Effect of Feeding *Lactobacillus* to Pigs Infected with *Salmonella typhimurium*

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Summary and Implications

Pigs were fed 24-hour cultures of *Lactobacillus* spp. daily for 34 days. On the 8th day of *Lactobacillus* spp feeding, animals were inoculated with 10^8 colony-forming units (CFU) of *Salmonella typhimurium*.

Based on these data, feeding *Lactobacillus* spp. cultures to pigs infected with *S. typhimurium* reduced the number of animals that were culture-positive for *S. typhimurium* by fecal and tonsil samples. Further, feeding *Lactobacillus* spp. cultures reduced the duration of *S. typhimurium* shedding from tonsil and fecal samples.

Introduction

Many strategies have been investigated for pre-harvest intervention of *Salmonella* in swine. Among these strategies is the oral administration of probiotics and competitive exclusion cultures (CEC). Previous studies have demonstrated reduced fecal shedding and cecal colonization in early-weaned pigs fed CEC and infected with *S. typhimurium* (1). Furthermore, probiotics containing more than one species of *Lactobacillus* have been used to reduce *Salmonella* colonization of tissues (2,3). The purpose of the current study was to examine the effect of administering one strain of *Lactobacillus* to 4-week-old-pigs challenged with *S. typhimurium.*

Materials and Methods

Trial 1: Ten 4-week-old pigs that were culture-negative for *Salmonella* were identified with a numbered ear tag and randomly assigned to each of two treatment groups. Each treatment group was repeated in trial 2. Group 1 was fed a 24 hour culture of *Lactobacillus* spp. strain GS-1. Group 2 was fed sterile medium. Rectal swabs were collected on days –12, 2, 4, 7, 9, 11, 14, 17, 19, 21, and 24 relative to challenge. Five 5-gram samples of feces were collected on the same days as rectal swabs and on day –1 relative to challenge. Palatine tonsil swabs were collected on days 15 and 21 relative to challenge. Blood was collected on days -1, 7, 14, 21, and 27 relative to challenge. Both groups were challenged intranasally with 3×10^8 (CFU) of *Salmonella typhimurium* (strain B) on day 8. Pooled pen fecal, rectal swabs and tonsil swabs were cultured for the presence of *Salmonella*. Briefly, samples were pre-enriched in buffered peptone water (BPW) at 37^oC for 18–24 hours. After pre-enrichment, 100 µl was transferred to 9.9 ml of Rappaport-Vassiliadis (RV) broth and incubated at 42^oC for 18–24 hr. After incubation in RV broth, a sterile cotton swab was used to transfer an aliquot of the cultures onto XLD agar plates. The agar plates were streaked for isolation and incubated at 37^oC for 24 hours. Colonies suspected to be *Salmonella* spp. were picked and transferred to Kliglers's iron agar, SIM semi-solid agar, phenylalanine agar, lysine iron agar, and trypticase soy agar slants and incubated for 37^oC for 24 hours. Presumptive *Salmonella* colonies were tested for "O" antigens by agglutination with typing antisera. Identification of *Salmonell*a from serogroup B were considered to be the challenge strain. Serum from blood samples was tested for antibody to *Salmonella* using the mix-ELISA. The trial was terminated 27 days after challenge.

Trial 2: Twenty-five 4-week-old pigs that were culture-negative for *Salmonella* were identified with numbered ear tags. Ten animals were randomly assigned to two treatment groups: group 1 and group 2 as in trial 1. Five animals were randomly assigned to a third group, group 3 (unchallenged control). Animals in the group 1 and group 2 administered *Lactobacillus* spp. strain GS-1 as in trial 1. Animals in group 3 were administered *Lactobacillus* spp. strain GS-1 as in group 1. Rectal swabs were collected on days –1, 2, 4, 7, 9 11, 14, 17, 19, 21, 23, 25, and 27 relative to challenge. Pen fecal samples were collected on the same days as rectal swabs except on day 23 when no pen fecal was collected. Tonsil swabs were collected on days –1, 2, 5, 7, 9, 11, 15, 21, 23, and 27 relative to challenge. Blood was collected on days –1, 7, 14, 21, and 27 relative to challenge. Animals in the group 1 and group 2 were challenged as in trial 1. Pooled pen fecal samples, rectal swabs, and tonsil swabs were cultured for the presence of *Salmonella* as in trial 1. Three and two animals from group 1 and group 2 were randomly selected and euthanized on day 2 and day 9, respectively. The trial was terminated 27 days after challenge.

Table 1 *Salmonella* isolation from pig feces

	Day (Challenge = 0)							
Group	2 *	4 *	7	9 *	11 *	14 *	21 *	24
1	5/12	1/12	1/12	2/10	1/10	0/10	0/10	0/5
2	12/2	8/12	4/12	9/10	8/10	5/10	5/10	1/5

***** P<0.05 for Fisher's exact test of proportions on a given day.

Table 2 *Salmonella* isolation from tonsil

	Day (Challenge = 0)							
Group	2	5	7	9	11 *	15 *	21 *	23
1	6/10	1/7	2/7	1/7	0/5	0/10	0/10	0/5
2	8/10	5/7	3/7	4/7	4/5	7/10	7/10	3/5

- P<0.05 for Fisher's exact test of proportions on a given day

Table 3 Summary of *Salmonella* isolation from pig feces and tonsil.

Group	Parameter	Number of animals	Average number of culture-positive days (std. err)
1	Pig feces	10	3.3 (1.42)[a]
2		10	17.5 (1.6) [b]
1	Tonsil	5	4 (1.4) [a]
2		5	21 (1.5) [b]

[a,b] Different letters indicate P<0.05 for difference between means.

Results and Discussion

Group 1 demonstrated reduced duration and number of animals culture-positive for *Salmonella* in the feces and tonsil compared with Group 2. No *Salmonella* was recovered from any of the fecal or tonsil samples cultured from group 3. In trials 1 and 2, 15 animals received *Lactobacillus* spp. strain GS-1 and were challenged. Of the 10 animals that remained until the study was terminated, *Salmonella* was never recovered from five animals on any day. In group 2, *Salmonella* was recovered from all of the animals on at least 1 day after challenge.

The results of this study indicate that administration of *Lactobacillus* spp. strain GS-1 reduced the duration and number of animals shedding *S. typhimurium* in the feces. Further, *Lactobacillus* spp. strain GS-1 reduced the number of days *S. typhimurium* was isolated from tonsils. The mechanisms of these effects may include immune stimulation, decreased pH of the gastrointestinal tract, competitive exclusion of nutrients, and luminal attachment sites within the gut (2,3).

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