Oral Sodium Chlorate, Topical Disinfection, and Younger Weaning Age Reduce *Salmonella enterica* Shedding in Pigs

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ABSTRACT

*Salmonella enterica* subsp. *enterica* can cause swine illness or human foodborne disease. Although nontoxic to mammalian cells, chlorate can be converted to cytotoxic chlorite by salmonellae. To test whether chlorate is effective at reducing *Salmonella* shedding in weaned pigs exposed to shedding dams, a chlorate-nitrate-lactate (chlorate) oral dose was administered daily for 5 days following weaning, and this treatment was evaluated in combination with two weaning ages and a topical disinfectant. A total of 80 pigs were weaned at 10 or 21 days of age. Half within each age group were topically disinfected at weaning. Piglets were selected from dams for which *Salmonella* was detected in feces shortly after giving birth. Chlorate treatment reduced *Salmonella* prevalence and estimated *Salmonella* concentration in feces, cecal contents, and ileocolic lymph nodes. Younger weaning age (10 days of age) was associated with reduced shedding (lower concentration and prevalence) in samples collected at 10 days postweaning (DPW) and later. Chlorate treatment reduced the concentration of *Salmonella* in fecal samples at 5 DPW and in cecal samples at 14 DPW. The protective effects persisted through the end of the study at 14 DPW, 9 days after the final administration of chlorate. Disinfectant treatment reduced shedding in fecal samples at 14 DPW. Interactions were detected between the effects of chlorate and disinfection and between chlorate and weaning age. Chlorate treatment, topical disinfection, and younger weaning age may be useful tools for reducing *Salmonella* shedding on farms that practice segregated weaning and where sow-to-piglet transfer of *Salmonella* is an important source of infection in nursery pigs.

*Salmonella* infection is one of the leading causes of foodborne illness (31) and has been estimated to cause 1.4 million cases of human disease in the United States (37). *Salmonella enterica* has been categorized into more than 2,400 serovars (17). Although only two of these, *Salmonella* Choleraesuis and *Salmonella* Typhimurium, are considered important pathogens of swine (25), a large number of serovars have been implicated as causing foodborne illness transmitted to the human food chain (9). Authorities in Europe regard all *Salmonella* serovars as risks for foodborne illness (10), and in the United States serovars have not been distinguished for the purpose of regulatory compliance (5).

*Salmonellae* are widely distributed in growing swine in most parts of the world. *Salmonellae* were detected in fecal samples from 61 (38.2%) of 160 swine herds in a national U.S study (4) and were detected among 24 of 30 midwestern U.S. herds at slaughter (13). From 1998 through 2003, *Salmonella* was detected in 4.3% of U.S. swine carcasses tested by regulatory authorities (7). In a study of nine abattoirs in Europe, *Salmonella* was detected on carcasses in four of the five participating countries.

Among pigs from these countries, *Salmonella* was detected on 3.8% of the carcasses tested (26). In Denmark, 22% of herds were *Salmonella* culture positive at slaughter (4).

Farms are thought to be the primary source of *Salmonella* that contaminates carcasses (25). *Salmonella* prevalence in farm-collected fecal samples was positively correlated with *Salmonella* detection in ileocecal lymph nodes (12, 13) and in feces and cecal contents at slaughter (12). Detection of *Salmonella* in mesenteric lymph nodes or feces was positively correlated with carcass contamination. (16). Elimination of *Salmonella* in groups of growing pigs should enhance pork food safety if recontamination and cross-contamination before and at slaughter could be prevented. For the same reasons, reduced concentrations of *Salmonella* shed by growing pigs should improve food safety if the reduction can be maintained through slaughter.

Segregated early weaning, i.e., the use of separate sites for the lactation and postweaning (nursery) periods in combination with early weaning (14 days of age or less), has been used to eliminate a variety of swine pathogens, with and without the use of antimicrobials (20). Elimination of *Salmonella* by segregated early weaning has been previously documented. In one study (19), *Salmonella* was eliminated from three groups of pigs maintained in narrow age-range groups in nursery barns physically segregated from the breeding herd, whereas in another study a similar approach was not effective in all groups (22). *Salmonella* Choleraesuis, a host-adapted serovar, was successfully eliminated by moving weaned piglets to a nursery remote from the sow herd site (32).
One unique metabolic trait of certain bacteria, including *Salmonella*, is the possession of an enzyme, nitrate reductase, that under anaerobic conditions reduces nitrate to nitrite. This enzyme also catalyzes the conversion of chlorate to cytotoxic chlorite, resulting in cell death. Mammalian cells and many bacteria cannot convert chlorate to chlorite (35) and thus are not killed. Both swine (1, 2) and poultry (18, 28, 30) that were orally challenged with *Salmonella Typhimurium* decreased shedding and/or harborage of this pathogen after administration of products containing chlorate salts. In experimentally challenged pigs, Anderson et al. (1) documented *Salmonella* reduction from approximately $10^3$ to $10^{0.5}$ CFU/g of cecal contents 16 h after chlorate treatment.

Integrating the above results, we hypothesized that chlorate treatment may be useful for reducing or eliminating *Salmonella* when that treatment is combined with segregated early weaning. Because fecal contamination of weaned piglets is expected in commercial settings, we also investigated whether topical disinfection, used separately or in conjunction with the other two treatments, would aid in *Salmonella* control. We tested these effects following *Salmonella* exposure from spontaneously shedding dams to more closely mimic patterns of transmission in commercial production facilities (14).

The objectives of this project were to determine whether administration of sodium chlorate, changes in age at weaning, and/or the application of a topical disinfectant modified or eliminated *Salmonella* shedding in weaned pigs derived from sows that shed *Salmonella* and to determine whether these treatments should be applied singly or in combination.

### MATERIALS AND METHODS

A swine herd at the Swine Research and Teaching Center (University of Wisconsin—Madison) was selected as the source of 80 weaned pigs based on a history of *S. enterica* infection in weaned pigs (unpublished observations), the commercially applicable genetic composition of the herd, and the use of commercially applicable production technology. Eight weaned pigs were taken from each of 10 litters. Eligible litters had at least eight viable piglets, the piglets were born during one of two 2-day periods corresponding to the two trial replicates, and *Salmonella* was detected in the sows’ feces within 2 days after giving birth. Nonviable pigs were defined as those whose weight was at least 30% below the mean weight for pigs in that litter. Among viable pigs, eight were randomly chosen per litter. One pig from each litter was randomly assigned to one of eight treatment combinations in a 2 × 2 × 2 randomized complete block study design. For each replicate, five litters provided 40 pigs (5 for each treatment). The objectives of this project were to determine whether administration of sodium chlorate, changes in age at weaning, and/or the application of a topical disinfectant modified or eliminated *Salmonella* shedding in weaned pigs derived from sows that shed *Salmonella* and to determine whether these treatments should be applied singly or in combination.

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Fecal samples collected from sows at 0 to 2 days after giving birth were tested for *Salmonella*. For each trial replicate, samples were tested from all sows giving birth on days when research facilities would be available for their piglets until at least five sows were identified that both shed *Salmonella* and gave birth within 2 days of each other. Conventional bacterial techniques with enrichment were modified from a published protocol (21). A 10-g sample of feces was homogenized in 100 ml of tetrathionate broth (Remel, Lenexa, Kans.) and incubated for 42 to 48 h. An aliquot of this broth (100 μl) was then transferred to 10 ml of R-10 broth (Remel), which was incubated for 18 to 24 h. This broth was streaked onto an XLT-4 agar plate (Remel) and incubated for 18 to 24 h. One suspect colony was streaked onto brilliant green agar (Remel), and *Salmonella* identity was confirmed using polyvalent *Salmonella* antibodies (Salmonella Polyvalent O Agglutinating Sera A-G, Remel). All incubations were at 37°C. One isolate from each dam was serotyped at a reference laboratory (Wisconsin Veterinary Diagnostic Laboratory, Madison).

Within a trial replicate, 40 pigs were weaned and transported at 7 or 14 (±1) days of age to an isolation facility (Charmany Instructional Facility, School of Veterinary Medicine, University of Wisconsin–Madison). One room was used for each treatment group (five pigs per room) to allow complete separation of pigs among the treatments. Upon arrival at the isolation facility, pigs in the chlorate+ treatment group were given 10 ml of aqueous solution orally in a syringe containing 100 mmol sodium chlorate, 2.5 mmol sodium nitrate, and 20 mmol sodium lactate. The addition of nitrate and lactate to the chlorate+ treatment was intended to ensure induction of nitrate reductase activity and to help ensure the presence of a fermentable substrate. The chlorate+ pigs were also provided the same aqueous solution as the sole source of drinking water for 5 days at a concentration expected to provide a sodium chlorate dose of 80 mg/day based on 80% anticipated ad libitum water consumption. The chlorate− pigs were given 10 ml of tap water orally by syringe on arrival at the facility and subsequently given free access to tap water. Treated water and tap water was provided for the remainder of the 24-h period. Pigs in the disinfect+ groups were coated with povidone iodine solution (1%) just before they were introduced to the isolation facility. The solution was applied with saturated paper towels to all external body surfaces except the conjunctiva. Eyes were protected by sterile ophthalmic salve. The pigs were then allowed to air dry under radiant heaters to prevent chilling. Pigs in the disinfect− groups were not treated topically.

Rooms were washed with disinfectant before entry of piglets. After the rooms were dried, four environmental swab samples from each room were cultured for *Salmonella*. Sterile cotton sponges (10 by 10 cm) were premoistened with buffered peptone water (BPW; Becton Dickinson, Sparks, Md.) and transferred to the air dry under radiant heaters to prevent chilling. Pigs in the disinfect− groups were not treated topically.

Dependent on the age at weaning, pigs were fed one of two diets (Table 1) designed to meet or exceed National Research Council nutritional requirements (6) and to mimic diets commonly used in U.S. commercial swine production facilities. The diets were formulated with maize, soybean meal, whey, lactose, and spray-dried plasma (Nutrapro animal plasma, APC, Inc., Ankeny, Iowa). Diet ingredients other than plasma proteins, vitamins, and...
trace minerals were irradiated with 20 to 50 kGray to kill salmonellae. Plasma proteins were irradiated with 10 to 20 kGray to prevent damage to the protein. The trace minerals and vitamins were not irradiated. At least one sample of each batch of plasma protein, trace mineral–vitamin products, and final feed was cultured for *Salmonella* (3). A 25-g sample was incubated in 225 lactose broth (Becton Dickinson) at 37°C for 24 h. One 0.1-ml aliquot of this culture was then transferred to R-10 broth (Becton Dickinson) and a second 0.1-ml aliquot was transferred to tetra-thionate broth (Remel); both aliquots were incubated for 24 h at 42°C. After streaking for isolation on XLT-4 and Hektoen enteric agars (Remel), suspect candidate *Salmonella* colonies were biochemically characterized and serotyped at a reference laboratory (Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin–Madison).

Pig fecal consistency was scored twice daily with a 4-point system for feces voided since the last observation: 1, normal; 2, partially formed; 3, unformed; and 4, watery. Pigs were weighed at weaning and again at termination of the study to calculate average daily weight gain.

Fecal samples were collected 2 days before weaning, at weaning, and at 5, 10, and 14 days postweaning (DPW). At 14 DPW, pigs were euthanized with an overdose of intravenous pentobarbital, and ileocolic lymph nodes and cecal contents were collected. Carcasses were destroyed following University of Wisconsin–Madison approved protocols and did not enter the human food chain. As described for sow fecal samples, conventional bacterial culture techniques were used to detect *Salmonella* in the ileocolic lymph node samples and fecal samples collected 2 days before weaning and at 10 and 14 DPW; however, the volume of tetra-thionate broth was reduced to 30 ml and the sample weight was limited to 3 g. Twenty isolates from fecal samples collected at weaning or at 14 DPW were serotyped at the reference laboratory (Wisconsin Veterinary Diagnostic Laboratory). One isolate was randomly picked from each treatment group in each replicate from samples collected at 14 DPW or from samples collected at weaning if no isolates were detected in the 14 DPW samples. Additional isolates were randomly selected to bring the total number of isolates tested to 20.

A most-probable-number (MPN) method (3) was used to estimate the *Salmonella* concentration (CFU per gram) for cecal contents and for feces collected at weaning and at 5 DPW. Up to 3 g total of each sample was assayed as three replicates of four 10-fold dilutions, 1 g per replicate. All sample dilutions were then processed following the procedure for bacterial culture described for the sow fecal samples except that the volume of tetra-thionate broth was 10 ml per tube. The MPN was computed with a spreadsheet program (15). After detecting *Salmonella* in all 12 tubes for several of the initial samples, two additional dilutions were added for all remaining assays, for a total of 18 tubes per sample.

Log-transformed concentration estimates (CFU per gram) were analyzed using the PROC MIXED procedure in the SAS software package (version 9.1, SAS Institute, Inc., Cary, N.C.). A value of 1.0 was added to all CFU estimates to allow log transformation before analysis. Only main effects and first order interactions were modeled. Random effects were included for replicate and birth litter. Concentrations could not be estimated for samples with all positive tubes; results for these tubes were recorded as the highest value that could be estimated by the spreadsheet, i.e., with 11 of 12 tubes positive. Qualitative outcomes were analyzed as log-odds using PROC GLIMMIX procedure of SAS 9.1. Water disappearance, fecal consistency score, and rate of weight gain were analyzed using SAS PROC MIXED. Treatment effects considered significant at \( P \leq 0.05 \) are reported.

**RESULTS**

*Salmonella* was detected in 5 of 13 and 9 of 20 sows in replicates 1 and 2, respectively. The *Salmonella* serotypes detected were Agona, Brandenburg, Mbandaka, and Meleagridis. The average parity of sows with study litters was 4.8, with a range of 1 to 7.

One piglet was removed from the study at 10 DPW after development of a superficial abscess. Terminal samples were collected from that piglet at that time. The removed pig had been weaned at 21 days of age and was in the chlorate−, disinfect+ treatment group. Samples from this piglet were analyzed as if they had been collected at 14 DPW even though they were collected at 10 DPW.

All feed and environmental room samples were culture negative for *Salmonella* before piglet placement. Two days before weaning, 56.3% of piglet fecal samples (45 of 80 samples) were positive for *Salmonella*. The average fecal sample weights were 1.4, 1.8, 2.9, 2.9, and 3.0 g at −2, 0, 5, 10, and 14 DPW. At weaning, *Salmonella* was detected in 71.3% of piglet fecal samples (57 of 80 samples), with a mean concentration of 10^{2.3} CFU/g. In piglet samples, *Salmonella* serovars Agona, Meleagridis, and Derby were detected.

Chlorate administration was associated with lower *Salmonella* concentrations in feces collected at 5 DPW and in cecal contents (Table 2). Chlorate+ treatment was associated with lower prevalence of *Salmonella* in feces collected at 10 DPW among disinfect+ pigs, in feces collected at 14 DPW among pigs weaned at 21 days of age, and in lymph nodes (Tables 2 and 3). Disinfect− treatment also associated with lower *Salmonella* prevalence in feces collected at 14 DPW. The younger weaning age (10 days of age) was associated with lower *Salmonella* prevalence in feces collected at 10 DPW and in lymph nodes and was associated with lower concentrations in cecal contents.

Across all chlorate+ groups and all treatment days, 4.1% of treated water was unconsumed and was therefore discarded. The effective consumed sodium chlorate dose in the first 24 h was 98 mg/kg/day for pigs weaned at 10 days of age and 90 mg/kg/day for those weaned at 21 days of age. Weaning at 21 days of age was associated with increased water disappearance during the treatment period. Average water disappearance was 0.75 liters per pig per day for pigs weaned at 10 days of age and 0.92 liters per pig per day for those weaned at 21 days. Neither chlorate nor disinfect treatments were associated with differences in water disappearance. No treatments were associated with changes in weight gain or fecal consistency scores.

**TABLE 1. Characteristics of diets provided to pigs weaned at 10 or 21 days of age**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Lysine (%)</th>
<th>Calcium (%)</th>
<th>Phosphorus (%)</th>
<th>Digestible energy (kcal/kg)</th>
<th>Crude protein (%)</th>
<th>Spray-dried bovine plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 days</td>
<td>1.5</td>
<td>0.9</td>
<td>0.7</td>
<td>3,567</td>
<td>22.0</td>
<td>9</td>
</tr>
<tr>
<td>21 days</td>
<td>1.4</td>
<td>0.8</td>
<td>0.7</td>
<td>3,566</td>
<td>20.6</td>
<td>6</td>
</tr>
</tbody>
</table>
TABLE 2. Salmonella enterica detection as a function of the main effects of treatments*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salmonella concn (log CFU/g)</th>
<th>Salmonella prevalence (% positive samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces (10 DPW)</td>
<td>Cecal contents (14 DPW)</td>
</tr>
<tr>
<td>Oral chlorate</td>
<td>1.95 b(^{b})</td>
<td>1.94 b(^{b})</td>
</tr>
<tr>
<td>Topical disinfection</td>
<td>1.21</td>
<td>1.50</td>
</tr>
<tr>
<td>Weaning age</td>
<td>1.18</td>
<td>1.05</td>
</tr>
<tr>
<td>10 days 1.14</td>
<td>0.87 b(^{b})</td>
<td>39.3 b(^{b})</td>
</tr>
<tr>
<td>21 days 1.26</td>
<td>1.68 b(^{b})</td>
<td>73.2 b(^{b})</td>
</tr>
</tbody>
</table>

* Topical disinfectant was applied at weaning. Oral chlorate (a mixture of sodium chlorate, sodium lactate, and sodium nitrate) was administered daily for the first 5 days after weaning. Significant difference is indicated by letters at \(P < 0.05\). Main effects are not reported for effects for which significant interactions were detected (see Table 3). DPW, days postweaning. \(b P \leq 0.01\). \(c 0.01 < P \leq 0.05\).

average rate of weight gain was 225 g/day, and the average fecal consistency score was 1.63.

**DISCUSSION**

Administration of the chlorate-nitrate-lactate solution reduced Salmonella shedding in feces (concentration and prevalence), carriage in cecal contents (concentration), and carriage in ileocolic lymph nodes (prevalence), demonstrating that this treatment combination may be useful for reducing Salmonella shedding and carriage in weaned pigs. This finding is consistent with a trial of weaned 26- to 29-day-old pigs reported by Anderson et al. (1), in which pigs were orally challenged with Salmonella Typhimurium. At 8-h intervals, these pigs were given oral doses of 10 ml of water containing 100 or 200 mM sodium chlorate, 2.5 mM sodium nitrate, and 20 mM sodium lactate. These authors reported that Salmonella concentrations were reduced at 16 h posttreatment but not at 24 h posttreatment. The 16-h effect in cecal contents was a difference of approximately 2 log CFU, with approximately 10^3 CFU/g in untreated and 10^0.5 CFU/g in treated pigs. In our study, reductions in Salmonella concentrations at the end of treatment (5 DPW) were similar to the maximum effect detected by Anderson et al. (1), and the effect was sustained for an additional 9 days without additional chlorate doses.

More than half of the pigs shed Salmonella at weaning, indicating that Salmonella can be readily transferred from sows to piglets. The high shedding rate observed in the current experiment is in contrast with a 5% prevalence reported by Funk et al. (23). Nollet et al. (33) also did not detect shedding in weaned pigs, even though some pigs were suckling sows that shed Salmonella in their feces. In both earlier reports, however, piglets samples were collected with rectal swabs, a practice that has been associated with a high proportion of false-negative results with conventional bacterial culture techniques (23). The higher prevalence of shedding in the current study may be explained in part by the relatively larger fecal samples collected and in part by the fact that only piglets from Salmonella-shedding sows were selected, virtually ensuring Salmonella exposure during lactation.

The Salmonella serovars detected (Agona, Brandenburg, Derby, Mbandaka, and Meleagridis) are not common causes of clinical disease in pigs (25); however, all have been cultured from human infections (8) and have been previously cultured from live pigs or pork carcasses (12, 24, 27, 29, 34, 36, 38). The average fecal consistency score indicated no diarrhea in the study pigs, which suggests that the infections observed have more relevance to pork food safety than to pig health.

Although reduced shedding in response to chlorate treatment has been documented in pigs challenged with a oral high dose (10^7 CFU) of Salmonella Typhimurium (1), this is the first report of a protective effect of chlorate in naturally exposed pigs from a farm environment representative of commercial production facilities. Thus, these treatments, which were simple and practical, appear to be applicable to commercial production environments and may be an effective preharvest food safety intervention for Salmonella.

Although effective at reducing shedding, the treatments tested were not sufficient to completely break the cycle of Salmonella transmission from mother to offspring. The study design ensured exposure by testing and selecting shedding dams, something not reported previously (22, 32). Even though segregated weaning techniques may be successful in certain cases, our findings suggest that this technique may not be effective if sows are shedding Salmonella during lactation.

Chlorate was administered for only 5 days, yet Salmonella concentrations and prevalence remained lower for samples collected 9 days later from chlorate+ pigs, suggesting that this intervention may have a sustained impact.

TABLE 3. Least square estimates of Salmonella enterica prevalence in pig feces

<table>
<thead>
<tr>
<th>Oral chlorate treatment</th>
<th>% Salmonella-positive fecal samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10 postweaning, topical disinfection</td>
<td>Day 14 postweaning, pigs weaned at:</td>
</tr>
<tr>
<td>Oral chlorate treatment</td>
<td>Day 10</td>
</tr>
<tr>
<td>+</td>
<td>9.3 A</td>
</tr>
<tr>
<td>−</td>
<td>81.9 B</td>
</tr>
</tbody>
</table>

* Results are shown for two outcomes (fecal prevalence at day 10 and day 14 post-weaning) for which statistical interactions between study factors were detected. Topical disinfectant was applied at weaning; oral chlorate (a mixture of sodium chlorate, sodium lactate, and sodium nitrate) was administered daily for the first 5 days after weaning. Percentages followed by different letters are significantly different (\(P < 0.05\)).
This finding could have practical ramifications for preharvest control of *Salmonella*, especially in near-market-weight pigs. If the protective effects are short lived, as has been reported (1), and because pigs are often reared in batches and then selected for slaughter over a 1- to 2-week period, chlorate administration would need to be repeated for each slaughter subgroup to be effective at reducing the potential for slaughter contamination. However, if the sustained effects documented in our study among weaned pigs also hold for older market-weight pigs, a single multiday treatment of all pigs in a batch or barn before the first pigs are slaughtered may be both simple and effective for reducing *Salmonella* shedding and carriage at slaughter. Further investigations of this extended protective effect are warranted.

In weaned pigs, the interaction detected between chlorate and disinfection effects for one outcome suggests that coupling these two procedures may more effectively reduce *Salmonella* than administering either treatment singly. The surface contamination of piglets possibly resulted in reinfection after termination of the chlorate treatment. Alternatively, these findings could be interpreted to indicate that disinfection is relatively less effective if the proportion of shedders is not reduced after disinfection. Similarly, the interaction detected between weaning age and chlorate suggests that these treatments may be more effective when applied together. Because interactions were found for only two outcomes, their interpretation is equivocal.

In summary, these findings suggest that chlorate should be considered as a preharvest intervention to reduce *Salmonella* shedding in swine. The chlorate treatment was ineffective in breaking the cycle of transmission at weaning, even when administered in combination with other treatments. The anti-*Salmonella* effect was prolonged through the 9 days of follow-up testing, which may allow for flexibility in the application of chlorate as a preharvest intervention. Topical disinfection and oral chlorate treatment may be useful for reducing *Salmonella* shedding that results from piglet exposure to shedding sows on farms that practice segregated weaning. However, topical disinfection and oral chlorate treatment at weaning would be effective as a food safety intervention only if the duration of protection is sustained through the growing period, a question that was not addressed in this study.

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