THESIS

EFFICACY OF ANTIMICROBIALS USING AN INNOVATIVE, NEW ELECTROSTATIC APPLICATION SYSTEM ON *SALMONELLA*-INOCULATED POULTRY PARTS

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ABSTRACT

EFFICACY OF ANTIMICROBIALS USING AN INNOVATIVE, NEW ELECTROSTATIC APPLICATION SYSTEM ON *SALMONELLA*-INOCULATED POULTRY PARTS

Two studies were conducted to evaluate efficacy of peroxyacetic acid (PAA) as an antimicrobial intervention treatment when applied electrostatically, in reducing inoculated populations of *Salmonella* serovars on chicken wings. The other objectives of these studies were: to determine critical operating parameters for reducing *Salmonella* serovars on poultry parts; to evaluate use of static electricity to maximize coverage of antimicrobial solutions applied electrostatically to poultry part surface areas while limiting volume to minimize weight gain; to evaluate use of vacuum to enhance absorption of antimicrobial spray into pores of poultry parts; and to determine optimal rotation speed of the Birko prototype application unit's containment drum to expose all poultry part surfaces during antimicrobial solution application.

Two different electrostatic spray systems (ES1 and ES2) were evaluated in two separate studies. For both studies, chicken wings were inoculated with nalidixic acid- and novobiocin-resistant *Salmonella* (5-strain mixture; 5-6 log CFU/ml of chicken wing rinse solution) sourced from poultry. Inoculated wings were either left untreated (control) or were treated with water or PAA.

In study 1, water and PAA (at a wt/wt concentration of 2000 ppm) were applied (30 s) with one of four application methods: (i) electrostatic spray (ES1), (ii) vacuum, (iii) ES1 + vacuum, or (iv) immersion. Chicken wings were then placed into Whirl-Pak bags containing Dey/Engley (D/E) neutralizing broth and sample rinsates were serially diluted and surface-plated

on both tryptic soy agar and tryptic soy agar supplemented with nalidixic acid (20 µg/ml) and novobiocin (25 µg/ml). Overall, least squares means for $\log_{10}$ *Salmonella* counts differed ($P < 0.05$) between all treated wings vs. the control. When PAA was applied, electrostatic spray was most effective ($P < 0.05$) at reducing *Salmonella* populations.

In study 2, treatment solutions of water and two concentrations of PAA (2000 ppm and 4000 ppm) were evaluated. These were applied (30 s) using two differing application methods [a Birko prototype application system (ES2) and immersion]. Sampling methods were the same as those used in study 1, with the exception that analysis of efficacy occurred at both 0 and 24 h. Untreated and treated chicken wings were placed in Whirl-Pak bags and held at 4°C for 24 h before sampling. For study 2, mean bacterial counts for all treatments differed ($P < 0.05$) from the control and there was a treatment and sampling time interaction. For both water and PAA, the immersion treatment was most effective ($P < 0.05$) at reducing *Salmonella* populations after 24 h storage.

Both electrostatic spray systems (ES1 and ES2) reduced ($P < 0.05$) bacterial populations of *Salmonella*, validating electrostatic application as a potential antimicrobial intervention method for chilled poultry parts.

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CHAPTER 1

Introduction

In recent years, consumers have become more steadfast in their search for knowledge regarding the foods they choose to eat (IFT, 2010). In fact, after the 2010 Consumer Food Safety Survey, Pat Conroy, Vice Chairman of Deloitte said, "Food companies are now dealing with an engaged consumer who actively seeks to understand the products they are looking to buy" (IFT, 2010). This is true for both quality and safety of food items and executives within the food industry realize that their number one goal, as well as their greatest challenge, is to protect consumers by delivering a safe product (Conroy et al., 2011). According to the Consumer Food and Product Insights Survey of 2011, the number of respondents that were more worried about the food that they ate grew 8% from the previous year's survey (Conroy et al., 2011). Due to increased media coverage, consumers have a heightened awareness of foodborne illness outbreaks and are more likely to do their research in order to make "good" purchasing decisions (Conroy et al., 2011). Consumers' concerns about foodborne illness are further supported by the large number of illnesses and outbreaks in the United States annually.

According to the Centers for Disease Control and Prevention (CDC), there are roughly 48 million foodborne illnesses annually and approximately 1 out of every 6 people in America suffers from foodborne illness each year (Benedict, 2013; CDC, 2016a). Furthermore, an estimated 125,000 hospitalizations and 3,000 deaths occur due to these foodborne illnesses (Benedict, 2013; CDC, 2016a). Foodborne illness has been a long-term concern for the food industry, and the meat industry in particular, because many foodborne pathogens are zoonotic; some of the major pathogens of concern are STEC/EHEC *Escherichia coli*, *Listeria*

monocytogenes, *Salmonella*, and *Campylobacter jejuni/coli* (FDA, 2014; Sofos, 2007). There have been myriads of foodborne outbreaks that have caused concern among consumers, the food industry, regulators, and scientists that include not only these pathogens, but also several others.

In particular, the meat industry has been implicated in many foodborne illness outbreaks because meat and poultry products are susceptible to contamination with enteric pathogens and, therefore, associated with pathogens causing foodborne illness (CDC, 2016a; Sofos, 2007). At the beginning of 1993, a multi-state outbreak was traced back to consumption of under-cooked hamburgers from Jack in the Box restaurants, leading to hundreds of *Escherichia coli* O157:H7 infections (Golan et al., 2004). Many developed hemolytic uremic syndrome, HUS, which destroys red blood cells in the kidneys and prevents the filtering of the kidneys (Flynn, 2009; NIH-NIDDK, 2015). Infections also led to the deaths of four children and long-term health effects in those who survived (Benedict, 2013). This outbreak has often been referred to as the "9/11 of the meat industry" and led new regulations (i.e. Pathogen Reduction and HACCP Systems Final Rule) and implementation of intervention methods to prevent foodborne pathogens from making people ill (Benedict, 2013).

Controlling foodborne pathogens has proven to be a difficult task for the meat industry over the years, especially as pathogens such as *Salmonella* have evolved (Sofos, 2007). Increased food safety measures have been implemented industry-wide since the 1990s to prevent, eliminate, and reduce potential food safety pathogens to acceptable levels using the principles of Hazard Analysis and Critical Control Points (HACCP) which were expanded by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in 1997 (FDA, 2014; NACMCF, 1998). These measures for prevention of foodborne illness include both the physical and chemical decontamination of meat and poultry products at U.S. processing plants and often

are part of a multiple hurdle system (Graves Delmore, 1998; Huffman, 2002; Leistner and Gorris, 1995; Milillo, 2011; Sofos, 2007; USDA-FSIS, 2016a). The Food Safety and Inspection Service of the United States Department of Agriculture (USDA-FSIS) defines a multiple hurdle system as one that involves "combinations of inhibitory factors that individually are insufficient to control microorganisms" (USDA-FSIS, 2016a). The USDA, as well as numerous others, have demonstrated that use of a multiple hurdle system with at least two decontamination treatments leads to a "synergistic or additive" effect on the reduction of microbial populations (Belk, 2001; USDA-FSIS, 2016a).

The mission of USDA-FSIS is to "protect consumers by ensuring that meat, poultry, and processed egg products are safe, wholesome, and correctly labeled and packaged" (USDA-FSIS, 2016b). For this reason, the Food Safety and Inspection Service, FSIS, implemented a Strategic Plan for fiscal years 2011 through 2016 to address concerns relating to foodborne pathogens in the food supply (USDA-FSIS, 2016b). The goals within the theme "Prevent Foodborne Illness" included matching inspection processes to existing and emerging risks, maximizing compliance with food safety policies, and strengthening collaborative efforts among stakeholders (USDA-FSIS, 2016b). Other goals crucial to meat safety were to "effectively use science to understand foodborne illness and emerging trends" and to protect public health by better training employees and emphasizing outreach to improve food-handling (USDA-FSIS, 2016b).

Because *Salmonella* is the primary bacterial foodborne pathogen causing illness and death in the United States, both FSIS and FDA have developed priority goals to reduce Salmonellosis cases in the United States as part of a "shared vision to reduce foodborne illness" (USDA-FSIS, 2013; USDA-FSIS, 2016b). In the U.S., it is estimated that over one million cases of Salmonellosis occur annually, with approximately 6% of cases being linked to an outbreak

(CDC, 2013a). According to a foodborne illness report from 2011, 94% of these one million foodborne Salmonellosis cases in the United States were caused by non-typhoidal *Salmonella*. (Scallan et al., 2011). In addition, non-typhoidal *Salmonella* serovars were reported as the leading cause of both hospitalizations (35%) and death (28%; Scallan et al., 2011).

While emerging issues, such as antibiotic resistance, are major topics of discussion, foodborne pathogens will continue to be problematic. In fact, pathogens of concern continue to emerge and evolve (Sofos, 2007). *Salmonella* serovars are among the pathogens that are evolving; incidence of foodborne infections associated with these bacteria alternates between remaining constant and growing (CDC, 2016b; Fratamico et al., 2005; Sofos, 2007). That said, continued research needs to be dedicated towards understanding these pathogens and their control. In 2014, USDA contributed over $70 million to the food safety effort through research, education, and extension programs and projects to aid in the development of a modernized public health system supporting the farm-to-fork model (USDA, 2015).

The primary focus of this thesis project was to investigate potential for using a new antimicrobial intervention application method in order to reduce both chemical and water usage while reducing bacterial populations on poultry parts. After a preliminary proof-of-concept study was conducted, these studies were designed to establish operating parameters when applying antimicrobials electrostatically in a prototype commercial system. Primarily, for both studies, our goal was to evaluate efficacy of a new electrostatic antimicrobial application system, in reducing inoculated populations of *Salmonella* serovars on poultry parts. For our purposes, peroxyacetic acid (PAA) was used; however, any antimicrobial compound could have been used within the Birko prototype application unit.

CHAPTER 2

Literature Review

2.1 Meat Safety

According to *The Principles of Meat Science*, meat science "is a component of all facets of the meat industry, beginning with animal production and ending with final preparation of meat for consumption" (Aberle et al., 2012). That said, the meat industry has faced plenty of adversity over the years as it has grown and expanded. Advancements in agriculture have been occurring for centuries, and the meat industry has seen a number of evolving problems and mechanisms to deal with those problems. Evidence includes passing of such acts as the Homestead Act of 1862, which promoted the establishment of individual family farms, and the Morrill Act of 1862, which led to the creation of State Agricultural Experiment Stations (Bray, 1997). Both of these pieces of legislation encouraged new developments in agriculture and have directly impacted the meat industry due to the expansion of animal production and, therefore, meat available to consumers both domestically and abroad (Bray, 1997). Expansion of railroads and the conception of refrigerated trucks and rail cars aided in the growth of the meat industry (USDA-FSIS, 2015). Developments in animal and meat production came with a responsibility to ensure that quality and safety of meat were not secondary concerns to profit, and the industry had to learn to respond to such concerns (Hulebak and Schlosser, 2002), whether real or imagined. European countries importing U.S. meat products began scrutinizing safety of U.S. exports in 1890, causing producers to request a federal inspection program that would prevent exports from being negatively affected as a consequence (Hulebak and Schlosser, 2002; USDA-FSIS, 2015).

Due to safety concerns and need for U.S. producers to compete in foreign markets, President Harrison passed the first law to require that meat products go through an inspection process in 1890 (USDA-FSIS, 2015). Originally, the USDA was only tasked to inspect salted pork and bacon destined for export; however, the law was amended a year later to require that all cattle and beef to be exported be inspected and certified by the USDA (USDA-FSIS, 2015). Unfortunately, this did not end consumers' worries about meat safety. In 1906, publication of Upton Sinclair's *The Jungle* brought forth scandalous information about the filthiness and unsanitary conditions of meat-packing facilities, which both infuriated and distressed the public (CRF, 2008; Hulebak and Schlosser, 2002). Sinclair described facilities in a negative light, shocking the average American with details about "diseased, rotten, and contaminated meat" that he found in the establishments (CRF, 2008). Public outcry led Congress to pass the Federal Meat Inspection Act (FMIA), the first law of its kind in the meat industry (Hulebak and Schlosser, 2002; USDA-FSIS, 2016c).

In response to *The Jungle*, the FMIA of 1906 was enacted to ensure that slaughter and meat processing occurred under sanitary conditions (USDA-FSIS, 2016c). It also banned sale of adulterated or misbranded meat and meat products along with requiring ante-mortem examination of all livestock, excluding poultry, and postmortem inspection of each carcass (Hulebak and Schlosser, 2002; USDA-FSIS, 2016c). The Bureau of Animal Industry (BAI) was responsible for enforcing the FMIA and, therefore, inspecting meat and meat products (USDA-FSIS, 2016c). Because poultry was considered a minor protein at the time the FMIA was passed, it was not covered by the act; however, as poultry production and consumption gradually increased, ante- and postmortem inspection became necessary (NRC, 1987). After an avian influenza outbreak in New York in the 1920s, demand for poultry inspection grew tremendously,

and local and state departments began to create inspection programs of their own (NRC, 1987). In 1926, the Federal Poultry Inspection Service (FPIS) was created to help smaller, local inspection programs (NRC, 1987).

The USDA, the New York Live Poultry Commission Merchants Association, and the Greater New York Live Poultry Chamber of Commerce worked in cooperation with the FPIS to conduct voluntary inspections of live poultry at railroad terminals and markets near New York City (NRC, 1987). Additionally, FPIS was given authority to conduct postmortem inspections and eviscerated poultry was inspected only upon purchaser request (NRC, 1987). Before 1940, inspection was sometimes conducted upon delivery and, sometimes, not conducted at all (NRC, 1987). While inspection up to this point had been voluntary, continued growth of production and consumption, as well as necessity of providing poultry from plants that met military sanitation requirements, led the USDA to implement protocols that served two purposes: (a) "ensuring the wholesomeness of poultry product" and (b) "promoting sales by enabling processors to ship their product into jurisdictions requiring certification" (NRC, 1987). These protocols eventually led to the Poultry Products Inspection Act (PPIA) passing in 1957 (NRC, 1987).

The PPIA mandated that all birds intended for human consumption be subjected to an inspection process before and after slaughter, and required inspection of processing plants; it also required verification of accuracy and truth for all labels (FDA, 2009; NCSL, 2016; NRC, 1987). Moreover, the act required inspection of any poultry products meant for interstate commerce, making such products subject to federal control (NRC, 1987). In 1967, the Wholesome Meat Act (WMA) became the most significant amendment to FMIA; the Wholesome Poultry Products Act (WPPA), an amendment to the PPIA, came a year later (MacDonald et al., 1996; NSCL, 2016; Worosz et al., 2008). These amendments established cooperative agreements between states and

the federal government mandating that all state inspection systems be at least as strict, if not more strict, as their federal counterparts (MacDonald et al., 1996; NSCL, 2016; Worosz et al., 2008). Although all of these laws are in place regarding inspection, contamination continues to be problematic for the meat industry.

2.2 Potential for Contamination

Bacterial contamination of live birds and raw poultry is most commonly linked to *Salmonella* and *Campylobacter jejuni* (Bryan and Doyle, 1995; Cheung and Kam, 2012; Heyndrickx et al., 2002; Whyte et al., 2002a). Multiple studies have shown that poultry can be contaminated with pathogens at a number of different steps in the production process, both pre- and post-harvest (Bryan and Doyle, 1995; Jones et al., 1991; Panisello et al., 2000; Whyte et al., 2002a). There is major potential for contamination of poultry and poultry products from farm-to-table (Bryan and Doyle, 1995; Panisello et al., 2000; Whyte et al., 2002a). Furthermore, broiler houses, and the feed and water in broiler houses, are frequently contaminated with pathogens (Heyndrickx et al., 2002). Upon processing, contaminants already present from birds can be spread through scalding, defeathering, evisceration, removing giblets, washing, and chilling (Bryan and Doyle, 1995; Stern et al., 2001). Mishandling raw and cooked poultry at the retail level, as well as in kitchens, also leads to cross-contamination and potential for consumption of undercooked poultry (Bryan and Doyle, 1995; Panisello et al., 2000; Whyte et al., 2002a).

Horizontal transmission of both *Salmonella* and *Campylobacter* is of more critical importance in the introduction of contamination onto poultry than is vertical transmission (Herman et al., 2003; Heyndrickx et al., 2002). In fact, several studies have shown that horizontal transmission from the environment is the main route by which flocks become infected with *Campylobacter* (Sahin et al., 2002). Risk factors associated with horizontal transmission

include poor cleaning and disinfection of broiler rearing houses between flocks, poor hygiene leading to contamination of feed, and contaminated transportation crates (Heyndrickx et al., 2002). As previously described, during slaughter and processing, any contamination already present can be spread and exacerbated in impact, causing meat to become positive for *Salmonella* or *Campylobacter*. Because risks for horizontal transmission are so high, many studies have evaluated incidence of pathogens in live birds and poultry carcasses (Jones et al., 1991; Stern et al., 2001).

Jones et al. (1991) investigated *Salmonella* prevalence in breeder and broiler houses, feed mills, hatcheries, and processing plants for two vertically-integrated broiler companies; *Salmonella* serovars were present in 33% of the samples collected from transportation trucks and in 21.4% of carcasses at processing facilities (Jones et al., 1991). The most commonly isolated serotype was *Salmonella* Typhimurium, which is an outbreak strain commonly known to cause foodborne illness (CDC, 2013b; Jones et al., 1991). In another study, samples were collected from 32 broiler flocks across eight farms in the U.S. in order to assess prevalence of *Campylobacter* (Stern et al., 2001). Overall, 87.5% of flocks tested positive for *Campylobacter* at some point and, once transported to the processing facility, scalded, and chilled, *Campylobacter* positives on carcasses ranged from 21 to 41% (Stern et al., 2001). Genigeorgis et al. (1986) studied three consecutive generations of live chickens across four ranches and found that *Campylobacter jejuni* was transmitted from generation to generation. Upon processing, flocks that previously were *Campylobacter*-negative resulted in contaminated meat; the researchers attributed this to lack of proper sanitation of harvest equipment (Genigeorgis et al., 1986).

A study conducted by researchers at the University of Arkansas examined survival and death rates for *Salmonella* Typhimurium and *Campylobacter jejuni* in water and on skin of chickens during scalding and chilling (Yang et al., 2001). Water used for both scalding and chilling was inoculated, as were chicken skins, and then treatments of scalding temperature at three levels – 50, 55, and 60°C and the chlorine concentration in chilled water – 0, 10, 30, and 50 ppm (Yang et al., 2001) were evaluated. *Salmonella* and *Campylobacter* populations were reduced in both scalding and chilled water; however, chlorinating chilled water did not reduce populations on chicken skins (Yang et al., 2001).

A review by Oyarzabal (2005) investigated use and efficacy of commercial antimicrobials during broiler processing; it listed key processing steps in poultry production, and provided information about reductions after each step. Before scalding, *Campylobacter* counts were reported to be between 5.4 and 7.5 log CFU/g (Berrang et al., 2000a; Kotula and Pandya, 1995; Oyarzabal, 2005). Additionally, when Kotula and Pandya (1995) looked at bacterial contamination before scalding, they found that the prevalence of *Salmonella* was between 27.5 and 75% and *Campylobacter jejuni/coli* was between 45 and 82.5%. After scalding at a temperature of 58°C or greater, reductions in *Campylobacter* counts were observed, but the defeathering process increases bacterial populations (Berrang and Dickens, 2000b; Izat et al., 1988; Oyarzabal, 2005). It is suspected that this was due to pressure that was applied to the carcass, which forced fecal matter out of the cloaca and then contaminated the carcass exterior (Oosterom et al., 1983; Oyarzabal, 2005). Evisceration also can lead to contamination of the carcass due to breakage of the intestinal lining, with *Campylobacter* counts often ranging between 2.8 and 3.7 log CFU/ml of rinse solution before a carcass wash occurs (Oyarzabal, 2004).

Bashor et al. (2004) evaluated different carcass wash systems across four large processing plants. At the first plant, samples were obtained before and after three separate carcass washers and after a post-chill in both spring and fall seasons, whereas samples were collected post-evisceration (before the first carcass washer), after the final carcass washer, after an antimicrobial treatment, where applicable, and after a post-chill tank in the other three plants (Bashor et al., 2004). Before carcass wash, *Campylobacter* counts on whole carcasses were between 4.7 and 4.9 log CFU/ml of rinse solution for all plants (Bashor et al., 2004; Oyarzabal, 2005). Although all counts were reduced post-wash, systems using chlorinated water between 25 and 35 ppm only reduced mean counts by 0.5 log CFU/ml of rinse solution (Bashor et al., 2004). Two of the processing plants used antimicrobials in a carcass wash as part of a multiple hurdles system; one plant used trisodium phosphate (TSP) and achieved an additional 1.03 log CFU/ml reduction after treatment, while the other used acidified sodium chlorite (ASC) which resulted in further reduction of 1.26 log CFU/ml (Bashor et al., 2004). Although *Campylobacter* populations were reduced after using multiple carcass washers, these reductions were minimal and, even after applying TSP or ASC, there were still over 3 log CFU/ml on carcasses, suggesting that other interventions were needed (Bashor et al., 2004; Oyarzabal, 2005).

Immersion chilling is another step in poultry processing, and since the 1950s, plants have been chlorinating water in the chiller in order to increase shelf life of products (Drewniak et al., 1954; Oyarzabal, 2005). Chilling is utilized to reduce carcass temperature rapidly (usually within a few hours) to less than 4.4°C in order to prolong shelf life by limiting growth of spoilage and pathogenic bacteria on carcasses (Oyarzabal, 2005). Oyarzabal et al. (2004) reported a reduction in *Campylobacter* counts after chilling, but quite a few carcasses tested positive for *Campylobacter* following enrichment. The researchers proposed that because *Campylobacter*

counts on carcasses are customarily high, immersion chilling alone was not an effective intervention (Oyarzabal et al., 2004). Additionally, immersion chilling step could potentially lead to cross-contamination of bacterial species (Mead et al., 1995; Whyte et al., 2002b). Whyte et al. (2002b) suggested that, due to the large numbers of carcasses subjected to commercial immersion chillers, there often is increased prevalence of pathogenic organisms such as *Salmonella* and *Campylobacter* on carcasses post-chilling. Cross-contamination among carcasses may be reduced by implementing a chlorinated system at higher levels into the tank of the immersion chiller (Whyte et al., 2002b; Yang et al., 2001).

These studies support horizontal transmission of pathogens, so the continued development of pre- and post-harvest decontamination interventions is absolutely necessary to reduce pathogens on poultry meat. *Salmonella* have been especially problematic for the food industry for years, and even though the *Salmonella* Action Plan was implemented by USDA, the 2015 Food Safety Report for the Healthy People 2020 goals showed that the rate of *Salmonella* infections in the population had not changed from years 2006-2008 which were the baseline years used for developing goals (CDC, 2016b; USDA-FSIS, 2016b).

2.3 *Salmonella* spp.

The genus *Salmonella* was discovered in 1885 by Theobald Smith, a member of the Bureau of Animal Industry (BAI) of the USDA, and Daniel E. Salmon, the chief of BAI at the time (Cima, 2013; Schultz, 2008; USDA-APHIS, 2015). Salmon and Smith were working on such problems as hog cholera, bovine tuberculosis, and Texas cattle fever in order to improve public health and reduce animal disease (Schultz, 2008). When Smith identified the first species of the genus, they were thought to be the cause of hog cholera and were, therefore, named

Salmonella choleraesuis; however, it was later discovered that cholera was a viral infection (Cima, 2013; Schultz, 2008).

Salmonella species (spp.) are motile, non-sporeforming, rod-shaped, gram-negative bacteria (FDA, 2012). They are classified into the *Enterobacteriaceae* family and the *Salmonellae* tribe (FDA, 2012). *Salmonella* spend the majority of their life cycles in animal hosts as they thrive in environments such as the gastrointestinal system (Coburn et al., 2007; Winfield and Groisman, 2003). The genus *Salmonella* can be divided into two species that lead to human illness: *S. enterica* and *S. bongori* (Coburn et al., 2007; FDA, 2012). *Salmonella enterica*, a facultative anaerobe, is the most significant concern to public health and is further divided into six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI; Coburn et al., 2007; FDA, 2012; Jones et al., 2008). Although *Salmonella* serotypes have similar genetics, they may vary significantly in pathogenic potential (Jones et al., 2008).

Over 60% of all identified *Salmonella* strains and 99% of serovars that cause disease in warm-blooded animals are estimated to belong to subspecies *enterica* (subspecies I; Chan et al., 2003). Somewhat unique to subspecies I serovars is the capability of infecting a wide array of hosts with diseases of highly variable severity (Chan et al., 2003). For instance, serovar Typhi (*Salmonella* Typhi) is known for causing typhoid fever, a systemic disease, in humans only, while many other serovars, including serovar Enteritidis, lead to gastrointestinal disease without bloodstream invasion in a variety of animals (Braden, 2006; Chan et al., 2003). As of 2007, 2,579 *Salmonella* serotypes had been reported, so the nomenclature system for the organism is complicated (FDA, 2012). It is important to note that, while there are a large number of serotypes, few are responsible for causing disease in humans and domestic animals (Porwollik et al., 2004).

The naming system for *Salmonella* strains began in 1934 when the Kaufmann-White typing scheme was published (FDA, 2012). This scheme differentiates strains based upon their surface and flagellar antigenic properties (FDA, 2012). Serovars within *S. enterica* subspecies I are designated by a name that usually corresponds to the geographic location where the serovar was isolated (Popoff et al., 2004). This is different from serovars within other subspecies, which are designated by subspecies followed by the antigenic formula (Popoff et al., 2004). Forty-six somatic (O) antigen groups have been recognized and when flagellar (H) antigens are detected, serotype identification is possible, which tremendously aids in epidemiological investigations of *Salmonella* (Fitzgerald et al., 2003). When Kaufmann first created the scheme, there were only 44 known serotypes, but that number skyrocketed since then, so it is critical that the scheme be updated to include new serotypes (FDA, 2012). The World Health Organization (WHO) Collaborating Center for Reference and Research on *Salmonella* is charged with updating the scheme to incorporate characterization of new serovars (Popoff et al., 2004).

According to the CDC, *Salmonella* serotypes are diverse in reservoirs and sources, and while Salmonellosis cases have not decreased over the years, strains/serotypes involved in causing infection have changed (CDC, 2015a; CDC, 2016b). Some common outbreak strains of *Salmonella* include Typhimurium, Enteritidis, Heidelberg, Montevideo, Newport, Uganda, Javiana, Saintpaul, Infantis, Agona, and Dublin (Jackson et al., 2013). *Salmonella* Typhimurium and *Salmonella* Enteritidis are generally the most common strains isolated in the United States with a higher rate of infection in summer months as compared to winter months (CDC, 2016c). Similarly, between the years 1998 and 2008, there were over 1,200 *Salmonella*-caused outbreaks with a confirmed serotype (CDC, 2013c). Among these confirmed serotypes, *S.* Enteritidis was responsible for 418 outbreaks making it the most common isolate (CDC, 2013c). After

Salmonella Enteritidis, the most common serotypes were Typhimurium (170 outbreaks), Heidelberg (102 outbreaks), and Newport (93 outbreaks; CDC, 2013c). Moreover, *Salmonella* serovars resulted in 4,034 of the 9,109 reported hospitalizations and resulted in the most deaths, followed by *Listeria* and STEC (CDC, 2013c; Scallan et al., 2011). Severity of disease and the type of illness can vary significantly from asymptomatic to very severe depending on the serotype (FDA, 2012; Jones et al., 2008).

There are two types of human illness caused by *Salmonella*: non-typhoidal salmonellosis and typhoid fever (FDA, 2012). Non-typhoidal salmonellosis is more common in the United States and is responsible for more hospitalizations and deaths than other infections (FDA, 2012; Scallan et al., 2011). Serotypes responsible for non-typhoidal salmonellosis are those other than *Salmonella* Typhi and *Salmonella* Paratyphi A (FDA, 2012; Jones et al., 2008). Non-typhoidal *Salmonella* are usually ingested orally through contaminated food or water, or through fecal particles; some of the common food items associated with *Salmonella* are meat and eggs, raw tree nuts, fruits and vegetables, and even spices (FDA, 2012). Only one cell is required to cause infection, depending on host health, age, and serotype, and infection can begin within 6 hours of exposure and up to 72 hours later (FDA, 2012). Symptoms related to non-typhoidal salmonellosis include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache, but the infection is usually self-limiting in otherwise healthy individuals, although it can present life-threatening complications in people with healthy immune systems as well (FDA, 2012). Generally, symptoms last four to seven days, but acute symptoms last a couple more days depending on the infection-causing serotype and a variety of host factors (FDA, 2012; Jones et al., 2008).

There can be complications related to non-typhoidal salmonellosis, one of which is dehydration from diarrhea and vomiting that leads to electrolyte imbalances; in children, the elderly, or other immunocompromised individuals, this can lead to death if not treated rapidly (FDA, 2012). Reactive arthritis, which may cause joint inflammation, urethritis, or conjunctivitis, also is a complication in 2% of confirmed *Salmonella* cases, and can develop three or four weeks following the onset of acute symptoms (ACR, 2015; CDC, 2016c; FDA, 2012). Additionally, if *Salmonella* escapes the gastrointestinal tract into the blood stream, septicemia may occur or the blood, internal organs, and joints can be infected, known as bacteremia (FDA, 2012). Mortality is generally low (less than 1%), but it can be higher for certain serotypes in susceptible populations (FDA, 2012; Jones et al., 2008). Among the non-typhoidal *Salmonella* serotypes, some of the more uncommon serotypes, such as *S.* Choleraesuis and *S.* Dublin, have been found to cause more severe infections that may lead to these complications, but differences in severity are not well understood (FDA, 2012; Jones et al., 2008).

The second type of illness, typhoid fever, is caused by *Salmonella* serotypes Typhi and Paratyphi A, which are found only in humans, and is a more serious illness with a higher mortality rate than non-typhoidal salmonellosis (FDA, 2012; Jones et al., 2008). These serotypes are relatively common in developing countries; however, less than 1% of *Salmonella* infections in the United States are caused by Typhi and Paratyphi A, and those that are tend to be associated with international travel (Jones et al., 2008). Typhoid fever is caused by ingestion of food or water contaminated with feces and the infective dose is fewer than 1,000 cells (Ericsson et al., 2005; FDA, 2012). Upon ingestion, onset of infection can occur in as early as one to three weeks, but in some cases, individuals take up to two months before showing any symptoms

(FDA, 2012). Symptoms include a fever of 39.4°C to 40°C, lethargy, abdominal pains, diarrhea or constipation, body aches, headache, general loss of appetite and, in some cases, a spotty rash with rose-coloring (FDA, 2012). This form of *Salmonella* illness usually lasts a couple of weeks to a month, but there can be complications such as septicemia or colonization of other tissues and organs that could lead to secondary infections like endocarditis (FDA, 2012).

Septic or bacterial arthritis is another complication associated with typhoid fever in which the joints are infected; this infection is difficult to diagnose and treat because symptoms such as fever and rigors are not always present (FDA, 2012; Schlapbach et al., 1990). In some complicated cases, the gallbladder also may become chronically infected, causing the individual to be a carrier after symptoms subside (FDA, 2012). One of the reasons that typhoid fever is problematic in developing countries is because carriers who do not exhibit symptoms, or those who have recovered from the illness recently, continue to excrete bacteria in large quantities; this leads to the spread of the bacteria and therefore creates endemic situations (Ericsson et al., 2005).

In the case of non-typhoidal *Salmonella*, bacteria are capable of passing from the lumen of the gut into the epithelium of the small intestine where inflammation materializes; there also is a possibility for enterotoxin to be produced within enterocytes (FDA, 2012). Likewise, penetration and passage of typhoid *Salmonella* organisms out of the lumen of the gut into the small intestine epithelium, and further, into the bloodstream can occur; this allows the organisms to travel to other tissues and organs where inflammation develops (FDA, 2012). In order to cause infections through these pathways, *Salmonella enterica* require several components, many of which are found on *Salmonella* pathogenicity islands (SPI), discrete regions of the chromosome (Morgan, 2007). *Salmonella* has evolved over time, and it is thought that, in the first phase of evolution, SPI-1 was obtained by the genome, likely in an ancestor to all *Salmonella* serotypes

(Bäumler et al., 1998). Logically, SPI-1 is found in all *Salmonella* (Bäumler et al., 1998). As of 2007, 14 SPIs had been identified and the differences in structure, function, and distribution of these SPIs among the subspecies and serovars influence host specificity and contribute to varying characteristics of serovars, along with virulence and pathogenicity (Morgan, 2007).

Pathogenesis and virulence are determined by both pathogenicity islands 1 and 2 (Coombes et al., 2005). *Salmonella enterica* possess both SPI-1 and SPI-2, while *Salmonella bongori* do not possess the second pathogenicity island (Bäumler et al., 1998). It is important to note that both SPI-1 and SPI-2 encode for their own type III secretion systems that introduce effector proteins (or virulence proteins) into the host (Coombes et al., 2005). *Salmonella* pathogenicity island 1 enables bacterial invasion of host epithelial cells, and is generally associated with intestinal infections, whereas SPI-2 helps intracellular bacteria replicate within membrane-bound vacuoles which contain *Salmonella*, and is more strongly affiliated with systemic infection or virulence (Coombes et al., 2005; Waterman and Holden, 2003). Using type III secretion, *Salmonella* organisms in contact with the epithelium are able to induce actin cytoskeletal changes at the contact site (Zhou and Galán, 2001). All of this considered, it is clear that *Salmonella* is a public health issue that will continue to cause foodborne illness due to changes in virulence factors and other components of the genome.

In 2016, there have been multiple *Salmonella* outbreaks caused by many serotypes and matrices; these include: *S.* Oranienburg in eggs; *S.* Reading, *S.* Abony, *S.* Muenchen, and *S.* Kentucky in alfalfa sprouts; *S.* Montevideo and *S.* Senftenberg in pistachios; and several outbreak strains related to live poultry in multiple states (CDC, 2016d). Across 45 states and eight separate outbreaks, 611 people were infected with *Salmonella* via contact with live poultry in backyard flocks leading to 138 hospitalizations and one death (CDC, 2016d). Although these

outbreaks are related to live birds and not poultry meat, there is potential for cross-contamination of meat already in consumers' homes, as well as potential for contamination of poultry meat from these flocks in the event that the birds are harvested. Two outbreaks in 2015 were linked to frozen, raw, stuffed chicken entrees produced by two different companies (CDC, 2015b).

Both of these outbreaks were associated with *Salmonella* Enteritidis; in the smaller outbreak, five individuals in Minnesota were infected with two Enteritidis strains after eating stuffed and breaded chicken entrees (CDC, 2015c). There were no reported deaths associated with this outbreak, but two people were hospitalized (CDC, 2015c). Upon investigation by USDA-FSIS, the CDC, and the Minnesota Department of Public Health, Aspen Foods recalled an estimated 1.9 million pounds of frozen product in July of 2015 in order to potentially prevent more people from being infected with *S.* Enteritidis (CDC, 2015c). In October, Aspen Foods expanded the recall to include an additional 561,000 pounds of potentially contaminated product (CDC, 2015c). The larger of the two outbreaks, which was not related to the Minnesota outbreak, spanned seven states and 15 people were infected with drug-resistant *Salmonella* Enteritidis after eating stuffed and breaded chicken products produced by Barber Foods (CDC, 2016b). During this outbreak, four hospitalizations and zero deaths were reported (CDC, 2016b). The National Antimicrobial Resistance Monitoring System (NARMS) laboratory tested the four isolates for resistance and found that all four were resistant to both ampicillin and tetracycline, which was concerning because of the increased risk of hospitalization, septicemia, and treatment failure in infected individuals (CDC, 2015b). There were several large recalls of these entrees, marketed under different brands, throughout the outbreak, and although both outbreaks were declared over in 2015, risk of infection remained due to the fact that the frozen entrees had a long shelf-life and could still be in people's freezers (CDC, 2015b; CDC, 2015c).

Another multistate outbreak associated with Foster Farms chicken began in March of 2013 and was not over until mid-2014 (CDC, 2014a). Seven outbreak strains of *S.* Heidelberg, which were found to be resistant to several common antibiotics, were isolated from 634 infected individuals in 29 states and Puerto Rico; 241 of these 634 infected people were hospitalized (CDC, 2014a). Also in 2014, a recall was implemented after a different outbreak associated with *S.* Heidelberg was investigated and linked to Tyson Foods, Inc. (CDC, 2014b). There were nine people infected with the outbreak strain after consuming mechanically separated chicken products at a correctional facility in Tennessee; two of these individuals were hospitalized (CDC, 2014b). Because of the outbreak, Tyson Foods, Inc. issued a recall of nearly 34,000 pounds of potentially contaminated mechanically separated chicken products (CDC, 2014b). It is clear that even with regulations, decontamination methods, and food safety education, there are still public health hazards associated with *Salmonella* serovars

In 1996, USDA-FSIS established the *Salmonella* verification program in the Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems Final Rule (9 CFR Part 417); this final rule provided performance standards, using national baseline studies, for *Salmonella* which could be used to verify process control in meat and poultry slaughter (USDA-FSIS, 2016d). These standards are now outdated because processes have changed and, with these changes, USDA-FSIS has made changes to the performance standards by utilizing risk assessment in order to "meet public health goals" and has released multiple federal register notices (FRN; USDA-FSIS, 2016d). Additionally, Supreme Court case Supreme Beef Processors Inc. vs. United States Department of Agriculture led to changes in how USDA-FSIS dealt with the data collected from inspections (PBS, 2014).

After failing USDA-FSIS testing three times in an eight month period, Supreme Beef was notified of USDA-FSIS' decision to remove inspectors from the facility (PBS, 2014). Supreme Beef took the matter to federal district court under claims that USDA-FSIS did not have authority to set limits on *Salmonella* levels in meat because they are naturally occurring bacteria (PBS, 2014). Furthermore, Supreme Beef argued that *Salmonella* in finished product "is not an adequate indicator of whether the pathogen control procedures employed in the plant are being properly implemented" because beef may be contaminated upon arrival to the packing plant (PBS, 2014). The company also argued that the *Salmonella* presence in meat is not a significant risk to consumers because the bacteria are "killed and rendered harmless when meat is cooked properly" (PBS, 2014). The Supreme Court ruled that the USDA was not authorized to shut down a meat-processing plant that repeatedly failed *Salmonella* contamination testing because presence alone did not directly correlate with product being "injurious to health" (PBS, 2014). While the USDA appealed the ruling, the decision was upheld by the appeals court in December 2001 (PBS, 2014).

The Modernization of Poultry Slaughter Inspection; Final Rule was published in 2014 in order to facilitate a reduction of pathogens on/in poultry products, while also improving efficiency of slaughter inspection and removing unnecessary regulations preventing innovation (USDA-FSIS, 2016d). The USDA-FSIS used this as an opportunity to remove the pathogen reduction standards for poultry (9 CFR 381.94(b)), so that new standards could be implemented (USDA-FSIS, 2016d). Early in 2015, FSIS released new *Salmonella* and *Campylobacter* performance standards for raw chicken parts and not ready-to-eat comminuted poultry products; it announced that routine sampling would be conducted throughout the year to determine whether processes were effective in reducing pathogens on poultry carcasses and products

(USDA-FSIS, 2016d). The new performance standards were implemented in July of 2016, so plants are now figuring out how to meet them.

The new FSIS program was created to reduce risk that consumers are exposed to contaminated poultry products by verifying that all establishments meet revised performance standards (USDA-FSIS, 2016d). Because *Salmonella* continues to cause outbreaks globally, the FSIS created two strategic goals: (1) the "All-Illness Measure" which sets goals on a quarterly basis to reduce the total illness caused by *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* and (2) the percent of young chicken establishments meeting the new performance standards (CDC, 2016b; USDA-FSIS, 2016b; USDA-FSIS, 2016d). These standards do not apply to individual products, but rather to the process control within processing plants; products are tested to measure efficiency of slaughter and grinding processes in limiting contamination (USDA-FSIS, 2016d). The standards were revised to hold the industry accountable for controlling *Salmonella* and, therefore, reducing likelihood of consumer exposure, but establishments are not required to hold or recall product based on the test results (USDA-FSIS, 2016d). It is estimated that 88% of establishments would meet the new performance standard, with the remaining establishments failing (USDA-FSIS, 2016d). Under these standards and this testing program, individual establishments would receive sample results for both *Salmonella* and *Campylobacter*, and FSIS would publish data once per quarter beginning in 2016 (USDA-FSIS, 2016d). To meet these new standards, it is critical that poultry establishments continue to evaluate decontamination methods used and to investigate new methods that could offer assistance in reducing pathogenic contamination of poultry.

2.4 Decontamination Methods

Some decontamination methods used today are the same methods we have been using for years, and multiple hurdles are implemented in most establishments (Leistner and Gorris, 1995). Sofos (2007) provided the following context:

> Decontamination with multiple interventions is the sequential application of animal hide cleaning, potentially followed or preceded by partial hair removal, chemical dehairing, knife-trimming and/or steam-vacuuming as the hide is removed, pre-evisceration carcass washing, final carcass spray-cleaning after "zero tolerance" inspection for visible soil, chemical and/or thermal decontamination, and carcass chilling.

Although these processes are more common in beef processing, it is important to note that multiple hurdles are successful and necessary for pathogen reduction, which is supported by countless studies over the years. A study conducted in the United Kingdom investigated surface pasteurization of chicken carcasses using hot water following some of the same methods that a previous study used to look at hot water wash treatments to reduce both pathogenic and spoilage bacteria on raw poultry available at retail stores (Corry et al., 2006; Purnell et al., 2003).

These studies looked at physical decontamination methods, some of which include: water rinses, sprays, or steam; ultrahigh pressure; irradiation; ultrasonic energy; UV light; and pulsed-field electricity (Bolder, 1997). The retail study, in particular, included a hot water wash combined with a spray chill; samples were analyzed for bacterial reductions. It was determined that a 70°C hot wash for 40 seconds followed by a spray chill of 13 seconds at 12-15°C reduced pathogens, without decreasing the quality, immediately following treatment, post-trussing, and after 8 days of storage as compared to controls (Purnell et al., 2003). James et al. (2007) also investigated steam or hot water as part of a multiple hurdle with rapid cooling, chilling, or freezing of carcasses at the surface level, concluding that crust freezing poultry directly

following a thermal decontamination treatment was an effective tool to reduce bacterial populations on carcass surfaces. However, this treatment was sometimes detrimental to appearance (James et al., 2007). The most effective treatment in the James et al. (2007) study was a 10 second steam treatment and then a rapid, crust freeze. In addition to physical decontamination methods, chemical treatments have become increasingly more popular in poultry production.

Implementation of chemical treatments is common practice in the poultry industry to reduce pathogens on carcasses and parts (Bauermeister et al., 2008; Bolder, 1997; Loretz et al., 2009). Such chemicals as chlorine, acidified sodium chlorate, acetic acid, lactic acid, trisodium phosphate, peroxyacetic acid, sodium carbonate, and cetylpyridinium chloride have been researched and implemented at various locations and steps within the poultry production process (Bauermeister et al., 2008; Bolder, 1997). Organic acids such as lactic acid (LA) and peroxyacetic acid (PAA) are two of the more common antimicrobial treatments used in the poultry production chain in pathogen reduction, as are chlorine and cetylpyridinium chloride (CPC; Bauermeister, 2008; Bolder, 1997; Scott et al., 2015; Waldroup et al., 2010).

The most widely used chemical decontaminants in the meat and poultry industry are organic acids solutions (Belk, 2001; Nagel et al., 2013). Of all of the organic acids evaluated to assess efficacy, as well as minimal negative effects on quality characteristics, lactic acid is one of the most widely accepted by consumers and utilized by industry members (Huffman, 2002). Lactic acid is commonly used in the industry and efficacy has been investigated in many studies (Bolder, 1997; Loretz et al., 2009). Its use between 1-2% has been shown to reduce bacterial populations immediately following slaughter, as well as throughout storage, without diminishing color and flavor characteristics (Bolder, 1997). When chicken breasts were immersed in 1%

lactic acid, a reduction of 2.0 log CFU/cm^2 in *Salmonella* serovars was observed (Loretz et al., 2009). A 1-2% solution of lactic acid applied during slaughter reduced *S.* Typhimurium by 1.8 to 2.2 log CFU/ml of rinse solution (Loretz et al., 2009). A study conducted at Washington State University evaluated use of a 2% lactic acid spray treatment as an alternative to chlorine (Killinger et al., 2010). Chicken wings were inoculated with a cocktail of *S.* Enteritidis, *S.* Typhimurium, *S.* Heidelberg, and *S.* Kentucky and then were either left untreated or treated with a three minute water rinse, a 50 ppm chlorine rinse, or a 2% lactic acid rinse (Killinger et al., 2010). Upon microbial analysis, there were 5.78 log CFU/wing on the untreated control samples, 5.81 log CFU/wing on the water rinsed samples, and 5.69 log CFU/wing on the chlorine rinsed samples (Killinger et al., 2010). Counts were not statistically different; however, samples after application of 2% lactic acid treatment differed and generated a final bacterial count of 0.39 log CFU/wing (Killinger et al., 2010).

In another study, researchers evaluated efficacy of lactic acid and lauricidin on the growth and survival of *Salmonella* Enteritidis (Anang et al., 2007). Chicken breasts were dipped in ethanol and passed through a flame in order to decontaminate surfaces before inoculation; they were allowed to cool and were then dipped in TSB broth containing around 9 log CFU/ml of *Salmonella* Enteritidis (Anang et al., 2007). After dipping, chicken breasts were given 20 minutes at room temperature for bacterial cell attachment before treatment (Anang et al., 2007). When compared to untreated control samples, significant reductions were observed for the chicken breasts dipped in four lactic acid concentrations (0.5, 1.0, 1.5, and 2.0%) at three time points (10, 20, and 30 minutes; Anang et al., 2007). While the reductions were statistically significant, bacterial populations were reduced by less than 1 log CFU/ml for the 10 minute dip at all concentrations at 0 hours of storage time (Anang et al., 2007). For this reason, the

researchers indicated a need for multiple hurdles with a combination of physical, mechanical, and chemical methods to implement an effective reduction strategy using lactic acid (Anang et al., 2007; Sofos, 2007).

Peroxyacetic acid (PAA) is another organic acid frequently used by the meat and poultry industries (Bauermeister et al., 2008; Scott et al., 2015). It is generally recognized as safe (GRAS) by the FDA, and is approved by USDA-FSIS for up to 2000 ppm in a post-chill immersion application (Bauermeister et al., 2008; Nagel et al., 2013; USDA-FSIS, 2016e). Researchers at Auburn University studied microbial and quality properties of poultry carcasses treated with peroxyacetic (peracetic acid) as an antimicrobial to validate its efficacy (Bauermeister et al., 2008). Poultry carcasses were inoculated with either *Salmonella* Typhimurium or *Campylobacter jejuni* and were assigned at random to a chilled chlorine solution at 0.003% or one of three PAA concentrations: 0.0025%, 0.01%, or 0.02% (Bauermeister et al., 2008). All three PAA levels effectively reduced counts of *Salmonella* Typhimurium compared to chlorine treatment; however, the 0.02% treatment was most effective (Bauermeister et al., 2008). Also of importance, uninoculated controls were negative, meaning that background *Salmonella* serovars were not present and, therefore, were not a factor in reductions observed (Bauermeister et al., 2008).

Scott et al. (2015) also evaluated efficacy of PAA against inoculated *Salmonella* serovars. Chicken wings were inoculated and then immersed in a 700 ppm solution of PAA for 20 seconds (Scott et al., 2015). Untreated controls were plated and found to have *Salmonella* levels of 5.5 to 5.7 log CFU/ml of rinse solution upon enumeration (Scott et al., 2015). When treated samples were plated on tryptic soy agar [TSA; for aerobic plate counts (APC)] and on xylose lysine deoxycholate agar supplemented with novobiocin and nalidixic acid (XLDNN; for selective

purposes), both 0 and 24 hour samples were statistically different from the control samples (Scott et al., 2015). Reductions of 1.7 log CFU/ml of wing rinsate and 1.9 log CFU/ml of wing rinsate were observed for 0 and 24 hour samples, respectively (Scott et al., 2015). Chlorine also is frequently included in carcass washes and immersion chillers (James et al., 2007; Yang et al., 2001).

Chlorine has been one of the more common antimicrobial treatments used in the United States for years; it has been used in multiple processing plants to decontaminate carcasses, and as a consequence, prevents cross-contamination of bacterial populations (McKee, 2011; Nagel et al., 2013). While chlorine has been a widely used antimicrobial historically, research suggested that efficacy of reducing bacterial counts is negatively impacted by an increase in pH, as well as an increase in organic loads (Byrd and McKee, 2005). In a study conducted to determine survival and death of both *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skins, researchers found that the as the age of chilled water increased, chlorine's reduction effect decreased (Yang et al., 2001). Chlorine levels in chilled water were 0, 10, 30, and 50 ppm; after treatment in 0-h chilled water with 10 ppm chlorine, *Salmonella* and *Campylobacter* populations were reduced by 0.7 log CFU/ml and 3.3 log CFU/ml, respectively (Yang et al., 2001). Resulting bacterial counts were below detection limit when higher levels of chlorine (30 and 50 ppm) were added to 0-h chilled water (Yang et al., 2001). At 8 hours, chlorinated chilled water was less effective, and with a chlorine level of 10 ppm, the reduction for both *Salmonella* and *Campylobacter* was less than 0.5 log CFU/ml (Yang et al., 2001). At 50 ppm and 8-h chilled water, reductions ranged from 4 to 5.5 log CFU/ml (Yang et al., 2001). Reduced efficacy of chlorine was attributed to age of chilled water, which is more than likely

related to increased organic load due to usage throughout the day (Byrd and McKee, 2005; Yang et al., 2001).

Bolder (1997) stated that while chlorine is frequently used for decontamination, it may not be effective enough at popular usage levels, which cannot exceed 50 ppm. At a level of 50 ppm, chlorine reduced bacterial loads by only 1 log cycle (Bolder, 1997; James, 1992). When the chlorine level was increased to 300 to 400 ppm, *Salmonella* serovars on poultry carcasses were effectively reduced to acceptable levels, but 50 ppm was found to be ineffective (Bolder, 1997; Teotia, 1975). One major problem is that regulated chlorine use is limited to between 20 and 50 ppm in poultry chillers, which has been shown to lead to a minimal reduction in bacterial counts (McKee, 2011). Because of this minimal reduction, continued research on chlorine use and other solutions using chloride may offer increased bacterial reduction effects (McKee, 2011). Chlorine dioxide (ClO_2) is one antimicrobial currently being evaluated for efficacy against *Salmonella* and *Campylobacter* (Berrang et al., 2011). In one study, application of ClO_2 was tested to determine whether 50 ppm applied during defeathering would aid in preventing *Campylobacter* counts on poultry carcasses from increasing to the degree that they normally would during this process (Berrang et al., 2011). When 50 ppm ClO_2 was used as a carcass spray, it was effective in mitigating the increase in bacterial populations during defeathering; it also was found that this application as an antimicrobial did not select for increased antimicrobial resistance (Berrang et al., 2011).

Another commonly used, and somewhat controversial, antimicrobial in poultry production is cetylpyridinium chloride (CPC); this compound is a commonly found in mouthwashes as antimicrobials (Kim and Slavik, 1995). In a study to assess CPC's efficacy against *Salmonella* attached to poultry skin, CPC was applied with two separate methods: a spray

or an immersion (Kim and Slavik, 1995). Cetylpyridinium chloride was sprayed at two temperatures (15°C or 50°C) at 0.1% for 1 minute, and was found to reduce bacterial counts by 0.9 to 1.7 log units; in general, the spray at 50°C resulted in better reductions (Kim and Slavik, 1995). Immersion consisted of three different treatments at 0.1% CPC: 1 minute, 1 minute plus a 2 minute dwell time, or 3 minutes (Kim and Slavik, 1995). Nearly equivalent to the CPC spray results, reductions after immersion ranged from 1.0 to 1.6 log units with no difference among the three treatment groups (Kim and Slavik, 1995).

Since Kim and Slavik (1995) was published, many other studies have tried to determine whether CPC is a viable intervention method when using the application parameters outlined in FSIS Directive 7120.1 Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products (USDA-FSIS, 2016e). According to FSIS, CPC as an immersion can be used to treat the surface of raw poultry carcasses or parts, both skin-on and skinless, and as a spray to treat the surface of raw poultry carcasses, giblets, or parts, both skin-on and skinless (USDA-FSIS, 2016e). It may only be used up to 0.8% by weight as a spray or an immersion and is only approved for a 10 second immersion; when application is not followed by an immersion chiller, it must be followed by a potable water rinse (which may contain up to 50 ppm chlorine; USDA-FSIS, 2016e). Since the approval of CPC as a GRAS compound in mouthwashes and poultry processing, studies have focused on applying CPC at the approved parameters.

In one study, CPC, under the brand name Cecure®, was sprayed on broiler carcasses after chilling at 0.25% and 0.4% for two to three seconds of direct contact time (Waldroup et al., 2010). At 0.25%, Cecure® was responsible for a 49% decrease in *Campylobacter* counts and the 0.4% treatment reduced counts by more than 2 logs (Waldroup et al., 2010). Scott et al. (2015) inoculated chicken wings with a 5-strain cocktail of *Salmonella* of poultry origin and applied

CPC to gauge its effectiveness against the inoculated populations. After inoculation, chicken wings were given time for bacterial attachment and were then immersed in a 4,000 ppm CPC solution for 10 seconds, followed by a water rinse (Scott et al., 2015). Upon enumeration, the researchers found that the CPC treatment under these parameters reduced aerobic plate counts by 0.7 log CFU/ml and inoculated *Salmonella* populations by 0.8 log CFU/ml (Scott et al., 2015).

Although all of these common antimicrobial treatments result in reductions of some sort, in order to meet performance standards recently outlined by FSIS, a major change in poultry processing needs to occur to meet the targets (USDA-FSIS, 2016d). For years, the industry has used some of the same physical and chemical treatments that lead to somewhat minimal reductions; therefore, the poultry industry needs to move forward in another direction to develop innovative intervention methods that could not only lead to greater bacterial reductions but also lead to less water and chemical usage.

2.5 Electrostatics

Electrostatics may be one of the innovations that the meat and poultry industries need in order to achieve increased pathogen reductions; however, it is something that is not widely understood for implementation in meat production facilities. According to Merriam-Webster (2016a), electrostatics is the physics that deals with phenomena due to attractions or repulsions of electric charges but not dependent upon their motion. Another definition states "of or relating to static electricity" or "of or relating to painting with a spray that utilizes electrically charged particles to ensure complete coating" (Merriam-Webster, 2016b). Additionally, the Khan Academy (2016) refers to electrostatics as "the study of forces between charges, as described by Coulomb's Law." The first known use of electrostatics was recorded in 1827 (Merriam-Webster, 2016a).

Jaworek and Sobczyk (2008) described electrospraying as "a method of liquid atomization by means of electrical forces." When an electrospray is utilized, liquid solution is forced by an electric field into fine droplets as small as nanometers (Jaworek and Sobczyk, 2008). Droplet size can be controlled by the flow rate determined by operators, as well as the voltage or charge at the nozzle of the sprayer (Jaworek and Sobczyk, 2008). One of the leaders in sprayers utilizing this technology for various purposes states that the droplets produced are 40 microns in size and the flow rate can be changed based on a variety of flow discs available to buyers (ESS, 2015). The company states that this aids in decreasing volume of solution used, which reduces water usage tremendously (ESS, 2015). This statement is supported by other research in the field which says that electric charge applied to droplets allows for better control of their motion due to the electric field; because charged droplets are self-dispersing in space, they do not coagulate (Jaworek and Sobczyk, 2008). Essentially, this means that more of the particles reach the target object than in spray applications without a charge (ESS, 2015; Jaworek and Sobczyk, 2008).

Electrostatics has been used for such things as nanoparticle production, thin film deposition, and for functional layer formation of properties (Jaworek et al., 2009). With the proper nozzle and pre-calculated flow rate, droplets can be submicron size with a narrow size distribution, which is beneficial in various industry applications (Jaworek et al., 2009). There are everyday processes and machines that utilize static electricity/electrostatic technology such as photocopiers, laser printers, paint sprayers, dust precipitators, and defibrillators (BBC, 2014).

Paint spraying with electrostatic technology has been used for years due to increased coverage of objects being painted (Hines, 1966). Hines (1966) stated that "the method has the very practical advantage of depositing almost all of the paint sprayed onto the workpiece that is

being painted." Contrastingly, when painting with a compressed air sprayer, up to 50% of the paint is wasted because it never reaches the object being painted (Hines, 1966). The process by which electrostatic spray painting occurs has changed, but in the form used in the mid-1960s, the system consisted of a charged knife edge spaced approximately 12 inches from the grounded object meant to be painted (Hines, 1966). Paint was fed into the knife edge at which point the electric field pulled the paint away from the electrode edge; the surface tension and free charge would cause the jets or nozzles to create droplets that would then get carried to the object by the electric field (Hines, 1966). This process resulted in nearly all of the paint reaching the object being painted as a consequence of using an electric field, which was economical from a resource standpoint in terms of paint used as well as time spent painting and cleaning up (Hines, 1966). Powder coating is yet another practical application of electrostatics used today.

Fairly recently, powder coating gained popularity in various industry groups due to its diverse application potential, as well as its environmental edge (reduction in resources) over common applications (Ye et al., 2002). Powder coating always utilizes electrostatic technology for a number of reasons, including the fact that it increases adhesion of particles to an object of interest (Bailey, 1998; Halim and Barringer, 2007). The motion of charged particles can be manipulated by combining electric and aerodynamic forces, which creates the benefit of distribution for thicker, more uniform coverage of the grounded object being coated (Ye et al., 2002). Studies were conducted to model the electrostatic process itself, as well as the powder coating process, which involves creating a simulation and experiment to determine the flow field and the particle deposition (Domnick et al., 2000; Ye et al., 2002). Researchers developed numerical models by simulating the electric field and particle phases while tracking particles, among other things, and found that the space charge played a major role in determining the spray

pattern (Ye et al., 2002). Understanding these models has helped the field of electrostatics grow to be one that is used in food industry applications as well (Halim and Barringer, 2007).

Due to the increased adhesion and uniformity that electrostatic technology provides, research in the food industry was conducted to determine if food matrices can benefit from the application (Halim and Barringer, 2007; Ricks et al., 2002). Particle size is one major factor in the degree of adhesion because smaller particles are known to adhere more strongly due to van der Waals and Coulombic forces, which are present not only in electrostatic adhesion, but also non-electrostatic adhesion (Halim and Barringer, 2007). In addition to particle size, relative humidity also plays a role in adhesion because as humidity increases, water is able to absorb onto the powder which, consequently, reduces adhesion in electrostatic applications (Halim and Barringer, 2007). Targets (potato chips, saltine crackers, white bread, aluminum foil, and pork rinds) in Halim and Barringer (2007) were coated both electrostatically and non-electrostatically to test the difference in adhesion between the two methods (Halim and Barringer, 2007). There was significant improvement of adhesion in foil, saltines, and bread when utilizing the electrostatic method; however, the non-electrostatic and electrostatic methods were equivalent for pork rinds and potato chips, which may have resulted because of higher oil content and, therefore, higher resistance toward the powder (Halim and Barringer, 2007).

Similarly, another study compared non-electrostatic powder coating to electrostatic powder coating in order to determine which resulted in better coverage of the final product, which aids in consumer acceptance of said product (Ricks et al., 2002). Ricks et al. (2002) applied 16 powders commonly used in the food industry both electrostatically and non-electrostatically and the amount of coating, as well as dust created during the process, were measured (Ricks et al., 2002). Electrostatic application improved coating by 68% and decreased

dust in the environment by 65% in most treatments; however, researchers were unsure whether this method could be applied across the food industry due to variation created by the wide array of powders used (Ricks et al., 2002).

While there is still some uncertainty in terms of the utilization of electrostatic spraying or powder coating in the food industry, there is clearly potential for use of this technology, particularly in meat and poultry products. Increased coverage of product due to atomization of particles in an electrostatic antimicrobial application could prove advantageous to industry from a pathogen reduction standpoint, as well as an economic and environmental standpoint due to reduced chemical and water usage.

2.6 Electrostatics in Meat and Poultry

Use of electrostatics to apply antimicrobial interventions in the meat industry began in the 1990s when testing started to determine whether activated lactoferrin (ALF) was effective against foodborne pathogens (Naidu, 2002). When applied electrostatically or using high pressure spray nozzles to beef carcasses, ALF was shown to be effective as an additional hurdle in the multiple hurdle system, as well as on subprimals during fabrication (Naidu, 2002). Activated lactoferrin is GRAS according to FDA's 21 CFR.170.36(f), and was approved by the USDA for use on fresh beef in December 2001 (Naidu, 2002). Applying ALF, or any antimicrobial, electrostatically was new to the meat industry at the time and has since gained traction.

Although use of electrostatics in meat and poultry production is relatively new, there is on-going research on the topic. One study conducted at Kansas State University utilized an electrostatic spray cabinet to evaluate and verify whether chemical and biological solutions could be applied to pre-chilled meat animal carcasses (Phebus et al., 2014). Objectives of Phebus et al.

(2014) required installation and calibration of an electrostatic cabinet in order to (i) test the chemical deposition by spraying fluorescent dye onto a carcass and (ii) determine whether the cabinet could uniformly apply a biological inoculum to a carcass. The team first applied a fluorescent dye to two pig quarters, as well as half a pig carcass, and utilized a black light to observe the uniformity of coverage of dye onto carcass surfaces (Phebus et al., 2014). In doing so, the researchers found that dye appeared to cover carcass surfaces, including the body cavity, split line, and hock areas, uniformly (Phebus et al., 2014). Furthermore, an inoculation study was conducted in which two strains of non-pathogenic *E. coli* biotype I were used; inoculum was sprayed onto two separate pig carcass sides and allowed time for bacterial cell attachment (Phebus et al., 2014). Upon sampling and enumeration, approximately 5-6 log CFU/cm^2 were recovered from 7 of 8 anatomic regions tested (Phebus et al., 2014). These tests, in combination with one another, suggested that electrostatic technology could greatly reduce chemical and water usage in antimicrobial interventions in processing facilities (Phebus et al., 2014).

In another study, efficacy of serial lauric arginate (LAE) against surrogates for shiga toxin-producing *E. coli* (STEC) was evaluated when applied electrostatically to chilled beef carcasses, loins, and trim (Sevart et al., 2016). Chilled carcass sides were inoculated with five strains of surrogate STEC and were treated electrostatically with 200 ml of 25% LAE solution; loins and trim were treated with 200 ppm/weight using the sprayed lethality in container (SLIC) method (Sevart et al., 2016). Trim was then ground and samples were obtained and held for an assigned period of time at 4°C before plating. Electrostatic application of LAE to chilled carcasses resulted in a reduction of approximately 0.7 log CFU/cm^2 (Sevart et al., 2016). Reductions of 1.0 log CFU/cm^2 and 0.6 log CFU/cm^2 were achieved on loin and trim/ground beef samples, respectively (Sevart et al., 2016). These applications, when used in conjunction

with hot carcass interventions, could be an additional hurdle against STEC and other bacteria at processing facilities (Sevart et al., 2016).

While there is not much published research in the field of electrostatic antimicrobial application to meat, potential for such application as a new intervention method is clear. Further research needs to be completed to evaluate efficacy, but the studies provided herein demonstrate proof-of-concept as an intervention method, as well as better surface coverage and reduced water and chemical usage. Any one of these items alone would be beneficial to the meat and poultry industry, but together, they provide basis for a major modification in the way processors view interventions.

CHAPTER 3

Efficacy of Antimicrobials Using an Innovative, New Electrostatic Application System on *Salmonella*-Inoculated Poultry Parts

Summary

Two studies were conducted to evaluate efficacy of peroxyacetic acid (PAA) as an antimicrobial intervention treatment when applied electrostatically, in reducing inoculated populations of *Salmonella* serovars on poultry parts. The other objectives of these studies were: to determine critical operating parameters for reducing *Salmonella* serovars on poultry parts; to evaluate use of static electricity to maximize coverage of antimicrobial solutions applied electrostatically to the poultry part surface areas while limiting volume to minimize weight gain; to evaluate use of vacuum to enhance absorption of antimicrobial spray into pores of poultry parts; and to determine optimal rotation speed of the Birko prototype unit's containment drum to expose all poultry part surfaces during antimicrobial solution application.

Two different electrostatic spray systems (ES1 and ES2) were evaluated in the two separate studies. For both studies, chicken wings were inoculated with nalidixic acid- and novobiocin-resistant *Salmonella* (5-strain mixture; 5-6 log CFU/ml of chicken wing rinse solution) sourced from poultry. Inoculated wings were either left untreated (control) or were treated with water or PAA.

In study 1, water and PAA (at a wt/wt concentration of 2000 ppm) were applied (30 s) with one of four application methods: (i) electrostatic spray (ES1), (ii) vacuum, (iii) ES1 + vacuum, and (iv) immersion. Chicken wings were then placed into Whirl-Pak bags containing Dey/Engley (D/E) neutralizing broth and sample rinsates were serially diluted and surface-plated on both tryptic soy agar and tryptic soy agar plates supplemented with nalidixic acid (20 µg/ml)

and novobiocin (25 µg/ml) plates. Overall, least squares means for $\log_{10}$ *Salmonella* counts differed ($P < 0.05$) for all treated wings vs. the control. When PAA was applied, electrostatic spray was most effective ($P < 0.05$) at reducing *Salmonella* populations.

In study 2, treatment solutions of water and two concentrations of PAA (2000 ppm and 4000 ppm) were evaluated. These were applied (30 s) using two application methods (ES2, a Birko prototype application unit, and immersion). Sampling methods were the same as those used in study 1, with the exception that analysis of efficacy occurred at both 0 and 24 h. Untreated and treated chicken wings for 24 h analysis were placed in Whirl-Pak bags and held at 4°C until sampling. For study 2, pathogen counts for all treatments differed ($P < 0.05$) from the control and there was a treatment and sampling time interaction. For both water and PAA, the immersion treatment was most effective ($P < 0.05$) at reducing *Salmonella* populations after 24 h storage.

Both electrostatic spray systems (ES1 and ES2) reduced ($P < 0.05$) bacterial populations of *Salmonella*, indicating that electrostatic application of PAA was an effective antimicrobial intervention against *Salmonella* serovars on chilled chicken wings.

Introduction

Each year, *Salmonella* serovars are responsible for an estimated one million foodborne illness cases in the United States, 6% of which are associated with outbreaks (CDC, 2013a). A 2011 foodborne illness report documented that non-typhoidal *Salmonella* were the leading bacterial cause of hospitalizations and deaths (Scallan et al., 2011). Several strains of *Salmonella* were implicated in outbreaks related to poultry products, including *S.* Enteritidis and *S.* Heidelberg in recent years (CDC 2014a, 2014b, 2015c, 2016b). In the largest of these outbreaks, 634 cases were reported and 241 individuals (38%) were hospitalized (CDC, 2014a). For this

reason, prevention of foodborne illness continues to be a top priority for industry leaders and the United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS, 2016b).

Use of sequential decontamination antimicrobial intervention systems on meat and poultry products is not a new concept; however, application of antimicrobials has changed over the years to better reduce pathogens and prevent foodborne illness (Bolder et al., 1997; Huffman et al., 2002; USDA-FSIS, 2016b). In an effort to address meat safety concerns, USDA-FSIS created a Strategic Plan for the years 2011 through 2016, which included goals and strategies for preventing foodborne illness (USDA-FSIS, 2016b). The first goal of the plan was to ensure that food safety inspection and existing and emerging risks matched each other in order to better reduce the risks of food safety hazards; other goals included public outreach for safe food-handling and the effective use of science to understand foodborne illness (USDA-FSIS, 2016b). New performance standards for poultry also were published in order to better address current food safety issues in poultry processing (USDA-FSIS, 2016d).

Because pathogens continue to evolve and regulations are becoming increasingly more stringent, scientific research must evolve as well. It is imperative that research pertaining to poultry processing and antimicrobial interventions remains a priority for all industry members. This research must include further investigation into pathogens of interest, as well as physical and chemical decontamination methods, in order for the poultry industry to advance and meet performance standards (Sofos, 2007; USDA-FSIS, 2016d). Understanding antimicrobials and different application methods should be one priority of this research. There are many requirements for antimicrobials in poultry production, which include effectively reducing bacterial populations (Loretz et al., 2009). Interventions must also be feasible to implement in

the production process, meaning that they must be safe, economically-focused, environmentally friendly, and not detrimental to organoleptic properties of products (Loretz et al., 2009). Utilizing antimicrobials in an electrostatic spray application has shown potential for reducing both chemical and water usage while reducing bacterial populations (Phebus et al., 2014; Sevart et al., 2016); however, limited research is available regarding this application. Therefore, the primary objective of these studies was to evaluate efficacy of electrostatic antimicrobial application at reducing inoculated populations of *Salmonella* serovars on chicken wings. Peroxyacetic acid (PAA) was used for these studies but other antimicrobials could also have been used. Secondary objectives included: (i) determine critical operating parameters at reducing *Salmonella* serovars on poultry parts, (ii) evaluate use of static electricity to maximize coverage of antimicrobial solutions on poultry parts while limiting volume to minimize weight gain, (iii) evaluate use of vacuum to enhance absorption of antimicrobial sprays into pores of poultry parts, and (iv) determine optimal rotation speed of the containment drum to expose all poultry part surfaces during antimicrobial solution application.

Materials and Methods

Preliminary studies. Before conducting these studies, two preliminary proof-of-concept studies were conducted. The objective for Study 1 (preliminary proof of concept study) was to evaluate peroxyacetic acid (PAA), Beefxide (BX), and lactic acid (LA) as antimicrobial intervention applications when applied electrostatically to determine the efficacy and critical operating parameters in reducing inoculated populations of non-pathogenic *Escherichia coli* biotype I, serving as surrogates for pathogenic *E. coli* and *Salmonella* serovars on beef trim and poultry parts. The objective for Study 2 (preliminary proof of concept study) was to evaluate PAA and LA as antimicrobial intervention applications when applied electrostatically to

determine the efficacy and critical operating parameters in reducing surrogate populations on poultry parts.

Both studies were designed as paired comparisons. The first preliminary study included 4 treatments with n = 3 per treatment for both beef and chicken samples. For the first study, BX was applied with a commercially available, hand-held electrostatic spray gun at 2.5% and microbial samples taken after 10 seconds of treatment. A 4% concentration of LA was applied with an electrostatic spray gun and microbial samples obtained after 10 seconds of treatment. Additionally, PAA was applied to poultry samples at 1200 ppm and beef samples at 400 ppm. Study 2 included 3 treatments with n = 3 per treatment. For the second study, LA was applied with an electrostatic spray gun at 10% and PAA was applied with a commercially available, hand-held electrostatic spray gun at 2200 ppm and microbial samples were obtained for each treatment. Data were analyzed using the Mixed Procedure of SAS version 9.3 and expressed as least squares means with differences reported using a significance level of $\alpha = 0.05$.

For Study 1, mean bacterial counts were reduced ($P < 0.05$) from 6.57 log CFU/g to 6.07 log CFU/g after treatment with 2.5% BX, 5.89 log CFU/g after treatment with 4% LA, and 5.5 log CFU/g after treatment with PAA. There was no significant difference between BX and LA, but these treatments were significantly different from the control. For study 1, all antimicrobial treatments were significantly different from the untreated control for poultry and all antimicrobial treatments were significantly different from untreated control besides BX applied at 2.5% for beef. For Study 2, after receiving either LA or PAA electrostatic spray treatment, bacterial populations were reduced ($P < 0.05$) when compared to the control samples. This information provided proof of concept for a new antimicrobial application method that could potentially save money and water due to better coverage of poultry parts and beef trimmings.

Preparation of *Salmonella* inoculum. Inoculum consisted of a five-strain mixture of *Salmonella* isolates of poultry origin, including *Salmonella* Montevideo JAB 03754 SL, *Salmonella* Typhimurium FFSRU ST NN, *Salmonella* Heidelberg JAB 13556 SL, *Salmonella* Enteritidis FFSRU SE NN, and *Salmonella* Newport JAB FSW07B2224N (provided by Dr. Thomas Edrington; Research Animal Scientist; USDA-ARS). Each of these isolates was resistant to nalidixic acid (20 µg/ml) and novobiocin (25 µg/ml); therefore, working cultures of the five *Salmonella* serotype strains were maintained on xylose lysine deoxycholate (XLD; Acumedia, Neogen Corp., Lansing, MI) agar supplemented with nalidixic acid and novobiocin (XLDNN). Strains were individually cultured (35°C, 24±2 h) in 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Franklin Lakes, NJ) supplemented with 20 µg/ml of nalidixic acid and 25 µg/ml of novobiocin (TSBNN). After incubation, broth cultures were subcultured by transferring 0.1 ml of the original culture into 10 ml of fresh TSBNN and incubating for 22-24 h at 35°C. Broth cultures of all five *Salmonella* serotypes were then combined and cells were harvested by centrifugation (15min, 4°C, 5590 x *g*, J2-MC Centrifuge, Beckman Instruments, Inc., Palo Alto, CA). Cell pellets were washed with 10 ml of phosphate buffered saline (PBS, pH 7.4; Sigma, St. Louis, MO), re-centrifuged, and resuspended to the original volume in PBS to obtain a concentration of approximately 8-9 log CFU/ml.

Inoculation of chicken wings. These studies evaluated use of electrostatic antimicrobial application to control *Salmonella* serovars on poultry parts. For study 1 and 2, whole, skin-on chicken wings (including the humerus, radius, ulna, and phalanges) of similar size and shape were collected at a commercial processing plant after a post-harvest chill and were shipped fresh and refrigerated to the Center for Meat Safety & Quality at Colorado State University (Fort Collins, CO). Chicken wings were randomly assigned to a treatment, inoculated by pipetting 100

µl of inoculum onto both the medial and lateral surfaces (200 µl total), and then spreading inoculum with sterile disposable spreaders to ensure that cells were evenly distributed across the surface (Berrang et al., 2014; Hawkins et al., 2016; Schambach et al., 2014). After inoculation, wings were left stationary for approximately 10 min/side (20 min total) before applying treatments to allow for bacterial cell attachment time similar to that expected in a normally-functioning, commercial processing facility (Berrang et al., 2014; Schambach et al., 2014). The target inoculation level before treatment was 5-6 log CFU/ml of sample rinse solution. After bacterial cell attachment, chicken wings were either assigned to a treatment or were left untreated to serve as the control samples for initial bacterial counts.

Antimicrobial treatment of chicken wings. On test days for study 1, inoculated poultry parts were left untreated (control) or were treated with water or peroxyacetic acid (PAA; Birko Corp., Henderson, CO) in the decontamination unit (Figure 3.1) or by immersion (Table 3.1). More specifically, four different application treatments (Electrostatic Spray, Vacuum, Electrostatic Spray with Vacuum, and an Immersion) were evaluated per antimicrobial intervention (water, 2000 ppm PAA). The study was conducted on two separate days with n = 10 per treatment (n = 5 per day). A total of 90 samples were used for this study. Additionally, 10 background (uninoculated and untreated) samples were analyzed to determine presence of contamination of wings upon arrival.

For the Electrostatic Spray, Electrostatic Spray with Vacuum, and Vacuum treatments, 10 chicken wings were inoculated, given time for bacterial cell attachment, and placed into the decontamination unit, a vacuum tumbler equipped with electrostatic nozzles. Settings were adjusted based on treatment and samples were treated for 30 seconds with an approximate flow rate of 100 ml/minute.

For the Immersion treatment, 10 chicken wings were inoculated, given time for bacterial cell attachment, and placed into Whirl-Pak bags containing 2 L of the antimicrobial for 30 seconds.

On test days for study 2, one set of inoculated poultry parts remained untreated (control), while the remainder were treated with water or PAA in the decontamination unit or by immersion (Table 3.2). More specifically, two different application treatments [Electrostatic Spray (ES2) and an Immersion] were evaluated using water, 2000 ppm PAA, and 4000 ppm PAA. The study was conducted on two separate days with n = 10 per treatment (n = 5 per day) for a total of 140 observations. Additionally, 10 background (uninoculated and untreated) samples were evaluated to determine presence of *Salmonella* before inoculated and treating.

Treatment applications for study 2 remained the same as those for study 1, with the exception that efficacy at two differing times post-treatment were evaluated (one sampling time at 0 hour and one at 24 hour).

Sampling and microbiological analysis of chicken wings. There were a total of 90 samples for study 1 (Table 3.1) and 140 samples for study 2 (Table 3.2). Samples were weighed before and after treatment to observe weight gain due to treatment, if any. Untreated (control) and treated samples for both studies were evaluated for microbiological loads using the shaking/rinsate method (Scott et al., 2015). More specifically, samples were placed into a sterile Whirl-Pak bag containing 150 ml of Dey/Engley neutralizing broth (D/E; Difco, Becton Dickinson, Franklin Lakes, NJ) and shaken vigorously by hand with a strong downward force for 1 minute to recover cells from the parts. Sample rinsates were serially diluted (10-fold) in 0.1 % buffered peptone water (BPW). Appropriate dilutions were surface-plated in duplicate (0.1 ml or 1 ml) onto tryptic soy agar (TSA; Acumedia, Neogen Corp., Lansing, MI) and tryptic soy agar

supplemented with nalidixic acid (20 µg/ml) and novobiocin (25 µg/ml) (TSANN). The TSA plates were enumerated following 72 hours incubation at 25°C. The TSANN plates were enumerated following 24 hours incubation at 35°C. For study 1, these sampling procedures occurred immediately after treatment (0 h). For study 2, these procedures occurred both immediately after treatment (0 h) and after 24 h storage at 4°C.

Statistical Analysis. Study 1 was designed as a paired comparison repeated on two days with a total of n = 10 per treatment. Treatment days were handled as block effects and separate analyses were utilized for each treatment to determine the effects on inoculated populations when compared to counts obtained from the untreated (control) samples. Bacterial populations recovered were analyzed using the Mixed Procedure of SAS version 9.4 (Cary, NC) with variables of day (random, block effect) and treatment (fixed effect). Counts were converted to base-10 logarithms and bacterial populations were expressed as least squares means with differences reported using a significance level of $\alpha = 0.05$.

Study 2 was designed as a completely randomized block design with a 7 x 2 factorial arrangement and was repeated on two days with a total of n = 10 per treatment. Treatment days were included in AOV models as random block effects and separate analyses were utilized for each treatment to determine effect of each contrasted against the untreated (control) samples. Additionally, counts were analyzed by treatment to determine whether a treatment x storage time interaction was present. Bacterial populations recovered from this study were analyzed using the Mixed Procedure of SAS version 9.4 (Cary, NC) with variables of day, treatment, and hour, and data were expressed as least squares means of log CFU per milliliter of wing rinsate with differences reported using a significance level of $\alpha = 0.05$.

Results and Discussion

Antimicrobial effect of treatments for study 1. In the first study, microbial populations from inoculated chicken wings were recovered from TSA and TSANN. When plated on TSANN, an initial bacterial count of 5.77 log CFU/ml of rinse solution was obtained from inoculated, untreated samples, and all antimicrobial treatments differed ($P < 0.05$) from the control (Table 3.4). The electrostatic spray treatment using water reduced ($P < 0.05$) initial counts from 5.77 to 5.08 log CFU/ml of rinse solution (Table 3.4). When vacuum was applied with water, counts were reduced ($P < 0.05$) to 5.15 log CFU/ml (Table 3.4). The combination of electrostatic spray and vacuum with water decreased ($P < 0.05$) counts to 4.87 log CFU/ml, whereas the immersion treatment counts were decreased ($P < 0.05$) to 4.81 log CFU/ml (Table 3.4). When PAA was applied at 2000 ppm, the electrostatic spray treatment reduced ($P < 0.05$) initial counts to 3.87 log CFU/ml of rinse solution (Table 3.4). Treatment with vacuum reduced ($P < 0.05$) initial counts to 4.96 log CFU/ml while treatment with PAA using an electrostatic spray and vacuum combination reduced ($P < 0.05$) counts to 3.88 log CFU/ml (Table 3.4). The 2000 ppm immersion treatment reduced ($P < 0.05$) counts from 5.77 to 3.92 log CFU/ml (Table 3.4). Electrostatic spray (2000 ppm PAA) was most effective at reducing bacterial populations. Pick-up weights were lowest when vacuum was utilized (Table 3.5). Although not discussed here, table 3.3 presents results of microbial populations recovered on TSA.

Antimicrobial effects of treatments for study 2. For the second study, microbial population from inoculated chicken wings were recovered on TSA and TSANN. When plated on TSANN, an initial bacterial count of 5.90 log CFU/ml of rinse solution was obtained from inoculated, untreated samples, and least squares mean plate counts for all antimicrobial treatments differed ($P < 0.05$) from the control at both 0 h and 24 h sampling intervals (Table

3.7). After an electrostatic spray treatment with water, initial mean bacterial counts were reduced ($P < 0.05$) to 5.32 log CFU/ml; however, least squares mean counts after 24 h of storage at 4°C did not differ ($P > 0.05$; Table 3.7). Additionally, immersion in water reduced ($P < 0.05$) counts from 5.90 to 4.99 log CFU/ml. After 24 h of storage at 4°C, mean bacterial counts were further reduced ($P < 0.05$) to 4.83 log CFU/ml, but this was not different ($P > 0.05$) from mean counts obtained at 0 h (Table 3.7). Electrostatic spray using PAA at 2000 ppm reduced ($P < 0.05$) bacterial populations from 5.90 log CFU/ml to 5.01 log CFU/ml (Table 3.7). After 24 h of storage, populations were reduced ($P < 0.05$) to 4.93 log CFU/ml, but there was no statistical difference ($P > 0.05$) between counts at 0 h vs. 24 h (Table 3.7). In addition, plate counts resulting from water immersion vs. electrostatic spraying (2000 ppm PAA) did not differ ($P > 0.05$; Table 3.7). When PAA concentration was increased to 4000 ppm, electrostatic spray treatment reduced ($P < 0.05$) initial counts to 4.47 log CFU/ml (Table 3.7). Final mean bacterial counts after electrostatic spraying (4000 ppm) and 24 h of storage were 4.39 log CFU/ml (Table 3.7). Immersion using PAA at 2000 ppm reduced ($P < 0.05$) initial counts from 5.90 log CFU/ml to 3.88 log CFU/ml, which was further reduced ($P < 0.05$) to 3.24 log CFU/ml after 24 h of storage (Table 3.7). When PAA concentration was increased to 4000 ppm, mean bacterial populations were reduced ($P < 0.05$) to 3.63 log CFU/ml, which did not differ ($P > 0.05$) from counts obtained following immersion in 2000 ppm PAA (Table 3.7). After immersion in 4000 ppm PAA and 24 h of storage, mean counts were reduced ($P < 0.05$) to 2.70 log CFU/ml (Table 3.7). Pick-up weights were lowest when utilizing electrostatic spray by itself or the electrostatic spray with vacuum (Table 3.8). Although not discussed here, table 3.6 presents results of microbial populations recovered on TSA.

Discussion of results. Mean counts were similar for TSA and TSANN for both studies. After analysis of study 1 data, it was determined that vacuum was not more effective in reducing bacterial populations than electrostatic spraying and, therefore, could be removed from the experimental design for study 2. Furthermore, the research team was interested in the potential effect of storage time. For this reason, a 24 h sampling interval was included in the experimental design of study 2.

In study 1, mean plate counts following application of PAA using electrostatic spraying, electrostatic spraying under vacuum, and immersion treatments did not differ ($P > 0.05$). In study 2, the immersion in 4000 ppm at both 0 h and 24 h was most effective in reducing bacterial populations.

Due to ease of operation and more consistent charge, the ES1 system was to be used in further studies. In the present studies, PAA was chosen as the antimicrobial due to published research suggesting its efficacy against *Salmonella* serovars in poultry processing (Bauermeister et al., 2008; Scott et al., 2015).

Peroxyacetic acid is approved for use in multiple species as per the FSIS Safe and Suitable Guidelines (2015); in poultry, it is approved as a spray, dip, immersion, or in chiller water up to 2000 ppm. Bauermeister et al. (2008) added PAA to chiller water and found that, at all levels (0.0025%, 0.01%, and 0.02%), counts of *Salmonella* Typhimurium on broiler carcasses were decreased. The 0.02% PAA (200 ppm) treatment level was most effective, with final counts of approximately 1.8 log CFU/sample (Bauermeister et al., 2008). Scott et al. (2015) also found that a 700 ppm PAA immersion decreased *Salmonella* populations by 1.5 log CFU/ml of rinsate at 0 h and 1.7 log CFU/ml of rinsate at 24 h. These studies do not directly correlate to the present

study due to differences in application method and PAA concentration used; however, efficacy was found to be similar despite these differences.

In the present study, two separate electrostatic systems were used address study objectives; ES1 for study 1 and ES2 for study 2. Electrostatics has become increasingly popular among other industries, but there is little published data available suggesting its efficacy as an intervention application in meat and poultry (Hines, 1966; Jaworek and Sobczyk, 2008). Phebus et al. (2014) conducted a fluorescent dye test as well as an inoculation test to determine whether electrostatic spraying could be used for increased coverage. Upon sampling and enumeration, approximately 5-6 log CFU/cm^2 were recovered from 7 of 8 anatomic regions tested, indicating that this application method results in more uniform coverage (Phebus et al., 2014). Additionally, a study using a 25% LAE solution applied electrostatically to inoculated beef achieved a 0.7 log CFU/cm^2 (Sevart et al., 2016). Overall, the electrostatic application of PAA performed as well as, and in some cases better than, current antimicrobial treatments, indicating its potential for use as an effective tool against *Salmonella*.

In the present studies, we evaluated PAA at the high end of compliance as well as a concentration greater than currently approved. Such a comparison should prove valuable as it is currently unknown what happens to antimicrobial solutions when they undergo the charging process. It is possible that this process results in a lower absolute volume and concentration on carcasses and meat products treated electrostatically. Additionally, water usage in electrostatic spraying treatments in the present studies was reduced by 95% when compared to traditional systems. Electrostatic spraying in the present studies utilized 100 ml of water per minute vs. a 2000 ml immersion. Across 10 JBS beef plants, approximately six billion gallons of water are used annually, with over 318 gallons per head used in most plants (Ritsema, 2016). In future

studies, different antimicrobials should be explored in addition to various concentrations of these antimicrobials.

Table 3.1. Antimicrobials used, application methods, and number of samples for study 1.

Antimicrobial	Antimicrobial Application Method	Number of Samples
Control (Untreated)	N/A	10
Water	Electrostatic	10
	Vacuum	10
	Electrostatic + Vacuum	10
	Immersion	10
PAA, 2000 ppm	Electrostatic	10
	Vacuum	10
	Electrostatic + Vacuum	10
	Immersion	10
Total Number of Samples		**90**

Table 3.2. Antimicrobials used, application methods, and number of samples for study 2.

Antimicrobial	Antimicrobial Application Method	Number of Samples	
		0 Hr	24 Hr
Control (Untreated)	N/A	10	10
Water	Electrostatic	10	10
	Immersion	10	10
PAA, 2000 ppm	Electrostatic	10	10
	Immersion	10	10
PAA, 4000 ppm	Electrostatic	10	10
	Immersion	10	10
Total Number of Samples		**140**	

Table 3.3. Least-squares means of bacterial counts (log CFU/ml) on Tryptic Soy Agar (TSA) from 5-strain *Salmonella* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Montevideo, *S.* Heidelberg) inoculated chicken wing samples*.

Treatment	**Treatment Application Method**	**log CFU/ml of rinse solution**
Control (untreated)	N/A	5.68[a]
Water	Vacuum	5.02[b]
	Electrostatic	4.96[b]
	Electrostatic + Vacuum	4.86[bc]
	Immersion	4.74[c]
PAA, 2000 ppm	Vacuum	4.89[bc]
	Immersion	3.90[d]
	Electrostatic	3.86[d]
	Electrostatic + Vacuum	3.85[d]
SEM		0.1096

[a,b,c,d] Means without a common superscript differ ($P < 0.05$).

*Antimicrobial applied for 30 seconds.

Table 3.4. Least-squares means of bacterial counts (log CFU/ml) on Tryptic Soy Agar supplemented with nalidixic acid and novobiocin (TSANN) from 5-strain *Salmonella* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Montevideo, *S.* Heidelberg) inoculated chicken wing samples*.

Treatment	Treatment Application Method	log CFU/ml of rinse solution
Control (untreated)	N/A	5.77^{a}
Water	Vacuum	5.15^{b}
	Electrostatic	5.08^{bc}
	Electrostatic + Vacuum	4.87^{cd}
	Immersion	4.81^{d}
PAA, 2000 ppm	Vacuum	4.96^{bcd}
	Immersion	3.92^{e}
	Electrostatic + Vacuum	3.88^{e}
	Electrostatic	3.87^{e}
SEM		0.1118

^{a,b,c,d,e} Means without a common superscript differ ($P < 0.05$).

*Antimicrobial applied for 30 seconds.

Table 3.5. Least-squares means of pick-up weight of treated chicken wings using a Birko-prototype application unit (ES1).

Treatment	Treatment Application Method	Average Weight Gain (%)	*P* Value
Water	Electrostatic	2.02^{bc}	0.0006
	Vacuum	1.35^{c}	0.0051
	Electrostatic + Vacuum	2.49^{ab}	0.0002
	Immersion	2.06^{bc}	0.0005
PAA, 2000 ppm	Electrostatic	2.28^{b}	0.0003
	Vacuum	1.86^{bc}	0.0009
	Electrostatic + Vacuum	2.49^{ab}	0.0002
	Immersion	3.07^{a}	<.0001

^{a,b,c} Means without a common superscript differ ($P < 0.05$).

Table 3.6. The interactive effect of treatment and storage time on least-squares means of bacterial counts (log CFU/ml) on Tryptic Soy Agar (TSA) from 5-strain *Salmonella* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Montevideo, *S.* Heidelberg) inoculated chicken wing samples*.

Treatment	**Treatment Application Method**	**log CFU/ml of rinse solution**	
		0 Hour	**24 Hour**
Control	N/A	5.90^{a}	5.90^{a}
Water	Electrostatic	5.33^{bc}	5.52^{b}
	Immersion	5.04^{de}	5.24^{cd}
PAA	Electrostatic, 2000 ppm	4.90^{e}	4.95^{e}
	Immersion, 2000 ppm	3.76^{g}	3.29^{h}
	Electrostatic, 4000 ppm	4.41^{f}	4.44^{f}
	Immersion, 4000 ppm	3.59^{g}	2.78^{i}
SEM		0.1053	

^{a,b,c,d,e,f,g,h,i} Means without a common superscript differ ($P < 0.05$).

*Antimicrobial applied for 30 seconds.

Table 3.7. The interactive effect of treatment and storage time on least-squares means of bacterial counts (log CFU/ml) on Tryptic Soy Agar supplemented with nalidixic acid and novobiocin (TSANN) from 5-strain *Salmonella* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Montevideo, *S.* Heidelberg) inoculated chicken wing samples*.

Treatment	**Treatment Application Method**	**log CFU/ml of rinse solution**	
		0 Hour	**24 Hour**
Control	N/A	5.90^{a}	5.77^{a}
Water	Electrostatic	5.32^{b}	5.34^{b}
	Immersion	4.99^{c}	4.83^{c}
PAA	Electrostatic, 2000 ppm	5.01^{c}	4.93^{c}
	Immersion, 2000 ppm	3.88^{e}	3.24^{f}
	Electrostatic, 4000 ppm	4.47^{d}	4.39^{d}
	Immersion, 4000 ppm	3.63^{e}	2.70^{g}
SEM		0.1270	

^{a,b,c,d,e,f,g} Means without a common superscript differ ($P < 0.05$).

*Antimicrobial applied for 30 seconds.

Table 3.8. Least-squares means of pick-up weight of treated chicken wings using a Birko-prototype application unit (ES2).

Treatment	Treatment Application Method	Average Weight Gain (%)	*P* Value
Water	Electrostatic	1.45[d]	0.0022
	Immersion	2.03[c]	0.0007
PAA, 2000 ppm	Electrostatic	1.48[d]	0.0021
	Immersion	2.51[b]	0.0004
PAA, 4000 ppm	Electrostatic	1.61[d]	0.0016
	Immersion	2.86[a]	0.0002

[a,b,c,d] Means without a common superscript differ ($P < 0.05$).

Figure 3.1. Birko-prototype decontamination unit (tumbler) used for both ES1 and ES2.

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