ABSTRACT OF THESIS

THE EFFICIACY OF VARIOUS *SALMONELLA* INTERVENTION METHODS APPLIED TO PORK CARCASSES DURING SLAUGHTER

Twenty-one market-age swine slaughtered in the University of Kentucky abattoir were inoculated with fecal slurry containing two strains of nalidixic acid resistant *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*, as there was no distinguishable difference in population. The shorter flame singe (10 s) was as effective as the 20 s application and the two chlorine solutions (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.

KEYWORDS: *Salmonella*, Intervention, Carcass Decontamination, Pork Slaughter

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THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science in the College of Agriculture at the University of Kentucky

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Chapter I

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 non-carrier 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. 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). The following experiment was designed to simulate pork carcass contamination subsequent to the dehairing process. Fecal slurry containing *S. typhimurium* was placed on the ham, belly, and jowl regions of pork carcasses. The efficacy of four pathogen intervention methods, hot water spray (53ºC @ 70 psi for 10 and 20 s), flame (1000ºC for 10 and 20 s), chlorine sprays (50, 100, 200 ppm @ 15 psi), and lactic acid sprays (1% and 2 % v/v). Also, the combination of these methods with a hot water rinse (53ºC) following evisceration and a 24 h chill (2ºC) was investigated. The remaining *S. typhimurium* on three pork carcass regions (ham, belly, jowl) was compared following treatments in order to learn more about the accessibility of the regions by decontamination strategies.

Chapter II

Literature Review

Pathogen Reduction Act

The incidence of beef and pork carcass contamination via *Salmonella* spp. and other enteric bacteria costs the meat industry and infected individuals billions of dollars each year. The economic and social losses are attributed to spending for medical care, decreased production due to absent workers and fatalities (Korber *et* al., 1997). Larger, more efficient meat plants have a greater chance of overcoming such dramatic profit losses than small to medium size processors but often product recalls and scared reputation are too costly to overcome. Hudson Foods had to recall 6 lots of frozen beef patties contaminated with *Escherichia coli* O157:H7 in 1997 (CDC, 1997). The company has since ceased all operations. Many production plants have inserted new sanitary technologies to prevent occurrences of carcass contamination. These facilities instantly reduce the probability of their final product being contaminated with *Salmonella* spp. and other enteropathogens.

The small meat production plants (10-499 employees) cannot compete with large (500 employees) production plants in areas such as head of livestock slaughtered (Fed. Register, 1996). However, pathogen intervention is one area where these smaller operations cannot afford to display inferiority. Recent data suggests that small pork processing plants have an increased incidence of *Salmonella* spp. on carcasses (Rose *et* al., 2002). These developments have prompted representatives from these plants to explore the efficacy of various

pathogen intervention methods. Some carcass decontamination methods currently being implemented in larger plants are providing insight for small processors. The most commonly used methods in industry include: Hot water wash, chlorine spray, organic acid sprays (citric acid, lactic acid, acetic acid), and trisodium phosphate washes (Dickson and Anderson, 1992). Many companies are also investigating the risks and benefits of using irradiation to decontaminate packaged raw meat products.

The USDA recommends the use of at least one intervention method in slaughter facilities as well as those facilities that produce raw ground meat (Fed. Register, 1996). A clear determination of the most effective methods has yet to be made. However, the importance of this determination is more urgent given the implementation of the "Pathogen Reduction" performance standards by the Food Safety Inspection Service (FSIS). These standards require random sampling of meat and poultry carcasses for generic *Escherichia coli* and *Salmonellae*. Generic *E. coli* has been characterized as a target organism as its presence indicates fecal contamination and therefore samples should be tested by the establishment at a frequency according to its production volume. The FSIS decided to require sampling of meat and poultry facilities for *Salmonellae* because "it is 1) the most common cause of bacterial food-borne illness; 2) FSIS baseline data show that *Salmonella* colonizes a variety of mammals and birds, and occurs at frequencies which permit changes to be detected and monitored; 3) current methodologies can recover *Salmonella* from a variety of meat and poultry products; and 4) intervention strategies aimed at reducing fecal

contamination and other sources of *Salmonella* on raw product should be effective against other pathogens" (Fed. Register, 1996).

Salmonella and Food-borne Illness

The high frequency of *Salmonella* induced illness, salmonellosis, makes it one of the most common sources of human and animal disease in the world (Anderson *et* al., 2000). More than 2,500 serotypes of *Salmonella* are known to cause this disease. Of the 2,500 serotypes, two are responsible for the majority of human food-borne salmonellosis cases – *S. typhimurium* and *S. enteritidis* (CDC, 2000). Some individuals experience complications with salmonellosis, mainly immuno-compromised individuals, due to the organism localizing in the epithelium tissue resulting in septicemia. Usually individuals without compromised immune systems require ingestion of 5 Log CFU/cm^2 or greater. Any serovar other than *S. typhi* is capable of causing septicemia. However, *S. typhi* is responsible for causing typhoid fever in humans; the most deadly *Salmonella* induced illness (Metcalf *et* al., 2000).

These facultatively anaerobic gram-negative bacteria are rod-shaped and usually motile – nonmotile exceptions being *S. gallinarum* and *S. pullorum* – without the ability to form spores (FDA, 2001). Typical cultures will produce acid and gas catabolized from D-glucose, while growing optimally at 37ºC. The bacteria are oxidase negative, catalase positive, grow on citrate as the sole carbon source, most strains produce hydrogen sulfide and decarboxylate lysine and ornithine, and they do not utilize urea. Such biochemical characteristics as

well as serological characteristics and DNA homology differentiate *Salmonella* spp. and establish taxonomical schemes.

The salmonellosis problem is the result of a continuous fecal-oral cycle, as this genus of organisms is able to survive with and without a host organism (Berends *et* al., 1996). *Salmonella* spp. are present in the gastrointestinal tracts and lymph tissues of many animals, mostly poultry and swine. Environmental sources include animal feces, raw meats, factory surfaces, and kitchen surfaces (FDA, 2001). Also, many serovars can survive for several months in the soil and on feed plants such as grasses.

The ubiquitous nature of *Salmonella* spp. is of concern to the meat and poultry industry. The inordinate amount of environmental reservoirs provides numerous opportunities for livestock infection. Some evidence of this can be observed in Dutch pig farms where about 2/3 of the animals were contaminated with *Salmonella* spp., and there is an 85% probability non-infected pigs will become infected on these contaminated farms (Berends, *et* al., 1996). In many cases, the carrier animals are asymptomatic and are therefore difficult to detect (Anderson *et* al., 2000). The carriers can then shed the pathogens in their feces, providing the potential for spread of the organisms. The National Animal Heath Monitoring System conducted a study in 1995 where 91% of the U.S. hog industry was monitored for *Salmonella* spp. shedding. The results showed that over 38% of the operations had infected pigs (NAHMS, 1996). Of the 10 most frequent *Salmonella* spp. isolates in this study, four were among the Centers for Disease Control and Prevention (CDC) list of top 10 *Salmonella* spp. isolated

from human cases of salmonellosis. These species are *S. enteritidis, S. typhimurium, S. heidelberg,* and *S. agona* (NAHMS, 1996). These *Salmonella* infections are most frequent in man and animals during periods of high temperature, June through October. However, a significant correlation between *Salmonella* infections in swine and season has not been found (Currier *et* al., 1985).

The world has become increasingly aware of the enteric pathogen *Salmonella* spp. over the past decade. This is due to several major outbreaks that have resulted in massive product recalls, company terminations, and human illnesses. As of 1993, the Federal Register reported that approximately half of the total food-borne illness cases (3,605,526-7,132,823) are attributed to contaminated meat and poultry. These numbers were similar in 1995 with the number of cases involving *Salmonella* tolling 348,000-2,880,000 (Fed. Register, 1996). These numbers are approximated due to the minimal number of mild cases reported. Soon after the number of cases related to salmonellosis were published government action set standards for meat and poultry plants to maintain standards to reduce pathogens. The ability of slaughter and raw ground meat processing facilities to uphold the *Salmonella* performance standards should result in dramatic decreases in food-borne salmonellosis.

Salmonella and Other Pathogens Associated With Pork Products

Humans have relied on animal derived foods for centuries, as they provide essential amino acids, fatty acids, vitamins, and minerals for survival. Around the world meat and poultry products make up a substantial percentage of the human

diet. Annually, the Economic Research Service (ERS) records the amount of food intake per capita in the U.S. The most recent numbers provided state that in 1999 individuals consumed approximately 200 lbs. of meat and poultry products (ERS, 2000). Some current trends in meat industry indicate a decrease in beef consumption over the past three decades while pork consumption has had a 12% increase (ERS, 2000). The increased consumption of pork coupled with the high prevalence of enteropathogens in the swine industry suggests a rise in food-borne illness cases can be expected.

Once transmitted to a food source, enteric bacteria can proliferate given a wide range of temperatures, water activities, and pH values. Because bacteria in the *Enterobacteriaceae* family are facultative anaerobes, organisms such as *Salmonellae*, *E. coli* O157:H7, and *Yersinia* spp. are able to survive in environments with little or no O_2 present such as in vacuum-packaged foods (Nissen *et* al., 2001). The lack of oxygen limits the amount of spoilage bacteria but does nothing to eliminate enteropathgens or disease-causing enteric bacteria. *Listeria monocytogenes* is not of the family *Enterobacteriaceae,* although it is a facultative anaerobe and is a psychotropic pathogen of concern in refrigerated anaerobically packaged meats (Devlieghere *et* al., 2001). Generally, foods are preserved by acidic conditions but some enteric has shown the ability to become resistant to low pH environments. Naturally acidic foods or foods treated with acid based antimicrobial solutions can no longer be assumed to be pathogen free. Similarities in acid tolerance characteristics are shared between *E. coli* O157:H7 and *Yersinia* spp. as they are naturally resistant to mild to low

pH levels (El-Ziney *et* al., 1995). In addition to acid tolerance, *Yersinia* spp. has shown to be capable of surviving a wide range of temperatures, with a preference for cooler temperatures (El-Ziney *et* al., 1997).

Most enteric bacteria emit distinct odor when they become densely populated in foods. However *Salmonella* does not release a distinct odor during growth. Without the hint of a stagnant odor a consumer would be entirely unsuspecting of a food product contaminated with *Salmonella*. Also, the ability of these bacteria to adapt to a varying range of environmental conditions, allows them to flourish within a wide variety of pork products. Documented survival of *Salmonella* in meat products has been observed among temperatures 5.2ºC-45ºC, water activities 0.945-0.999 and pH values 4.5-9.5 (Portocarrero, 2000). These ranges of extrinsic conditions would satisfy optimum growth requirements for *Salmonella* spp. and other enteropathogens. Once enteropathogens assume a high growth rate under ideal conditions they can quickly reach high enough numbers to cause illness. For example ingestion of enterhemorrhagic *E. coli* O157:H7 populations 3 Log CFU/cm ^{2} is sufficient for clinical symptoms to arise in healthy individuals.

Outbreaks reported involving food-borne *Salmonella* spp. as the causative organism occurs each year. The annual summary of 1997 conducted by the CDC identified 105 outbreaks in the U.S. of which 12% were represented by *Salmonella* spp. Preliminary data from the CDC 2001 surveillance suggests *Salmonella* is responsible for 38% of laboratory-diagnosed food poisoning cases with *Campylobacter* causing approximately 35% of the cases. Although not as

frequently identified in food poisoning cases, *E. coli* O157:H7 and *Yersinia* spp., are potentially more lethal than *Salmonella* and *Campylobacter* (CDC, 2002). Gram positive pathogens commonly associated with contaminated meat products include *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Bacillus cereus*, and Group D *Streptococci*.

Cured meat products such as country hams decrease the amount of water available for microbial growth, as NaCl concentrations in excess of 5% are required. Many microorganisms cannot endure the osmotic stresses that are encountered with high salinity and low water activity; therefore cured products are rarely carriers of pathogenic bacteria. However, *Salmonellae* are some of the more tolerable pathogens to these conditions. The ability of this organism to survive in an environment with a low water activity increases with an increase in temperature (Tamblyn, 1995). A popular product in Spain, Serrano variety cured ham, has been the source of four outbreaks of *Salmonella typhimurium* (Gonzalez-Hevia *et* al., 1996). During the curing process the temperature is maintained near optimal (35ºC) for *Salmonellae* as the product ages, thus aiding in the adaptation of the organism to the extreme environment. Another pathogen that has shown the ability to grow and produce enterotoxin during ham curing is *Staphylococcus aureus*. Enterotoxins (SEA, SEB, SEC_1, SEC_2, SED and SEE) can be produced at a minimum water activity (a_w) of 0.86 and at 10% NaCl concentrations (Bergdoll, 1983).

Cooked ham products have been shown to harbor some enteropathogens for an extended period of time. *Salmonella* spp. was responsible for infecting 36

individuals in northeast and west Ireland after consuming cooked ham (Coshgriff, 2000). Another cooked meat product was implemented in an outbreak of listeriosis where 38 individuals were infected. Hot dogs were contaminated with *L. monocytogenes* while manufactured at Bil Mar Foods (CDC, 1998). Simulating contamination during packaging Stiles, *et* al. inoculated cooked and pasteurized ham luncheon meats with five enteropathogens, *Salmonella typhimurium*, *Bacillus cereus*, *E. coli*, *Clostridium perfringens*, and *Staphylococcus aureus*. The vacuum packaged ham products contained *S. typhimurium* and *S. aureus* after 30 days of ideal and abusive storage conditions, while *E. coli* had variable results. After storage at 4ºC or 10ºC for 30 days neither *B. cereus* nor *C. perfringens* were detected (Stiles and Ng, 1979). Thus, given a nutrient rich environment some bacterial pathogens can survive and even proliferate under unfavorable growth temperatures.

Fermented meat products have long been considered an inhospitable environment for pathogenic bacteria given their low pH. However, there have been several strains of *Salmonella typhimurium* isolated and found to be acid resistant. An outbreak of salmonellosis occurred in Pennsylvania in 1995 from a fermented Lebanon bologna product (Ellajosyula *et* al., 1997). A bacterium even more acid resistant is *E. coli* O157:H7 as it has been identified in an outbreak associated with consumption of apple cider. It was also responsible for infecting individuals in Washington after ingesting fermented salami (CDC, 1995). Any failure in reaching and maintaining adequate temperatures and pH for a sufficient amount of time during the fermentation process will allow *S. typhimurium* or *E.*

coli O157:H7 to survive. Other outbreaks from consumption of dry and semi-dry fermented sausages have prompted the FSIS to require manufacturers of processed meats to validate processes to ensure a 5 Log CFU/unit reduction of *Escherichia coli* O157:H7. As processors validate these processes they are ensured at least the same reduction in *Salmonella typhimurium* numbers because it is less viable in those types of products than *E. coli* O157:H7 (Ihnot *et* al., 1998).

Swine Slaughter

Many meat processing facilities slaughter livestock in addition to preparing a product for packaging and distribution. Other processing facilities choose to have an outside company perform the slaughtering and just have the raw meat brought in. These slaughter facilities are now under close watch by the FSIS to be certain that *Salmonella* populations are being reduced. Because swine are capable, healthy carriers of *Salmonella* it is important maintain sanitary conditions during the slaughter process (Berends *et* al., 1996).

The first step in swine slaughter is to restrain the animal using a series of chutes and restrainer conveyers. Once the animal is immobilized it is stunned or shocked by one of three methods. A significant current of electricity, a mechanical method consisting of a .22 caliber gun shot to the brain, or chemical stunning involving CO_2 as an anesthesia can be used (Romans, 1994). The mechanical method of stunning is often not the preferred method due to brain splattering and possible lead or steel contamination. The electrical stunning method, commonly used in the U.S., requires an electrical current of 70-90 volts

for 2-10 seconds (Romans, 1994). The stunned animal can then be suspended and exsanguinated, resulting in animal death. To minimize the amount of blood splattering via ruptured capillaries, the time between stunning and exsanguination should be minimized (Romans, 1994). The animal is bled by inserting a 6" knife in front of the sternum and directing it up towards the tail, lacerating the carotid arteries and jugular veins (Romans, 1994).

Next the animal is scalded by either hanging or by being placed in a hot water (60ºC) vat. This step is used to loosen the hair so the following dehairing step is more efficient. The high water temperature causes the protein in the hair follicles to denature and loosen the hair (Romans, 1994). Scalding for too long will denature the follicles then set the hair again as the skin tightens.

The carcass is then placed on a machine that consists of rotating drums and scraper blocks that removes the hair (Borch *et* al., 1996). A flaming or singeing process follows to loosen any hair that remains. Then any remaining hair is scraped off or polished manually with a knife or brush. The animal is then dressed or eviscerated removing the stomach with the intestinal tract and then the plunk set (esophagus, liver, trachea, lungs, kidneys, diaphragm, heart, and tonsils) (Borch *et* al., 1996). The head is removed from the dressed carcass which is then split in half and sent into chill room. The chill room temperature is usually kept around 1-2ºC (Romans, 1994).

Carcass Contamination

During the slaughter process there are several modes for which the carcasses can become contaminated. The skin and hair of livestock harbor

many bacteria as well as the gastrointestinal tract and digesta (Korber *et* al., 1997). Some of these bacterial species can pose a threat to livestock as well as humans if ingested. The pathogenic or disease-causing gastro-intestinal inhabitants of the greatest concern are *Campylobacter jejuni/coli, Escherichia coli,* and *Salmonella* species (Fed. Register, 1996). The pathogens are transported through the digestive tract into the environment through defecation. While in holding pins, livestock will inevitably walk and/or lie in fecal matter. Once attached to the hides of the animals, the bacteria have a greater possibility contaminating the muscle tissue. Ransom *et* al. analyzed fecal, hide, and carcass samples from cattle for the presence of *E. coli* O157:H7 and *S. typhimurium*. They found 36.7% of lots were positive (detected from the hide) for *E. coli* O157:H7 compared to 70% for *S. typhimurium*. A carcass sample did test positive for *S. typhimurium* indicating a horizontal transmission of the pathogen from the hide (Ransom *et* al., 2002).

Once employees in the slaughter facility come into contact with livestock they become the vehicle for the spread of contamination if sanitary standard operating procedures are not followed. A reduction of bacterial counts occurs during scalding but pathogens can be reintroduced to the carcass via employees and/or via the dehairing machine (Borch *et* al., 1996). Also, the meat can become contaminated if the animal is scalded for too long, essentially cooking the skin off (Romans, 1994). Throughout the entire process instruments used such as knives and brushes are vehicles of contamination if not cleaned appropriately.

The pathogenic enterics can also contaminate the carcass surface during harvesting of the animal when exterior flesh comes into contact with the muscle tissue (Belk, 2001). Another mode of pathogen transport is from feces to the carcass via leakage from the intestines (Tamplin, *et* al., 2001). Leakage of the large intestine or colon can occur during evisceration. These organs become ruptured when incisions are made to the abdomen and through the gut of the animal, thus resulting in muscle contamination (Borch *et* al., 1996). Commonly the animals are not fed several hours before slaughter so that the stomach is not swelled, decreasing the chance of becoming punctured.

Another primary reservoir for *Salmonella* spp. in market-ready pigs is the lymph tissues which are handled during evisceration (Anderson, *et* al., 2000). These bacteria are able to survive and replicate inside macrophages and leucocytes. So, there is a distinct possibility that the lymph tissue is contaminated with *Salmonella* if the animal has had been infected with the organisms sometime during its lifetime. In such cases, the individual dressing the animal unavoidably contaminates the carcass from contact with the tonsils and pharynx (Borch *et* al., 1996).

In many cases, employees practice GMPs while handling the carcasses through the slaughter process just to have the product recontaminated during processing such as grinding or cutting (Korber *et* al., 1997). When employees have a better understanding of surface-borne and carcass-borne pathogens, the incidences of recontamination and cross-contamination will be limited (Korber *et* al., 1997).

Salmonella Intervention Methods

There are methods in addition to hygienic processing practices that can aid in the reduction of pathogen populations, preventing them from coming into contact with the final product. The FSIS encourages livestock and poultry slaughtering facilities to apply at least one intervention method to the carcasses. However some individuals have theorized that non-pathogenic meat flora will out-compete pathogens and thus no intervention strategies would be necessary. Nissen *et* al. looked at the growth of *Salmonella enteritidis* on chicken that had been decontaminated (natural flora removed) and untreated. The growth was unaffected as there was no difference in *S. enteritidis* populations on treated and untreated chicken (Nissen *et* al., 2000). Much research is being conducted to evaluate the ability of pathogen intervention methods to reduce enteric pathogen levels during slaughter and meat processing while satisfying sensory concerns. Such methods include organic acid sprays, chlorine solution sprays, trisodium phosphate washes, flaming, steam vacuuming, chemical dehairing, irradiation, and hot water rinses (Dickson and Anderson, 1992).

Organic acids provide meat and poultry industries with a way of deterring bacterial growth on animal carcasses by lowering pH and disrupting the outer membrane of gram negative bacteria (Alakomi *et* al., 2000). Only the acids that are able to penetrate the hydrophobic membranes of the bacterium affect its permeability. This effect has been elucidated on with lactic acid. The undissociated form of lactic acid largely gives it the ability to penetrate the cytoplasmic membrane and reduce the intracellular pH of the bacterium (Alakomi

et al., 2000). In a study conducted by Alakomi *et* al., the antimicrobial effects of lactic acid and acetic acid on *S. typhimurium* were compared and the strongest response resulted from lactic acid. It is also known that in lower pH environments, organic acids are more undissociated thereby more effective as an antimicrobial agent (Goddard *et* al., 1995).

Chlorine is commonly used in the food industry as a sanitizing agent and can be used on food contact surfaces as it is a Generally Recognized as Safe (GRAS) compound. When chlorine is added to water it forms hypochlorous acid (HOCl) and becomes a bactericidal agent. The neutral charge allows it to penetrate the bacterium cell membranes and interrupt enzyme functioning (Tortora, 1995). Sodium hypochlorite (NaOCl) is the active ingredient in commercial bleach and is commonly used in the food industry as a disinfectant. Its mode of action is not completely understood but it is known to disrupt the integral membrane functionality. While chlorine containing compounds are effective antimicrobial agents they are often the cause of off odors in and corrosion of processing equipment. They also are relatively ineffective antimicrobial agents when applied to organic objects such as foods.

A compound gathering more popularity in the poultry industry for its efficiency in decontamination of carcasses is trisodium phosphate (TSP). The antimicrobial action of TSP is understood to interfere with the stability of the cell wall by forming complex linkages of bivalent metals required by the cell (Pohlman *et* al., 2002). The work by Pohlman *et* al. elucidated the effectiveness of TSP treatments in the meat industry as pathogen reduction was monitored as well as

sensory color and odor characteristics. They found that TSP, in fact improved color extension and stability in ground beef with no negative odor characteristics, while reducing *E. coli*, *S. typhimurium*, coliforms, and aerobic bacteria (Pohlman *et* al., 2002).

The efficacy of water rinses to remove fecal contamination has been compared at various pressures and temperatures. Water rinses at temperatures ranging from 70ºC to 80ºC are documented to be more effective than cooler water temperatures and organic acids (Cabedo *et* al, 1996). Hot water interventions applied at higher pressure (psi) reduce bacterial counts more effectively (Dorsa *et* al., 1995). The application of hot water under pressure aids in the removal of dirt and fecal matter on the carcass skin while extended exposure of bacterial cells to hot water denatures integral membrane proteins. Some researchers have questioned the effectiveness of single hand-held nozzle sprayers at distributing water rinses. Although hand-held nozzle sprayers work well under experimental practices, the single stream of water can easily miss contaminated areas of the carcass (Dickson *et* al., 1992).

Some suggest that intervention methods that include high pressure sprays will remove some fecal material while simply spreading remaining fecal matter over other areas of the carcass (Dorsa *et* al., 1995). With this in mind, the steam-vacuum was designed. The steam-vacuum applies hot water (>82ºC) and steam to the carcass then a vacuum removes any loose material on the carcass (Dorsa *et* al., 1995). Dorsa *et* al. applied this treatment to beef carcasses in addition to other water and steam interventions. The steam-vacuum treatment

succeeded in displaying the most significant reduction of bacterial contamination (Dorsa *et* al., 1995). Ironically, another study indicated that the steam-vacuum spread bacterial contamination from the inoculated area to neighboring tissue and this treatment had to be coupled with either a hot water or lactic acid treatment to reduce bacterial populations (Castillo *et* al., 1999).

The irradiation of packaged raw meat is being considered more often by meat producers as an effective way to ensure a reduction in pathogen populations. Due to the high amount of physical variability between bacterial species and the population that is present in a given food, the amount of radiation (kGy) necessary to kill food-borne bacteria is not absolute. In the case of the commonly isolated pathogens from meat and poultry products; *Salmonella* spp. requires approximately 3.7-4.8 kGy, *Staphylococcus aureus* requires 1.4-7.0, and 1.0-2.3 kGy for *E. coli*. A dose of 3.0 kGy is sufficient in eliminating *Campylobacter jejuni* in vacuum-packaged packaged pork loin. Similar results were published by Fu *et* al. in which pork chops inoculated with 5-6 Log CFU/g *S. typhimurium* and *L. monocytogenes* were undetected after medium doses (1.8 to 2.0 kGy) of irradiation (Fu *et* al., 1995). It is theorized that the resulting ionization of the microbial cell environment by radiation causes it to cease function. The same ionizing effect can take place in food molecules, causing free radicals to form in lipids and proteins. These reactions cause the food to take on undesirable color, odor, and flavor characteristics. Assays conducted following pork loin irradiation indicated no change in TBA, Hunter L (lightness), or Hunter b

(yellowness) values but had consistently higher Hunter a (redness) values (Lebepe, *et* al., 1990).

An alternative dehairing process involving sodium sulfide to dissolve the hair on the hides and H_2O_2 for neutralization has been evaluated for its ability to reduce bacterial loads on beef carcasses (Castillo, *et* al., 1998). Not only is it effective in removing hide hair, it has shown the capability to reduce 5 Log CFU/cm^2 counts of *E. coli* and *S. typhimurium* to undetectable levels (Castillo, *et* al., 1998). If implemented this method could replace dehairing machines and the possibility for recontamination of carcasses during the dehairing process.

Protective Barriers Promoting Pathogen Survival

Food-borne pathogens rely on organic materials such as grains, dirt, and feces for transportation and protection from harsh environmental or industrial conditions. The common vehicle of transportation of enteropathogens to meat and poultry products is fecal matter. The consistency of freshly shed feces allows it to attach to surfaces easily, thereby spreading pathogenic bacteria to these fecal contact surfaces. The high moisture content and near neutral pH of fecal matter allows it to be a desirable reservoir for many bacteria. In fact, Wang, *et* al. found that bovine feces inoculated with *E. coli* O157:H7 (3 Log CFU/g) and held at 5ºC for 70 days still had detectable amounts of the pathogen. The low temperatures allowed the fecal matter to retain a moisture content of 74% and an a_w of 0.98. Incredibly, feces incubated at 37ºC had detectable amounts of the pathogen after 56 days. The theory presented was that even with an overall a_w of 0.36 the center of the fecal sample retained enough moisture for growth

(Wang *et* al., 1996). Evidence such as this indicates that feces offer some protection for bacteria as well.

Some suggestions have been made as to what specific characteristics is the cause of increased bacterial survival on animal tissue. There has been some indication that bacteria become entrapped in muscle fibers, rendering them inaccessible to antimicrobial treatments (Pohlman *et* al., 2002). Dorsa *et* al. discussed how fecal contamination on carcasses protects the included bacteria by providing additional moisture which affects the collagen, lipids, and proteins on the carcass (Dorsa *et* al., 1995). The extra moisture on the carcass surface expands muscle fibers allowing tiny crevices to form, preventing exposure of bacteria to sanitizers.

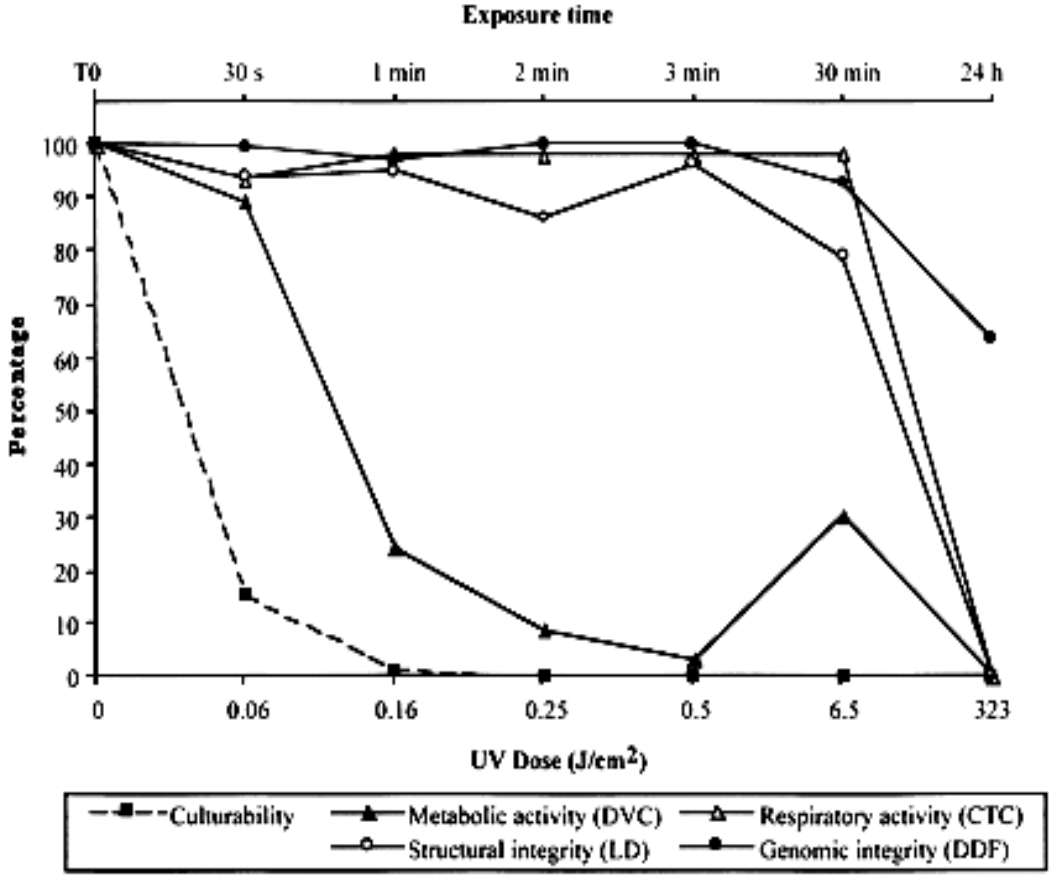
Sanitary standard operating procedures (SSOPs) are ineffective when microbes targeted by sanitizing agents cannot be reached in small crevices on foods or food equipment. Some pathogens have the ability to add another dimension of protection from harsh conditions by producing a biofilm. These organisms adhere to inert surfaces such as stainless steel by releasing a glycoprotein layer that enhances the surface tension necessary for attachment. Complex polysaccharides continue to be released, capturing other bacteria and organic material. The bacteria within the biofilm continue to grow and toxin-producing bacteria can release toxins within the biofilm. The ineffectiveness of sanitizing agents on *Listeria monocytogenes, Salmonella* spp., and *E. coli* O157:H7 biofilms relative to the surface roughness has been documented. The biofilm produced within the smallest crevices tested (0.15 mm width) provided the

greatest protection from TSP in terms of the number of surviving bacteria (Korber *et* al., 1997). Crevices with widths only fractionally larger must allow for greater accessibility and penetration of biofilms. However, any pathogen cells surviving on food-contact surfaces have the opportunity to either enter a food product and proliferate or reattach to the processing equipment.

In the food production environment, bacterial cells will enter an altered physiological state if they have not been killed after being exposed to sanitizing agents and extreme temperature fluctuations (Smith *et* al., 1994). The exposure of harsh chemicals and temperatures will injure or stress those cells that are not killed, presenting a unique situation for quality assurance employees as the bacteria become undetectable by standard bacteriological enumeration techniques. These cells deemed viable but not culturable (VBNC) cannot be isolated by using selective media (Brackett, *et* al., 1994). VBNC cells are intact and alive when test for enzyme activity, photosynthesis, respiration, and/or energy charge but do not undergo cell division in or on routinely employed bacteriological media (Roszak and Colwell, 1987). Such sublethally stressed cells are still potentially hazardous because they have the ability to repair the damage they suffered (Van Netten *et* al., 1984). The food environment provides the perfect medium for damaged bacterial cells to resuscitate, thus all injured cell counts should be included when sampling food products for surviving pathogens. Some investigation into the matter of retaining pathogenicity while in the VBNC state has revealed *S. typhimurium* cells in the VBNC state lose their ability to cause disease in mice (Caro *et* al., 1999). Presently the question revolving

around VBNC pathogenic bacteria is whether they can regain pathogenicity after being allowed to repair injury or resuscitate. A study involving the pathogen *Vibrio vulnificus* has shown its ability to resuscitate *in vivo* from the VBNC state and cause disease (Oliver, 1995). It is quite possible that other enteropathogens can regain pathogenicity as well, making it extremely hazardous to neglect VBNC cells.

Figure 2.1. Loss of culturability and physiological deterioration (as determined by DVC, CTC, LD, and DDF methods) of *S. typhimurium* according to the time of exposure to UV-C in seawater (Caro *et* al., 1999)



Some have purposed the installment of a direct viable counting method (DVC) (Figure 2.1) developed by Kogure *et* al., to enumerate injured and non-injured cells responsive to substrates (Roszak and Colwell, 1987). This method would provide the food industry with a more accurate representation of the number of pathogenic bacteria present after exposure to decontamination strategies.

Research Needs

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). 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.

Chapter III

Materials and Methods

Isolation of Salmonella from Swine Feces

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 H_2S 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

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. e*nterica* 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.

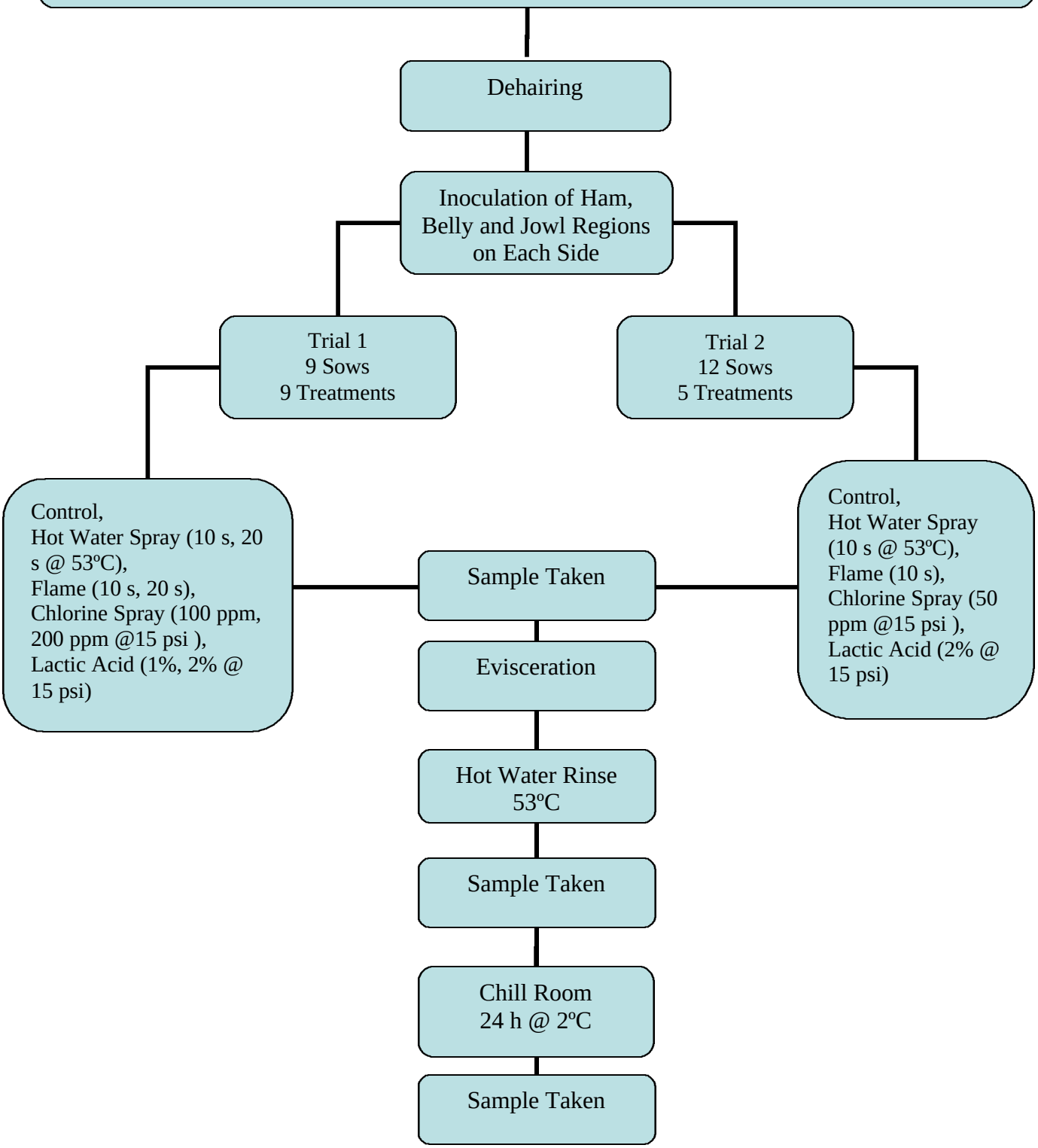
Prior to experimental procedures, the *S. typhimurium* cultures were grown in TSB with nalidixic acid (50 µg/mL) and then plated on XLD agar containing the same antimicrobial agent. Both strains produced distinctive black colonies and had similar resistance to nalidixic acid on the agar plates after 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 12 in Trial 2 were used in the experiment and each 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 cm^2) 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 cm^2) segmented into three 50 cm^2 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 (Figure 3.1).

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

Figure 3.1. Decontamination procedures during swine slaughter



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 12 pork carcasses/24 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.

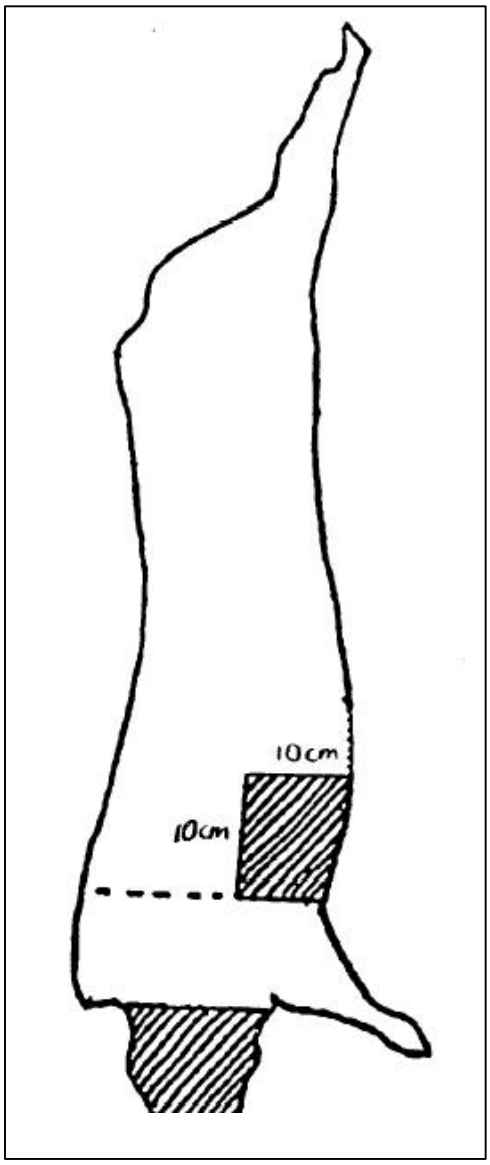
During the experimental procedure, hot water treatments (53ºC) were applied for 10 s and 20 s, a flame was used for 10 s and 20 s, a chlorine spray (25ºC) was prepared at 50 ppm, 100 ppm and 200 ppm, and a 1% v/v and 2% v/v lactic acid solution (25ºC) was prepared for spraying. A manual polyethylene 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 (Figure 3.2) 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

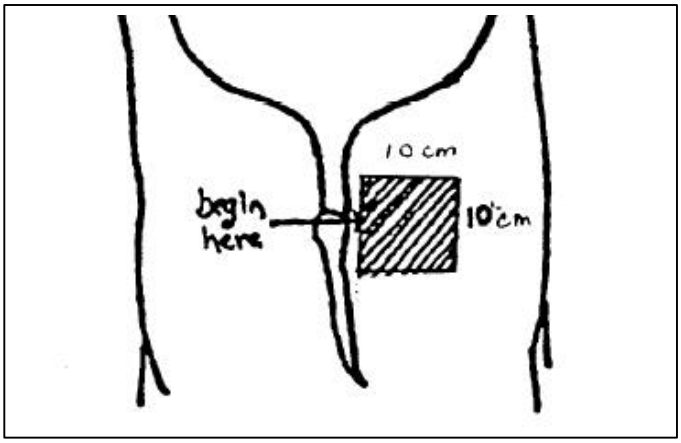
Figure 3.2. Inoculation and sampling regions of pork carcasses



Belly region

Jowl region

Ham region



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

$$\frac{\text{(Inoculum (Log CFU/cm}^2\text{) – Bacteria (Log CFU/cm}^2\text{) following treatment)}}{\text{Inoculum (Log CFU/cm}^2\text{)}} \times 100$$

Chapter IV

Results and Discussion

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.

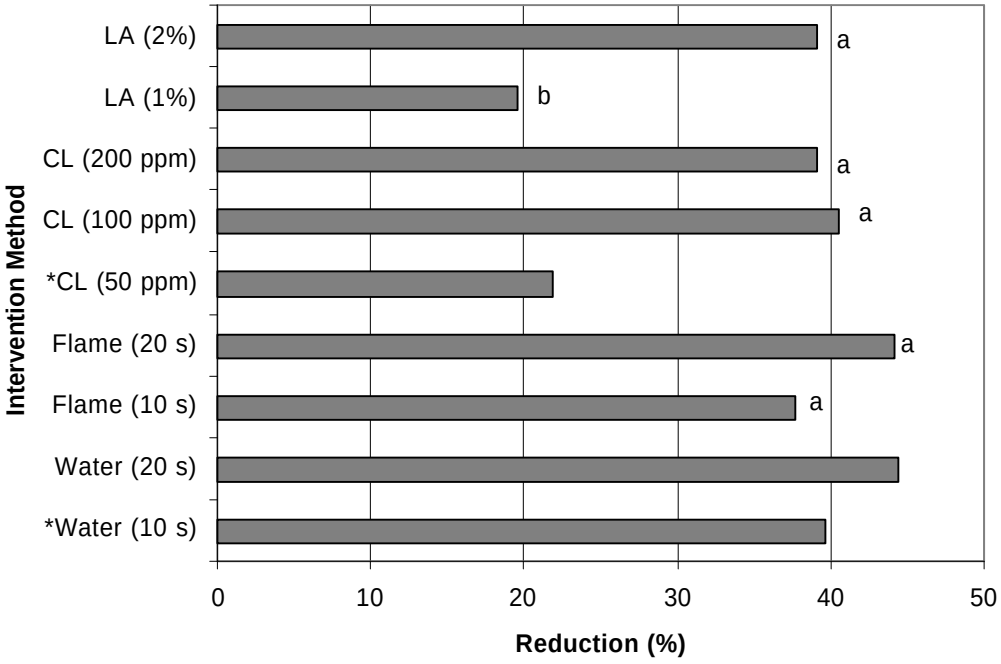
Trial 1: Determination of Treatment Level

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/cm^2) 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/cm^2, was reduced by 38% and 44% (Figure 4.1) 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/cm^2 (Figure 4.1) 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/cm^2 (Table 4.1). 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/cm^2, while the 2% lactic acid spray lowered the pathogen count to 4.31 Log CFU/cm^2.

Figure 4.1. Efficacy of varying levels of intervention methods at removing a *S. typhimurium* inoculated fecal slurry from pork carcasses



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).

The 2% lactic acid treatment was more ($P<0.05$) effective, as it proved lethal to nearly 20% (Figure 4.1) 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 4.1), 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 4.1).

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/cm^2, 4.23 Log CFU/cm^2, and 4.71 Log CFU/cm^2, 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

Table 4.1. *S. typhimurium* population (Log CFU/cm^2) 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 4.2. 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.

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/cm^2 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 re-contaminate 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/cm^2 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 4.2). The population on the jowl was 0.88 Log CFU/cm^2 higher than on the pig belly.

The difference ($P<0.1$) between the jowl and ham was 0.75 Log CFU/cm^2 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 4.3) were 1.50 Log CFU/cm^2, 1.43 Log CFU/cm^2, and 1.43 Log CFU/cm^2 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.

Table 4.3. The combined effect of an intervention method, post-treatment rinse, and chill on reducing *S. typhimurium* (Log CFU/cm^2) 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).

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 4.3). 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 4.1). Similarly to Trial 1 (Table 4.4), no significant interaction (Table 4.5) 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 4.2). 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 4.2), from 5.45 Log CFU/cm^2 to 3.23 Log CFU/cm^2, 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 4.4. Model effects for *S. typhimurium* reduction during swine slaughter in trial 1

Source	DF	F value	Pr > F
Treatment	6	2.34	0.1459
Area	2	6.19	0.0057
Treatment*Area	12	1.26	0.2940
Time	1	426.62	<0.0001
Treatment*Time	6	5.02	0.0018
Area*Time	2	0.90	0.4215
Treatment*Area*Time	12	1.24	0.3395

Treatments: Hot water (20 s), Flame (10 s), Flame (20 s), Chlorine (100 ppm), Chlorine (200 ppm), Lactic Acid (1%), Lactic Acid (2%).
Areas: Samples taken from the pig belly, ham, and jowl.
Times: Samples taken after treatment and after treatment + hot water rinse.

Table 4.5. Model effects for *S. typhimurium* reduction during swine slaughter in trial 2

Source	DF	F value	Pr > F
Treatment	4	28.64	<0.0001
Area	2	3.62	0.0301
Treatment*Area	8	0.86	0.5558
Time	2	1218.62	<0.0001
Treatment*Time	8	15.12	<0.0001
Area*Time	4	1.92	0.1115
Treatment*Area*Time	16	1.19	0.2828

Treatments: Control, Hot water (10 s), Flame (10 s), Chlorine (50 ppm), Lactic Acid (2%)
Areas: Samples taken from the pig belly, ham, and jowl
Times: Samples taken after treatment, after treatment + hot water rinse, and after treatment + hot water rinse + a 24 h chill

(Table 4.3) 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 4.3). 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 4.3 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/cm^2 (Figure 4.2). Surprisingly, the rinse had the least success on carcasses that had received a hot water rinse, prior. However some reports have indicated that readministration 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 4.6) 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 (Table 4.8) was also significant, unlike in Trial1 (Table 4.7). 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.

Table 4.6. The mean effect of the intervention, post-treatment rinse, and chill strategies on reducing *S. typhimurium*

	Average Reduction per Strategy[a]		
	Treatment	Rinse	Chill
Trial 1	37.80	38.59	ND
Trial 2	25.94	29.49	41.52

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

Table 4.7. Fixed effects of variables on *S. typhimurium* reduction following interventions and post-treatment rinse in trial 1

Source	DF	F value	Pr > F
Treatment	6	2.02	0.1153
Area	2	2.51	0.1092
Treatment*Area	12	1.03	0.4614

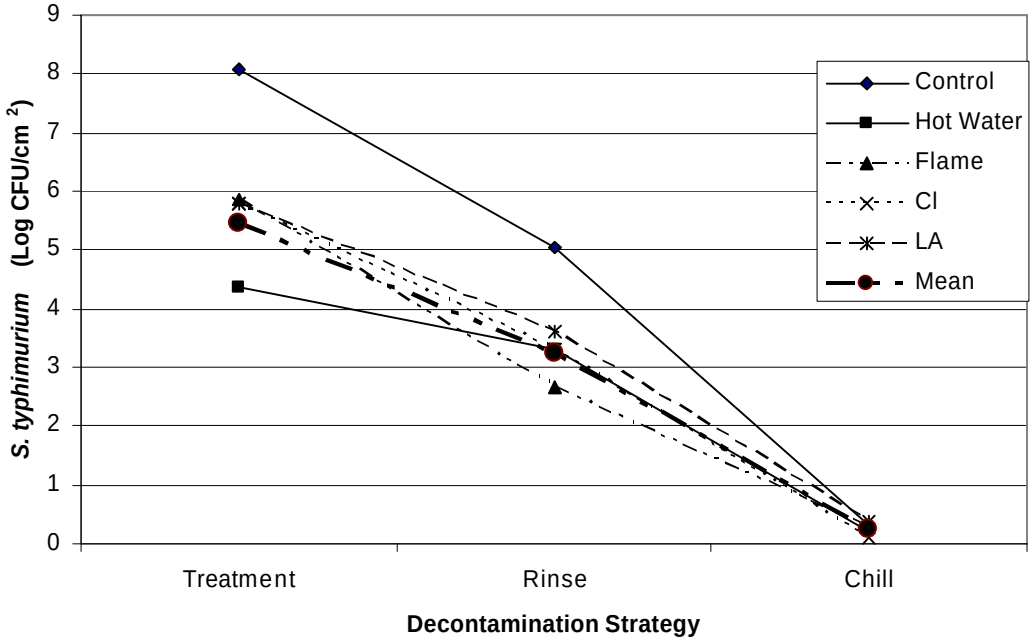
Treatments: Hot water (10 s), Flame (10, 20 s), Chlorine (100, 200 ppm), Lactic Acid (1, 2%)
Areas: Samples taken from the pig belly, ham, and jowl.

Table 4.8. Fixed effects of variables on *S. typhimurium* reduction following interventions and post-treatment rinse in trial 2

Source	DF	F value	Pr > F
Treatment	4	23.66	<0.0001
Area	2	9.26	0.0005
Treatment*Area	8	1.40	0.2288

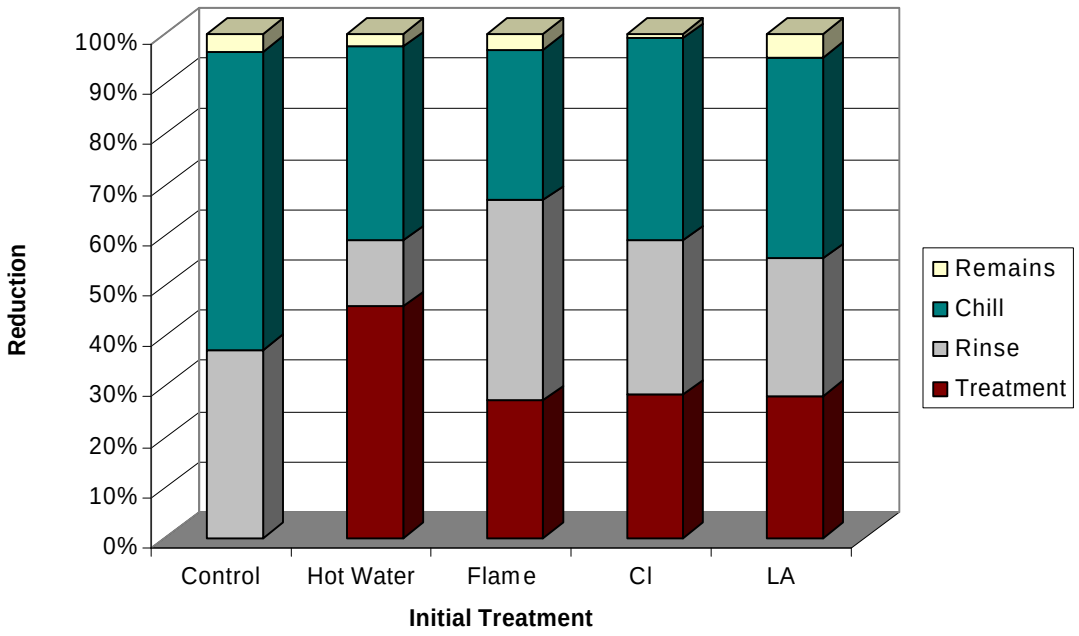
Treatments: Control, Hot water (10 s), Flame (10 s), Chlorine (50 ppm), Lactic Acid (2%)
Areas: Samples taken from the pig belly, ham, and jowl.

Figure 4.2. Effectiveness of varying combinations of decontamination strategies in removing *S. typhimurium*



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).
Samples taken subsequent to treatment (Treatment), after treatment + hot water rinse (Rinse), and after treatment + hot water rinse + 24 h chill (Chill).
Statistical data recorded in Table 4.3.

Figure 4.3. Efficiency of individual decontamination strategies on reducing the *S. typhimurium* inoculum 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).

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 4.2). 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 perhaps be effective when 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/cm^2, not including the control data (Table 4.3). The effect of the chill treatment was markedly higher than both the intervention methods and the post-treatment 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 4.3). 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 cm^2 (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/cm^2 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/cm^2.

The *S. typhimurium* inoculum of 8.07 Log CFU/cm^2 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 (Table 4.5) 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 4.3) 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/cm^2 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*.

Chapter V

Conclusions

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/cm^2). High pressure interventions have been thought to spread contamination to adjacent carcass areas. However, Gorman and coworkers sampled carcass areas adjacent to the inoculated area subsequent to hot water spray washes and discovered no spreading (Gorman *et* al., 1995). A possible negative effect of using high pressure sprays to remove soiled carcasses is that the tight stream of liquid would be more likely to neglect contaminated surface areas. In this experiment, the areas of contamination were know and easily targeted by the high pressure water spray. Nevertheless, moist heat treatments have been reported to be more or equally effective as organic acids and chlorine treatments in reducing bacterial populations on carcasses (Dorsa *et* al., 1995).

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). The inoculum was reduced by 29% after a 2% v/v lactic acid spray was applied. These results almost mirrored those recorded for the chlorine (50 ppm) spray treatment as 25% more *S. typhimurium* was enumerated from the lactic acid and chlorine treated carcasses than those washed with hot water. 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. Some negative physical and odor characteristics would likely result, though if the compounds were allowed to be absorbed by the carcass skin. Therefore, an extended duration, albeit not the entire duration of the chilling period (24 h), of lactic acid interaction with a contaminated carcass surface should be investigated.

The combination of decontamination strategies in this experiment was essential in reducing approximately 8.0 Log CFU/cm^2 of *S. typhimurium* to < 1.0 Log CFU/cm^2, 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).

It was after the post-treatment hot water rinse when the effect of carcass area was significant. The main contributing factor to the overall significance was that the jowl area retained much greater amounts of the fecal slurry than the belly and ham regions. This suggests that the high pressure hot water rinse had difficultly removing contamination from a carcass area with some undulation. The belly region proved to be the most accessible region for the post-treatment rinse as less ($P<0.05$) *S. typhimurium* was enumerated from that area than the other two areas. This region of the pig is relatively smooth and flat and can be treated with a high pressure spray on a straight plane. However, the jowl region should receive a post-treatment rinse of moderate to low pressure over a longer duration. A positive note reported by Tamplin and coworkers is that the jowl or neck area of swine carcasses had the lowest incidence of *Salmonella* spp. contamination (Tamplin *et* al., 2001). Therefore, the likelihood of leaving a substantial amount of pathogenic bacteria on the jowl area after an antimicrobial spray treatment would not be great.

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. The bacteria were probably more exposed to the cold conditions upon fecal slurry removal.

There was not an attempt to resuscitate the *S. typhimurium* cells that may have been sublethally injured thus rendered unculturable. From that vantage point it was unclear whether cells remained that could not be enumerated because of acid or heat injury or if such injured cells could simply not survive the 24 h duration at 2ºC. The large reduction in *S. typhimurium* calculated after the chill period was probably mostly accurate with a small percentage of injured cells surviving. Nevertheless, more emphasis should be placed on recovering stressed pathogens targeted by decontamination strategies in order to reduce false low recoveries and get a true understanding of the efficacy these strategies possess (Van Netten *et* al., 1984). A non-selective medium should be used

when culturing stressed cells (Brackett *et* al., 1994). The majority of enumeration techniques for fecal indicator and pathogenic bacteria require culturing with selective media thereby compromising the detection of VBNC cells (Smith *et* al., 1994). The injured or stressed *S. typhimurium* cells should still be considered hazardous even though it is unclear whether they can regain pathogenicity after being resuscitated. It has been reported that *S. typhimurium* loses pathogenicity when cells become unculturable due to extrinsic stresses (Caro *et* al., 1999).

The main objective of this experiment was to determine the most efficient *S. typhimurium* intervention method suitable for 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 (Berends *et* al., 1997). 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/cm^2 *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. The effect of near-freezing temperatures immediately following a hot water rinse on pathogens associated with livestock carcass contamination should be elucidated using essential bacteriological techniques to enumerate VBNC cells.

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