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Evaluation of three intervention strategies to reduce the transmission of *Salmonella* Typhimurium in pigs

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ABSTRACT

Despite current control measures, *Salmonella* in pigs remains a major public health concern. In this in vivo study, the effect of three intervention strategies on *Salmonella* Typhimurium transmission in pigs was evaluated. The first intervention was feed supplemented with coated calcium-butyrate (group A); the second comprised oral vaccination with a double-attenuated *Salmonella* Typhimurium strain (group B), and the third was acidification of drinking water with a mixture of organic acids (group C). After challenge at 8 weeks of age, animals were individually sampled for 6 weeks (blood once per week; faeces twice per week) and then were euthanased at 14 weeks of age. Post-mortem ileum, caecum, ileocaecal lymph nodes, and tonsils were sampled, along with ileal, caecal and rectal contents, and tested for the presence of *Salmonella* spp. Transmission was quantified by calculating an 'adjusted' reproduction ratio 'R_a' and its 95% confidence interval (CI).

The proportion of pigs that excreted *Salmonella* spp. via the faeces was significantly higher in group C (58%, $P < 0.0001$) and the positive control group (41%, $P = 0.03$), compared to group B (15%), and the proportion in group C was also significantly higher than in group A (23%, $P = 0.01$). Group A had the lowest proportion of positive post-mortem samples (18%), followed by group B (31%), the positive control group (41%) and group C (64%) ($P < 0.03$). The highest transmission was seen in the positive control group and group C ($R_a = +\infty$ with 95% CI [1.88; $+\infty$]), followed by group B ($R_a = 2.61$ [1.21; 9.45]) and A ($R_a = 1.76$ [1.02; 9.01]). The results of this study suggest that vaccination and supplementation of the feed with coated calcium-butyrate limited *Salmonella* transmission in pigs and might be useful control measures.

Introduction

Salmonella infection in pigs is a major concern in the European Union (EU),[1] and in the past 10 years contaminated pork has been the second most important source of human salmonellosis in many EU countries (Hauser et al., 2010). Infection or contamination can occur at many different levels of the pig production chain such as via the feed, at the primary production site, in the slaughterhouse, and during pork processing. *Salmonella* infection and/or contamination at the primary production site plays a key role in this chain, as positive associations have been demonstrated between within-herd *Salmonella* seroprevalence and carcass contamination (Sørensen et al., 2004; Baptista et al., 2010), and reducing pre-slaughter *Salmonella* infections increases pork safety, i.e. fewer infected lymph nodes and intestinal contents (Hurd et al., 2002).

Unfortunately, controlling *Salmonella* infections in pig herds is difficult. The pathogen is common, persists in the environment, and infections with most serovars occur without any obvious symptoms (Davies et al., 2004; EFSA, 2008). Although hygiene and biosecurity on-farm are of paramount importance in decreasing *Salmonella* seroprevalence in slaughterhouses (Hotes et al., 2011), *Salmonella*-free housing cannot be obtained simply by cleaning and disinfection regimens at farm level (Mannion et al., 2007; McLaren et al., 2011). Hence, such regimens should be combined with other measures as part of an overall strategy to control *Salmonella* on-farm (Wales et al., 2009).

Several studies have evaluated the effect on *Salmonella* control of on-farm treatment of feed or water with acids (Letellier et al., 2000; van der Wolf et al., 2001; Lo Fo Wong et al., 2004; Canibe et al., 2005; Farzan et al., 2006; Creus et al., 2007; Poljak et al., 2008; De Busser et al., 2009; Taube et al., 2009; Tanaka et al.,

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[1] See: Regulation (EC) No. 2160/2003.

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2010). The results varied greatly between studies, possibly because of the large differences in the acidification process and dosage used (O'Connor et al., 2008). Of the different acidification methods and products, the use of coated butyric acid appears promising, as it decreased *Salmonella* shedding significantly in several studies (Van Immerseel et al., 2005; Boyen et al., 2008b; Guilloteau et al., 2010).

Another possible method of on-farm control of *Salmonella* infections in pigs is vaccination. In most studies the use of *Salmonella* Typhimurium vaccines significantly decreased clinical signs and excretion of *Salmonella* (Springer et al., 2001; Roesler et al., 2004, 2006; Eddicks et al., 2009; Farzan and Friendship, 2010; Hotes et al., 2011; Hur et al., 2011). However, Denagamage et al. (2007) concluded in their review that the design and reporting deficiencies in many studies (e.g. little detail on population type, sample size, type of vaccine, dose and dosing regimens) meant that the association between vaccination and *Salmonella* reduction in finisher swine was promising, but not proven. Furthermore, currently available serological tests do not differentiate between commercial-vaccine-induced and infection-induced antibodies, so vaccine use may be compromised in countries where serology is used for *Salmonella* surveillance (e.g. Denmark, Germany, UK, Belgium; EFSA, 2011).

To our knowledge, the ability of such intervention measures to prevent the spread of *Salmonella* among pigs has not yet been investigated via transmission experiments. A great advantage of such experiments is that the reduction of both the infectivity and susceptibility of treated animals can be quantified, whereas traditional challenge studies only demonstrate the effect of reduced susceptibility (Springer et al., 2001; Roesler et al., 2004; Tanaka et al., 2010). The aim of the present study was to evaluate, through the estimation of an adjusted reproduction ratio, the influence of three different intervention strategies, namely, (1) feed with coated calcium-butyrate, (2) vaccination, and (3) acidified drinking water, on the transmission in pigs of *Salmonella enterica* subspecies *enterica* serovar Typhimurium – the most prevalent *Salmonella* serotype in pigs in Belgium and Europe (CODA-CERVA, 2010; EFSA, 2012).

Materials and methods

This experiment was approved by the ethical committee of the Scientific Institute of Public Health and the Veterinary and Agrochemical Research Centre IPH-VAR (100412-02).

Herd selection

For the initial survey, pig herds in the national *Salmonella* monitoring programme were selected, based on consistently low sample-to-positive (S/P) ratios (S/P < 0.20) in three consecutive blood samples taken from grower-finishing pigs in the preceding year. These herds were then visited, bacteriological and serological samples were taken, and the supply farm was selected based on hygiene, management and sample results.

Piglet selection

In order to select *Salmonella*-negative piglets, six sows from each selected herd were chosen through a bacteriological and serological screening process, which was repeated three times. From each sow, three piglets were screened as well. Finally, the sows with the lowest S/P ratios were selected to provide the experimental piglets. The average S/P ratio for the sampled piglets (at the time of the third screening at the age of 8 days) was 0.07 ± 0.10 (standard deviation).

A total of 69 piglets from six different sows were selected, weaned at 19 days of age and then transported to the experimental animal facilities of CODA-CERVA in a thoroughly cleaned and disinfected trailer.

Study design

Upon arrival, the piglets were randomly assigned into five groups: Group A ($n = 2 \times 8$) received feed supplemented with 0.3% m/m coated calcium-butyrate salt (Greencab-70, Sanluc International) (see Table 1 for details), group B ($n = 2 \times 8$) was orally vaccinated at 22 and 43 days of age with 5×10^8–5×10^9 colony forming units (CFU) of a double-attenuated histidine–adenine auxotrophic *Salmonella* Typhimurium vaccine (Salmoporc, Impfstoffwerk Dessau-Tornau), and group C ($n = 2 \times 8$) received drinking water adjusted to pH 3.6–4.0 using 0.09% v/v of a mixture of formic, propionic, acetic and lactic acid (Agrocid Super, CidLines) (see Table 1 for details). This water was checked daily with a pH-meter (pHep + ATC, Hanna Instruments). A positive control group (infected/untreated; $n = 2 \times 8$) and a negative control group (uninfected/untreated; $n = 5$) were also included. All treatments were applied from arrival in the experimental facilities (3 weeks of age) until the end of the experiment (14 weeks of age).

Table 1
Composition of the feed (group A) and water (group C) supplement.

Ingredients	% in supplement	‰ in feed (1) or water (2)
(1) Feed supplement in group A		
Butyrate anion	±70.0	±2.1
Calcium (organic)	±14.0	±0.4
Free fatty acids	±10.0	±0.3
C16:0	7.5–9.0	0.23–0.27
C18:0	0.5–1.0	0.02–0.03
C18:1	0.5–1.0	0.02–0.03
C14:0	<0.35	<0.01
(2) Water supplement in group C	(pH 2.0–3.5)	(pH 3.6–4.0)
Formic acid	>50	>0.044
Propionic acid	>10	>0.009
Acetic acid	>10	>0.009
Lactic acid	<5	<0.004

Group A was given deodorized calcium-butyrate salt coated with plant oils in the pig meal, while group C received a mixture of organic acids in the drinking water.

All animals were fed the same meal without antimicrobials throughout the study, except for group A where the feed was supplemented as described above. Every group was housed in a different room with two similar pens (2×8 pigs), which were separated with solid 1 m high partitions. The stocking density was 0.42 m^2 per pig.

At 57 days of age, two pigs in each pen (except for those in the negative control group) were moved to a separate room and were orally challenged (Day −1) with 10^8 CFU of a nalidixic acid-resistant *Salmonella* Typhimurium strain 112910a, previously isolated from a pig without clinical signs of salmonellosis by Boyen et al. (2008a). Twenty-four hours after this challenge, these 'seeder' pigs were replaced into their original pens (Day 0). All pigs were euthanased and autopsied at 95 days of age (Day 37).

Sampling

The sampling scheme is shown in Table 2. From 3 weeks of age (Day −39) until euthanasia at 14 weeks of age (Day 37), blood samples were obtained from all 69 pigs once a week to detect *Salmonella*-specific antibodies via ELISA. From 3 weeks of age (Day −39) until challenge (Day −1) pooled faecal samples were taken weekly. After challenge, individual faecal samples were collected from all pigs twice a week. At autopsy (Day 37), samples of ileocaecal lymph nodes, ileum, ileal content, caecum, caecal content, faeces and tonsils were taken, and examined bacteriologically.

Bacteriological examination

Faecal examination was initiated within 2 h of collection. *Salmonella* was isolated using the ISO 6579 Annex D method (ISO 6579:2002). Briefly, samples were inoculated in buffered peptone water (BPW, Bio-Rad) in dilution 1:10 and incubated aerobically for 16–20 h at 37 °C. Of this solution, 0.1 mL was inoculated on a modified semi-solid Rappaport Vassiliadis plate (MSRV, Bio-Rad) and incubated aerobically at 42 °C for 48 h. Growth halos were plated on a xylose lysine deoxycholate agar plate (XLD, Bio-Rad) and a brilliant green agar plate (BGA, LabM) – the latter supplemented with 20 μg/mL nalidixic acid – and then incubated aerobically for 21–27 h at 37 °C. One *Salmonella*-suspected colony on these XLD plates (challenge or vaccine strain) or on the BGA plates (only the challenge strain) was inoculated in triple sugar iron agar (TSI, Bio-Rad) and lysine decarboxylase bouillon (Oxoid) and incubated for 18–24 h at 37 °C for final identification.

For samples from group B, when growth of *Salmonella* bacteria was only observed on XLD and not on BGA, the sample was additionally tested with medium A and B (*Salmonella* Diagnostic Kit, IDT) to confirm that the isolated bacterium was the vaccine strain. Quantification of *Salmonella* spp. was performed on faecal samples 3, 7 and 24 days post infection (DPI) using standard enumeration protocols (Boyen et al., 2008a).

The tissue samples taken at necropsy were first rinsed with phosphate buffered saline, then sliced, suspended in BPW (dilution 1:10) and homogenized in a stomacher (BagMixer, Interscience) for 1 min. Further isolation was performed as for the faecal samples.

Table 2
Experimental design of the transmission study with the taken samples/actions and the used diagnostics/products, in function of the age of the pigs.

Sampling/action (frequency)	Age of the pigs (weeks)												Diagnostic method/product
	3	4	5	6	7	8	9	10	11	12	13	14	
Pooled faeces per pen (once/week)	x	x	x	x	x	x							Isolation[a]
Individual rectal faeces (twice/week)							x	x	x	x	x	x	Isolation[a]
Blood (once/week)	x	x	x	x	x	x	x	x	x	x	x	x	ELISA[b]
Supplementations in feed (A) and water (C) (ad libitum)	x	x	x	x	x	x	x	x	x	x	x	x	Feed: coated calcium-butyrate salt (Greencab-70, Sanluc International) Water: mixture of organic acids (Agrocid Super, CidLines)
Vaccination (B) (primer + boost)	x			x									Commercial *Salmonella* Typhimurium vaccine (Salmoporc, IDT)
Challenge (two pigs/group)						x							Nalidixic acid resistant *Salmonella* Typhimurium strain 112910a
Rectal faeces, ileum + content, caecum + content, ileocaecal lymph nodes, tonsils (autopsy)												x	Isolation[a]

[a] According ISO 6579 Annex D: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:314:0012:0013:NL:PDF.
[b] Commercial ELISA kit (HerdChek Swine *Salmonella*, IDEXX Laboratories).

Serological examination

Blood samples were allowed to clot for 24 h at 15 °C and were then centrifuged for 15 min at 1200 *g*. Serum was diluted 20-fold and analysed for *Salmonella*-specific antibodies with a commercial ELISA-kit based on lipopolysaccharide (LPS) antigens (HerdChek Swine *Salmonella*, IDEXX), following the manufacturer's instructions. Optical densities (OD) were determined by photospectrometry with a 650 nm filter. The outcome was expressed as a 'sample-to-positive' ratio:

$$S/P = (\text{OD of sample} - \text{mean OD of negative kit control})/(\text{mean OD of positive kit control} - \text{mean OD of negative kit control})$$

Samples with an S/P ratio $\geqslant 0.25$ (or OD% $\geqslant 10$) were considered positive.

Statistical analysis

The number of positive faecal samples collected after challenge were compared between the groups using generalized estimating equations (GEE) (PROC GENMOD and lsmeans; SAS version 9.2). A logit link function, a binomial distribution and an autoregressive first order correlation matrix was assumed with repeated measures. A one-way ANOVA test was used to assess differences in the number of *Salmonella* spp. ($\log_{10}$ normalized data) quantified in the faeces on Day 3, 7 and 24.

The S/P ratios obtained by the ELISA were analysed by repeated measures ANOVA using (PROC MIXED and lsmeans; SAS). Both pig and pen were considered random effects. An autoregressive first order correlation structure of was used for the within-subject correlation. In all models the Bonferroni correction was used to account for the use of multiple comparisons.

The transmission of *Salmonella* Typhimurium in each group was estimated on the basis of the stochastic 'susceptible-infectious' (SI) infection model using an 'adjusted' reproduction ratio 'R_a'. This ratio expresses the mean number of secondary infections caused by one typically infectious animal in a fully susceptible population during a defined time period (3–14 weeks of age in this study) (Meyns et al., 2004). An Ra value of, for example, 1.76 implies that an infected pig from a group infects, on average, 1.76 pen mates during the observed period. These R_a values were calculated via maximum likelihood estimation using each of the following definitions of infection. At least one positive sample was necessary to define an animal as *Salmonella*-positive based on: (1) individual faeces; (2) ileum and/or ileal content and/or caecum and/or caecal content; (3) ileocaecal lymph nodes and/or tonsils; (4) all tissue and/or all faecal samples.

Different definitions were used to assess the most suitable matrix for this estimation. For each of these four possible definitions the number of contact-infections was thus determined as variable 'X_i'. The following equation was used for this estimation:

$$R_{a,mls} = \max_{R_a} \prod_{i=1}^{m} F(X_i, R_a | N, S_0, I_0)$$

in which $F(X_i, R_a|N, S_0, I_0)$ is the likelihood function for the observed X_i-value, if N (total number of piglets), S_0 (number of susceptible piglets), I_0 (number of infectious piglets) and m (number of experiments) are given (Meyns et al., 2004).

Results

Bacteriological examination

All faecal samples taken prior to challenge were negative for *Salmonella* spp. No *Salmonella* spp. were isolated from the negative control group at any timepoint, supporting the conclusion that all other positive isolates were from infection with the introduced challenge strain. From challenge until euthanasia, the number of excreting pigs in group C (58%) was significantly higher than in group A (23%, $P = 0.01$) and B (15%, $P = 0.0001$). Likewise, the number of shedding pigs in the positive control group (41%) was significantly higher than in group B ($P = 0.03$), while no significant differences were observed between group C and the positive control group (Fig. 1). These results were consistent with the *Salmonella* bacterial counts on Day 3, 7, and 24. Log_{10}-values (CFU/g faeces) were 2.5, 1.1, and 0.7 for group A, 1.0, 0.3, and 1.1 for group B, 3.6, 2.3, and 1.6 for group C and 2.1, 1.2, and 1.3 for the positive control group, with only significant differences at Day 3 and 7 when group C was compared with group A ($P = 0.0066$) and B ($P = 0.0002$).

Of all organ samples, 18%, 31%, 64% and 41% were found to be positive for *Salmonella* in groups A, B, C and the positive control group, respectively (Table 3). Group C had significantly more infected tissue samples than all other groups ($P < 0.01$). In group B, 31% of all organ samples contained the vaccine strain, with the proportion of vaccine-positive tonsils being higher than all other tissues ($P < 0.01$) (Fig. 2).

The adjusted reproduction ratios R_a for the different infection definitions are shown in Table 4. Overall R_a values were lower in group B ($R_a = 2.6$ [1.21; 9.45]) and A ($R_a = 1.76$ [1.02; 9.01]) than group C and the positive control group (both $R_a = +\infty$ [1.88; $+\infty$]).

Serological examination

All pigs were seronegative before the start of the study. The evolution of *Salmonella*-specific antibody titres is illustrated for all groups in Fig. 3. During the entire experiment, titres in the negative control group remained very low (S/P ratios below 0.015). In group B, an increasing mean *Salmonella*-specific antibody response was observed from Day −19 until Day −1 (day of challenge) and also subsequently until the end of the experiment. In groups A and C and the positive control group, *Salmonella*-specific antibodies increased from 2 weeks after challenge; no significant differences

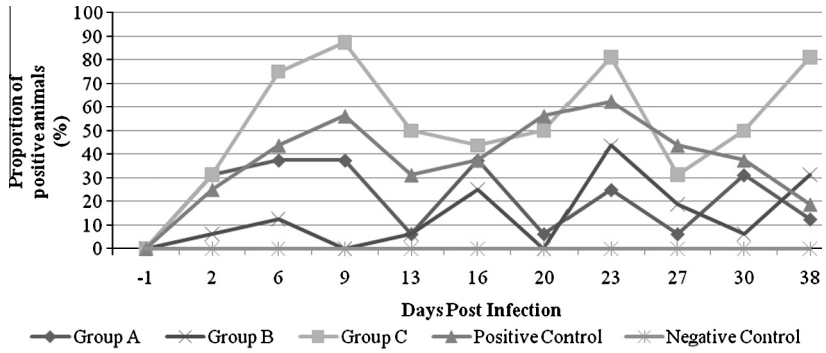

Fig. 1. Isolation of the inoculated *Salmonella* strain in individual faeces (ISO 6579 Annex D) twice a week: proportion of positive animals in function of the days post infection in group A (fed with coated calcium-butyrate salt), group B (vaccination), group C (water with organic acids), and in the positive and negative control group. Day −1: Oral infection with 10^8 CFU *Salmonella* Typhimurium of two seeder pigs per group of eight pigs. Day 0: Reintroduction of seeders in their original pen. Day 38: euthanasia and necropsy.

Table 3
Number of tissue and content samples positive for the challenge strain, *Salmonella* Typhimurium 112910a, in the four groups.

Group	Different autopsy samples						All autopsy samples
	Ileum	Ileal content	Caecum	Caecal content	Ileocaecal lnn	Tonsils	
A (n = 16)	4/16 (25%)	2/16 (13%)	2/16 (13%)	3/16 (19%)	3/16 (19%)	3/16 (19%)	17/96 (18%)
B (n = 16)	7/16 (44%)	4/16 (25%)	3/16 (19%)	6/16 (38%)	7/16 (44%)	3/16 (19%)	30/96 (31%)
C (n = 16)	9/16 (56%)	5/16 (31%)	10/16 (63%)	13/16 (81%)	10/16 (63%)	14/16 (88%)	61/96[a] (64%)
D (n = 16)	9/16 (56%)	7/16 (44%)	6/16 (38%)	8/16 (1%)	6/16 (38%)	3/16 (19%)	39/96 (41%)
All groups (n = 64)	29/64 (45%)	18/64 (28%)	21/64 (33%)	30/64 (47%)	26/64 (41%)	23/64 (36%)	147/384 (38%)

A, feed with coated calcium-butyrate salt; B, vaccination; C, water with organic acids; D, positive control.
[a] Significant difference between group C and the other groups ($P < 0.03$).

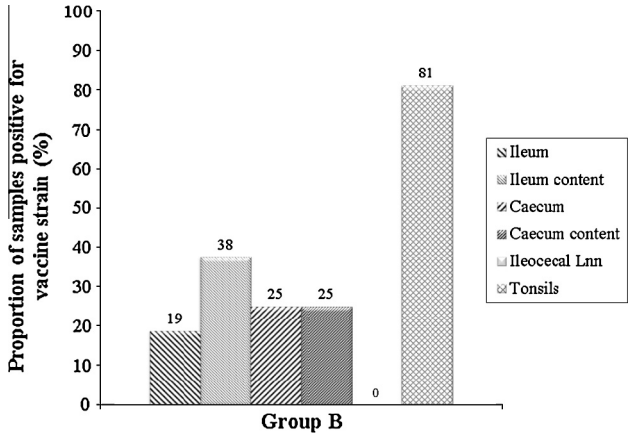

Fig. 2. Isolation of the *Salmonella* vaccine strain in different tissue samples (ISO 6579 Annex D + IDT *Salmonella* Diagnostic Kit): proportion (%) of vaccine-positive samples from ileum/ileal content/caecum/caecal content/ileocaecal lymph nodes/tonsils of group B (vaccination).

in titre were detected between these groups. The mean antibody titre of group B was significantly higher than all other groups.

Discussion

Current control measures are not sufficient to prevent the impact of pig-related *Salmonella* on public health. The present study investigated three intervention strategies to reduce the transmission of *Salmonella* Typhimurium in pigs. Supplementation of feed with coated calcium-butyrate and oral vaccination with an attenuated strain both limited *Salmonella* Typhimurium transmission; however, water acidified with various organic acids was shown in this study to be non-effective.

Using the present 'transmission' design, where treatment was given prior to challenge (treated seeders were brought together with treated contact pigs), an adjusted transmission ratio R_a was calculated which quantified the effects of the treatments on both infectiveness and susceptibility. This is an important contrast to efficacy studies in which it is susceptibility that is principally tested, and not the combined effect (Springer et al., 2001; Roesler et al., 2004; Tanaka et al., 2010). A similar approach has been used in previous transmission experiments with other pathogens (Laevens et al., 1998; Dewulf et al., 2001; Velthuis et al., 2003; Heres et al., 2004; Meyns et al., 2004).

When observing the obtained transmission ratios, higher R_a values were found in the group treated with acidified water (C) and in the untreated control group (D), independently of the definition of infection. This suggests a lower transmission of *Salmonella* in the treated groups A and B when compared to groups C and D. Yet, the R_a value in both group A and B – based on the detection of *Salmonella* in faeces – was >1, indicating that there was still spread of the infection during the study period.

In the present trial, a coated calcium-butyrate supplement was chosen as one strategy, since this has been shown to be beneficial in previous experiments (Boyen et al., 2008b; Guilloteau et al., 2010), though not with the current transmission design. In the digestive tract, butyrate can accelerate the renewal of necrotic areas, down-regulate *Salmonella* virulence, suppress intestinal inflammation and diminish pathogen invasion (Hamer et al., 2008). To reach the colonization sites of *Salmonella* spp. (ileum, caecum and colon) in their active form, however, supplemented short-chain fatty acids need protection or 'coating' from the intestinal environment (Van Immerseel et al., 2005; Boyen et al., 2008b) because they are generally quickly metabolized and then absorbed by gastro-intestinal epithelial cells (Louis et al., 2007). The results

Table 4
The adjusted reproduction ratio R_a for the three intervention groups A, B, C and the positive control group D.

Group	All individual faeces	Ileum/content/caecum/content	Ileocaecal lnn/tonsils	All organs/all faeces
A (n = 16)	1.62 [0.85; 8.43]	0.77 [0.35; 3.80]	0.32 [0.12; 2.94]	1.76 [1.02; 9.01]
B (n = 16)	1.16 [0.56; 3.95]	1.03 [0.56; 3.95]	1.19 [0.70; 7.18]	2.61 [1.21; 9.45]
C (n = 16)	3.53 [1.88; 11.70]	2.52 [1.67; 24.87]	3.53 [1.88; 11.65]	+∞ [1.88; +∞]
D (n = 16)	3.53 [1.88; 11.70]	1.91 [1.22; 24.78]	0.89 [0.47; 4.23]	+∞ [1.88; +∞]

A, feed with coated calcium-butyrate salt; B, vaccination; C, water with organic acids; D, positive control; lnn, lymph nodes.
A pig was considered infected if at least one sample was positive during the transmission period.

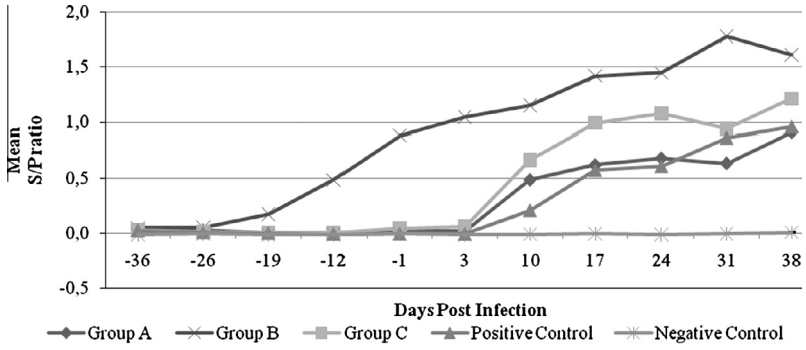

Fig. 3. Weekly *Salmonella*-specific antibody detection in serum (ELISA): Mean S/P ratio in function of the days post infection in group A (feed with coated calcium-butyrate salt), group B (vaccination), group C (water with organic acids), and in the positive and negative control group. Day −36 and Day −15: primer and booster vaccination in group B, respectively. Day −1: oral infection with 10^8 CFU *Salmonella* Typhimurium of two seeder pigs per group of eight pigs. Day 0: reintroduction of seeders in their original pen. Day 38: euthanasia and autopsy.

of our study showed a tendency towards reduced *Salmonella* transmission in this group, in comparison with the acidified water group and the positive control group. Therefore, we conclude that coated calcium-butyrate may be helpful in the reduction of *Salmonella* infections in pigs.

In contrast, the addition of a mixture of formic, acetic, propionic, and lactic acid (Agrocid Super, CidLines) to the drinking water did not reduce the transmission rate of *Salmonella* Typhimurium in comparison with the positive control group. A possible explanation is that this acid shock induced a stress response system enabling *Salmonella* Typhimurium to survive in an extreme low acid environment (an acid tolerance response or 'ATR'; Foster and Spector, 1995; Suk Baik et al., 1996). Previous reports on the use of organic acids via drinking water have produced inconsistent results. Van der Wolf et al. (2001) observed a reduction in *Salmonella* when a 0.2% v/v organic acid mixture was administered during the entire finishing period. In contrast, no reductions were observed with the same concentration applied for a shorter time (2 weeks pre-slaughter) (De Busser et al., 2009), or using a lower concentration administered to weaners (0.02% v/v in Letellier et al. (2000)). However, the comparison of these study results is hindered not only by the different duration of application or concentration, but also by the fact that different organic acid mixtures were used in all these studies.

The vaccine used in this study (Salmoporc, IDT) has been shown to reduce both faecal shedding and colonization of the intestinal tract of piglets (Springer et al., 2001). In the present transmission study, this vaccination resulted in the lowest number of shedding pigs and the lowest R_a (faecal results). However, vaccination also increased the *Salmonella*-specific antibodies and resulted in a proportion of tissue samples colonized by *Salmonella* that was comparable to that in the positive control group (31% vs. 41%, respectively).

Although the vaccine and wild type strains can be distinguished using the *Salmonella* Diagnostic Kit provided by the manufacturer of the vaccine, the commercial LPS-ELISA cannot differentiate between vaccination- and infection-induced antibodies in serum or meat juice. Vaccination may thus hamper the use of serology for *Salmonella* monitoring programmes. Programmes that are based on bacteriology, new diagnostic tests that do not detect vaccine-induced antibodies (Selke et al., 2007), or new vaccines that are not detected by current ELISA methods (Leyman et al., 2011) are necessary if vaccination is to become a routine part of *Salmonella* control.

Conclusions

Both feed supplementation with coated calcium-butyrate and, particularly, vaccination with an attenuated vaccine, decreased *Salmonella* Typhimurium transmission in pigs. Further studies are needed to assess the practical issues related to the implementation of these promising interventions. For example, more data are needed to determine the best age groups and treatment regimens for the coated calcium-butyrate and to learn how to overcome the problem of *Salmonella*-specific antibodies in vaccinated pigs. Alternative bacteriology-based monitoring programmes, diagnostic tests that do not detect vaccine-induced antibodies or new vaccines that are not detected by current antibody detection methods are required.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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