RESEARCH REPORT
PORK SAFETY

Title: Evaluation of the efficacy of various intervention methods used by small processors for pork carcasses contaminated with Salmonella spp. - **NPB # 02-108**

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Date Received: June 10, 2003

Abstract: Thirty-three market-age swine harvested at the University of Kentucky abattoir were inoculated with fecal material containing two strains of *Salmonella typhimurium* on the ham, belly, and jowl regions on each side of the carcass. Trial 1 revealed that a 10 s hot water spray was just as effective as the 20 s spray in removing *S. typhimurium*. The shorter flame singe (10 s) was as effective as the 20 s application and the two chlorine solution sprays (100, 200 ppm) had similar results. The 2% lactic acid spray reduced *S. typhimurium* populations significantly more than the 1% treatment.

Trial 2 compared the four most efficient levels of each intervention method. Efficacy of the intervention methods was observed in the following order: Hot water (10 s) > Chlorine (50 ppm) = Lactic acid (2%) > Flame (10 s). The effect of carcass area was significant following the post treatment hot water rinse. The jowl area was least accessible by the high pressure water spray. However after the treatment applications, hot water rinse, and 24 h chill (2ºC) there was no significant difference between treated and untreated carcasses or between carcass areas indicating that all treatments followed by proper washing and chilling were acceptable intervention methods.

Introduction: Meat and poultry slaughter facilities have been challenged by the United States Department of Agriculture (USDA) to meet pathogen performance standards in order to reduce the prevalence of *Salmonella* spp. on raw products (Fed. Register, 1996). This enteric pathogen has consistently been one of the main causative agents of bacterial food-borne illness worldwide. Livestock are common carriers of *Salmonella* spp. and can easily transmit the pathogen to noncarrier animals through fecal shedding (Berends *et* al., 1996). Pigs often harbor these pathogens but rarely display clinical symptoms, making it impossible to quarantine the infected animals (Swanenberg *et* al., 2001). Subsequently, the host animals spread infection during holding and/or during the slaughtering process.

In order to maintain safe levels of *Salmonella* spp. on meat carcasses, slaughter facilities implement a variety of decontamination strategies.

By establishing performance standards for *Salmonella* prevalence on meat and poultry carcasses, the FSIS expects to reduce the high incidence of this pathogen in slaughter facilities and ultimately prevent the frequency of salmonellosis. The small slaughter establishments (<500 employees) have had more difficulty maintaining these standards than the larger facilities, particularly pork facilities (Rose *et* al., 2002). Although these small plants process a smaller volume of pork, they supply a wider niche market, often directly to consumers. In addition, most intervention technologies have targeted large scale plants, making it difficult to determine which methods are appropriate for small processors.

By incorporating more *Salmonella* intervention methods into the swine slaughter regimen the smaller facilities can considerably reduce the frequency of carcass contamination. Several varying decontamination strategies (e.g. lactic acid + hot water + chilling) administered to a carcass in succession can remove soil from carcasses and injure or kill any remaining bacterial pathogens present (Bacon *et* al., 2000). For these reasons, it is essential to discover efficient and inexpensive methods of limiting *Salmonella* prevalence on pork carcasses.

The most efficient of these strategies is still in question although much research has been conducted comparing the antimicrobial activity of commonly used sanitizing compounds. Such strategies include: water rinsing, chlorine, organic acids, and hydrogen peroxide (Dickson and Anderson, 1992). After investigating possible biological hazards during hog slaughtering, Berends *et* al. discovered that the dehairing and evisceration processes re-introduced microbes to the carcasses (Berends *et* al., 1997). Dehairing machines roll the animals thereby coming into contact with the entire hide surface which allows contamination from the machine to contact the carcass. Evisceration should be closely monitored as lymph tissues, liver, and the lower digestive tract are organs where *Salmonellae* commonly reside (Borch *et* al., 1996). Contact between these contaminated organs and sterile muscle tissue is a common mode of pathogen transmission onto the carcass (Gill *et* al., 1996).

Objective: Determine the efficacy of various methods of intervention on pork carcasses contaminated with fecal material.

Materials & Methods: Fecal matter was gathered from finishing pigs and sows at the Animal Science Department at the University of Kentucky as well as at the Bluegrass Stockyards, Lexington, Kentucky. Fecal samples (25 g subsamples) were gathered subsequent to defecation and stomached (Seward-Stomacher 400, England) for 60 s with 225 mL of lactose broth (Becton Dickinson, Sparks, MD) for the initial enrichment. The enrichment was incubated at 35ºC for 24 h. A Salmonella spp. selective enrichment was then conducted using Tetrathionate (TT) (Difco, Detroit, MI) broth and Rappaport-Vassiliadis (RV) (Difco, Detroit, MI) broth. An aliquot of 0.5 mL was taken from the initial enrichment and transferred to the TT broth. The RV broth was inoculated with a 1 mL aliquot of the initial enrichment. The TT and RV broths were then incubated at 42ºC for 24 h. After which, the broths were streaked onto Bismuth Sulfite (BS) (BioPro, Redman, WA), Hektoen Enteric Agar (HEA) (Difco, Detroit, MI), and Xylose Lysine Deoxicholate (XLD) (Becton Dickinson, Cockeysville, MD) agar plates and placed in a 35ºC incubator for 24 h. Typical Salmonella spp. colonies release H2S and appear completely black or have black centers when plated on BS, HEA, and XLD agar plates. Atypical colonies may appear yellow on XLD and HEA and green, clear or mucoid on BS plates. Colonies indicative of Salmonella spp. were selected and stabbed into a Triple Sugar Iron agar (TSI) slant and 26 incubated at 35ºC. Once a Salmonella spp. characteristic bacterium (alkaline slant and black butt) had been isolated a slide

agglutination test was performed. For this procedure, a sterile stick was used to collect the culture from the TSI slant and inoculate 1 drop of Salmonella polyvalent O antiserum (Difco, Detroit, MI). A positive test for agglutination would indicate the presence of serogroups A through I and Z. Any Salmonella strain isolated was tested for resistance to nalidixic acid (50 µg/mL) (Sigma, St. Louis, MO). This antibiotic was used in plating media (50 µg/mL) to increase selectivity for the target bacterium.

Bacterial Cultures and Inoculum Preparation

Two strains of nalidixic acid resistant Salmonella enterica subsp. enterica serovar Typhimurium (Salmonella typhimurium) (296 NAL and 298 NAL) were used in this study. The cultures were obtained from the culture collection of the Food Science Section, Department of Animal Science, University of Kentucky. Each culture was transferred to Tryptic Soy Broth (TSB) (Becton Dickinson, Sparks, MD) containing 50 µg/mL nalidixic acid and incubated at 35ºC. Manure was collected from finishing pigs at the Animal Science Department at the University of Kentucky. The manure was tested for any nalidixic acid resistant bacteria by plating on XLD agar and Tryptic Soy Agar (TSA) (Difco, Detroit, MI) containing nalidixic acid (50 µg/mL), spread with sterile bent rods, and incubated at 35ºC. Salmonella negative fecal slurry (1:1) was made in which 20 mL of each broth culture was combined with 40 g feces and stomached (Seward-Stomacher 400, England) for 60 s. This ratio provided the necessary consistency to be adequately spread onto the swine carcass surface. The inoculum load was determined subsequent to sampling untreated (control) carcasses. Prior to experimental procedures, the S. typhimurium cultures were grown in TSB with nalidixic acid (50 µg/mL) and then plated on XLD agar containing the same antimicrobial agent. Both strains produced distinctive black colonies and had similar resistance to nalidixic acid on the agar plates after 24 h incubation at 35ºC. Only such black colonies or colonies with black centers were identified as S. typhimurium during the plate count of each sample.

Experimental Design

A total of 9 market-age swine in Trial 1 and 24 in Trial 2 were slaughtered and dressed in the university abattoir according to USDA guidelines. The three sampling locations (ham, belly, and jowl) selected are described by the USDA, Food Safety Inspection Service for Salmonella testing of swine carcasses as part of the 1996 Pathogen Reduction; HACCP systems; Final Rule (Federal Reg., 1996). Prior to inoculation, three templates (50 cm2) were drawn corresponding to each sampling area, using a 10 cm stainless steel blade and an edible ink stamp pad. The end result was a rectangle (150 cm2) segmented into three 50 cm2 boxes painted on the ham, belly, and jowl regions. Fecal slurry aliquots (1.23 mL) were distributed to the three sampling areas on each side of the carcass using a ¼ teaspoon and a spatula. The inoculation occurred after the dehairing process. The sides of the pork carcasses were randomly assigned to receive either a lactic acid (1% and 2% v/v at 25ºC), a chlorine (50, 100, 200 ppm at 25ºC), a hot water (53ºC for 10 s, 20 s), a flame singe (10 s, 20 s), or control treatment. Previous studies have indicated that singe treatments performed for 10 s raises the surface temperature of the carcass to 100ºC (Borch et al., 1996). Trial 1 was designed to eliminate the least effective concentration or application time for each intervention method. It consisted of the previously mentioned treatments (except 50 ppm chlorine spray) being applied to 8 pork carcasses/16 sides with the control and background flora analyses conducted on 1 pork carcass/2 sides. Each treatment was replicated on a total of two carcass sides. The treatments were applied once the fecal material had been attached for 5 min. The carcasses then proceeded through the standard harvesting steps and prior to entering the chill room the carcass received a

final hot water rinse (53ºC). The carcasses were held in the chill room (2ºC) for 24 h. Samples were gathered before (control) and after the initial treatment and following the hot water rinse. Trial 2 was carried out similarly to Trial 1, only with analysis of the following treatments: Hot water (10 s), flame singe (10 s), chlorine spray (50 ppm), and lactic acid (2% v/v). A total of 24 pork carcasses/48 sides were used with each treatment repeated five times. An additional sample was gathered from each pig side following the 24 h chill. Control sides were sampled with no treatment, after the hot water rinse, and following the 24 h chill. The control treatments were replicated four times.

Carcass Treatments

The inoculated carcass surface was treated with four intervention methods with the objective of significantly reducing *Salmonella typhimurium* populations. During the experimental procedure, hot water treatments (53ºC) were applied for 10 s and 20 s, a flame was used for 10 s and 20 s, a chlorine spray (25ºC) was prepared at 50 ppm, 100 ppm and 200 ppm, and a 1% v/v and 2% v/v lactic acid solution (25ºC) was prepared for spraying. A manual polyethylene compressed air sprayer (7.6 L) (Hudson, Hastings, MN) with Teflon coated O-ring and gaskets was used to apply (10-15 psi) the chlorine and lactic acid spray treatments. The chlorine and lactic acid solutions were applied to the inoculated locations until the fecal contamination was no longer visible. The 50 ppm, 100 ppm, and 200 ppm chlorine solutions were prepared by adding 2 mL, 4 mL, and 8 mL Clorox®, respectively, with 1 g Sodium Dodecyl Sulfate and bringing it up to 1 L with distilled water (BAM, 2001). The 1% and 2 % lactic acid solutions were prepared by bringing 12 mL and 24 mL of 85% lactic acid (Fisher, Fairlawn, NJ) up to 1 L distilled water, respectively.

Enumeration

The ham, belly, and jowl template areas were sampled using a sterile specimen sponge contained in a Whirl-Pak™ (Nasco, Ft. Atkinson, WI) bag. The sponge was hydrated with 25 mL sterile Butterfield's phosphate buffer prior to sampling each location. Each specimen sponge was handled with sterile latex gloves which were changed between samples. Background samples were taken prior to inoculation with the fecal slurry and plated on Plate Count Agar (PCA) and Petrifilm® (3M Corp., St. Paul, MN). The control samples had no intervention applied and therefore established the inoculation level of the slurry. Samples were then collected to enumerate populations of *S. typhimurium* on each carcass area before and after the intervention methods, subsequent to the final rinse, and after being refrigerated for 24 h. All samples were plated, in duplicate, on XLD agar containing nalidixic acid (50 µg/mL), spread with sterile bent rods, and incubated at 35ºC. The XLD agar plates were then observed for any colonies distinctive of the two *S. typhimurium* strains used in the fecal slurry.

Statistical Analysis

Data were analyzed by PROC MIXED procedures of SAS (SAS Institute Inc., Cary, NC). The initial mixed model included effects of treatment, area, time and all interactions. Side (treatment) was treated as a random effect. Time was treated as a repeated effect, and a first order autoregressive variance-covariance structure was adopted. The Satterthwaite adjustment was used for degrees of freedom. Results were supported by the model fitting criteria calculated by the MIXED procedure. Because a significant time*treatment interaction was observed in the initial analysis, separate analyses were subsequently conducted for each level of time. Least squares means

were calculated. Differences (P<0.05) between intervention treatments were obtained using the PDIFF option of PROC MIXED. Microbiological count data were transformed into logarithms before statistical analyses were conducted. Percent reduction was calculated by (Inoculum (Log CFU/cm2) – Bacteria (Log CFU/cm2) following treatment) x 100 Inoculum (Log CFU/cm2)

Results:

***Salmonella* spp. Wild-type Fecal Isolate**

The attempt to isolate a *Salmonella* spp. from swine feces was successful. The fecal samples from randomly selected animals were grouped together for the primary enrichments, so the number of animals tested was estimated. Fecal matter from approximately 20 animals was analyzed and only one fecal isolate of *Salmonella* spp. was obtained. A nalidixic acid resistant strain was a necessary attribute for the inoculum organism. The antibiotic prevents competitive microbes naturally present in the feces from growing on the XLD plating media. However, the isolate was sensitive to nalidixic acid (50 µg/mL) and would not be properly identified as the inoculum organism when added to feces during the following trials. Two *Salmonella typhimurium* nalidixic acid resistant strains were selected instead of the environmental isolate. These laboratory strains could be positively identified against the background flora in the fecal slurry, during each experimental trial.

Trial 1: Determination of Treatment Level

The objective of Trial 1 was to screen the intervention methods to determine the most promising treatment level. The least significant difference (LSD) procedure was conducted to compare *Salmonella typhimurium* mean populations (Log CFU/cm2) between the levels of each treatment. Every treatment effectively reduced (P<0.05) the inoculum population. The comparison of the population means after the 10 s flame singe and the 20 s flame singe was not significant at the 5% level. The pathogen inoculum, 7.08 Log CFU/cm2, was reduced by 38% and 44% (Figure 1) for the 10 s and 20 s flame singe, respectively. The 10 s flame singe intervention was selected for Trial 2 because it was not significantly different from the longer singe treatment. Thus, the shorter singe treatment was found to be as effective as the longer singe in reducing the pathogen population while expending less time and fuel. The difference between the chlorine 100 ppm and 200 ppm treatments was not significant at the 5% level. The inoculum population was reduced by 41% to 4.21 Log CFU/cm2 (Figure 1) after the 100 ppm solution was applied to the carcass. The 200 ppm chlorine solution reduced the *S. typhimurium* population by 39% to 4.31 Log CFU/cm2 (Table 1). A third chlorine spray (50 ppm) was introduced in Trial 2 because the 100 ppm chlorine solution was so successful in Trial 1. Also, due to the tendency of chlorine to produce off odors (Tamblyn, 1995) it would be beneficial to investigate the efficacy of a lesser concentration. Alternatively, the use of lactic acid mixture in decontamination of beef loins has been shown to not adversely affect meat and fat color or odor attributes (Goddard *et* al., 1996). So, there is an obvious preference of lactic acid over other organic acids in industrial decontamination strategies because of this reason (Smulders, 1995). The population means were markedly different between the 1% v/v lactic acid solution and the 2% v/v solution. The 1% lactic acid spray reduced the initial *S. typhimurium* inoculum to 5.69 Log CFU/cm2, while the 2% lactic acid spray lowered the pathogen count to 4.31 Log CFU/cm2. The 2% lactic acid treatment was more (P<0.05) effective, as it proved lethal to nearly 20% (Figure 1) more *S. typhimurium* cells. It was selected for further analysis in Trial 2. Some human experimental error prevented an accurate comparison of hot water treatments. Samples taken after a 10 s

hot water spray were incorrectly maintained. The 20 s hot water application was very effective in lowering the pathogen population. The bacterial counts were reduced by over 44% (Table 1), considerably greater than any other intervention method tested. Despite problems gathering data following the 10 s hot water spray, a trial with this treatment level was conducted during Trial 2 and compared to the results gathered for the 20 s treatment (Figure 1). Additional analyses were conducted to compare the reduction of *S.typhimurium* per swine carcass sampling region. The population of the pathogen was compared at the ham, belly, and jowl regions for each intervention method. The mean population remaining on the ham, belly, and jowl areas was 4.26 Log CFU/cm2, 4.23 Log CFU/cm2, and 4.71 Log CFU/cm2, respectively. A difference ($P<0.10$) between the amounts of *S. typhimurium* residing on these regions was distinguished. The analysis indicated that the jowl area was the most difficult region for each treatment to access. Average populations were higher on that region than either the ham or belly (Table 4.2). Previous studies conducted with beef carcasses indicated no differences between microbial populations relative to carcass region (Castillo *et* al., 1999). However, the shape of the jowl area on swine carcasses can prevent an equal distribution of an antimicrobial spray to be applied. Thus, only a portion of the jowl region can receive the adequate pressure (psi) and volume of the antimicrobial treatment. The post treatment hot water rinse was able to reduce the *S. typhimurium* counts by another 2.56 Log CFU/cm2 or 36%, overall. Combination treatments have been reported to produce significantly larger reductions of coliform and *E. coli* populations compared to single wash treatments (Dorsa *et* al., 1995).

Additional treatments are needed in facilities that use lactic acid decontamination because it has shown to induce acid-adaptation in pathogens which can then recontaminate meat products (Van Netten *et* al., 1998). Previous studies have also indicated that acid-adapted organisms are more sensitive to halogen containing compounds (Leyer *et* al., 1996). The most dramatic drop in bacterial cell count was seen after the 10 s flame singed carcasses were rinsed. An average drop of 3.96 Log CFU/cm2 or 56% occurred on those carcasses. This drastic reduction caused the *S. typhimurium* population to be less ($P<0.05$) in comparison to the other carcasses. After the intense heat treatment was administered the fecal matter had a blackish crispy appearance. The hardening of the fecal material after the singe treatment may have allowed for easier removal via the hot water rinse. A reduction of 31% was seen after the 20 s flame singe carcass was rinsed. However, no notable differences were observed between it and the other treatments following the hot water rinse. The jowl region had a greater ($P<0.1$) quantity of the inoculum remaining than the belly and ham regions (Table 2). The population on the jowl was 0.88 Log CFU/cm2 higher than on the pig belly. The difference ($P<0.1$) between the jowl and ham was 0.75 Log CFU/cm2 with the greater amount also remaining on the jowl.

Trial 2: Efficacy of Decontamination Strategies

The hot water (53ºC) intervention was applied for 10 s and had very similar bactericidal results to the 20 s application in Trial 1. The *S. typhimurium* population was reduced by 40% compared to 44% for the longer application time (Figure 4.1). The difference between the population means after the two applications was not major. Also, the better overall consistency of the fecal slurry in this trial proved to be more difficult to remove compared to the less moist slurry inoculum used in Trial 1. For example, the average reduction of all the interventions (treatment) was 38% and 26% in Trial 1 and 2, respectively. The comparison of the hot water intervention (10 s) to the three other treatments did yield significant differences. Mean populations (Table 3) were 1.50 Log CFU/cm2, 1.43 Log CFU/cm2, and 1.43 Log CFU/cm2 higher after the

flame singe (10 s), chlorine spray (50 ppm), and lactic acid (2% v/v) spray, respectively. These results compliment previous studies that indicate hot water sprays are more effective than some organic acids, trisodium phosphate, and hydrogen peroxide (Cabedo *et* al., 1996 and Gorman *et* al., 1995). Specifically, modest pathogen reductions after chlorine and lactic acid decontamination sprays have been reported as well (Dickson and Anderson, 1992). No other statistically significant differences between *S. typhimurium* populations were observed after comparing the flame singe, chlorine spray, and lactic acid results.

Nonetheless, by comparing statistically significant and non-significant differences between mean populations an order of efficiency (largest *S. typhimurium* reduction) was determined to be: Hot water (10 s) > Chlorine (50 ppm) = Lactic acid (2% v/v) > Flame (10 s) (Table 3). A chlorine (100 ppm) spray treatment should be revisited as it reduced the inoculum by 41% in Trial 1 while the chlorine spray (50 ppm) in Trial 2 only showed a 22% reduction (Figure 2). Similarly to Trial 1 (Table 2), no significant interaction occurred between the treatments and carcass areas so the effect of the treatments was the same for each area. When the carcass areas alone were analyzed for any effects on the treatments none were found. A pattern seemed to develop as the jowl area retained more of the inoculum after the carcasses were treated, similar to Trial 1 (Table 2). In addition to the curvature of the jowl region, the downward (45º) angle at which the spray treatments had to be applied to that area could have deterred their efficiency. Alternatively, the belly region of the carcass could be accessed by spray treatments at a level (180º) angle. The remaining inoculum was therefore less on the pig belly than either the ham or jowl.

As expected, the post-treatment hot water rinse further reduced the inoculum remaining on the treated pork carcasses. An overall reduction (Figure 2), from 5.45 Log CFU/cm2 to 3.23 Log CFU/cm2, occurred after the rinse step. The control (untreated) carcasses all had higher ($P<0.05$) amounts of the pathogen than the carcasses that had received a previous treatment. On average the control carcasses carried 23% more *S. typhimurium* than the treated (Table 3) carcasses.

As observed in Trial 1, the carcasses receiving the flame singe (10 s) intervention showed the greatest reduction (61%) in *S. typhimurium* populations following the post-treatment hot water rinse. The reductions of the hot water rinse following the chlorine, lactic acid, and hot water interventions were 53%, 49%, and 53%, respectively (Table 3). With the application of the hot water rinse step the highest post-treatment population, flame singe (10 s) treatment, became the lowest ($P<0.05$) remaining population. Figure 2 illustrates how much more effective the hot water rinse was in removing the bacteria after the flame treatment than following the other three treatments. The rinse step removed an average of almost 2 Log CFU/cm2 (Figure 2). Surprisingly, the rinse had the least success on carcasses that had received a hot water rinse, prior. However some reports have indicated that re-administration of moist heat does not have any additional effect on removing fecal contamination (Dorsa *et* al., 1995). In rehashing the suggestion that the slurry in Trial 2 was more established when introduced to the carcass skin than the slurry in Trial 1 *S. typhimurium* reductions were calculated. The average reduction (Table 3) recorded after the hot water rinse in Trial 1 and 2 was 39% and 29%, respectively.

The effect of carcass area after the post-treatment rinse was also significant, unlike in Trial1. The reason for the difference between trials has to be due to the difference of fecal slurry consistency. Because the slurry used in Trial 1 was more easily removed from the carcass, the overall effect of carcass area was not significant.

The belly region had a lower ($P<0.05$) quantity of the enteropathogen than the jowl and ham regions in Trial 2. Thus, the pig belly was the most accessible area on the

carcass for the hot water rinse while the jowl area was the least accessible area (Table 2). The combined effect of the hot water rinse following a flame singe, chlorine spray, or lactic acid spray cannot be denied, however if the rinse was conducted prior to the treatments a greater reduction might be observed. Castillo *et* al. have indicated that hot water followed by a lactic acid spray reduces pathogens by at least 4.5 Log cycles. This organic acid treatment would be effective carried out as the final sanitizing step because its antimicrobial effect seems to be extended through storage (Castillo *et* al., 1998).

The final step in diminishing the *S. typhimurium* inoculum remaining on the carcasses following the hot water rinse was a 24 h chill (2ºC). The populations on the carcasses, including the controls, were not significantly different. The 24 h chill had reduced the average remaining population of the organism by 3.37 Log CFU/cm2, not including the control data (Table 3). The effect of the chill treatment was markedly higher than both the intervention methods and the posttreatment rinse. More than likely, *S. typhimurium* cells remained on some carcass areas that could not be detected without resuscitation or enrichment procedures. Such viable but not culturable (VBNC) *S. typhimurium* cells have been reported to lose pathogenicity in a mouse model (Caro *et* al., 1999). The VBNC cells possibly present after the 24 h chill would pose a negligible health risk to humans unless they were resuscitated and reassumed pathogenicity. In a food environment, sublethally injured cells have a surplus of nutrients available to aid in repairing the damage inflicted by organic acids, halogen-based sanitizers, or heat (Van Netten *et* al., 1984). It is not known whether *Salmonella* spp. are able to reassume pathogenicity subsequent to resuscitation. However, *Vibrio vulnificus* has shown the ability to resuscitate *in vivo* from the VBNC state and cause disease (Oliver, 1995).

Intervention methods alone had an average reduction of 26% while the bacterial population was reduced by another 24% following the post-treatment rinse (Figure 2). Bacon *et* al. reported similar total plate count reductions on beef carcasses after intervention strategies while post-chill reductions were approximately 2.0 Log CFU/100 cm2 (Bacon *et* al., 2000). The untreated carcasses had greater ($P<0.05$) amounts of *S. typhimurium* than treated carcasses after the intervention step as well as after the rinse step. A drop of 4.77 Log CFU/cm2 or 59% on the control carcasses following the 24 h chill step produced an undistinguishable difference between untreated and treated carcass populations. A larger percentage of *S. typhimurium* cells remaining on control carcasses entering the chill room allowed for greater reductions following chill. Lethal cold shock probably ensued when cells exposed to the near freezing temperature subsequent to 53ºC water. Cold shock ensues when rapidly growing cultures are effectively killed when suddenly chilled (Ingraham, 1987). Similar *S. typhimurium* counts were recorded for each pig region after the 24 h chill, also. The average detectable population following the chill step was a mere 0.57 Log CFU/cm2.

The *S. typhimurium* inoculum of 8.07 Log CFU/cm2 had been reduced by 93% after treatment, hot water rinse, and 24 h chill. This multiple treatment or “hurdle” strategy was essential in reducing heavy contamination loads and pathogens that may be tolerant to antimicrobial treatments. Although a least significant difference analysis between the intervention, rinse, and chill means was not performed a significant interaction between treatment and time was observed as in Trial 1. Time indicates the stages at which a specific decontamination strategy was applied, e.g. intervention, rinse, chill. Therefore, each of the decontamination strategies played a major role in removing the fecal remnants.

The application of any of the above mentioned interventions without any following decontamination methods (rinse, chill) would not satisfactorily remove pathogen containing soil from animal carcasses. The hot water spray treatment

average reduction of the inoculum (Table 3) was more than 1.5x greater than that of the other three interventions, but 60% of the inoculum remained on the carcasses. The flame singe treatment performed the least efficiently of the treatments at sanitizing the contaminated carcass areas, although when it was combined with the hot water rinse it showed the greatest reduction from the point of inoculation. The combination of only two "hurdles" was effective in removing nearly 5 Log CFU/cm2 of the initial pathogen population. Bacterial cells surviving the decontamination applications become sublethally injured and thereby more sensitive to fewer or lower hurdles (Leistner, 1995). Several industrial slaughter facilities use between four to six sequential decontamination strategies to consistently achieve adequate bacterial reductions in compliance with USDA performance standards (Bacon *et* al., 2000). The implementation of three sequential decontamination strategies in this experiment removed nearly 8 log cycles of *S. typhimurium*.

Discussion: The application of a hot water (53ºC) spray at a high pressure (70 psi) was very efficient in removal fecal contamination from swine carcasses during slaughter. The hot water intervention was the most efficient while the flame (10 s), chlorine (50 ppm), and lactic acid (2%) interventions all reduced ($P<0.05$) the *S. typhimurium* inoculum (8.0 Log CFU/cm2). The main advantage of using high pressure hot water sprays is to remove the pathogen containing soil from the carcass. Alternatively, lactic acid and chlorine treatments physically attack the outer membrane of gram negative pathogens, such as *S. typhimurium*. The result of such membrane attacks either kills or sublethally injures the bacteria. Lactic acid solutions have shown to be very effective in eliminating *S. typhimurium* in previous studies (Alkomi *et* al., 2000). A possible explanation for the less efficient performance of the two antimicrobial sprays is that they did not have sufficient time to act. The time between the intervention applications and post-treatment hot water rinse was approximately 10 min. A reverse scenario of a lactic acid or chlorine treatment following a hot water spray, prior to entering the cooler would allow the antimicrobials an extended period to attack pathogens present on carcasses while they were chilled.

The combination of decontamination strategies in this experiment was essential in reducing approximately 8.0 Log CFU/cm2 of *S. typhimurium* to < 1.0 Log CFU/cm2, overall. This multiple-sequential treatment or "hurdle" approach is implemented by many meat and poultry slaughter facilities in their HACCP systems as it has been proven to be an effective means for reducing microbiological contamination (Bacon *et* al., 2000). The best illustration of the effect of multiple treatments in reducing carcass contamination during this experiment was when carcasses were flamed then rinsed with hot water. The singed carcasses initially showed least reduction of *S. typhimurium*, but following the post-treatment rinse the same carcasses had significantly less remaining contamination than the other carcasses. The flame-hardened slurry was more easily removed by the hot water rinse than when the slurry had been initially treated with a moist spray (i.e., lactic acid or chlorine).

The probability of any contamination remaining after a routine overnight chilling of the pork carcasses was reduced considerably. Chilling of the carcasses at 2ºC for 24 h lowered the amount of culturable *S. typhimurium* more dramatically than any individual intervention spray, flame, or post-treatment rinse with the exception of the 10 s hot water spray. Previous studies have also observed significant reductions between the final wash step and after the chill step after enumerating total plate count and total coliform count (Bacon *et* al., 2000). The carcasses (control) that have previously not received a treatment, but only a hot water rinse before chill, showed the greatest decrease (59%) in *S. typhimurium* population subsequent to the chill step. It was

obvious that only such a large decrease could be noted on control carcasses because they had more cells on them prior to entering the chill room than treated carcasses. Nevertheless, these results indicate that *S. typhimurium* cells were extremely sensitive to the cold temperatures following removal of fecal slurry via hot water rinse.

Lay Interpretation: The main objective of this experiment was to determine the most efficient *S. typhimurium* intervention method suitable for small scale industrial implementation. Ultimately, the specific intervention method used did not matter as no distinguishable differences were observed between inoculated carcasses that received different intervention treatments, a standard hot water rinse and 24 h chill step. A general intervention should be implemented following dehairing and evisceration because both processes present the possibility for re-contamination. The hot water (53ºC) sprayed at 70 psi was the most effective individual intervention but it was essential that the carcasses also be rinsed or receive some antimicrobial treatment after evisceration and chilled to achieve a near complete reduction of 8.0 Log CFU/cm2 *S. typhimurium*. Also, some caution should be heeded when applying high pressure sprays during swine slaughter as the jowl area was found to be the least accessible area to these treatments. The combination of various decontamination strategies during livestock slaughter is encouraged by the FSIS as they have been proven to reduce enteropathogen populations on carcasses (Fed. Register, 1995).

Salmonella spp. prevalence since HACCP was implemented has dropped mainly due to several meat and poultry establishments incorporating antimicrobial treatments during processing in order to meet FSIS performance standards (Rose *et* al., 2002). There is an abundance of research currently being conducted with the objective of finding the most efficient combination of antimicrobial treatments while in most cases, neglecting the significance of the chill step. The chill step had the greatest influence on the *S. typhimurium* strains used in this experiment. For further information contact Dr. Benjy Mikel, University of Kentucky, (859-257-7550) or (wmikel@uky.edu)

Table 1. *S. typhimurium* population (Log CFU/cm2) for each level of treatment and post-treatment rinse in trial 1.

Intervention	Remaining bacterial population[c]	
	Treatment	Rinse
Hot Water (20 s)	3.93 ± 0.31[a]	0.97 ± 0.57[ab]
Flame Singe (10 s)	4.41 ± 0.26[a]	0.57 ± 0.44[a]
Flame Singe (20 s)	3.96 ± 0.29[a]	1.71 ± 0.51[b]
Chlorine (100 ppm)	4.21 ± 0.26[a]	2.29 ± 0.44[b]
Chlorine (200 ppm)	4.31 ± 0.26[a]	2.09 ± 0.44[b]
Lactic Acid (1%)	5.69 ± 0.26[b]	2.03 ± 0.44[b]
Lactic Acid (2%)	4.31 ± 0.26[a]	1.98 ± 0.44[b]

s = seconds, ppm = parts per million

[a,b] Mean in the same column with different superscripts differ ($P<0.05$) as per LSD procedure.

[c] Samples taken subsequent to treatment (Treatment) and after treatment + hot water rinse (Rinse); *S. typhimurium* inoculum was 7.08 Log CFU/cm^2.

Table 2. Total *S. typhimurium* population reduction following treatment, post-treatment rinse, and chill per carcass area for each trial

		Reduction After Decontamination Strategy[e]		
		Treatment[f]	Rinse[g]	Chill[f]
Trial 1				
	Ham	40.07[a]	79.27[a]	ND
	Belly	39.92[a]	81.23[a]	ND
	Jowl	33.41[b]	68.67[b]	ND
	Mean	37.80	76.39	ND
Trial 2				
	Ham	26.27[c]	54.65[c]	97.65[c]
	Belly	25.90[c]	59.48[d]	97.03[c]
	Jowl	25.65[c]	52.17[c]	96.16[c]
	Mean	25.94	55.43	96.95

ND = No Data

[a,b] Trial 1 means in the same column with different superscripts differ as per LSD procedure.

[c,d] Trial 2 means in the same column with different superscripts differ as per LSD procedure.

[e] Samples taken subsequent to treatment (Treatment), after treatment + hot water rinse (Rinse), and after treatment + hot water rinse + 24 h chill (Chill); Units in % reduction.

[f] Significant at levelP<0.05.

[g] Significant at level P<0.10.

Table 3. Combined effect of intervention methods, post-treatment rinse, and chill on reducing *S. typhimurium* (Log CFU/cm2) in trial 2.

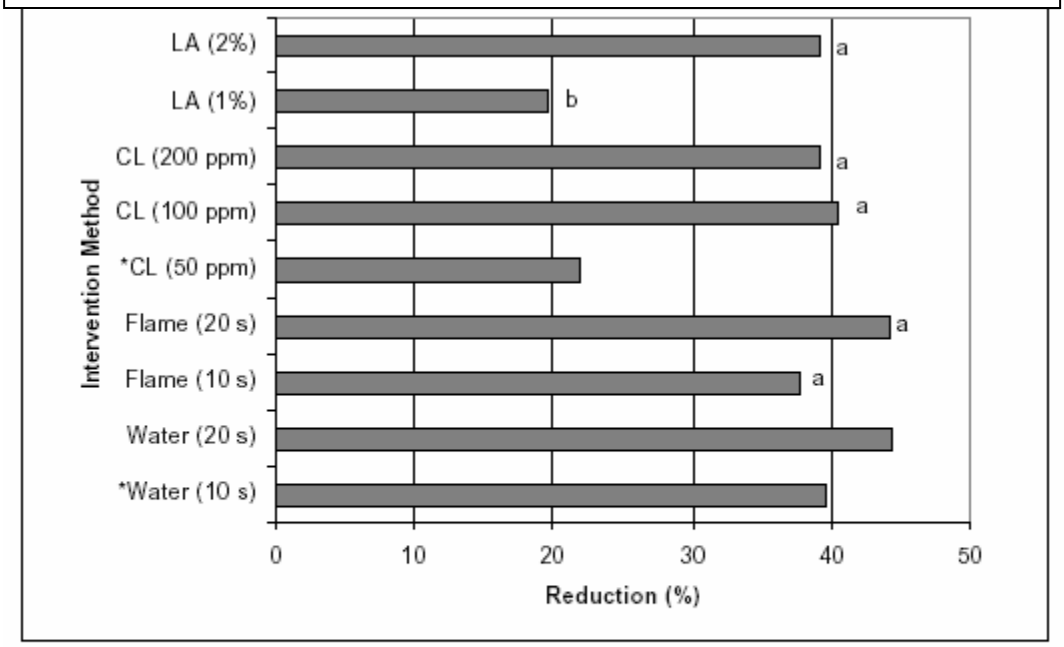
Intervention	Remaining bacterial population[d]		
	Treatment	Rinse	Chill
Control (no treatment)	8.07 ± 0.29[a]	5.06 ± 0.19[a]	0.29 ± 0.17[a]
Hot Water (10 s)	4.36 ± 0.26[b]	3.32 ± 0.17[b]	0.22 ± 0.15[a]
Flame Singe (10 s)	5.87 ± 0.26[c]	2.67 ± 0.17[c]	0.26 ± 0.15[a]
Chlorine (50 ppm)	5.79 ± 0.26[c]	3.32 ± 0.17[b]	0.09 ± 0.15[a]
Lactic Acid (2%)	5.80 ± 0.26[c]	3.61 ± 0.17[b]	0.38 ± 0.15[a]

s = seconds, ppm = parts per million

[a,b,c] Mean in the same column with different superscripts differ (P<0.05) as per LSD procedure.

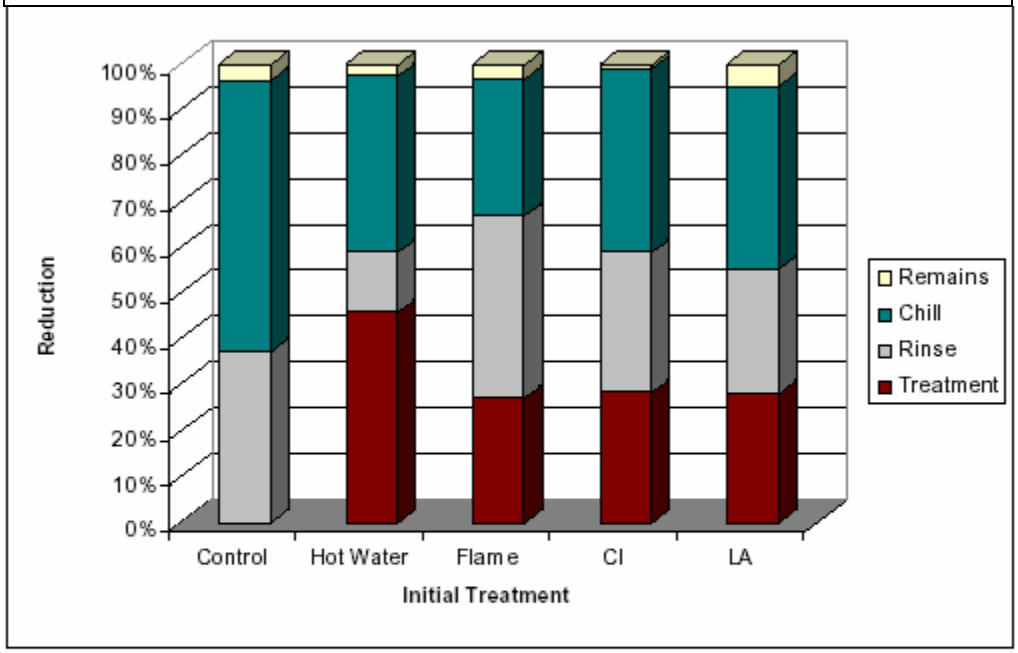
[d] Samples taken subsequent to treatment (Treatment), after treatment + hot water rinse (Rinse), and after treatment + hot water rinse + 24 h chill (Chill).

Figure 1. Efficacy of various levels of intervention on pork carcasses in trial 1.



Percent reduction compared to inoculum (Log CFU/cm^2).
* Data collected in Trial 2 and not statistically compared to Trial 1 treatments.
LA = lactic acid spray (15 psi @ 25ºC), CL = chlorine spray (15 psi @ 25ºC).
Flame temperature reported @ 1000ºC; Water temperature reported to be 53ºC (70 psi).
Levels of the same intervention with different letters (a,b) are significantly different (P<0.05).

Figure 2. Efficiency of various decontamination strategies on reducing *S. typhimurium* in trial 2.



LA = lactic acid spray (15 psi @ 25ºC), Cl = chlorine spray (15 psi @ 25ºC).
Flame temperature reported @ 1000ºC; Water temperature reported to be 53ºC (70 psi).
S. typhimurium (Log CFU/cm^2) values and statistical data are recorded in Table 4.3.
Samples taken subsequent to treatment (Treatment), after treatment + hot water rinse (Rinse) and after treatment + hot water rinse + 24 h chill (Chill).