

CONTROL OF *SALMONELLA* IN SWINE BY USE OF PROBIOTICSLETELLIER, A.¹, MESSIER S.¹, QUESSY S.²

Competitive exclusion for the prevention of intestinal colonization by *Salmonella* is an attractive approach that showed promise in some animal species. Nurmi and Rantala demonstrated that the susceptibility of broiler chicks to colonization by *Salmonella* spp. was due to the delayed establishment of intestinal microflora in chickens. They also showed that *Salmonella* spp. infections could be prevented by feeding the chicks with anaerobic cultures of normal adult fowl flora (Nurmi *et al* 1973). Although the efficacy of competitive exclusion has been demonstrated in chickens, little work has been done with other species. Mucosal competitive exclusion was recently used to control *Salmonella* in swine and tends to reduce the presence of *Salmonella* in tissues (Fedorka-Cray *et al*, 1996). Other studies have used well characterized lactic acid bacteria. Shanhani *et al* (1977) reported that lactobacilli could inhibit the growth of *Salmonella in vitro*. Competitive exclusion of *E.coli* and other pathogens by lactobacilli and their cell wall fragments has been demonstrated on human cells in culture by several other workers (Chan *et al* 1985, Coconnier *et al* 1992). The most commonly used and reported probiotics include lactobacilli (*L. acidophilus*, *L. casei*, *L. bulgaricus*), and bifidobacteria (*B. bifidum*, *B. longum*, *B. breve*, *B. infantis*) (Saavedra J.M. 1995). One of the proposed mode of action of such microbial preparations is that the component organisms may colonize the intestine, inhibit the growth of pathogenic bacteria such as salmonellae, and establish a more favourable environment in the host animal (Jin L.Z. *et al* 1996). Carrier state of *Salmonella* in swine productions may result in contamination of meat. Efforts are now being made to control *Salmonella* infection at farm-level and probiotics seems to be a practical and safe approach. The objective of this experiment was to determine if probiotics used as feed additive influence the colonization of tissues and the shedding of *Salmonella typhimurium* in experimentally infected pigs.

METHOD

Animals: Twenty early-weaned 12 day-old *Samonella* free piglets were randomly assigned to either control or treatment groups. Each group was composed of 5 pigs. Clinical signs were monitored twice daily throughtout the experiment.

Probiotics supplementation: The probiotic preparation (Ferlac-2, Rosell Institute, Montreal, Canada) used in this study is a mixture of five microorganisms including *Lactobacillus acidophilus* (4%), *L. rhamnosus* (65%), *L. bulgaricus* (0.1%), *Enterococcus faecium* (25%) and *Streptococcus thermophilus* (5.9%). This preparation was used in a pasty

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form to standardize the supplementation dose. Treated piglets were fed individually for 20 days with 2×10^9 C.F.U. of this preparation per day. Control groups were not supplemented and housed in separate controlled facilities.

Challenge: Salmonella typhimurium, a clinical isolate for septicemic pig (Faculty of Veterinary Medicine, University of Montreal) and kept frozen at -70°C , was used. *S. typhimurium* was inoculated into Nutrient broth (NB, Difco, Detroit, MI) and incubated at 37°C for 18 h. This starting culture was used to inoculate fresh NB tube (1:100). This culture was incubated and bacteria in log phase were used for the challenge. Two doses were given (10^5 and 10^7 C.F.U. *per os*) to the different groups of animals ten days after the beginning of the supplementation.

Necropsy and bacteriology: Rectal swabs were collected every two days before and after the challenge with *Salmonella* and processed as described below. Thirty days post-supplementation, pigs were euthanatized and necropsied. Tissues collected for bacteriology were tonsil, liver, spleen, middle ileum 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 48 h at 37°C , for selective enrichment of *Salmonella* spp. Then, one loopful ($10\ \mu\text{L}$) of the selective enrichment media was inoculated in Brilliant green sulfa agar (BGS, Difco) containing novobiocin (Sigma Chemical Co., St-Louis, MO) at $20\ \mu\text{g/mL}$ 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 against polyvalent O-antisera (Poly A1-Vi, Difco) and *Salmonella* isolates were serotyped under the supervision of Dr. C. Poppe, Agriculture and AgriFood Canada Laboratories in Guelph, Ontario, Canada. Quantitative bacteriology was done on MLN. Dilutions of homogenized tissues (NB) were done in PBS and number of C.F.U. was evaluated by plating dilutions on BGS agar. Colonies typically corresponding to *Salmonella* were identified as describe above.

RESULTS

Clinical signs were not detected in any pigs. As shown in Table 1, for the control groups, *S. typhimurium* was found in tissues of most animals, mainly in MLN and tonsils. *S. typhimurium* inoculated at 10^7 C.F.U. induced a higher number of animals with *Salmonella* in tissues. No *Salmonella* was recovered from tissues of animals supplemented with the probiotic preparation, excepted in MLN of one animal. Quantitative evaluation of *Salmonella* in MLN was negative in all supplemented pigs and ranged between 100-200 C.F.U./g in 3 pigs in the control groups. Recovery of *Salmonella* from rectal swabs was variable. However, pigs supplemented shedded sporadically *S. typhimurium* while in control pigs, only one pig shedded one day during the experimental period.

Table 1. Effect of Ferlac-2 on *Salmonella typhimurium* colonization of tissues in infected pigs

<u>Groups</u>	<u>Dose (C.F.U.)</u>	<u>Shedding</u>	<u>MLN</u>	<u>Spleen</u>	<u>Liver</u>	<u>Tonsils</u>	<u>Ileum</u>
control	10 ⁵	0/5	3/5	0/5	1/5	1/5	0/5
	10 ⁷	1/5	4/5	1/5	1/5	3/5	0/5
Ferlac-2	10 ⁵	5/5	0/5	0/5	0/5	0/5	0/5
	10 ⁷	5/5	1/5	0/5	0/5	0/5	0/5

CONCLUSION

Our findings suggest that the use of this combination of bacteria may prevent the invasion of tissues by *Salmonella* but would not impair the spread of *Salmonella* in the environment. These results demonstrated a reduction of carrier state in swine treated with probiotics. Further studies are now being performed to identify possible mechanisms by which probiotic agents might procure their protective effect against *Salmonella*. Many mechanisms may be proposed, including competition with other flora for nutrients (Simon G. 1984), acidification of luminal intestinal contents, production of inhibitory substances (Saavedra J. M. 1995), competition for receptors or adhesion to the intestinal mucosa (Jin L.Z. 1996) and immunomodulation (Hatcher G.E. 1993). Since Ferlac-2 prevented the tissues invasion by *Salmonella* but gave limited effect on shedding, one could consider the use of this product combined with an adjunct treatment to control shedding such as supplementation with fructooligosaccharides or mannose (Oyofe *et al* 1989).

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