EFFECT OF CLEANING AND SUBTHERAPEUTIC CHLOR TETRACYCLINE ON SALMONELLA

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Abstract The objective of this study was to evaluate the effect of stringent cleaning and subtherapeu tic chlortetracycline (CTC) on Salmonella enterica (SE) prevalence in market age swine. Twenty-four finisher barns from one farm were enrolled in a 2X2 factorial study design. Treatments included 1) assignment to a “stringent” or standard cleaning protocol and 2) inclusion of 50g of CTC/ton of feed or no feed antimicrobials. Barn swab samples were obtained pre- and post-cleaning for SE detection. Post-cleaning aerobic plate counts (APC) were collected. Feces were collected to determine SE prevalence pre-market. The stringent cleaning protocol resulted in decreased mean APC post-cleaning, but there was no difference in isolation of SE from the barn environment between cleaning protocols. Pigs housed in barns that received CTC had decreased odds of being SE positive, but there was no difference in the odds of a pig being SE positive between cleaning protocols.

Introduction Contamination of the barn environment with Salmonella enterica (SE) has been suggested to be an important risk factor for SE infection in swine. All-in, all-out production practices with cleaning and disinfection between groups is often utilized to aid in the control of production impairing illnesses in swine. However, the effectiveness of these practices on the prevalence of SE is not clear. Relatively high SE prevalence has been reported on US farms that utilize all-in, all-out pig flow with cleaning and disinfection between groups.(Davies et al., 1998; Davies et al., 1997; Funk et al., 2001; Funk and Gebreyes, 2004)

Subtherapeutic antimicrobial use, in particular for the purpose of growth promotion, is under increasing scrutiny regarding its contribution to antimicrobial resistance in human pathogens. It is currently unclear what the effect of subtherapeutic antimicrobial use has on the prevalence of SE. Few on-farm clinical trials evaluating the effect of subtherapeutic antimicrobials on SE prevalence have been conducted. Previous research regarding SE shedding and antimicrobial resistance subsequent to antimicrobial therapy have been conducted in laboratory facilities involving experimental infection with S. enterica (Abou-Youssef et al., 1979; Baggesen et al., 1999; Dealy and Moeller, 1976; Delsol et al., 2003; Ebner and Mathew, 2000; Evangelisti et al., 1975; Girard et al., 1976; Gutzmann et al., 1976; Jacks et al., 1988; Jones et al., 1983; Mathew et al., 2002; Wilcock and Olander, 1978; Williams et al., 1978). The goal of this study is to determine the effect of stringent cleaning and the use of subtherapeutic CTC on the prevalence of SE in market ready swine.

Materials and Methods One production company with 24 finisher barns at six different production sites (4 barns per site) was selected. Entry criteria was willingness to participate and proximity to the laboratory as substantial investment in travel and labor were required. The study was a 2X2 factorial design. Treatments included 1) assignment to a “stringent” or standard cleaning protocol and 2) inclusion of 50g of CTC/ton of feed or no feed antimicrobials.

Prior to the cleaning of any barns 30 environmental swabs were taken to get a baseline of SE contamination in the environment. These samples were taken using a 4x4 swab that was moistened in 30ml of buffered peptone water (BPW). The samples originated from surfaces throughout the barn including; gates, feeders, waters, overhead pipes and air vents, fans, the floor and the entry door. Samples were transported to the lab on ice and cultured for SE using standard methods. Briefly, 100ml of BPW was added to each sample and incubated at 37°C for 18-24 hours. Following incubation 100µl was transferred to 9.9ml of Rappaport-Vassiliadis Broth (RV) and incubated at 42°C for 18-24 hours. Finally samples were struck onto XLT-4 agar and compared to control strains. The suspected positive samples were confirmed using Triple Sugar Iron agar slants and urea broth incubated at 37°C for 18-24 hours.

Following the environmental sampling barns were then cleaned according to their randomly assigned protocol. The standard protocol, which was conducted by the farm personnel, included a cold water soak, followed by cold water pressure wash (2500 psi), and ended with the application of Virkon®-S applied using the pressure washer. The farm personnel estimated that the standard protocol required a single person 12-13 hours to complete.

The stringent protocol, conducted by laboratory personnel, started with the same cold water soak, which was then followed with a hot water (180-190°F) high pressure wash (4000 psi). Barn equipment such as gates, feeders, and waters were hand scrubbed during this same time period.
using boot brushes. After the completion of the pressure wash and hand scrubbing the barn was rinsed with cold water, and Virkon®-S was applied using a low pressure applicator. After drying, Virkon®-S was applied to the barn environment by thermal fogging. The stringent protocol required 3 people approximately 12 hours to complete.

After the cleaning and disinfecting of the barns was complete 30 environmental samples were taken in an identical fashion to those taken prior to cleaning. Also to determine the bacterial load post-cleaning 15 RODAC® contact plates containing D/E Neutralizing agar were collected and incubated overnight at 37°C.

Pre-harvest 96-10 gram fecal samples were collected from each barn and cultured for SE using standard methods. Briefly, 90 ml of Tetrathionate broth (TTB) was added to each 10g fecal sample and incubated at 37°C for 48 hours. Following incubation 100µl was transferred to 9.9ml of RV and incubated at 42°C for 18-24 hours, then struck onto XLT-4 and suspect colonies were confirmed using TSI and urea broth. All suspect SE were sent to National Veterinary Service Laboratory (NVSL) for serotyping. The producer involved in the study kept records for average daily gain, feed efficiency, and mortality rates. This data was provided for analysis upon completion of the study.

Comparison of the SE prevalence on barn swabs, APC between cleaning protocols and comparisons between production parameters were conducted using the χ² test. To evaluate the effect of chlortetracycline and cleaning protocols on SE prevalence, a multilevel logistic regression model was constructed (MLwiN, v. 2.1a) with an individual pig’s SE status as the dependent variable and the cleaning and chlortetracycline treatments as fixed independent variables. For the variance structure, the lowest level was pig, the second level was barn, and the third level was site.

Results Environmental SE contamination and APC: For the standard protocol, 3 of 12 barns and 4.7% of environmental swabs were positive for SE prior to cleaning. For the stringent protocol, 2 of 12 barns and 0.57% of all swabs were SE positive pre-cleaning for the stringent protocol. Post cleaning, 0.27% were positive in barns cleaned using the standard protocol and 0.87% of swabs were positive in barns cleaned using the stringent protocol. All SE isolates from the environmental samples were serovar Typhimurium var Copenhagen. There was no significant difference in the SE isolation rate from environmental swabs between cleaning protocols. There was a significant decrease in the APC for barns cleaned using the “stringent” protocol as compared to the standard protocol (p<.001) with a median values of 24 cfu/cm² and 176 cfu/cm² respectively.

Pre-harvest SE prevalence: The overall prevalence of SE was 3.6%. Five of the 6 sites (83.3%) and 13 of 24 barns (54.2%) had at least one SE positive pig. Prevalence in SE positive barns ranged from 1% to 31.3%. Pigs fed chlortetracycline were at a decreased risk (OR 0.29, 95% CI 0.12-0.67) to be SE positive as compared to barns that did not receive chlortetracycline. There was no association (OR 0.70, 95% CI 0.29-1.67) between cleaning protocol and SE status of a pig. The distribution of the model variance was such that most of the variance was associated with pig level variance (57%), with the barn level contributing 10% of the variance and the site contributing 33% of the variance of the odds of being SE positive. The serovars of SE isolated from fecal samples were Infantis (68 isolates), Seftenberg (2 isolates), and Alachua (2 isolates).

No differences in production parameters (average daily gain, feed efficiency and mortality) were identified between treatments (cleaning or CTC inclusion in the diet).

Discussion Successful control of SE on swine farms is likely to require multiple interventions, which results in a great difficulty in conducting field trials to evaluate the benefits of individual potential interventions. In this study the barns that were cleaned by the stringent protocol did provide a cleaner environment for the pigs to enter into, as indicated by aerobic plate counts, but there was no difference in the isolation of SE. Interestingly the serovars of SE identified in the pigs pre-harvest were not the same as those serovars isolated from the environment prior to pig placement. This would suggest that the SE shed at the time of marketing were introduced from a source other than the barn environment at placement. There was also no difference in production efficiency that would be required to justify the additional cost of equipment and the additional labor needed to implement the stringent cleaning protocol.

A previous study from our laboratory found no effect of subtherapeutic CTC on SE prevalence in growing swine (Funk et al., 2003). One potential explanation for this discrepancy is methodological, as a greater fecal sample weight was used for culture in this study, which may have increased the sensitivity for detection sufficiently to allow discernment of a difference in prevalence. Another may be farm specificity of the effect, as the trials were conducted on different
farms than the one included in this study. Nonetheless, these results present a challenge for evaluation of the potential benefits and risks posed by the use of subtherapeutic antimicrobials.

**Conclusion** Although a “stringent” cleaning and disinfection protocol may provide a cleaner environment, this increased effort in both labor and equipment costs did not result in a benefit for SE prevalence or growth performance over standard cleaning protocols. This would suggest that at least for this farm, a greater investment in barn cleaning using the “stringent” protocol described in this study over the current standard procedures is not warranted. The decreased prevalence associated with subtherapeutic CTC is to the best of our knowledge this first report of decreased SE prevalence associated with growth promotant doses of CTC in on-farm trials, and is in disagreement with a previous study from our group. This may suggest that the effect of sub-therapeutic CTC ion SE prevalence may be farm specific.

**References**


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