The Effect Of Feed Withdrawal On The Shedding Of Salmonella Typhimurium By Swine

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Abstract

This project was designed to determine if feed withdrawal in conjunction with transportation-related stress caused increased shedding of Salmonella by carrier pigs. In this experiment, 48 pigs were challenged orally with S. typhimurium after weaning and allowed to grow under typical production practices. Antibiotics were not included in feeds. At monthly intervals, fecal and serum samples were collected from each pig. All pigs shed the challenge organism at least once during the experiment. By the sixth month, most pigs were negative for the challenge organism when cultured from feces. When pigs reached market weight (~240 pounds), they were split into 4 groups and subjected to one of the following feed withdrawal protocols: group 1 had no feed withdrawal, groups 2-4 had feed withdrawn at 6, 12 or 24 hours, respectively. Pigs in each group were transported ~225 kilometers (4 hours), returned to the production facility and necropsied. Contents at the ileal-cecal junction were collected and cultured for the test organism. There was a direct correlation between the time of feed withdrawal and the number of pigs with S. typhimurium in intestinal contents. Pigs that had no feed withdrawal had the fewest number of positive pigs, whereas pigs that were off feed for 24 hours had the highest numbers of positive pigs. Serum samples were assessed using a mixed ELISA to detect antibodies against Salmonella. All pigs had detectable antibodies. Over the various bleedings, most pigs exhibited increases in anti-Salmonella antibodies over time. Some had modest drop-offs at the later times. In about 10% of pigs, there was no increase or decrease in anti-Salmonella antibodies. In these non-responding pigs, the specific titers were lower than responding pigs, and above background levels. The results from this experiment complement our previous work and demonstrated that feed withdrawal when in conjunction with shipping contributes to shedding of Salmonella by pigs. Most pigs were not shedding the challenge organism just prior to slaughter, yet most (>80%) had persistent infections. This indicates that the detection of infected, asymptomatic carriers using conventional culture methods is difficult and could complicate plans to develop programs to certify Salmonella-free pigs.

Introduction

Of the food borne pathogens, the United States Department of Agriculture has identified Salmonella to receive its highest priority (4). The consumption of pork products contaminated with Salmonella is a frequent cause of disease. Bryan (3) demonstrated, for example, that 11% of Salmonella outbreaks were associated with the consumption of pork, while Bean and Griffin (1) have described numerous outbreaks where pork was identified as the source of contamination. S. typhimurium is one of the leading causes of salmonellosis in man and is the second most commonly isolated serotype from swine (2, 5). Animals exposed to Salmonella generally become persistently colonized for the remainder of their lives and can serve as reservoirs to contaminate other animals and food products. In a study by Wood, et al (8), it was shown that animals challenged with S. typhimurium continued to shed S. typhimurium until they reached market weight 28 weeks later. In that study, carrier animals were identified because they persistently shed low levels of the challenge organism.

Current data is consistent with the hypothesis that pigs are infected early in life and become persistently infected. Stresses, particularly those associated with shipping, are believed to increase the shedding of Salmonella by carrier animals. Williams and Newell (7) showed that shipment of pigs led to altered (increased) shedding patterns of Salmonella. Thus, as a result of shipping, carrier animals begin to shed higher levels of Salmonella that may be spread within the herd during shipment, at packing plants, and during the processing to finished products. In our previous experiments it was shown that pigs experimentally challenged with S. typhimurium exhibit increased shedding after transportation (6). In the initial experiments, it was further shown that feed withdrawal prior to transporting the pigs also was an important factor in the shedding process. However, contrary to what was expected, feed withdrawal actually was correlated with decreased shedding of S. typhimurium in shipped pigs, rather than an increase. The experiments described here were designed to determine more specifically the effect of feed withdrawal on the shedding of S. typhimurium in pigs that were experimentally challenged and subjected to shipping. To assess the immune response to experimental challenge, a mixed ELISA was performed using sera obtained from the same pigs.
Materials and Methods

Forty-eight pigs were obtained and after weaning (4-5 weeks of age) each was challenged orally with 1 ml of *S. typhimurium* strain 798 containing $2 \times 10^4$ viable cells. The strain used was resistant to nalidixic acid. Four weeks post challenge the pigs were re-challenged with the same number of organisms. Fecal samples were obtained from each pig on a monthly basis starting one week post challenge. When the pigs reached an average weight of 240 pounds, they were divided into four groups of equal size. Pigs in group 1 were allowed access to feed for the 48 hour period prior to slaughter while pigs in groups 2, 3 and 4 were taken off feed at 6, 12, or 24 hours prior to slaughter, respectively. All pigs were transported by truck-trailer for 167 miles (approximately four hours in duration) and slaughtered. Contents from the intestinal tract at the ileocecal junction were collected from each animal.

One gram samples of feces or ileocecal contents were added to 10 ml tetrathionate broth, mixed, and four serial 1:10 dilutions were prepared into four additional tubes containing 10 ml tetrathionate broth. All tubes were incubated 24 hours at 37°C. One ml from each tube was added to 10 ml of Rappaport medium, incubated 24 hours at 37°C, and then plated on brilliant green plates containing nalidixic acid (50 µg/ml). After another 24 hour incubation period at 37°C red colonies from the brilliant green plates were picked and plated on LB agar containing nalidixic acid (50 µg/ml) to confirm that the colonies were the nalidixic acid resistant challenge organism. Random colonies were picked and confirmation that they contained *Salmonella* was performed using API20E strips. The relative concentration of challenge *S. typhimurium* per sample was defined as the highest $\log_{10}$ dilution yielding growth.

The mixed ELISA protocol was as developed by Dr. Paula Fedorka-Cray (personal communication). Antigen was prepared from four organisms (*S. typhimurium*, *S. choleraesuis*, *S. enteritidis*, and *S. anatum*). The antigen was prepared by heating cultures to 60°C for 1 hour, the supernatant was clarified by centrifugation and then filtered through a filter (0.22µm). This antigen was added to the wells of microtiter plates (4 µg protein/well of total antigen). Serum was serially diluted (2-fold) with a starting dilution of 1:40.

Results

It was previously shown that transportation of pigs led to the increased presence of *S. typhimurium* in the ileocecal junction of pigs challenged with *S. typhimurium* (6). However, when pigs were also subjected to feed withdrawal (24 hours), fewer pigs were *S. typhimurium* positive than if they were not subjected to feed withdrawal. To more carefully assess the effect of feed withdrawal on the presence of *S. typhimurium*, the following protocol was designed. Pigs were orally challenged with *S. typhimurium* and at production weight were subjected to feed withdrawal of 0, 6, 12, or 24 prior to transport and slaughter.

Following the challenge, the presence and quantity of *S. typhimurium* per pigs was assessed. The results are shown in the following figures. Over time the number of pigs actively shedding the challenge organism declined to a point just before slaughter when only 2 pigs were actively shedding *S. typhimurium*. While the number of positive pigs never exceeded 80% at any sampling time, all pigs were positive for the challenge organism at some time during the experiment. The average concentration of the challenge organism decreased during this time. Of the two pigs that were positive for *S. typhimurium* just prior to slaughter, one had 1 $\log_{10}$ per gram of feces and the other had 2 $\log_{10}$ per gram of feces.

The results of sampling ileocecal contents from pigs in the four groups is shown in the figure below. There appeared to be a direct correlation between the time of feed withdrawal and the number of positive pigs. It should be noted that even though none of the pigs were positive prior to transport (via fecal culture), 18% were positive even in the pigs that remained on feed after transport.

Figure legend. Time of feed withdrawal (hours): 1=0 hours, 2=6 hours, 3=12 hours, 4=24 hours.
The results of the serologic examination indicated that all pigs produced antibodies against *Salmonella* at some time during the experiment. Over the various bleedings, most pigs had increases in anti-*Salmonella* antibodies over time. Some did show declines at the later times. However, about 10% of pigs exhibited constant titers during all times sampled. In each case, the specific titers from these low responding pigs was low compared to the other pigs but were higher than baseline levels (measured 1 week after challenge).

**Discussion**

The results of this study demonstrated several very important points about *Salmonella* in swine. Firstly, by following the shedding patterns of *Salmonella* by pigs, it is clear that upwards of 80% or greater of challenged pigs became persistent carriers. While all pigs shed the challenge organism at some time during the 5 month experiment, just prior to slaughter only 2 pigs could be identified as still shedding (and the 2 pigs were shedding the challenge organism at low levels). However, based on the samples at necropsy, greater than 80% of pigs that were subject to feed withdrawal for 24 hours and then transported, were positive for the challenge organism. While there is concern about the true correspondence between fecal culture and culturing intestinal contents, this observation is striking. It probably underestimates the number of *Salmonella* positive pigs. This observation is even more important within the context of developing testing methods to identify carrier pigs that could be part of a certification program or to simply test the effects of intervention programs to reduce infected animals (and as a result reduce the risk of contaminating pork products post slaughter). If carrier pigs cannot be satisfactorily identified, then any programs that require detection of such animals will miss large number of carrier pigs.

A second important point is the apparent correlation between the length of feed withdrawal with the detection of the challenge organism in pigs. This experiment demonstrated that the longer the time of feed withdrawal, the greater the number of *Salmonella*-positive pigs. This result is consistent with what one might predict if stress responses are responsible for increased numbers of *Salmonella*-positive pigs and if feed withdrawal causes stress. However, this result is in direct contrast to what was seen in our previous studies where feed withdrawal (24 hours) actually was correlated with reduced numbers of *Salmonella*-positive pigs (6). Collectively, the results of each of the experiments indicate that both feed withdrawal and transportation can, in a non-predictable way, lead to increased shedding of *Salmonella* by pigs. This conclusion is consistent with these results and suggests that additional, yet unidentified, factors also contribute to this phenomenon.

Thirdly, the results of serology indicate that most pigs responded to the oral challenge by producing circulating anti-*Salmonella* antibodies. Most of the pigs in the study exhibited clear-cut increases in antibody titers after challenge. Four pigs did not show obvious increases in antibody titers post challenge but these animals did have antibody titers that were above baseline (established 1 week post challenge). The antibody levels in these pigs, while above baseline were low and did not increase or decrease over time. Thus, individual pigs respond differently. There was no correlation between the level of colonization or the length of time of colonization and specific trends in antibody responses. Therefore, while this test can measure exposure, it is not necessarily useful in the identification of carrier animals or even animals that are shedding at any given time.

**References**


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