Assessment of different treatments to reduce *Salmonella* in swine.

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**Abstract**

In this study, different strategies to reduce carriage of *Salmonella* in pigs were evaluated. Probiotics, prebiotics, vaccination, and acidification of drinking water were assessed as means of reducing *Salmonella*. Following an experimental infection, a reduction of *Salmonella* in mesenteric lymphatic nodes was observed with the use of Flavomycin<sup>TM</sup> and vaccination by SC54<sup>TM</sup>. A reduction in *S. Typhimurium* shedding was also observed after supplementation with FOS in drinking water. Acidification of water, use of egg-yolk specific immunoglobulins, and vaccination with Endovac<sup>TM</sup> cannot reduce *Salmonella* in swine.

**Materials and Methods**

*Animals*: Early-weaned 12 day-old *Salmonella* free piglets, as verified by fecal and tonsils swabs, were randomly assigned to either control or treatment groups.

Each group was initially composed of 10 pigs. Clinical signs were monitored daily throughout the experiment.

*Pig Treatments*: Water acidification (0.02% formic acid; Fisher, Nepean, Ont. Canada), Ferlac-2<sup>TM</sup> (2 x 10<sup>6</sup> CFU/day, composed of *Lactobacillus acidophilus* (4%), *L. rhamnosus* (65%), *Enterococcus faecium* (25%), *Streptococcus thermophilus* (5.9%) and *L. bulgaricus* (0.1%) in feed, Rosell Institute, Montreal, Canada), Flavomycin<sup>TM</sup> (0.5 kg/ton of feed; Hoechst Canada, Regina SK), FOS (1% in water or feed; Encore Technologies US) and egg-yolk specific immunoglobulins (1g/piglet in the feed; Vetco Inc. Canada) were used in different groups from day 0 (at twenty-one days of age) to day 28. Two other groups were also vaccinated with Endovac<sup>TM</sup> (2 mL i.m.; Bayer, USA) or SC54<sup>TM</sup> (2 mL, intranasal; Boehringer, Iowa, US) at day 0. The control group was not supplemented and each group was housed in separate controlled facilities.

*Challenge*: A clinical isolate of *Salmonella Typhimurium*, isolated from a septiceptic pig (Faculté de Médecine Vétérinaire, Université de Montréal, Québec, Canada) was used. This strain was inoculated into Nutrient broth (NB, Difco, Detroit, MI) and incubated at 37°C for 18 h. The starting culture was used to inoculate fresh NB tube (1:100). This culture was incubated and log phase bacteria were used for the challenge. A dose of 10<sup>7</sup> C.F.U. was given orally to each piglet in the different groups, 14 days after the beginning of the treatment (Day 14).

**Bacteriology and Necropsy**: Rectal swabs were collected every two days before and after the challenge with *S. Typhimurium* and processed as described below. Fourteen days after the challenge (Day 28), pigs were euthanized and necropsied within 20 minutes to avoid post mortem bacterial invasion of tissues. Tissues collected for bacteriology were tonsils, liver, spleen, middle ileum, colon and...
mesenteric lymphatic nodes (MLN). One gram of each tissue or feces was homogenized in 9 mL NB and incubated 18 h at 37°C. One mL of NB of each specimen submitted to the primary enrichment was inoculated into 9 mL of Tetrathionate Brilliant Green (BBL, Cockeysville, MD) and incubated for 24-48 h at 37°C, for selective enrichment. Then, one loopful (10 μL) of the selective enrichment media was inoculated in Brilliant green sulfa agar (BGS, Difco) containing novobiocin (Sigma Chemical Co., St-Louis, MO) at 20 μg/mL and incubated for 24-48 h at 37°C. Lactose negative colonies were submitted to biochemical testing by urease and Triple sugar iron media (Difco). Colonies typically corresponding to *Salmonella* spp. were tested by agglutination using polyvalent O-antisera (Poly Al-Vi, Difco) and *Salmonella* isolates were serotyped under the supervision of Dr. C. Poppe, Health Canada in Guelph, Ontario, Canada. Quantitative bacteriology was done on MLNs. Dilutions of homogenized tissues (NB) were done in PBS and C.F.U. were evaluated by plating dilutions on BGS agar. Colonies typically corresponding to *Salmonella* were identified as described above.

**PFGE analysis.** Pulsed-field gel electrophoresis (PFGE) was carried out to compare the DNA profiles from *Salmonella* strains isolated from each animal to the profile of the experimental strain of *S. Typhimurium*. DNA was extracted and digested with SpeI as described previously (13). DNA fragments were separated by electrophoresis in a 1.2% agarose gel at 200 V with a linear ramp switch time of 5 to 25 sec for 18 h with a Gene Navigator apparatus (Pharmacia, Sweeden).

### Results

Following the experimental infection, 70% of the control pigs became colonized by *S. Typhimurium* in the gut and 60% were infected in mesenteric lymphatic nodes (MLN) (Table 1). No significant difference was observed in quantitative evaluation of *S. Typhimurium* in MLN (data not shown) in the different groups. Bacterial counts under 30 CFU were observed in MLN.

No beneficial effect was observed when Endovac™ was used as vaccine. On the contrary, an increase in the colonization by *S. Typhimurium* in the gut and MLN was detected. On the other hand, using SC54™ vaccine, a reduction of the presence of the bacteria in the MLN was noted in comparison to control group. No change was observed in the shedding of the bacteria in feces. However a significant reduction of *Salmonella* prevalence in ileum (p<0.05, Fisher test) was noted but distribution in other tissues was similar to the control group. Acidification of drinking water and egg-yolk specific immunoglobulins directed against *S. Typhimurium* were not efficient to reduce *Salmonella* infection in swine.

Prebiotics such as fructooligosaccharides (FOS) and Flavomycin™ were able to reduce shedding of *S. Typhimurium* in feces. Flavomycin™ reduced the shedding of *S. Typhimurium* only for few days after the experimental infection but reduced the presence of the bacteria in MLN. Using FOS, we noted that administration in drinking water considerably reduced the level of shedding (Figure 1) but

### Table 1. Recovery of *S. Typhimurium* from tissues and fecal material after experimental infection of pigs receiving various treatment (percentage of positive animals).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Spleen</th>
<th>Tonsils</th>
<th>MLN</th>
<th>Ileum</th>
<th>Colon</th>
<th>Feces*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10%</td>
<td>0%</td>
<td>20%</td>
<td>60%</td>
<td>70%</td>
<td>10%</td>
<td>60%</td>
</tr>
<tr>
<td>Endovac</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>90</td>
<td>30</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>SC54</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>20</td>
<td>0 b</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>Water acidification</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>90</td>
<td>80</td>
<td>40</td>
<td>70</td>
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<tr>
<td>FOS in water</td>
<td>50</td>
<td>30</td>
<td>10</td>
<td>60</td>
<td>50</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>FOS in feed</td>
<td>40</td>
<td>10</td>
<td>70</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Ferlac-2 in feed</td>
<td>0</td>
<td>11</td>
<td>44</td>
<td>44</td>
<td>89</td>
<td>33</td>
<td>89</td>
</tr>
<tr>
<td>Ferlac-2 + FOS in feed</td>
<td>10</td>
<td>0</td>
<td>40</td>
<td>100</td>
<td>70</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Flavomycin in feed</td>
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<td>0</td>
<td>40</td>
<td>30</td>
<td>50</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Egg-yolk in feed</td>
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<td>60</td>
<td>50</td>
<td>70</td>
<td>10</td>
<td>80</td>
</tr>
</tbody>
</table>

*Percentage of positive animals during one moment or another during the infection period

*Significantly different of control group (p<0.05, Fisher test)*
this effect was not observed when FOS was given in feed. Probiotics such as Ferlac-2™ was efficient to slightly reduce presence of bacteria in MLN and other tissues. However it was without effect on shedding and only a slight reduction of carrier state was noted when given in feed. The use of FOS and Ferlac-2™ together in feed had no effect on Salmonella infection in swine (Table 1).

Clinical signs were not observed in pigs throughout the experiment. PFGE analysis was done in order to compare the DNA profiles from Salmonella strains isolated in each infected animal to the profile of the challenge strain of S. Typhimurium. To conclude that animal became carrier of the experimental infection strain of Salmonella, the DNA profile has to be the same. The DNA profiles of all but two strains recovered from infected animals were identical to the profile of the challenge strains. The animal from which another Salmonella DNA profile was observed was not considered in the compilation of the results. This DNA profile was identical to Salmonella Derby serotype.

Discussion

Pigs leaving the farm are considered to be the main source of abattoir contamination by Salmonella (14). It is thus believed that pigs represent an important reservoir of Salmonella spp. and can spread the bacteria throughout the abattoir environment. It was shown that carrier pigs are positive for Salmonella in mesenteric lymph nodes, tonsils, colon or feces (15,16). Development of control measures to reduce fecal or lymph nodes carriage of Salmonella in live animals would thus be an important step in the reduction of this pathogen in meat products.

Salmonella spp., are facultative intracellular pathogens that can avoid humoral immunity in the intracellular environment, suggesting that a strong cellular response is needed for the elimination of this pathogen (17). Vaccination of pigs with SC54 vaccine™, a S. Choleraesuis var kunzendorf attenuated strain, has been reported to be beneficial in protecting pigs against the development of clinical disease from infection by virulent S. Choleraesuis (18). In this study, the use of SC54™ before experimental infection with S. Typhimurium resulted in a decrease of the bacteria in the MLN suggesting that the use of an avirulent, live S. Choleraesuis vaccine can prevent the colonization of MLN by S. Typhimurium. The significant reduced prevalence observed in ileum would also suggest that a stimulation of local defense mechanism, such as an increase of immunoglobulins A, could also be involved in the vaccine mechanism of action.

It is recognized that prebiotic and probiotic supplements promote the colonization by beneficial microorganisms (19). The mechanism by which probiotic and prebiotic supplements affect the microbiology of the intestines has been recently studied. This new knowledge on the interactions of mixed microbial populations allows speculation on effects that these supplements may have. It includes competition for nutrients (20), production of inhibitory substances or antimicrobials which inhibits growth of certain enteropathogens (21), competition for receptors or adhesin of the intestinal mucosa (19) and finally immunomodulation

Figure 1. Shedding of S. Typhimurium in feces following the experimental infection for each group.

![Graph showing shedding of S. Typhimurium in feces following the experimental infection for each group.](image-url)
such as macrophage activation (22), increase of IgA production (23), cytokine production and increase of T and B cells (24).

In a previous study (25) Ferlac-2, used as a paste was found to be more efficient to reduce colonization of MLN and other tissues by S. Typhimurium, in comparison to administration in feed. This way of administration provided a constant concentration of probiotic bacteria in the gut and competitive exclusion. In this mechanism, microflora develop and adhere to the mucosal surfaces of the intestinal tract. This prevents pathogenic bacteria from colonizing and thus excludes them from lining the intestine. However, since the shedding of Salmonella was not reduced by probiotics, as it was observed by Parraga et al. (26), it can be suggested that this exclusion mechanism is not sufficient to avoid the survival and replication of Salmonella in mucus blanket without massive colonization of the cells of the mucosa.

FOS added to water reduced considerably the shedding of S. Typhimurium in feces and changes were observed in gut microbial composition of selected sections of the intestinal tract. Changes in microbial composition may be achieved by different mechanisms. Colonization of the mucosa by Salmonella can be reduce by direct antagonism mediated by antibacterial agents produced and secreted by the microflora organisms (27). The combination of FOS with Ferlac-2™ was not efficient suggesting that probiotics and prebiotics, given together became antagonists. Flavomycin™, a bambermycins agent used to control pathogen bacteria in poultry, swine and cattle, has been investigated in salmonella challenge models in swine and results showed that Flavomycin™, significantly reduced the rate and the magnitude of Salmonella Typhimurium shedding in pigs (28). In the present study, Flavomycin™ at this concentration, was efficient to slightly reduce the presence of Salmonella in MLN but not the shedding. The supplementation of feed by egg-yolk immunoglobulins directed to S. Typhimurium was not efficient to reduce shedding or colonization of MLN by this bacteria. It is thus probable that immunoglobulins from eggs were altered by the swine digestive tract and failed to recognize Salmonella before its penetration of the mucosa.

In conclusion, vaccination with SC54 vaccine™ gave good results in reducing the carrier state of S. Typhimurium in swine. Field experiments should be done to evaluate the potential efficiency of treatments in natural Salmonella contamination level. Resistance to the infection was also noted in 30-40% of experimentally infected pigs, suggesting a natural host resistance to Salmonella infection in some animals. A comprehensive study on host response to SC54™ vaccine, probiotics and prebiotics is needed to better understand the protection against Salmonella infection achieved by these treatments.

References


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