CHLORATE AND DISINFECTANT MODIFY *SALMONELLA ENTERICA* SHEDDING IN WEANED PIGS

Prapas Patchanee*, Peter B. Bahnson[1], Thomas D. Crenshaw[2]
*University of Wisconsin-Madison, School of Veterinary Medicine, Dept. of Comparative Biosciences, 2015 Linden Drive, Madison, WI 53706-1102, Phone: (608) 263-5878 Fax: (608) 263-3926, prapas@vetmed.wisc.edu, [1]University of Wisconsin-Madison, School of Veterinary Medicine, [2]University of Wisconsin-Madison, Department of Animal Sciences

Abstract The effects of chlorate administration, age at weaning (10 and 21 days), and topically applied disinfectant on *Salmonella enterica* shedding were evaluated in 80 weaned pigs naturally exposed to *Salmonella* positive dams. *Salmonella spp.* were qualitatively detected in samples collected on post-weaning days (PWD) -2, 10 and 14 and the concentration estimated for samples collected PWD 0, 5 and for PWD 14 cecal content.

Lower *Salmonella* prevalence was found in chlorate treated pigs for all post-treatment qualitative samples. An interaction was detected between chlorate and disinfectant for PWD 10 fecal samples and between age at weaning and chlorate for PWD 14 fecal samples. Chlorate reduced number of *Salmonella spp.* in PWD 5 fecal samples and in PWD 14 cecal samples. Early weaned pigs had a less *Salmonella* in cecal content and lower prevalence in ileocecal lymph nodes than did late weaned pigs.

Introduction *Salmonella spp.* have been commonly detected in U.S. swine herds. In a study of 160 randomly selected herds, *Salmonella spp.* were detected fecal samples from 61 herds (38.2%) (Anonymous, 1995). In Midwest U.S. herds, 80 of 134 (60%) had one or more culture positive ileocecal lymph node sample (Bahnson *et al.*, 2003). *Salmonella spp.* have been detected in 4.3% of carcasses tested by regulatory authorities in the U.S during the period 1998-2003 (Anonymous, 2004). If recontamination of live pigs and carcasses can be prevented, on-farm elimination or reduction of *Salmonella* in the pig gut would enhance pork food safety.

Elimination of *Salmonella spp.* by segregated early weaning has been previously documented. *Salmonella* were eliminated from groups of pigs maintained in narrow age ranges in segregated nurseries. However, the technique was not effective in all groups (Fedorka-Cray *et al.*, 1997). *Salmonella choleraesuis*, a host-adapted serovar, was successfully eliminated by offsite weaning from a single herd of pigs. (Nietfeld *et al.*, 1998)

Administration of a single dose of sodium chlorate reduced the concentration of *Salmonella* Typhimurium by 1.4-2.7 logs in cecal content of challenged pigs. (Anderson, *et al.*, 2001). Consequently, chlorate may be useful to reduce or eliminate *Salmonella spp.* in conjunction with segregated early weaning. The objectives of this study were to determine whether age at weaning, application of topical disinfectant, and/or administration of sodium chlorate modify the shedding of *Salmonella spp.* among pigs exposed to naturally infected dams during lactation.

Materials and Methods A farm was selected as the source of weaned pigs based on a history of *Salmonella spp.* detected in weaned pigs. Fecal samples (10g) were tested for *Salmonella* from sows 0-2 days after giving birth. Litters from sows with *Salmonella*-positive fecal samples and with at least eight viable pigs were eligible for selection. Non-viable pigs were defined as being at least 30% lower weight than their littermates. 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 2x2x2 randomized block study design. The treatments were: 1) early- (10 days of age, EW) or late- (21 days of age, LW) weaning 2) administration of chlorate in water or plain water (CLOR), and 3) application of a topical disinfectant (DIS). Upon arrival to an isolation facility, pigs in the chlorate treatment group were given 10ml water with sodium chlorate (100mMol), sodium nitrate (2.5mMol), and sodium lactate (20mMol). These pigs were also provided the chlorate/nitrate/lactate mixture in the drinking water for five days. The concentration was formulated such that total chlorate consumption was expected to be 80 mg/kg/day, as previously described (Anderson *et al.*, 2004). Povidone iodine was applied topically to pigs in disinfectant+ groups by drenching pigs as they arrived at the isolation facility.

Pigs were transported to a sanitized isolation facility at either 10 or 21 days of age. Five pigs were housed together in each of eight rooms. The study was replicated once, for a total of 80 pigs. To minimize the potential transfer of *Salmonella* between rooms, separate coveralls and

boots were kept at each room. In addition, personnel used disposable gloves and hairnets, discarding these between rooms.

Fecal samples were collected on post-weaning days (PWD) -2, 0, 5, 10, and 14 relative to weaning. In addition, on PWD 14, samples of ileocecal lymph nodes and cecal content were collected after humane euthanasia using an overdose of pentobarbital. Conventional bacterial culture techniques were used for qualitative detection of *Salmonella* in fecal samples collected on PWD -2, 10 and 14, and for the ileocecal lymph node samples. Briefly, 3g of feces were homogenized in 30 ml of tetrathionate broth (TT), then incubated at 37°C for 42-48 hours. A 100 µl aliquot of TT broth was transferred and incubated in 10 ml of R-10 broth at 37°C for 18-24 hours. A loopful of R-10 broth was streaked onto an XLT-4 (Xylose-Lysine-Tergitol-4) plate and then incubated at 37°C for 18-24 hours. One suspect colony was plated onto a brilliant green (BG) plate. Identity as *Salmonella spp*. was confirmed using polyvalent *Salmonella* antibodies (*Salmonella* Polyvalent O Agglutinating Sera A-G, Fisher Scientific Ltd®.)

A most probable number (MPN) method (Blodgett, 2003) was used to quantify the number of *Salmonella spp*. in PWD 0 and 5 fecal samples and for PWD 14 cecal content. Three grams of each sample was assayed using three replicates of four ten-fold dilutions. All sample dilutions were then processed following the bacterial culture described above, except that the volume of TT was 10ml per tube. The MPN was computed using a spreadsheet program (Blodgett, 2003). After finding several samples with all 12 tubes with positive growth in the first set of samples collected, two additional dilutions were added for a total of 18 tubes per sample for all remaining samples. Log transformed MPN estimates were analyzed by using SAS PROC MIXED (Version 9.1, SAS Inst., Inc., Cary, NC, USA) with random effects for litter and reported as log MPN per gram of sample. Qualitative outcomes were analyzed as log-odds (logit) using SAS PROC GLIMMIX (Version 9.1, SAS Inst., Inc.). All treatment effects with statistical significance at $p<0.05$ are reported.

Results Two days prior to weaning, 56.3% (45/80) of all fecal samples were *Salmonella*-positive. Although not statistically different, among EW pigs 62.5% (25/40) and among LW pigs 50% (20/40) had *Salmonella* culture-positive fecal samples. At weaning, the mean MPN of *Salmonella*/g feces was $10^{2.29}$ CFU/g. A lowered risk of PWD 10 fecal culture positive status was associated with chlorate (CHLOR+ 43.6%, 17/39 vs. CHLOR- 71.4%, 28/39), earlier weaning age (EW 48.7%, 19/39 vs. LW 66.7%, 26/39) and topical disinfection (DIS+, 43.6%, 17/39 vs. DIS-, 71.8%, 28/39). A treatment interaction was also detected between chlorate and disinfectant; the proportion of *Salmonella*-positive pigs was 15.79% (3/19) for CHLOR+, DIS+, 70.0% (14/20) for CHLOR+, DIS-, 73.68% (14/19) for CHLOR-, DIS+, and 70.0% (14/20) for CHLOR-, DIS-.

Chlorate was associated with lower MPN for PWD 5 feces, with 101.88 colony forming units per gram (CFU/g) detected in CHLOR- and 100.35 CFU/g among CHLOR+ pigs. Chlorate was associated with decreased MPN in cecal content. The mean CFU for CHLOR- pigs was 101.93 and among CHLOR+ pigs was 100.48. In cecal content EW pigs had lower (100.77 CFU/g) numbers of *Salmonella* when compared to LW pigs (101.63CFU/g).

For PWD 14 fecal samples, the proportions of Salmonella-positive pigs in CHLOR+/- groups were 45.0% (18/40) vs. 79.6% (30/39), respectively. Culture prevalence in DIS+ groups was 46.2% (18/39) vs. 75.0% (30/40) in DIS- groups. A interaction was detected between chlorate and age at weaning for PWD 14 fecal samples; the prevalence was 55.0% (11/20) for CHLOR+, EW pigs, 35.0% (7/20) for CHLOR+, LW pigs, 60.0% (12/20) for CHLOR-, EW, and 94.74% (18/19) for CHLOR-, LW pigs.

Both CHLOR+ and EW groups were at decreased risk for *Salmonella* carriage in ileocecal lymph nodes. Salmonella were detected in 45% (18/40) of CHLOR+ pigs and 66.7% (26/39) in CHLOR- pigs. The proportion of pigs with *Salmonella*-positive ileocecal lymph nodes in the EW and LW pigs was 40% (16/40) vs. 71.8 % (28/39), respectively.

Shedding of *Salmonella* at weaning was associated with the age at weaning. The concentration of *Salmonella spp*. at weaning for EW pigs was $10^{1.63}$ and was $10^{0.77}$ CFU/g among LW pigs.

Discussion Chlorate substantially reduced the concentration and prevalence of *Salmonella spp*. shed in feces, demonstrating that chlorate administration can be effective in reducing the shedding of *Salmonella* in weaned pigs. This effect has previously been observed in experimental *Salmonella* challenge model that demonstrated a 1.4-2.8 log reduction using a single dose of chlorate (Anderson, *et al.*, 2001). The current study documents that this effect can also be realized

with natural exposure to shedding dams in a commercial-style farm setting.

Although effective at reducing shedding, the treatments tested were insufficient to break the cycle of *Salmonella* transmission from mother to offspring. In contrast to reported cases, the current study ensured exposure by testing and selecting shedding dams, something not reported in prior work (Fedorka-Cray, *et al*., 1997, Neitfeld, *et al*, 1998).

In combinations, the treatment effects were additive in reducing the concentration of *Salmonella* in all tested samples and additive in reducing log-odds of shedding in all qualitative samples except PWD 10 and 14 fecal samples. For PWD 10 fecal samples, a meaningful reduction in prevalence was found only for pigs that were given chlorate and disinfected, and for PWD 14 fecal samples, only for pigs given chlorate and weaned at 21 days of age. These interactions were not detected for all sample outcomes. The reasons the apparent inconsistent findings is not clear.

Both the duration of the chlorate treatment and the duration of follow-up testing were relatively short in the current study. Although not detected 10 days after discontinuing treatment, *Salmonella* shedding might later rebound in treated pigs. Consequently, longer term study of the residual effects is justified. Long-term administration and changes in dose of sodium chlorate should be assessed.

Conclusions Sodium chlorate, used alone or combination with early weaning and/or topical disinfection is effective in reducing *Salmonella* concentration in feces and cecal content, and in reducing *Salmonella* prevalence in feces and ileocecal lymph nodes. These findings may aid in the development of techniques to reduce *Salmonella* shedding in nursery pigs and may be considered in programs designed to reduce or control *Salmonella* as a potential foodborne pathogen.

Acknowledgements Funding was provided by the U.S. National Pork Board and the University of Wisconsin-Madison. Technical assistance was provided by Andrew Bonhoff, Jeremy Page, Amy Sekorski, Jammie Verstoppen, Lynn Volk and Ann Zielinski.

References

Anderson, R.C, Buckley, S.A, Callaway, T.R, Genovese, K.J, Kubena, L.F, Harvey, R.B, Nisbet, D.J, 2001. Effect of sodium chlorate on *Salmonella* Typhimurium in the pig gut. *J Food Prot*. 64; 2, 255-258

Anderson, R.C., Hume, M.E., Genovese, K.J., Callaway, T.R., Jung, Y., Edrington, T.S., Poole, T.L., Harvey, R.B., Bischoff, K.M., Nisbet, D.J. 2004. Effect of drinking water administration of experimental chlorate ion preparations on *Salmonella enterica* serovar Typhimurium colonization in weaner and finished pigs. *Vet Res Commun*. 28:179-189

Anonymous, 1995. Shedding of *Salmonella* by Finisher Hogs in the U.S. Info Sheet, USDA:APHIS Veterinary Services, Centers for Epidemiology and Animal Health USDA:APHIS:VS

Anoymous, 2004. Progress Report on *Salmonella* Testing of Raw Meat and Poultry Products, 1998-2003. http://www.fsis.usda.gov/PDF/Salmonella_Progress_Report_1998-2003.pdf, accessed August 28, 2004

Bahnson, P.B., Omran, L.M., Fedorka Cray, P.J. Ladely, S.R.,Troutt, H.F., 2003. Repeated observations on the *Salmonella* culture status of Midwest U.S. herds. The Fifth Symposium on the Epidemiology and Control of Foodborne Pathogens in Pork. October 1-4, 2003, Hersonissos, Heraklion, Crete, Greece. Poster # PE-10, pp 248-250

Blodgett,R., 2003.The Bacteriological Analytical Manual online, Appendix 2: Most Probable Number from Serial Dilutions, January 2001, revised July 2003. U.S. Department of Health and Human Services. U.S Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA/CFSAN), http://www.cfsan.fda.gov/~ebam/bam-a2.html, accessed October, 2003

Fedorka-Cray, P.J., Harris, D.L., Whipp, S.C.,1997. Using isolated weaning to raise *Salmonella*-free swine. *Vet Med*. 92: 4, 375-382

Nietfeld, J.C., Feder, I., Kramer, T.T., Schoneweis, D., Chengappa, M.M., 1998. Preventing *Salmonella* infection in pigs with offsite weaning. *J Swine Health Prod*. 6: 1, 27-32