RESEARCH REPORT
PORK SAFETY

Title: Effect of chlorate treatment on transmission of *Salmonella* in swine during lairage and transport. - **NPB #02-074**

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Abstract: Each year more than 1.3 million human cases of Salmonellosis are reported in the United States. Swine can be a reservoir of *Salmonella* that can be transmitted to human consumers of pork products. *Salmonella* have the ability to respire anaerobically by reducing nitrate to nitrite via the intracellular enzyme nitrate reductase (NR). However, NR does not differentiate between nitrate and its valence state analog chlorate, which can be converted within the bacterium to cytotoxic chlorite. When added to pure and mixed cultures of bacteria, chlorate killed both *E. coli* and *Salmonella* within 24 h. Preliminary in vivo studies indicated that chlorate supplementation reduced *E. coli* O157:H7, wild-type *E. coli* and *Salmonella* in cattle, sheep and swine, respectively. Therefore, an experimental chlorate-containing product (XCP) has been developed for use in food animals. The current study was undertaken to evaluate the effectiveness of XCP during the short-term lairage period immediately prior to harvest. Pig manure (10 kg) was inoculated with 10^3 CFU/g *Salmonella* Typhimurium and was spread throughout pens housing pigs (n=20) to simulate the introduction of swine to dirty lairage facilities. After 2 h, pigs were given *ad libitum* feed (controls) or feed supplemented with XCP (XCP) for 6 h. Animals were humanely sacrificed and tonsils, ileocecal lymph nodes, cecal and rectal contents were collected. Fewer pigs treated with XCP had *Salmonella*-positive tonsils, but not unexpectedly due to the continuous exposure to *Salmonella*-contaminated feces this difference was not significant ($P>0.05$). No differences were noted in lymph node or intestinal content *Salmonella* status, likely due to the very short duration of XCP treatment. However, in a follow-up study using pigs (n=20) naturally colonized with *Salmonella*, XCP treatment significantly ($P < 0.05$) reduced natural cecal *Salmonella* colonization. Thus, these results indicate that XCP could be a viable pre-harvest intervention strategy to reduce *Salmonella* concentrations in swine, however further research is needed to optimize the effectiveness of XCP during lairage and transport to the slaughter facility.

Introduction: Swine can be carriers of the food borne pathogenic bacteria *Salmonella*. Pigs may become colonized with *Salmonella* at the farm of origin, the finishing floor, during transport or lairage immediately prior to slaughter. Recent studies have indicated that the lairage period plays an important role in *Salmonella* colonization of swine.

Sodium chlorate kills bacteria equipped with the enzyme nitrate reductase; this includes *Salmonella* species. Chlorate is very effective in vitro against Salmonella species, and has been used previously to reduce *Salmonella* populations in finishing pigs previously. However, in all previous studies the chlorate supplementation periods were always $\geq$16 h before slaughter.

Therefore, this study was designed to examine the usefulness of chlorate in treating pigs in the short-term lairage period immediately prior to slaughter to reduce *Salmonella* populations in the gut. Also, this study was designed to determine if chlorate treatment would affect *Salmonella* transmission from the environment (dirty holding pens) to the animal.

Objectives: The objectives of this project were: 1) Test and evaluate the effects of sodium chlorate treatment on the incidence and intestinal populations of *Salmonella* in finished swine during the transport and/or lairage period; and 2) Determine the effectiveness of chlorate treatment on the transmission of *Salmonella* from the environment to swine during lairage.

Materials and Methods: To address **objective 1**, pure cultures of *Salmonella* typhimurium were treated with various concentrations of sodium chlorate. *S*. typhimurium cultures were grown anoxically in TSB, and growth was measured via change in optical density (600 nm). This data determined the necessary concentration of chlorate needed to kill *Salmonella* as an estimate for use in the pig gut.

Transport studies proposed were not performed due to the untimely passing of our collaborator, Dr. Julie Morrow-Tesch. The specially modified and equipped transport trailers and behavior expertise was no longer available to us following her death.

Swine (n=10) were purchased from a single source that has traditionally had problems with *Salmonella* on-farm. When pigs arrived at FFSRU all pigs were positive for *Salmonella* via rectal swabs. At 22:00 one group (chlorate-treated) was given *ad libitum* drinking water supplemented with 30 mM Chlorate, and were given feed containing an experimental chlorate product (ECP); Control pigs were given access to unsupplemented drinking water and feed. At 14:00 (16 h after initiation of chlorate treatment), pigs were humanely sacrificed, and tonsils, ileocecal lymph nodes, and tissues and contents from the cecum and rectum were collected. Tissue samples were enriched in tetrathionate and RV broth and were streaked on Brilliant Green Agar supplemented with novobiocin (for selection of *Salmonella*) to qualitatively determine the presence of the inoculated bacterial strain. This experiment was to verify that chlorate could reduce intestinal populations of *Salmonella* in pigs.

Swine (n=100) were purchased from the Texas Department of Criminal justice and were transported to FFSRU. Swine tested negative for naturally-occurring Salmonella. Pigs were divided into “seeder” and “contact” groups. “Seeders” were inoculated via oral gavage with 6 x 10^9 CFU/ml *Salmonella* typhimurium resistant to Novobiocin and Nalidixic acid. The Seeders were kept in a separate pen from the Contact pigs for 24 h. After 24 h, their feces were examined and they were found to always be shedding more than 10^3 CFU/g feces *S*. typhimurium. Seeders (2/pen/experiment) were moved into pens containing Contact pigs (8/pen/experiment).

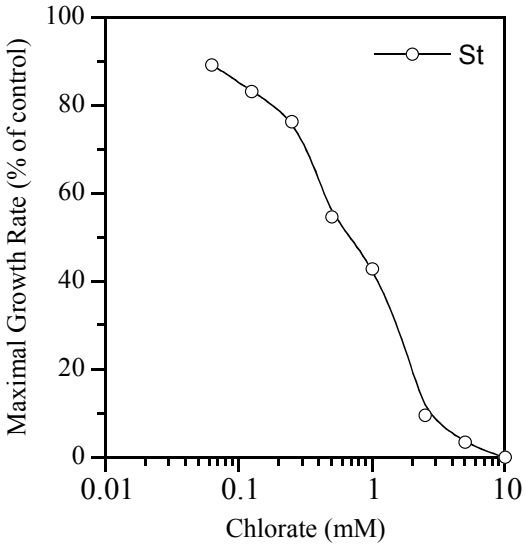
Each experiment consisted of a control and a chlorate-treated group (20 pigs/experiment). The length of the exposure between Seeders and Contacts was increased in each subsequent study due to the increasing length of chlorate treatment necessary to affect *Salmonella* levels. Initially, the Contact/Seeder exposure period was 4 h, but following 2 replications, this time period was not sufficient to obtain reproducible infection of Contacts as had been demonstrated previously. Therefore the Contact/Seeder exposure period was increased to 8 h, 16 h, and eventually to 24 h. Initially, the chlorate exposure time was 2 h, but that was increased in subsequent replications to 4, 6, 8, and 16 h. Intestinal contents *Salmonella* populations were quantitatively and qualitatively determined using the same methods as described above.

To address **objective 2**, pigs (n=20) were purchased and transported to FFSRU and were housed in 2 group pens (n=10 each) overnight, and had free access to water and feed until 00:00 h. Feed and water were withdrawn to enhance consumption during the subsequent treatment period. At 06:00, pig manure from farms in IA and TX (not farms of experimental pig origin) was inoculated with a *Salmonella* Typhimurium strain marked with antibiotic resistance (novobiocin/ nalidixic acid resistant) and mixed thoroughly. The final concentration of *Salmonella* in the spread manure was with $3 \pm 1.5 \times 10^3$ CFU/g feces. Unfortunately, no natural *Salmonella* strains were isolated from enrichments of this manure. The manure was spread evenly (by weight) in pens, and the pigs immediately began playing with/consuming it. At 08:00 (2 h after manure/*Salmonella* addition) one group (chlorate-treated) was given *ad libitum* drinking water supplemented with 30 mM Chlorate, and were given feed containing an experimental chlorate product (ECP); Control pigs were given access to unsupplemented drinking water and feed. At 14:00 (8 h after manure/*Salmonella* addition, and 6 h after initiation of chlorate treatment), pigs were humanely sacrificed, and tonsils, ileocecal lymph nodes, and tissues and contents from the - and rectum were collected. Tissue samples were enriched in tetrathionate and RV broth and were streaked on Brilliant Green Agar supplemented with novobiocin and nalidixic acid to qualitatively determine the presence of the inoculated bacterial strain.

In follow up studies, pigs (n=20) were transported to FFSRU. Again, the manure that was utilized did not contain any naturally occurring *Salmonella*. All pigs were placed in a pen containing the manure spiked with 4×10^3 CFU/g of the antibiotic resistant *Salmonella* strain. Following 2 hrs of *Salmonella* exposure, the pigs were moved to 2 clean pens (containing no added manure), and one group was given access to chlorate-supplemented water and feed, and the control group was given unsupplemented feed and water. Swine were slaughtered at 8 h after manure addition, and 6 h after chlorate treatment initiation, as in the previous *in vivo* study.

Results: Addition of several concentration of chlorate to in vitro incubations of *Salmonella* Typhimurium demonstrated that chlorate inhibited growth of *Salmonella* (Figure 1). This result indicated that the food-borne *Salmonella* strains that were used in the subsequent in vivo studies were chlorate-sensitive and a concentration of approximately 1 mM was necessary to cause a 50% inhibition in growth rate. Therefore, we calculated an approximate dose of chlorate necessary to yield a chlorate concentration in the effective range.

Figure 1. Effect of chlorate on *Salmonella* Typhimurium growth rates.



In addressing **Objective 1**, we found that the addition of chlorate to pigs naturally infected with *Salmonella* reduced cecal populations of *Salmonella* significantly (Table 1). Indicating that the dose of chlorate utilized in this study was sufficient to reduce *Salmonella* in the gut, and that the ECP was functional in the pig gut when given at least 16 h prior to slaughter.

Table 1: Number of pigs containing naturally-occurring *Salmonella*.

	Tonsils	Cecum	Rectum
Control	0/5	5/5	3/5
Chlorate	0/5	0/5	1/5

Typical results from experiments addressing the use of chlorate to reduce gastrointestinal populations of *Salmonella* are presented in Table 2.

Table 2: Number of pigs containing challenge strain of *S*. Typhimurium when exposure time to *Salmonella* "Seeders" was 16 h, and a chlorate treatment length of 6 h.

	Tonsils	IC Lymph	Cecum	Rectum
Control	7/8	5/8	3/8	2/8
Chlorate	6/8	3/8	2/8	3/8

Other results from other replicates are not presented for brevity's sake. However the following conclusions were gathered from these several studies: 1) in this experimental design, at least 8 h exposure between Seeders and Contact pigs was necessary for a majority of rectal and cecal samples to be *Salmonella* positive; 2) At least 16 h of chlorate treatment is necessary for there to be reduction in *Salmonella* in the gastrointestinal tract. These "failed" studies are important to the understanding of the spread of *Salmonella* in lairage pens, but do not provide any evidence to prove the hypothesis tested.

In final studies, the exposure time to seeders was expanded to 24 h, and chlorate treatment duration was 16 h.

Table 3: Number of pigs containing challenge strain of *S*. Typhimurium when exposure time to *Salmonella* "Seeders" was 24 h, and a chlorate treatment length of 16 h.

	Tonsils	IC Lymph	Cecum	Rectum
Control	8/8	3/8	7/8	7/8
Chlorate	8/8	1/8	2/8	3/8

We had proposed transport and behavior studies in our original grant proposal. However, these studies were not performed due to the untimely passing of our collaborator, Dr. Julie Morrow-Tesch. The specially modified and equipped transport trailers and behavior expertise was no longer available to us following her death.

To address **objective 2**, pigs were placed into pens containing manure that contained $3\pm1.5 \times 10^3$ CFU ST/g feces. Pigs were exposed to manure for 2 hours prior to initiation of chlorate treatment. Chlorate (or control) treatment lasted for 6 h prior to slaughter; results are presented in table 4

Table 4: Number of pigs containing challenge strain of *S*. Typhimurium when exposed to manure containing $3\pm1.5 \times 10^3$ CFU ST/g feces.

	Tonsils	Cecum	Rectum
Control	10/10	2/10	0/10
Chlorate	7/10	1/10	1/10

In a follow up study, pigs were placed into pens containing manure containing 4×10^3 CFU ST/g feces. Pigs were exposed to manure for 2 hours prior to initiation of chlorate treatment, and were moved from the dirty pen, to clean pens. Chlorate (or control) treatment lasted for 6 h prior to slaughter; results are presented in table 5.

Table 5: Number of pigs containing challenge strain of *S*. Typhimurium when exposed to manure containing 4×10^3 CFU ST/g feces. Pigs in this study were moved from dirty pens to clean pens at initiation of chlorate/control treatment.

	Tonsils	Cecum	Rectum
Control	8/10	1/10	0/10
Chlorate	4/10	0/10	0/10

Discussion: Based on our results, it does not appear that chlorate had a significant impact on *Salmonella* contamination of pigs when used exclusively during the short-term lairage period (**Objective 1**). However, these results are not surprising, given the fact that the pigs were continuously exposed to *Salmonella* by the "Seeder" pigs in the pens up until the moment of slaughter. This result is not unexpected and is very logical. Also, the fact that chlorate must 1) reach the lower gut in sufficient quantities and 2) be present a period of time to affect killing is a logical and expected result. Unfortunately, it appears to limit the treatment with chlorate to the period at least 16 h prior to slaughter; even though the chlorate would be fed before the lairage period, it would be functional (killing pathogens) inside the gut during the lairage period.

A similar result was seen in the environmental contamination studies in the fact that any tissue that was cleaned of *Salmonella* by chlorate could immediately be dirtied again by repeated exposure to *Salmonella* in the dirty pens (**Objective 2**). Therefore, we modified our approach to examine a more limited environmental exposure to *Salmonella* immediately prior to the entry to lairage. Because of the potential for constant recontamination of the tonsils and cecum with ingested *Salmonella* (even following chlorate treatment), we investigated the effects of chlorate on animals exposed during lairage to environmental contamination, but housed in cleaner pens than those previously examined.

Interestingly, in our environmental contamination studies, even though the manure used was inoculated with 10^3 CFU/g *Salmonella* (novobiocin/nalidixic acid resistant), none of the tissues of any of the pigs other than tonsils in this study tested positive for the inoculated *Salmonella*. This result does not agree with the previously published results of Hurd et al. This difference is hypothesized to simply be due to a difference in ST strain used or diet used in the studies. However a repeated failure to repeat those results is significantly disappointing.

These results indicate that: 1) inoculation of animals via contaminated feces is not a clear-cut, immediate process; 2) the movement to clean facilities significantly reduced tissue contamination by *Salmonella*; 3) it appears that chlorate reduced cecal contamination with naturally-occurring *Salmonella*, although because of the relatively small number of animals involved to date this result is not statistically significant. However, this result is encouraging because if swine are brought into clean lairage facilities, then chlorate could potentially reduce total carcass contamination before slaughter.

Lay Interpretation: Chlorate can be used to reduce *Salmonella* in pigs. However, it must reach the lower gut in order to kill *Salmonella*. Therefore, although chlorate will work to reduce *Salmonella* during the lairage period, it must be fed to the animal approximately 16-24 h prior to slaughter in order to reap any benefit from chlorate treatment. Thus, we cannot recommend the use of chlorate as a treatment specifically in the short-term (6 h) lairage period, although it does appear beneficial when used a short period of time (16-24 h) before slaughter. Additionally, these results indicate that proper facility sanitation is crucial to the effectiveness of this, and any, intervention strategy. We thank the National Pork Board and our nation's pork producers for their generous financial assistance in exploring this important avenue of improving food safety.

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