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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.
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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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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.

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