THE ROLE OF TRUCK WASH PRACTICES IN DISSEMINATION OF *SALMONELLA* AND *CAMPYLOBACTER* IN COMMERICIAL SWINE PRODUCTION.

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Summary This study investigated the sources of two foodborne pathogens, *Salmonella* and *Campylobacter* in a commercial swine production system. Pathogens were characterized using conventional culture and isolation techniques and antibiograms. Four swine herds were selected and followed from late nursery to slaughter along with the four truck washes servicing the system. Increase in *Salmonella* prevalence with increasing age from late nursery to slaughter was found. Prevalence of *Campylobacter* fluctuated in different age groups throughout the production period. All truck washes, except truck wash D, showed a reduction in contamination from pre to post wash. It was found that trucks remain a potential source of *Salmonella* and *Campylobacter* even after washing and disinfection. The type and extent of multi-drug resistance varied by stage of production and environmental source. Genotypic profiling is underway to determine the clonality of isolates from pigs, trucks and environmental samples to better characterize important sources of cross contamination.

Introduction *Salmonella* and *Campylobacter* are the most important food borne pathogens in many countries including the United States. These bacteria have been responsible for more than 500 and 124 deaths each year, respectively (Mead *et al.,* 1999). In the U.S. *Salmonella* serovars of public health importance are common in swine in North Carolina. Identification of factors that may contribute to introduction and dissemination of these food borne pathogens is a very important issue facing today's pork industry, especially large integrated systems where there are many moving parts within the same system. This project proposes to study the role of trucks and truck washing systems, housing environment, holding pens at processing facilities, and the evisceration process as potential factors that contribute to the dissemination of these foodborne pathogens and their contribution to the prevalence and profiles of multi-drug-resistant (MDR) isolates. The long-term goal of this study is to identify those factors responsible for introducing antimicrobial resistant foodborne pathogens into pork products and implementation of preventive measures that result in reduced pathogens in the food supply. The specific aims of this study include the following: First, to compare and evaluate different truck wash practices in the pork production system, particularly using recycled lagoon water with that of clean water washing systems. Second, to compare *Salmonella* and *Campylobacter* isolates identified from pigs and the environment (trucks, lagoon water, barn flush, and floor swab) using phenotypic and genotypic approaches. Third, to determine the role of environment for the introduction and dissemination of *Salmonella* and *Campylobacter*.

Materials and Methods A total of four, three site production flows within the same integrated system were chosen for this study. Also, within this system, four truck wash stations were chosen for evaluation. Sixty fecal samples were taken from each location of study pigs at each stage of production. The sampling distribution includes late nursery (just prior to relocation into the finisher), early finish (two weeks into the finisher), late finish (just prior to relocation to the slaughter plant), and slaughter (60 cecal and 60 mesenteric lymphnode post-evisceration samples – note: no slaughter samples were obtained for "farm 3" due to early shipment of the finisher pigs). Furthermore, throughout the production period, environmental samples were obtained which include pre-load nursery transport trailer swabs (5 swabs per truck), pre-fill finisher pen drag swabs (10 pens), and finisher lagoon samples (inlet, outlet, and pooled samples). From the truck wash stations one sample was taken from the lagoon outlet, lagoon inlet, pooled lagoon sample, the pump lifter, and hose. In addition, 10 trailers were sampled at each station both pre and post wash. Five samples were taken from each pre and post wash trailer. All samples were processed for *Campylobacter* and *Salmonella* as previously described (Gebreyes *et al.*, 2000). A maximum of

3 colonies for *Cambylobacter* and 5 colonies for *Salmonella* were selected from positive Campy cefex and XLT4 plates and were subjected to further biochemical testing for confirmation of genus as previously described. The Kirby Bauer method was used for subsequent antimicrobial susceptibility testing using the following antibiotics as previously published: ampicillin (A; 10 µg), amoxicillin-clavulanic acid (Ax; 30 µg), amikacin (An; 30 µg), ceftriaxone (Cro; 30 µg), cephalothin (Cf; 30 µg), chloramphenicol (C; 30 µg), ciprofloxacin (Cip; 5 µg), gentamicin (G; 10 µg), kanamycin (K; 30 µg), streptomycin (S; 10 µg), sulfasoxazole (G; 250 µg), and tetracycline (T; 30 µg) (Gebreyes *et al.*, 2005).

Results In this study, prevalence of *Salmonella* and *Campylobacter* at different stages of swine production as well as environmental samples including trucks, lagoon water, barn swabs, holding pens at slaughter and slaughter specimens including cecal content and mesenteric lymph nodes were assessed. Increase in *Salmonella* prevalence with increasing age from late nursery to slaughter was found (Figure 1). This trend is seen in all three farm flows which increased in prevalence from nursery to slaughter from 6.4% to 56.7%, 5% to 65%, and 0% to 50% in farms 1, 2 and 4 respectively.

On the other hand, prevalence of *Campylobacter* fluctuated in different age groups throughout the production period. This has been consistent across all the three groups as shown on Figure 2.

Four truck wash stations were also evaluated in this study for *Salmonella* and *Campylobacter* contamination both before (pre wash) and after (post wash). These stations were conveniently selected based on their differences on the specific practices of using different types of chemicals and sources of water. Truck wash A used recycled water with Vercon disinfectant and had a post wash *Salmonella* and *Campylobacter* prevalence of 0%. Truck wash B used recycled water with a phenol disinfectant and had a post wash *Salmonella* and *Campylobacter* prevalence of 20% and 20% respectively. Truck wash C used fresh water, followed by soap, and a phenol disinfectant and had a post wash *Salmonella* and *Campylobacter* prevalence of 45% and 90.9% respectively. Truck wash D also used recycled water with a phenol disinfectant and had a post wash *Salmonella* and *Campylobacter* prevalence of 100% and 100% respectively. All truck washes, except truck wash D, showed an improvement in the amount of contamination from pre to post wash. At this cleaning station the recovery of *Salmonella* and *Campylobacter* after washing was increased from 70% to 100% and from 20% to 100% respectively.

The antimicrobial resistance profiles for the *Salmonella* isolates obtained from farm flow 1 revealed a number of cephalosporin resistant isolates present on both the pre-load nursery truck and in the nursery fecal sample. Of the isolates recovered from these samples 54% and 23% respectively, were resistant to cephalothin. These fecal isolates from the nursery also showed the greatest percentage of multi-drug resistance (100%) among the animal samples obtained (using 3 or greater antibiotics as a cutoff). The environmental samples in the finisher (lagoon, and pen samples) showed the highest frequency of MDR (100%) among the environmental samples. The greatest variability shown in the type of resistance displayed was in the samples obtained from the mesenteric lymph nodes. At least one isolate was present in each of the antibiotic resistance categories.

Discussion The increase in *Salmonella* prevalence throughout the production period may be due to the constant exposure to contaminated environments such as trucks, pens, etc… However, the dramatic increase in prevalence which is seen in the cecal and mesenteric lymph node samples obtained from the slaughter plant may be a result of stress shedding induced by the trucking process (Isaacson *et al.*, 1999) in addition to potential cross contamination during transport and holding. On the other hand, *Campylobacter* prevalence was not in constant increase as seen in *Salmonella*. Though, the risk factors and epidemiology of this organism has not been studied much, this may be a result of early exposure, intermittent shedding, and re-exposure. There is a dramatic difference in the pre and post truck wash prevalence of *Salmonella* and *Campylobacter* among the four truck washes analyzed. The combination of recycled water flush followed by Vircon disinfection appeared to be the best. However, among the two truck washes that had the same procedure of using a recycled water flush followed by disinfection with a phenol, the post wash difference in prevalence was dramatic. This may be a result of variability in the completeness of the washing and disinfection process. Further study with a larger sample size and sampling approaches is needed to reach a conclusive results. The cephalosporin resistance which is seen in the preload nursery

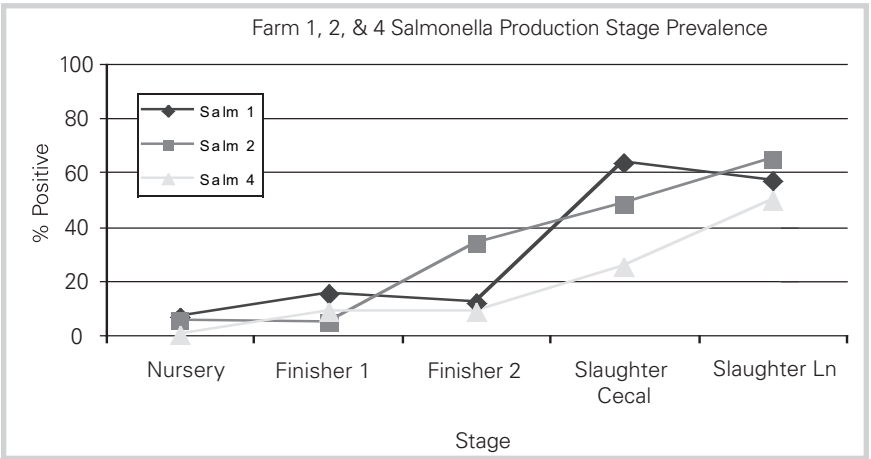

Figure 1. Prevalence of *Salmonella* in various production stages and processing specimens (cecal contents and mesenteric lymph nodes) in three pig flows.

truck isolates very closely mimics the cephalosporin resistance which is found in the fecal samples of the nursery pigs. However, with time, the number of isolates showing resistance to cephalosporins goes down. This may be a function of increased use of cephalosporins in the early nursery phase and there subsequent replacement with other strains because of lack of selective pressure in the finishing stage on this particular farm flow. Genotypic profiling needs to be completed to determine the genetic relationship of these isolates, and to better identify sources of infection.

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References

Mead, P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M., and Tauxe R.V., 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607-625

Gebreyes, W.A., Thakur, S., 2005. Multidrug-resistant *Salmonella enterica* Serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. *Antimicrobial Agents and Chemotherapy* 49(2): 503-511.

Gebreyes, W.A., Davies P.R., Morrow W.E.M., Funk J.A., Altier C., 2000. Antimicrobial resistance of *Salmonella* isolates from swine. *Journal of Clinical Microbiology* 38(12): 4633-4636.

Isaacson R.E., Firkins L.D., Weigel R.M., Zuckermann F.A., DiPietro J.A., 1999. Effect of transportation and feed withdrawal on shedding of *Salmonella* Typhimurium among experimentally infected pigs. *Am J Vet Res* 60(9):1155-8.

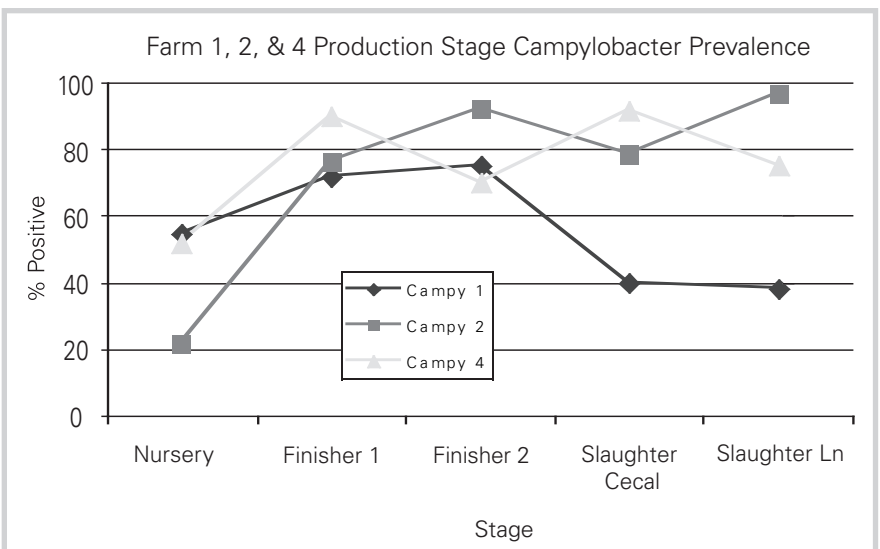

Figure 2. Prevalence of *Campylobacter* in various production stages and processing specimens (cecal contents and mesenteric lymph nodes) in three pig flows.