparison with previous studies must be applied carefully owing to inherent experimental
327 differences (Ruggeri et al., 2015; Davies et al., 2016), our results confirm that vaccination of sows

328 can reduce the prevalence of *Salmonella* infarrow-to-finish pig herds. In addition, these results
329 highlight an important reduction in environmental contamination in the farm environment.

330 There are a number of strategies that may be used when implementing vaccination of pigs against
331 *Salmonella* (Wales and Davies, 2017). For instance, immunization of sows to protect their offspring
332 (Roesler et al., 2006; Ruggeri et al., 2015; Davies et al., 2016) or vaccination early in the pig's life
333 (Hur and Lee, 2010; Schwartz et al., 2011; De Ridder et al., 2014; Ruggeri et al., 2015), during
334 suckling (Hur et al., 2001), after weaning (Merialdi et al., 2008; Berends et al., 1996; Kranker et al.,
335 2003) or during fattening (Arguello, 2013). It has been reported that when sows were vaccinated,
336 the prevalence of *Salmonella* shedders, as well as the prevalence of seropositive pigs within the
337 progeny, was reduced and it was suggested that vaccination with an injectable vaccine for
338 breeding sows could be an easy-to-apply and economic way to reduce *Salmonella* transmission to
339 progeny and enhance maternal immunity. Other studies have suggested that additional
340 vaccination of sucking piglets and weaners would provide additional benefits, but this is less easy
341 and economic to carry out in many farming systems (Roesler et al., 2006; Andres and Davies, 2015;
342 Ruggeri et al., 2015). Vaccination of sows only would avoid the possibility of the vaccine strain
343 being present in the lymphoid tissue of slaughtered progeny (Wales et al., 2011; Wales and Davies,
344 2017). Vaccinal protection of sows is particularly relevant in farrow-to-finish pig herds where
345 breeders and finishing pigs are housed in the same environment and weaned pigs present a
346 continuous source of environmental contamination with ST or mST. (Lurette et al., 2009). The
347 carriage of *Salmonella* by piglets is readily demonstrated from the farrowing accommodation
348 onwards (Wales et al., 2011). According to Kranker et al. (2003), *Salmonella* is predominant in
349 weaners, growers, and finishers. Nevertheless, once all sows were vaccinated, a reduction in
350 *Salmonella* prevalence was observed in all these stages of pig production, mainly in finishers,
351 hence, reducing the total *Salmonell*a burden before slaughter, at the beginning of the pork-based

352 food chain. This time lag seen with reductions in shedding by growing pigs on the farrow-to-finish
353 pig herds is also consistent with enhanced passive immunity, clearance of infection and reduced
354 carriage of infection by weaners, eventually maturing into growers and finishers (Davies et al.,
355 2016). Although, previous findings have shown that pigs born from vaccinated sows show reduced
356 *Salmonella* faecal shedding (Roesler et al., 2006; Matiasovic et al., 2013). The reduction in
357 environmental contamination and re-cycling of infection is also important (Davies et al. 2016).
358 Collectively, our data suggest that maternal vaccination can significantly reduce carriage of
359 *Salmonella* in the progeny of vaccinated pigs, as well as environmental contamination.

360

361 However, the *Salmonella* prevalence reduction observed in the vaccinated farms was not
362 observed in all herds, and this is consistent with other studies. De Ridder et al. (2014), using the
363 same vaccine, observed response variability in three farrow-to-finish pig herds. In our study, in
364 two herds, vaccination did not reduce the the faecal or environmental prevalence of *Salmonella*.
365 Importantly, in these two farrow-to-finish pig herds acute outbreaks of salmonellosis occurred
366 shortly before the start of the vaccination program, which may have presented an overwhelming
367 challenge for the vaccine within the timescale of the study. In both of these herds staff reported a
368 marked decline in clinical salmonellosis following the start of vaccination. It is known that live
369 attenuated *Salmonella* Typhimurium vaccines can help prevent clinical salmonellosis, reducing
370 tissue colonization and faecal shedding (Roesler et al., 2004; Gradassi et al., 2013). In one of these
371 farrow-to-finish pig herds, peak prevalence occurred after the start of vaccination in the later
372 stages of pig production. Specifically, prevalence increased from 6.3%, 7.5% and 0% to 46.9%,
373 82.5% and 36.7%, for weaners, growers and finishers, respectively. However, at the final visit,
374 prevalence level for weaners was 0%, but growers and finishers retained high prevalence (87.8%
375 and 44.7%, respectively). This is likely to represent a delayed effect of the vaccine in the face of

very high levels of infection at the start of the study, but it may also suggest other underlying precipitating factors relating to management of contamination in grower and finisher accommodation. In the other farrow-to-finish pig herd no effect after vaccination was observed throughout the study. Each pig farm is unique in terms of location, facilities, management, host susceptibility, and other influential factors (Andres and Davies, 2015)' and there are several plausible possible explanations for the variability in the vaccinal effect. Under field conditions, pigs are infected at different points in time, with a herd-dependent and even batch-dependent variability in both infection pressure and host response (Beloeil et al., 2003; Lo Fo Wong et al., 2004; Rostagno et al., 2012). Similarly, the presence of herd-specific *S*. *enterica* strains might have affected the impact of vaccination (Van Parys et al., 2013). There may also have been interactions, whereby (for example) vaccination may not have been effective if threshold levels of farm risk factors control were not achieved resulting in a high level of environmental contamination or risk of transmission of infection between batches of pigs (Davies et al., 2016). As example of natural variability, a significant reduction in *Salmonella* prevalence was observed in one non-vaccine farm. It should be noted that this farm had the second lowest prevalence level of all farms at the beginning of the study. A plausible explanation may be that existing farm hygiene and biosecurity controls were being better implemented and maintained ot that the management systems in place involved exposure to infection at times that could maximise the development of natural herd imunity (Knetter et al., 2015) (Davies et al., 2016), or some farm-resident strains may theoretically lose virulence over time (Hayden et. al, 2016) . ~~Nonetheless, analysis excludes data from these farrow-to-finish pig herds suggests an underlying effect of vaccination that is in the same way than that shown by the complete dataset (about 50%).~~ The overall study findings demonstrate that it is reasonable to postulate that maternal vaccination strategy at least contributed to the observed improvements in *Salmonella* control, especially since these farms were challenging in terms of farm design and possibilities for effective hygiene control and did not

apply any other interventions during the study period. The validity of the findings is supported by the fact that, independently of the sample type, pooled faecal samples, individual faecal samples and environmental sample, present the similar reductions in *Salmonella*-positivity, even though pooled pen faecal samples e are highly sensitive sample types and the culture method used can identify small numbers of organisms (Fedorka-Cray et al., 2000) and a low within-group *Salmonella* prevalence thus maximising detection (Arnold and Cook, 2009). A less sensitive sampling and detection method may have resulted in more apparently negative samples and therefore a greater apparent vaccinal effect, however, *Salmonella* counts in representative positive faeces selected for quantification were similar between non-vaccinating and vaccination herds. ~~Direct comparison is not possible because information available is very limited.~~ In another field study, Davies et al. (2016) reported a reduction in *Salmonella* counts in faeces from the pigs born to vaccinated dams. In an experimental trial, Jordan et al. (2013) also reported a reduction in *Salmonella* counts twenty days after vaccine administration in growing pigs. More research is required to fully elucidate the impact of vaccination on *Salmonella* counts in faeces.

Although 23 serovars were isolated, many of these serovars were likely to have been transient. Shedding was generally low and none of the other serovars found have a similarly high pathogenic importance for humans as ST/mST. No significant control of non-ST/mST *Salmonella* serovars following vaccination was observed, probably due by the limited cross-protection against non-target serovars provided by current vaccines (Wallis 2001; Foss et al., 2013; Foss et al., 2013; Bearson et al., 2016), despite the fact that it has been reported that vaccination against *S*. Choleraesuis can cross-protect pigs against *S*. Typhimurium (Nnalue and Stocker 1987; Maes et al., 2001). The significant reduction in isolation of 'all salmonellas' over time reflects the high prevalence of *S*. Typhimurium and monophasic variants isolates in UK pigs and in this study

(Davies et al., 2016). The most important finding is that serovars that pose the highest risk to humans within the UK pig reservoir could be potentially better controlled by using the vaccine evaluated in this study, especially when combined with good biosecurity, management and farm hygiene practices (Andres and Davies, et al., 2015).

Potential economic benefits could be achieved through better herd performance, for example, by reducing salmonellosis and the need to medicate pigs at weaning, improving feed conversion efficiency and daily live weight gain (Andres and Davies, et al., 2015). In this context, previous studies have demonstrated average daily gain benefits as a result of vaccination of pre-weaned piglets (Farzan and Friendship, 2010; De Ridder et al., 2014). In contrast, Husa et al. (2009), in an experimental trial, reported that the growth rate was lower in piglets vaccinated with a commercial *S*. Choleraesuis/*S*.Typhimurium live vaccine than in unvaccinated piglets due to adverse reactions after vaccination, but the vaccine was protective against subsequent challenge. In our study, vaccination did not have any significant effect on piglet and sow performance, including average daily liveweight gain for piglets, as reported by the farmers' farm records, but this was not independently measured in this study. In a similar experimental design (piglets born from vaccinated sows), Ruggeri et al. (2015) showed a beneficial effect on the average daily live-weight gain, although the differences did not reach statistical significance. De Ridder et al. (2014) also found that vaccination was associated with improved daily live-weight gain in experimentally-infected pigs and suggested that feed conversion efficiency may have been improved, but it is likely that *Salmonella* infection depressed the appetite of the non-vaccinated study pigs , but significant improvements in growth parameters were not observed in the current study.

Taken together, our results provide evidence that maternal vaccination as a *Salmonella* Typhimurium reduction strategy on farrow-to-finish pig herd with a salmonellosis problem seems

to be a suitable measure to reduce clinical salmonellosis in weaned piglets as well as both faecal and environmental prevalence of *Salmonella*, especially for serovars S. Typhimurium and its monophasic variants. *Salmonella* vaccines therefore have the potential to reduce prevalence of *Salmonella* in pigs and result in a reduction of human cases attributed to pork. However, more research is required to quantity the impact throughout the pig meat production chain.

Conflict of interest

The authors declare no conflicts of interest.

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590 10.1016/j.prevetmed.2016.12.004.
591

592 Table 1. Example schedule for a vaccination program on a weekly-farrowing system with 23
593 batches of pigs.

594

595

Week	Event	Sampling
0	**Initial sampling visit**	**Full set of animal samples** **Environmental samples**
1	1st vaccination of first batch of sows (6 weeks ante-partum)	
4	2nd vaccination of first batch of sows (3 weeks ante-partum)	
7	First batch of sows farrow	
21	**1st Mid study sampling**	**Full set of animal samples** **Environmental samples**
23	1st vaccination of last batch of sows	
25	Pre-2nd farrowing booster to 1st batch of sows	
26	2nd vaccination of last batch of sows	
28	Second farrowing, 1st batch of sows	
29	First farrowing, last batch of sows	
33	Pigs from first farrowing of 1st batch of sows go to slaughter	
46	Pre-3rd farrowing booster to 1st batch of sows	
47	Booster vaccination of last batch of sows (3 weeks prior to 2nd farrowing)	
49	Third farrowing, 1st batch of sows	
50	Second farrowing of last batch of sows	
54	Pigs from 2nd farrowing of first batch of sows go to slaughter	
55	**2nd Mid study sampling (all finishers on farm from vaccinated sows)** **Pigs from 1st farrowing of last batch of sows go to slaughter**	**Full set of animal samples** **Environmental samples**
68	Booster vaccination of last batch of sows (3 weeks prior to 3rd farrowing)	
69	**Final sampling visit**	**Full set of animal samples** **Environmental samples**

Table 2. Bacteriological results from the pooled and individual faecal samples and environmental samples collected on-farm for the evaluation of the protection against *Salmonella* Typhimurium and its monophasic variants conferred by licensed live *Salmonella* Typhimurium vaccine administered to sows on eight commercial farrow-to-finish pig herds and compared to eight control farms. *Salmonella* vaccination commenced between the first and second visit.

***Salmonella*-positive (%)**

Visit (days*)	Farms	Pooled samples						Individual samples						Environmental samples					
		N	Vaccine	N	Control	SE	P value	N	Vaccine	N	Control	SE	P value	N	Vaccine	N	Control	SE	P value
1 (0)	8	1,297	30.8	1,169	36.2	6.94	0.591	1,430	19.1	1,062	21.9	5.47	0.722	238	34.6	160	53.0	8.37	0.143
2 (161-182)	8	1,268	28.2	1,240	32.0	7.64	0.731	1,429	20.0	1,382	26.9	5.81	0.415	201	29.2	159	47.4	8.82	0.162
3 (308-402)	8	1,279	26.1	1,178	31.4	6.87	0.588	1,394	20.6	1,360	26.8	5.24	0.412	188	31.3	228	40.6	9.26	0.489
4 (514-569)	8	1,288	19.8[b]	1,028	41.0[a]	6.64	0.041	1,423	13.4[b]	1,425	32.0	4.94	0.018	208	21.2	212	42.8	7.87	0.073

***S.* Typhimurium and monophasic variants -positive (%)**

Visit (days*)	Farms	Pooled samples						Individual samples						Environmental samples					
		N	Vaccine	N	Control	SE	P value	N	Vaccine	N	Control	SE	P value	N	Vaccine	N	Control	SE	P value
1 (0)	8	1,297	26.6	1,169	31.3	6.69	0.624	1,430	17.8	1,062	21.7	5.17	0.603	238	30.1	160	46.3	9.20	0.234
2 (161-182)	8	1,268	26.4	1,240	26.0	7.70	0.962	1,429	19.5	1,382	23.2	5.55	0.639	201	28.7	159	40.8	9.39	0.378
3 (308-402)	8	1,279	24.2	1,178	27.8	7.09	0.727	1,394	18.7	1,360	23.8	5.13	0.498	188	27.2	228	36.6	9.22	0.480
4 (514-569)	8	1,288	19.1	1,028	34.3	6.35	0.112	1,423	13.2	1,425	27.0	4.78	0.060	208	20.64	212	35.8	8.42	0.220

* Number of days. N: total number of samples. SE: standard error. a,b Data in the same row for each group are with uncommon letters are different (P<0.05).

605 Table 3. Bacteriological results from the pooled and individual faecal samples and environmental samples collected 6 farm where was observed *a*
606 positive effect conferred by licensed live Salmonella Typhimurium vaccine administered to sows on farrow-to-finish pig herds. *Salmonella*
607 vaccination commenced between the first and second visit.
608
609 * Number of days. N: total number of samples. SE: standard error. a,b Data in the same row for each group are with uncommon letters are different

***Salmonella*-positive (%)**

Visit (days*)	Pooled samples							Individual samples							Environmental samples						
	N	Vaccine	SE	N	Control	SE	P value	N	Vaccine	SE	N	Control	SE	P value	N	Vaccine	SE	N	Control	SE	P value
1 (0)	955	36.2	7.34	990	40.2	6.80	0.703	955	22.7	6.40	883	23.7	5.93	0.907	185	44.7	9.02	148	52.2	8.35	0.551
2 (161-182)	934	30.1	8.28	1062	36.2	8.17	0.622	934	21.8	6.70	1,202	30.4	6.20	0.368	163	31.9	9.67	147	53.3	8.95	0.133
3 (308-402)	947	26.6	7.98	988	35.7	7.39	0.415	947	19.7	5.90	1,180	30.4	5.46	0.210	147	30.6	10.10	218	45.0	9.33	0.317
4 (514-569)	974	15.5[b]	6.41	812	46.5[a]	5.93	0.005	974	11.9[b]	5.45	1,246	35.5[a]	5.04	0.009	164	22.7[b]	8.02	188	48.9[a]	7.42	0.035

***S.* Typhimurium and monophasic variants -positive (%)**

Visit (days*)	Pooled samples							Individual samples							Environmental samples						
	N	Vaccine	SE	N	Control	SE	P value	N	Vaccine	SE	N	Control	SE	P value	N	Vaccine	SE	N	Control	SE	P value
1 (0)	955	30.8	7.49	990	34.6	6.94	0.72	955	21.0	6.05	883	23.6	5.60	0.766	185	39.7	10.39	148	44.6	9.62	0.735
2 (161-182)	934	28.0	9.27	1062	29.2	8.58	0.922	934	21.2	6.55	1,202	26.3	6.07	0.580	163	31.2	10.96	147	45.5	10.15	0.361
3 (308-402)	947	24.8	8.52	988	31.6	7.89	0.569	947	17.4	5.88	1,180	27.0	5.45	0.253	147	29.6	11.01	218	40.4	10.20	0.486
4 (514-569)	974	14.5[b]	6.50	812	38.8[a]	6.02	0.019	974	11.6[b]	5.54	1,246	29.7[a]	5.13	0.035	164	22.0	9.44	188	41.0	8.74	0.168

610 (P<0.05).
611

612 Table 4. Mixed-effects logistic model to test for association between vaccination and the presence
613 of Salmonella and *Salmonella* Typhimurium and its monophasic variants, whilst accounting for a
614 priori variables, from a controlled trial of 16 pig farms.
615

		***Salmonella*-positive**		**ST/mST-positive[a]**	
		Odds ratio	**p-value**	**Odds ratio**	**p-value**
Farm type	Non-vaccinated	Ref.			
	Vaccine	0.726	<0.001	0.706	<0.001
Visit x Farm type	1 x Farm type	Ref.			
	2 x Farm type	1.070	0.492	1.311	0.008
	3 x Farm type	1.028	0.775	1.043	0.667
	4 x Farm type	0.512	<0.001	0.613	<0.001
Sample type	Individual	Ref.			
	Pooled	2.697	<0.001	2.558	<0.001
Season	Winter	Ref.			
	Spring	1.090	0.070	1.119	0.025
	Summer	1.214	0.004	1.198	0.013
	Autumn	1.069	0.268	1.130	0.047
Pig type	Gestation	Ref.			
	Boars	1.496	0.564	1.842	0.381
	Farrowing	0.559	<0.001	0.610	<0.001
	Weaners	6.292	<0.001	6.995	<0.001
	Growers	5.349	<0.001	6.119	<0.001
	Finishers	3.261	<0.001	3.732	<0.001
	Gilts	1.733	<0.001	2.069	<0.001
	Environmental	4.252	<0.001	4.987	<0.001
	Dry sows	2.269	<0.001	3.061	<0.001
	Mixed	3.252	<0.001	3.640	<0.001
Visit	1	Ref.			
	2	0.783	0.001	0.721	<0.001
	3	0.890	0.086	0.934	0.326
	4	1.095	0.193	1.045	0.539

616
617

Table 5. Summary of serial dilution results for determination of *Salmonella* $\log_{10}$ estimations per gram of individual faecal sample,

Farm type	Visit	N	Proportion of samples with that concentration (%)					
			<1	1-10	1-10^2	10^2-10^3	10^3-10^4	10^4-10^5
Vaccine	1	188	60.1	16.5	11.2	9.6	1.6	1.1
	2	221	57.0	23.1	11.8	6.8	0.9	0.5
	3	242	54.1	24.4	15.3	2.9	2.1	1.2
	4	274	60.9	15.3	9.5	7.7	4.0	2.6
Non-vaccinated	1	163	59.5	20.0	13.5	5.5	1.2	0.0
	2	251	40.2	31.1	14.3	9.6	3.2	1.6
	3	247	53.8	19.0	15.4	10.9	0.4	0.4
	4	175	58.9	24.0	7.4	5.7	4.0	0.0

N: total number of samples.

Table 6. Summary of herd performance data from vaccinated and non-vaccinated farms collected at the first and final study visit.

Performance determinations	Visit	Vaccine		Non-vaccinated	
		N	Mean	N	Mean
Piglets born	1	8	13.4	7	13.0
	4	8	14.3	5	13.1
Piglets weaned	1	8	11.4	7	11.4
	4	8	12.0	5	11.3
Pigs sold per sow per year	1	8	26.3	7	23.5
	4	7	26.0	5	23.9
Slaughter live weight (Kg)	1	7	117.0	6	101.7
	4	7	115.0	5	106.4
Litters per sow	1	8	2.4	7	2.3
	4	8	2.4	5	2.3
Sow mortality (%)	1	8	6.0	6	2.9
	4	7	11.0	2	3.0
Replacement sows (%)	1	8	48.0	7	45.6
	4	8	72.0	4	58.5
Sow parity maximum	1	7	7.0	6	6.3
	4	7	7.0	3	5.3
Pre-weaning mortality (%)	1	8	16.9	7	11.5
	4	8	17.1	5	13.8
Weaning age (days)	1	7	27.0	4	25.7
	4	7	27.0	5	25.3
Post-weaning mortality weaners (%)	1	8	5.5	6	3.5
	4	7	5.7	3	4.8
Post-weaning mortality growers (%)	1	8	5.5	6	2.7
	4	7	5.7	3	4.4
Post-weaning mortality finishers (%)	1	8	5.5	6	3.0
	4	7	5.7	4	4.5
Daily live weight gain* (g)	1	8	830.0	5	729.8
	4	7	938.0	4	685.0
Feed conversion rate*	1	5	2.5	4	2.4
	4	4	2.7	2	2.4

N: total number of farms.

Figure 1. Results of shedding *Salmonella* in faecal pooled samples of piglets born from vaccinated sows at weaners, growers and finishers rearing states. Data are expressed as means ± standard error. a,b Grouped bar with uncommon letters are different (P<0.05).