THE EVALUATION OF LIVE PRODUCTION AND PROCESSING INTERVENTIONS AND THEIR IMPACT ON *SALMONELLA* REDUCTION IN BROILERS

A Dissertation

by

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DOCTOR OF PHILOSOPHY

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ABSTRACT

Salmonella is a zoonotic bacteria threatening food safety, especially in the poultry industry. There are thousands of serovars, but *S.* Kentucky, *S.* Heidelberg, *S.* Enteritidis, and *S.* Typhumirum are the most commonly detected serovars in poultry. The poultry industry invests much time and money in preharvest and post-harvest interventions to reduce the prevalence of *Salmonella.* We assessed boot swab samples taken at the farm along with hot rehang, prechill, post chill, and chicken part samples collected at two geographically processing facilities to determine the effect of preharvest and post harvest interventions on *Salmonella prevalence*. Carcass rinse samples were taken using the rinsates Buffered Pepton Water (BPW) and Neutralizing Buffered Peptone Water nBPW to collect hot rehang, prechill, post chill, poultry parts, and boot swabs. The samples were analyzed for *Salmonella* presence or absence using Polymerase Chain Reaction (PCR). We found that *Salmonella prevalence* was significantly associated with the season and the location of the poultry processing facility. We also found that some hatchery vaccines were significantly associated with a reduction in *Salmonella* prevalence. When preharvest interventions were used individually, such as gentamicin, the prevalence of *Salmonella* was reduced. When used in conjunction with Megan Vac 1, there was no reduction or change in the prevalence of *Salmonella.* Using a treatment in the water (PWT®, Aquaprime®, litter (PLT®), and feed (OptiBac®) contributed to a reduction of *Salmonella* prevalence. Post harvest interventions like PAA aid with continuous reduction of prevalence, especially in post chill samples and finished products. We saw a significant reduction in *Salmonella* prevalence from hot rehang samples to post chill samples. Although the prevalence of *Salmonella* is high at hot rehang, this research provides evidence that investing in some preharvest interventions may provide a reduction in *Salmonella.*

DEDICATION

I dedicate this dissertation to my heartbeats, my daughters. My daughters are my joy and inspiration. Every breath I take, the choices I make, dreams I dream, and decisions made are with them in mind. Throughout my matriculation, I hope they realize quitting is never an option, and there are no obstacles too big that should hinder you from achieving your goal. Without their love and unwavering support, I would not be where I am today.

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Be anxious for nothing, but in everything by prayer and supplication, with thanksgiving, let your request be made known to God. Philippians 4:6

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This work was supported by a dissertation committee consisting of Dr. John Carey (advisor) of the Department of Poultry Science, Dr. T. Matthew Taylor of the Department of Animal Science, Dr. Keri Norman of the Department of Veterinarian Integrative Biosciences, and Dr. J. Allen Byrd.

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NOMENCLATURE

APHIS	Animal and Plant Health Inspection Services
BGS	Brilliant Green Sulfa Agar
BPW	Buffered Peptone Water
nBPW	Neutralizing Buffered Peptone Water
CDC	Center of Disease Control
CFR	Code of Federal Regulations
CPC	Cetylpyridinium Chloride
DOA	Dead on Arrival
DMILA	Double Modified Lysine Iron Agar
EFLA	Enzyme Linked Fluorescent Assay
FDA	Food and Drug Administrations
FSIS	Food Safety and Inspection Service
HACCP	Hazard Analysis Critical Control Point
mL	milliliters
MLG	Microbiological Laboratory Guidelines
MSRV	Modified Semi-Solid Rapport Vassiliadis
NARMS	National Antimicrobial Resistance Monitoring System
NPIP	National Poultry Improvement Plan
NCC	National Chicken Council
PAA	Peracetic Acid
PCR	Polymerase Chain Reaction
PLT®	Poultry Litter Treatment

PPM	Parts Per Million
PWT®	Poultry water treatment
TT	tetrathionate broth
µL	micro liters
USDA	United States Department of Agriculture
XLT4	Xylose Lysine Terigitol

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CHAPTER I
INTRODUCTION

Non-typhoidal *Salmonella enterica* in the broiler industry is one of the most discussed topics in poultry production and harvest, and the focus on reducing its prevalence is ongoing. Many factors play a vital role in the distribution of *Salmonella* in the broiler industry. However, no one aspect of animal production or handling has shown a more significant impact over another (Koutsoumanis et al., 2019). When selecting the most effective method for reducing *Salmonella* prevalence in live production complexes and processing facilities, it is essential to understand its functionality, genetic makeup, and effects on broiler complexes.

Salmonella can be transmitted vertically or horizontally to broilers. Vertical transmission passes agents from parent to offspring by contaminating the egg or yolk (Linden, 2012). Horizontal transmission is passed from bird to bird (Holt et al., 1998). Emerging evidence shows that implementing antimicrobial interventions during pre-harvest, including the bird grow-out stages, aids in reducing *Salmonella* prevalence during post-harvest handling (Dorea et al., 2010; Van Immerseel et al., 2005). *Salmonella* preharvest controls include arduous biosecurity measures placed on the farms, vermin (i.e., rodents, insects, pests), and sanitary feed, among other factors (Alali & Hofacre, 2016).

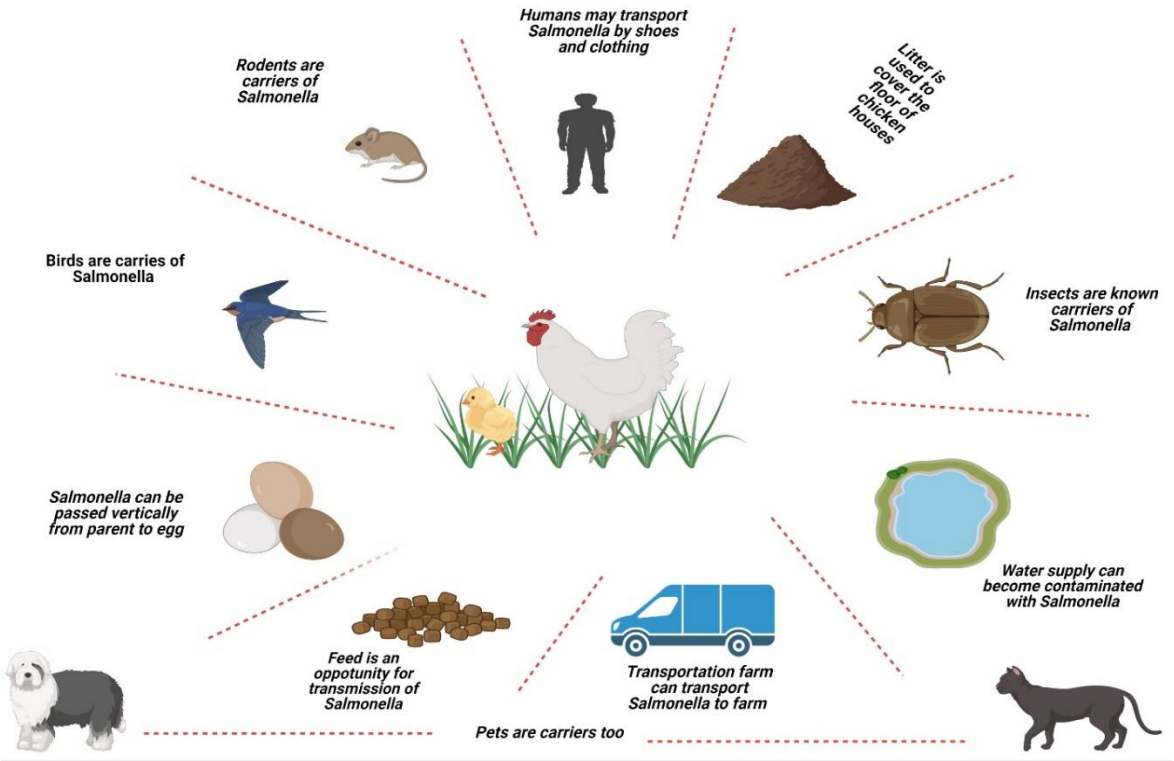

Figure 1. Transmission of *Salmonella* to broiler farms. Created with BioRender.com

Salmonella is a gram-negative, anaerobic bacillus and non-spore-forming bacterium that affects the intestinal tract and is a member of the *Enterobacteriaceae* family. The bacteria *Salmonella* is known to call salmonellosis in humans. In addition to *Salmonella* causing salmonellosis in humans, it is capable of causing bacteremia, enterocolitis, and focal infections (Darwin & Miller, 1999). *Salmonella* is omnipresent and appears worldwide in animals and humans. (Giannella, 1996). It is most frequently transmitted when consuming food or water or in contact with the feces of animals and their natural habitat (Center for Disease Control and Prevention, 2013). *Salmonella* can grow in environments and dwell in many animals'

gastrointestinal tracts, complicating foodborne salmonellosis (Dawoud et al., 2017). In humans, *Salmonella* will develop into gastroenteritis and, in worst cases, enteric fever (Giannella, 1996). Approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths are caused by *Salmonella* in the United States annually (Center for Disease Control and Prevention, 2022). *Salmonella* remains a concern for public health as a significant foodborne illness in the United States.

This research examines if *Salmonella* antimicrobial interventions, meteorological factors, and preharvest interventions, including feed, water, and litter treatments, influence used in farmhouses and hatcheries will decrease the prevalence of *Salmonella* in chickens at slaughter. The three objectives include 1) providing evidence that temperature, humidity, seasons, climate, bird wetting, and processing play a role in *Salmonella* prevalence in broilers; 2) analyzing data and the factors that differentiate two U.S. geographical regions for *Salmonella* prevalence in broilers; 3) evaluating the efficacy of interventions used during preharvest to corroborate a reduction of *Salmonella* from hot rehang to post-chill samples while including carcass parts (breast, wings, and thighs).

CHAPTER II

LITERATURE REVIEW

2.1 Most common *Salmonella* serovars found in broilers

Salmonella is a part of the two *Salmonella species*: *Salmonella bongori* and *Salmonella enterica*. *S. enterica* comprises >2,500 serovars (Mumy, 2014). *Salmonella* serovars are determined by the bacterium's distinct antigenic structures, its outer lipopolysaccharide (LPS), and flagella. The outer layer of the bacterium is called the O-antigen, also known as the somatic antigen. The O-antigen is a repetitive oligosaccharide made up of lipopolysaccharides attached to and present on the outer side of a gram-negative bacterium membrane. Due to the complexity of the O-antigen, it is most helpful in serotyping gram-negative bacteria (Wang et al., 2010). The O-antigen determines the serovar identity of a specific *Salmonella* isolate. O-antigens determine the specific strain of *Salmonella* by the sugar sequence on the cell. The H-antigen is the flagellar antigen. *Salmonella Enteritidis* and Typhimurium are two serovars that produce flagella. The H-antigen can be both monophasic and diphasic. Of the 2,500 serovars, *S.* Kentucky, *S.* Enteritidis, *S.* Heidelberg, and *S.* Gallinarum concerned the poultry industry, and the serovars impact the industry differently. Among the *Salmonella* serovars, *Salmonella Kentucky* has been commonly found in food animals within the United States (Haley et al., 2019). Additionally, *S.* Kentucky has been one of the most identified serotypes discovered in poultry processing facilities in the United States and Europe (Salehi et al., 2017). From 1997 to 2007, *S.* Kentucky's prevalence increased from 25% to 50%, according to National Antimicrobial

Resistance Monitoring System (NARMS) (Salehi et al., 2016)USDA-FSIS reported in 2016, approximately 15% of broiler, turkey, and ground beef was found with both *Salmonella Enteritidis* (S.E.) and Kentucky (FOODS, 2019a). Another report showed that 60.8% were positive for *S.* Kentucky, 13.6% for *S.* Enteritidis, 7.7% for *S.* Typhimurium, 6.5% for *S.* Infantis, and 3.4% for *S.* Heidelberg (FOODS, 2019b).

The serotype *S.E.* is commonly found in avians and transmitted through eggs (Hammack & Andrews, 1999). *S.E.* is passed from the hen through the cloaca and attacks tissue within the bird (GAST & BEARD, 1993). Secondly, *S.E.* colonizes within the ovaries of hens more than any other animal (GAST & BEARD, 1993). Additionally, it can infect eggs more than any other *Salmonella* serovar or human pathogen (Raspoet et al., 2014). Lastly, contamination can occur through the egg yolk, shells, albumen, or reproduction organs (Gantois et al., 2009). An interesting fact regarding *S.E.* is that growers or bird handlers will not recognize a contaminated flock because this strain does not present signs of sickness. Therefore, a flock can become infected with *S.E.,* which is unknown until an outbreak. The reason is that *Salmonella is* not known to be harmful to chickens (Guard-Petter, 2001)

S.E. is one of the most frequently reported human serotypes and was responsible for several salmonellosis outbreaks in the 1980s (Sher et al., 2021) (Gantois et al., 2009). S.E. affiliation has more human sickness related to egg contamination than other serovars (Guard-Peter J, 2001b). It has been considered that *S.E*. has a more significant impact on eggs because it can penetrate the deep muscle tissues (Keller et al., 1997). In the 1990s, *S.E*. exceeded S. Typhimurium as the most commonly recovered serovar from humans in the United States (Patrick et al., 2004). Moreover, this strain of *Salmonella* is most commonly detected in humans worldwide (Raspoet et al., 2011).

Salmonella Heidelberg is among the top five *Salmonella* infections in humans in North America (Vincent et al., 2018). It is the most common serotype in North America versus any other country. The strain produces myocarditis and bacteremia cases and seems virulent (Santin et al., 2017). In 2016, *Salmonella Heidelberg* was listed third among the Canadians, but the United States reporting is higher. The United States Department of Agriculture (USDA) National Veterinary Service Laboratory (NVSL) collected data from 1968 to 2010, revealing that 71% of *S.* Heidelberg isolates originated from poultry-related sources. (Center for Disease Control and Prevention, 2013)The serovar *S.* Heidelberg is known to live in the gut of birds. The gut accommodates the living needs of *S.* Heidelberg and other serovars because it is polymicrobial (Kaldhone et al., 2017). Polymicrobial means being composed of many infections or diseases. The ceca are the area most fitting for the development of this bacterium. However, the crop is where *Salmonella* and even *Campylobacter* occur (J. A. Byrd et al., 2001). Most recently, antimicrobial-resistant strains of *S.* Heidelberg have been recognized or detected in the G.I. tract. (Lynne et al., 2009). In 2014, 12.5% of the human S. Heidelberg infections were caused by antimicrobial resistant strains that became resistant to an antibiotic known as cephalosporin, according to the NARMS report (Deblais et al., 2018).

Salmonella Typhimurium is one of the top causes of foodborne infections in the western hemisphere and impacts the United States by causing approximately a million cases of salmonellosis a year (Jørgensen et al., 2013). According to the CDC, there were around 356 people linked to a single *S.* Typhimurium outbreak. Approximately 76% reported having contact with live poultry, and 95% purchased live poultry (Center for Disease Control and Prevention, 2013).

Many serovars were not mentioned but had an impact on the world. The various serovars may be more common in other animals, such as pigs, beef, fruits and vegetables, and so forth, but may still affect humans. However, the serovars discussed in this section were those related to the poultry industry.

2.2 Environmental factors that impact *Salmonella prevalence* in broilers

Exposure to *Salmonella* in live birds requires more attention at the pre-harvest level (Bailey, 1993). According to research, *Salmonella*'s life cycle can be sustained due to farm environmental factors (J. Byrd et al., 2003; Guard-Petter, 2001; Jones- Hamilton Agricultural Division, n.d.; Liljebjelke et al., 2005). Environmental factors impacting *Salmonella* prevalence in the poultry industry include litter management (wet or dry litter, or the composition of litter, etc.) and meteorological factors such as temperature, humidity, and seasons (Jones et al., 2005). Meteorological factors are considered uncontrollable natural phenomena. Studies show flooding and natural disasters can be environmental factors that impact *Salmonella* (Volkova et al., 2009).

Additionally, research shows weather and seasons affect the growth of *Salmonella*, especially when temperatures are warmer (Hwang et al., 2020; Wales et al., 2007). One study's results show an increase in pathogen growth and development during the summer months compared to the winter months (Wales et al., 2007). The discovery observed in this experiment with caged and free-range layer flocks was further observed during this research. The inconsistent climate change, especially in the southern states, including Mississippi, has been attributed to the spread of Salmonella (Akil et al., 2014). The data show pathogen spreading

was more prevalent in the summer months because it is drier and hot, while the winter months are cool and wet (Wales et al., 2007). These factors are the perfect environment for pathogen harborage (Akil et al., 2014). The genetic makeup of today's birds shows that sweltering temperatures cause many abnormalities and degradation of the birds' immune system, gut, and productivity (Syafwan et al., 2011; Varasteh et al., 2015). As a result of extreme temperatures, especially during higher temps, the gut becomes colonized with *Salmonella* due to the imbalance caused by the heat stress (Arojona et al., 1988). With many environmental factors in play and their role in increasing *Salmonella* prevalence, the industry forces to implement interventions to reduce this problem**.**

2.3 Interventions that affect *Salmonella* in broilers during pre-harvest

Pre-harvest is the period on the farm where processes are implanted before birds go to slaughter, also known as the grow-out period. Pre-harvest prevents bacteria contamination from the beginning life stages of the bird to the end of its cycle (Alali, 2018) Post-harvest is any process that happens at the processing facility beyond the bird's life. Interventions are used in pre-harvest and post-harvest environments to assist with reducing *Salmonella*. Using interventions during pre-harvest is most beneficial for an extended period and covers a large area where birds will nest (Buncic & Sofos, 2012). This study will evaluate interventions used during pre-harvest, including litter and water treatment and vaccines, to determine their effectiveness during this growth phase. Moreover, during post-harvest, antimicrobials are used as an intervention.

2.3.1 Litter Treatment

Poultry litter comprises rice hulls, wood shavings, peanut shavings, and manure (Topalcengiz et al., 2021). It is vital to maintain healthy and dry litter. When litter is dry, it reduces the number of ammonia burns that could impact the welfare of the bird's overall health (Barnie, 2012). The goal of ammonia levels is to be at a pH of less than 6.0 because it reduces the amount of ammonia and other decaying matter taking place. However, if the levels are lower, such as 5.0, it can increase the chances of *Salmonella* growth (J. A. Byrd et al., 2001). At this level, poor litter can cause poor performance in birds and high feed conversion(Huff et al., 1984). Therefore, the need for suitable litter and ammonia reduction is critical. Various treatments have been developed to ensure bird health and litter management to aid this concern in the industry. Poultry Litter Treatment is one of the treatments developed.

Poultry Litter Treatment (PLT®) is a sodium bisulfate acid that controls ammonia inside poultry houses by reducing the pH. PLT®is used for pest management, litter acidification, and treatment to support HACCP programs (Terzich et al., 1998). Most litter treatments are designed to lower the pH and remove ammonia from farmhouse air. It is assumed that applying acid to the litter will reduce bacteria, including *Salmonella* (Pope & Cherry, 2000). According to a study conducted using PLT®, the results showed through statistical analysis there is a reduction of bacteria during the grow-out phase but not in the processing facilities. More specifically, for *Salmonella*, the PLT®treatment reduced bacteria in the litter, but there was not a statistical difference between litter, treated and nontreated (Pope & Cherry, 2000).

2.3.2 Water Treatments

Providing clean drinking water is a priority for flocks and essential in raising commercial flocks. Contaminated water and water lines affect bird health and ultimately infect litter with other bacteria if moisture increases (Jacobs et al., 2020). In addition, to feed withdrawal, poultry producers have been thinking of innovative ways to maintain bird health and weight while reducing *Salmonella.* The addition of an organic acid has been a great discovery. The organic acids that make up short-chain fatty acids may be used in place of antibiotic growth promoters (Scicutella et al., 2021). The term antibiotic growth promoter is a medicine that may impede bacteria growth. The use of organic acids further provides conclusive results that lead to healthy birds and immense performance in binds microbial functions can be impacted by at least two mechanisms, cytoplasmic acidification and accumulation of toxic acid levels (Mani-López et al., 2012). Organic acids that reduce *Salmonella* growth in broilers include acetic acid, lactic acid, and formic acid (Byrd et al., 2001). When using acetic, lactic, or formic acids in drinking water during preharvest, it has been discovered that the pH of the crop decreases, and the presence of *Salmonella* is minimal. Studies have shown using 0.5% of an organic acid can reduce the prevalence of *Salmonella* if used in conjunction with feed withdrawal (Byrd et al., 2001). This reduction can be observed at the processing facilities during pre-chill sampling (Harris et al., 2019). Adding organic acids helps maintain homeostasis in the gut flora (ElKatcha et al., 2018). Organic acids bring balance to the gut, but it also helps with the overall anatomical activity, enhancing the immune system and reducing the pH (Mustafa et al., 2021). The negative impact of adding acids to the water is the decrease in water intake from the birds during feed withdrawal (J. A. Byrd et al., 2001).

Aquaprime® is a disinfectant that reduces the pH in poultry and other livestock farming drinking water. Even though this chemical's development reduces pH levels, it has reduced biofilm growth in water lines and water (Wang et al., 2013). Biofilms are a cumulation of microorganisms found in the waterline of poultry farms. Biofilms are pivotal in *Salmonella's* presence on poultry farms and chicken slaughterhouses (Wang et al., 2013).To aid in the removal of biofilms, poultry producers added a disinfectant to the drinking water. Disinfectants applied must be safe for birds and the consumer. Several drinking water disinfectants have been developed to remove biofilm, acidic compounds, biocides, aldehyde-based, caustics, chlorine, and hydrogen peroxide. More disinfectants to consider are iodine, isothiazolinones, ozone, peracetic acid, phenols, biguanides, and surfactants (Merino et al., 2019). Studies show the usage of peracetic acid did not remove biofilm on specific surfaces or sodium hydrochlorate (Merino et al., 2019). Another study determined that Aquaprime® did not reduce microbial growth but improved the birds' body weight and overall welfare(Jacobs et al., 2020).

2.3.3 Feed Treatments

In 2006, the European Union prohibited antibiotics used for animal health which caused industry producers and growers to seek other methods but still obtain the same results (El-Hussein et al., 2008). Prebiotics and probiotics were discovered to replace growth promotion: probiotics and other live bacteria with added benefits to producing a healthy gut flora. Probiotics are natural microorganisms found in yeast and bacteria to balance the gut and enhance feed effectiveness. Probiotics are also known as direct-fed microbial. In poultry, using probiotics oppositely to antibiotics has added benefits to the industry. It has become beneficial because it

removes the apprehensiveness of consumers who fear antibiotics used in the industry (dos Santos et al., 2018). Prebiotics are considered non-digestible feed that prompts healthy gut activity. Both prebiotics and probiotics increase healthy immune systems in birds. (Jha et al., 2020).

Bacillus spp. is an endospore-forming bacteria including *B.subitilus* and other nonpathogenic species that alleviates gastrointestinal problems but heightens growth performance. Other bacteria, *Lactobacillus* and *Bifidobacterium*, are more commonly incorporated because they are capable of enduring extreme circumstances such as heat, storage, and dehydration (dos Santos et al., 2018). Probiotics such as *Bacillus lichenifomis* are supplementary feed, allowing them to inhibit dysbacteriosis (Huvepharma, 2018). Dysbacteriosis s best described as inflammation and imbalance of the microflora in the gut (Meng et al., 2020). OPTI-BAC® is a commonly used probiotic used for poultry. This probiotic is a sprayed-dried spore-forming bacterium (Huvepharma, 2018).

2.3.4 Antibiotics

An antibiotic can be defined as a drug or medicine provided to humans and animals to reduce the spread of certain bacterial infections. In the poultry industry, antibiotics can be used to prevent avian diseases. A commonly used antibiotic in the poultry industry was gentamicin. Gentamicin is an antibiotic given to day-old chicks during this study. This antibiotic has no longer been a part of grow-out operations in recent years. However, Garasol, a gentamicin brand, was used during this study. The study's results will show the impact it had on *Salmonella* prevalence.

According to CDC, antibiotics attack the bodies of both humans and animals to ward off infections or diseases caused by viruses or bacteria (Centers for Disease Control and Prevention, 2021). Using antibiotics unnecessarily increases the opportunity to develop antibiotic resistance (AMR). In poultry, antibiotics have been used for decades to treat various diseases and other treatments poultry (Cardoso et al., 2006). Gentamicin is an antibiotic used to treat bacterial infections in humans and animals. It is made up of an aminoglycoside that treats aerobic gram-negative bacilli diseases. Gram-negative bacteria include, at a minimum, *Salmonella enterica*, *E. coli*, Enterobacteriaceae Meningococcus, Chlamydia trachomatis, and Campylobacter jejuni.

2.3.5 Vaccines

Pre-harvest vaccines are essential when it comes to the growth of birds. The purpose of vaccines is to create a stamp or memory marker that will allow the body to develop an immunity, eventually reducing the prevalence (Zhang-Barber et al., 1999). Vaccinating broilers and breeders provide additional protection against *Salmonella* (Young et al., 2007). According to the European Food Safety Authority, a study revealed that vaccines decreased S.E. serovars by l88% when given to laying hens ("Report of the Task Force on Zoonoses Data Collection on the Analysis of the Baseline Survey on the Prevalence of *Salmonella* in Broiler Flocks of Gallus Gallus, in the EU, 2005-2006 - Part A: *Salmonella* Prevalence Estimates," 2007). *Salmonella* vaccines can be live-attenuated or inactivated. A live-attenuated vaccine is a lesser form of bacteria used to eradicate a pathogen. A live vaccine created directly from a direct *Salmonella* serovar generates substantial protection against disease (Aehle & Curtiss, 2017; Young et al., 2007).

According to the CDC, a live attenuated vaccine is designed to lessen the virus. This method is designed in a laboratory setting by conducting multiple cultures. The development of this type of vaccine is more effective on young birds if given by spray or water. The reason is that response activates a cell-mediated response in the host that destroys the cytotoxic T cells or intracellular organisms by macrophages (Farmer & Dietert, 2013). A live vaccine is vital at this age of their life because the immune system is still developing. While the immune system matures, the live vaccines mature with the system. McReynolds and Van Immerseel suggested that introducing vaccines at an early age protected chicks if given at 48hours or earlier when given a wild type of *Salmonella* (McReynolds et al., 2007) (van Immerseel et al., 2005). The first live vaccine developed and licensed was Megan®Vac-1. Megan®Vac-1 is a double-stranded gene-altered from S.T. for broilers (Burns, 2004). Megan®Vac-1 is evaluated during my study.

Another vaccine used is the inactive vaccine known as a killed vaccine. An inactive vaccine is produced as a whole cell but must include an adjuvant for its effectiveness (Aehle & Curtiss, 2017) (Rabie & Amin Girh, 2020). The cell's killing is conducted by acetones, alcohols, formaldehyde, heat treatments, or radiations (Rabie & Amin Girh, 2020). The focus has been on treating carcasses post-harvest, but more recently, treating during pre-harvest has been incorporated. Studies show that *Salmonella* species have been traced back to the farm and the parent flock (Bailey et al., 2002). By providing vaccinations during pre-harvest, it is the intent that there will be a reduction of *Salmonella* in the processing facilities (Young et al., 2007).

2.4 Interventions that affect *Salmonella* in broilers during post-harvest

2.4.1 Processing Aids

Processing aids are approved by the Food and Drug Administration (FDA) detailed in 21 CFR 100.100(a)(3) United States Department of Agriculture, 2008. Processing aids, frequently referred to as antimicrobials, are chemicals used to inhibit the growth of microorganisms. The following qualifications must apply for the authorization of antimicrobials for food safety. The antimicrobial must be approved, effectively researched, level and contact time acceptable, cost-effective, and impact quality (Bauermeister et al., 2008). Processing aids are used throughout processing facilities, including inside-outside bird washers, pre-chillers, main chillers, and post-chillers (Vaddu et al., 2021). For decades, chlorine has been the choice for many processing facilities for bacterial reduction. Nonetheless, facilities replaced chlorine with peracetic acid (PAA) due to not reducing the organic load and increasing pH above 7.0 (Lillard, 1979). PAA is a mixture of acetic acid and hydrogen peroxide. The effectiveness of PAA is due to its acid and oxidizing agents (Nagel et al., 2013). Additionally, peracetic acid and hydrogen peroxide combined result in an enhanced quality product (Bauermeister et al., 2008). Processors benefit from this antimicrobial because it does not affect the quality, flavor, or color (Bauermeister et al., 2008)(Blankenship, 1990) used in chillers and dips (Kumar et al., 2020). This is due to greater contact time in the chiller than being sprayed for a short period of 50ppm-2000 parts per million(USDA, 2021a). However, the product's quality will be affected if antimicrobials are not mixed with some hydrogen peroxide(Blankenship, 1990). Cetylpyridinium Chloride (CPC) (Cecure; SafeFoods Corp., Little Rock, AR) is a chemical used in oral hygiene products such as

mouthwash, toothpaste, and throat lozenges but helps reduce *Salmonella* on poultry. Zee Company (Chattanooga, TN) is a PAA product used as a processing aid in poultry facilities. According to FSIS, all antimicrobials used in poultry facilities target a range of 50-2000 ppm of PAA (USDA, 2021a).

CHAPTER III

METHODS AND MATERIALS

3.1.1 Process of collecting birds for Salmonella testing

Birds selected for this study are Ross 708 female and a yield + + (Y+P) male birds and said birds grew for approximately 48-50 days. The birds were raised in two geographical locations labeled as locations 1 and 2 to protect the identity of the farms. The grow-out process was evaluated from the hatchery to the processing facility while describing each step related to the study. The study will use January 2017 through December 2019 (Table 1).

Table 1. Total Number of Samples Collected from Locations 1 and 2.

Locations	Samples
Location 1	8,064
Location 2	8,761
Total	16, 825

Birds were obtained from a commercial hatchery and identified by a unique number. Birds were inspected and sprayed with a vaccine cocktail (Merck's Mildvac® C2M (Rahway, NJ), Merck'sMildvac® Ark, Elanco's AviPro® ViBursa CE (Greenfield, IN), and Elanco's AviPro® Megan® (Greenfield, IN) including *Salmonella,* before being placed on a farm. The live-modified *Salmonella* vaccine (AviPro®Megan®Vac1) reduces certain *Salmonella* strains, specifically *Salmonella* serovars Enteritidis, Heidelberg, and Typhimurium, in the

gastrointestinal tract of broilers. The vaccine was combined with other vaccines such as Newcastle (NDV-18). At 14 days of age, a second dose of the live *Salmonella* vaccine (AviPro®Megan® Vac 1) is administered to the birds in the drinking water. After 48-50 days of growth, birds were caught manually by a catch crew, placed in metal transport cages, and loaded onto a truck. Misters, fans, and wetting were applied to help birds to help them remain cool while being loaded onto a truck or staged at a processing facility beginning at an ambient temperature of 65°F or greater, depending on the bird's comfort.

After birds are caught, a trained person will take and handle boot swab samples. A modified version of the National Poultry Improvement Plan procedure collects boot swabs(United States Department of Agricultural, 2019). Boot swabs samples are taken after the catch but not to exceed 12 hours from the sale of birds. The boot swabs were tied around plastic shoe cover worn by personnel and walked the length of the house. The walking method is provided by Northwest Arkansas Laboratory (Springdale, AK). After the farmhouse was walked, swabs were removed from the shoe and placed in a sterile bag in the cooler with cold packs. Boot swabs were shipped to a lab for analysis (United States Department of Agriculture, 2014). Below is the list of interventions used in the hatchery and during preharvest (Table 2).

Table 2. Interventions, Locations, and Dates of Preharvest Interventions

	Dates	Locations	Interventions
Start	7/20/2018	Location 2	AviPro® Megan®
Stop	1/4/2020		
Start	11/30/2016	Location 1	AviPro® Megan®
Stop	7/24/2018		
Start	6/4/2018	Location 2	PWT®
Stop	Currently in use		
Start	4/2/2018	Location 1	PWT®
Stop	7/30/2018		
Start	4/16/2018	Location 2	Opti-Bac®
Stop	11/26/2018		
Start	ND	Locations 1,2	Gentamicin
Stop	4/22/2019		
Start	8/1/2018	Location 1	AquaPrime®
Stop	Currently in use		Location 1

*N.D
Not Determined
* AviPro®Megan Vac® Elanco
(Greenfield, IN)
* Opti Bac® Huvepharma
(Peach City Tree, GA)
* AquaPrime® Neogen (Lansing, MI)
* PWT® Poultry Water Treatment Jones- Hamilton (Waldridge, OH)

Meanwhile, birds arrived at the processing facility in metal cages. Birds were held under a shed from catch to slaughter not to exceed 12 hours (National Chicken Council, 2017). While birds are kept under the holding shed, fans or misters may have been used depending on the temperature or humidity. Birds were emptied onto a belt, manually caught by the feet, and placed into shackles to begin euthanizing. Birds were electrically stunned before euthanasia was performed by exsanguination. Next, euthanized birds were de-feathered and hocks removed. After this process, a hot-rehang carcass rinse sample is collected after the de-feathering process. A hot-rehang sample is taken prior to the evisceration of the birds. The samples are rinsed in 400 mL of Buffered Peptone Water (BPW) (3M ™) broth. Before pre-chill rinses, carcasses were introduced to different antimicrobials after evisceration. The birds were washed with peracetic acid (PAA) (Safe Foods™) to prepare carcasses for chilling. Prior to birds entering the chiller, a pre-chill rinse sample was taken and rinsed in 400 mL of Neutralizing Buffered Peptone Water (nBPW) (Hardy Diagnostics, Santa Maria, CA) rinsate. Once carcasses enter the chiller, the dwell time can range from 1.5 to 2 hours. After birds exit, the chiller and post-chill carcass rinses are taken using the Microbiological Laboratory Guidelines (MLG) 4.5.5 procedure (USDA, 2021b).

3.1.2 Boot Swabs/Drag Swabs analysis

Modified Semi-Solid Rappaport-Vassiliadis (MSRV) (Hardy Diagnostics, Santa Maria, CA), tetrathionate broth (T.T.) (Hardy Diagnostics, Santa Maria, CA), Xylose-Lysine-Tergitol 4 (XLT4) agar (Hardy Diagnostics, Santa Maria, CA), and Brilliant Green Sulfa agar (BGS) (Edge Biologicals, Memphis, TN) media was used to detect *Salmonella spp.* Before use, swabs will come to room temperature and be plated directly onto MSRV. After a colony grows on the

MSRV plate, a colony is taken and enriched in tetrathionate. An inoculated sample was enriched in a 1:10 dilution and incubated at 37 or 42°C for 20 to 24 hours. Samples are transferred to 100 ml of enriched culture onto an MSRV plate and incubated right side up to 42°C for 24 hours. Presumptive positives are plated on BGS or XLT4 selective media. Boots swabs are shipped to a 3rd party laboratory for analysis, according to the National Poultry Improvement Program (NPIP); (United States Department of Agriculture, 2014).

3.1.3 Carcass rinse analysis

One carcass rinse was collected every 22,000 carcasses per directive 10,250.1 (United States Department of Agriculture, 2021). Ten to twelve rinses were collected during a single day at hot-rehang, pre-chill, and post-chill. A random number generator determines the carcass sample times collected daily. Chicken parts, including breasts, wings, tenders, thighs, or drumsticks, were taken after the last intervention when the antimicrobial was applied (United States Department of Agriculture, 2021) per parts sampling protocol regulation. Briefly, carcass rinses were collected by cleaning and sanitizing the surface of the sampling cart before collecting and placing the carcass into a sterile bag (United States Department of Agriculture, 2021). The sample bags will have 400 mL of one of the following broths added to each bag, Buffered Peptone Water (BPW) (Neogen®, Lansing, MI), hot-rehang, and nBPW (Hardy Diagnostics, Santa Maria, CA) for pre-chill and post-chill in the carcass cavity. The rinse bags were manually shaken for one (1) min, and then 400 mL of rinse solution was poured back into the container without touching the inside of the bag. The final step is rinsing the carcass with potable water and placing it back onto the processing line. Samples will be transported to a laboratory for incubation and analysis. Samples were incubated for 37±2° for 20-24hours (MLG 4.10).

For rinsing parts samples, 3lbs. to 4 lbs. of chicken part types are collected, including legs, breasts, thighs, wings, or drumsticks. The chicken parts were placed inside a sterile bag on a clean and sanitized sampling cart. A total of 400 mL of nBPW rinse was poured into the bag and manually shaken for one min. After one min, the 400 mL rinse solution was poured back into the original container. There was 30±0.6 mL of rinse solution used for *Salmonella* *analysis*. Samples were enriched with an additional 30±0.6 mL of BPW broth to ensure homogenization. Samples are incubated for 37±2° for 20-24hours (MLG 4.10). After incubation, samples are analyzed for *Salmonella* by PCR.

3.1.4 Microbiological Testing Tempo® Enterobacteriaceae (E.B.)

Tempo® (bioMeriéux, Durham, NC) was developed to calculate enterobacteria within 22-27 hours. The samples are prepared using the manufactures instructions. Samples are prepared using a 1:40 dilution of BPW, which gives a $4.9x10^4$ CFU/mL of rinsate. Sterile water and a dispenser are used to dispense 3 mL of water into empty vials. A sterile pipette will transfer 1 mL of filtered carcass rinsate to regenerate the cultured medium prep from the manufacturer, resulting in 4 mL. The sample rinsate was mixed for 3 sec, creating a 1:40 dilution. The equipment to homogenize samples is a vortex. Six (6) sample vials and cards per rack are filled synchronously. Racks were placed in a Tempo® Filler (bioMeriéux, Durham, NC), and the rinsate sample will absorb into the card, taking up to 3 min to complete. Cards are removed from Tempo Filler, and checks are conducted to ensure all medium has been absorbed from the vial. Rinsate cards incubate for 22-27 hours at 35±1°C. The results are determined by the luminescence shown on the cards in the reader. The system automatically identifies the sample with the correct test and dilution. The results are imported into the laboratory

information management system (LIMS; Thermo Fisher Scientific). Cards are disposed of after testing is complete (Tempo E.B.).

3.1.5 Microbiological Testing for Salmonella

VIDAS®Up *Salmonella (*bioMeriéux, Durham, NC) is an automated system that conducts qualitative tests to detect *Salmonella* on human and animal food products and environmental and product samples. The VIDAS is an enzyme immunoassay instrument that uses Enzyme-Linked Fluorescent Assay (EFLA) to detect *Salmonella.* The testing strip used for the VIDAS is coated with a protein designed explicitly for *Salmonella detection*. The test is conducted using the 'manufacturer's instructions. While preparing for the test, enrichment broth was preheated prior to transfer. Chicken carcass rinsate is placed in a sterile Whirl pack (Nasco, Fort Atkinson, WI) bag containing 30 mL of carcass rinsate and 30 mL of BPW. The BPW is preheated at 42±1°C. The *Salmonella Supplement* (item # 42650) and AOAC Official Method of Analysis (No. 2013.01) approved protocols state to add 0.25 mL to the sample and mix for two min, followed by incubation for 20-24 hours at 42±1°C (Australian Government Department of Agriculture, n.d.). The VIDAS® Heat and Go (Bibby Scientific, Staffordshire ST15 the 0SA United Kingdom) was used for a heating mechanism using 0.5 mL of enrichment broth in a well on the strip. The samples will warm for 5±1 min inside the sample strip and cool for 10 min. After cooling, samples are ready to run on VIDAS®. The steps follow the AOAC approved method (2013.02)(Australian Government Department of Agriculture, n.d.).

3.1.6 DuPont BAX ®Salmonella

The DuPont BAX® (Hygiena, Camarillo, California) system is used to detect *Salmonella in* real-time using a Polymerase Chain Reaction (PCR) assay. The PCR has a target with a marked genetic sequence that indicates presence. It is best used to detect *Salmonella in* food and environmental samples. The results of the system can be available within 1 hour.

The samples are enriched according to the MLG 4.10 protocol. After enrichment and incubation, samples are analyzed using the DuPont BAX® system. DuPont BAX ® heat blocks must be preheated to 37 and 95°C and have a cooling block available with a temperature of 2-8°C. Secondly, samples are labeled and entered into the system synonymously. Next, 12 mL of lysis buffer bottle is mixed with 150 µL protease followed by 200 µL of lysis reagent to each cluster tube. Lastly, 5 µL of the enriched sample was added to the cluster tubes. Samples are heated for 20 minutes at 37°C, followed by 10 minutes at 95°C and cooling for 5 minutes at 2-8°C.

Once samples cool for 5 min, samples are placed (PCR tubes) on a cooling rack and covered. Strips used for PCR include a pellet inside the well of the strip. A de-capping tool will remove caps from tubes, retrieve 30 µL of lysate into PCR tubes, and reseal. The steps are repeated until pellets inside of the strips have dissolved. PCR tubes will rest on a cooling block from 10-20 min but not exceed 30 min before being loaded on the thermocycler by DuPont BAX®. PCR will run for a period depending on the number of samples in the machine. Color-coded indicators will show on the screen for results, green for negative, red for positive, and yellow for inconclusive or signal error. All presumptive positives will undergo a confirmation step.

Presumptive positives are cultured after screening. Using the original enrichment, 0.5±0.05 samples are transferred in 10 mL of tetrathionate broth (T.T.) followed by 0.1 ±0.02 mL of (Hajna) broth into a 10 mL MSRV broth. Samples will incubate for 22-24hr at 42±0.5°C. After incubation, samples are mixed for homogenization and streaked on BGS and DMILA agar plates with a loop of 10 µL of a sample. Plates will incubate at 35±2°C for approximately 18-24 hours. The technique was conducted following the MLG 4, Section 4.7 method (USDA, 2021).

3.1.7 Statistical Analyses

Statistical analyses were conducted using the statistical platform Stata (version 16.1 StataCorp, College Station, TX). Descriptive statistics compared *Salmonella* positives and negatives between locations. Statistical charts and graphs are used to depict the relationship between the average daily temperature and relative humidity between locations. The dependent variables explored were *Salmonella* prevalence at hot rehang, *Salmonella* prevalence at prechill, *Salmonella* prevalence at post chill, *Salmonella* prevalence of chicken parts, and *Salmonella* prevalence of boot swab samples. Bivariable logistic regression models were used to assess the association between location and seasons and the dependent variables.

Additionally, bivariable logistic regression models were used to assess the association between preharvest interventions and hatchery vaccines and *Salmonella* prevalence for hot rehang samples, prechill samples, and boot swab samples. Bivariable logistic regression was used to assess the association between fans, misters, bird wetting, and *Salmonella* prevalence for hot rehang samples. Multivariate logistic regression models were used to explore further associations for multiple independent variables that were found to be significant in bivariable

logistic regression. Marginal means graphs were plotted from multivariate logistic regression models to visualize *Salmonella* prevalence. For chicken part samples, the parts were categorized into FSIS tested, and non-FSIS tested parts. FSIS tested parts included thighs, drumsticks, wings, tenders, breast, and leg quarters; non-FSIS tested parts included giblets and necks. Chicken parts were categorized into bone-in, boneless, and giblets.

CHAPTER IV

RESULTS

4.1.1 Descriptive Statistics

All samples in the study were collected from two commercial poultry complexes. The birds in this study were grown in similar environments, received similar treatments, and were processed similarly. The meteorological data was collected using www.wunderground.com (accessed 4/2/2020). All temperatures and humidity were calculated using the daily average. While collecting temperature and humidity data, correlating seasons were added to the data. Fall, winter, spring, and summer are determined as shown on the annual calendar. The temperatures in the geographical region determined the usage of fans, misters, and wetting to maintain the comfortability of the birds. The average daily temperature ranged from 25.8° to 93° Fahrenheit, with an average of 70.9°F (Figure 2). The average daily relative humidity ranged from 0 to 100%, with an average of 72.2% (Figure 3).

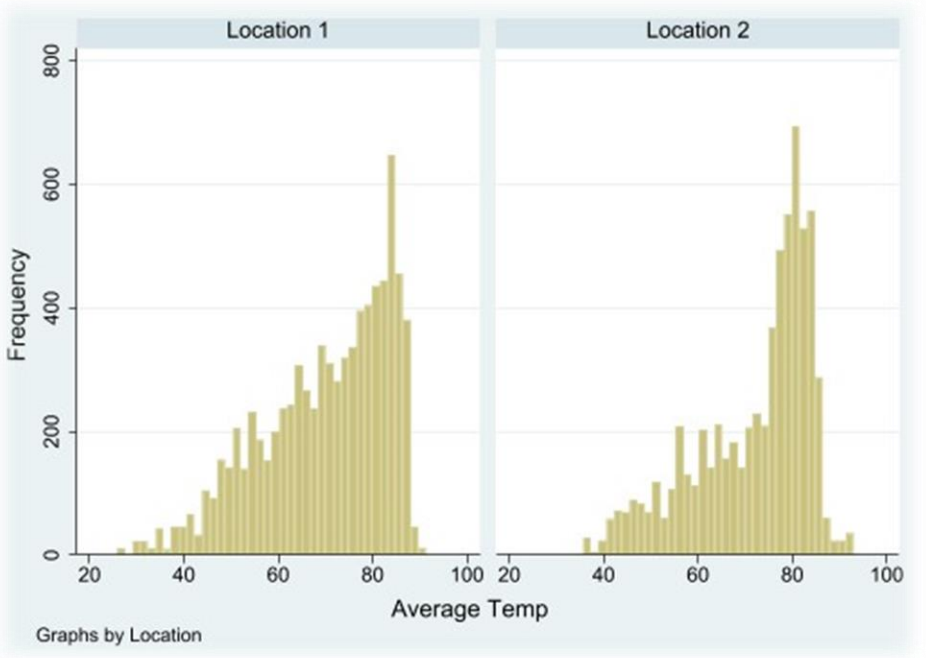

Figure 2. Average daily temperatures for the two geographical locations from January 2017-December 2019 (www.wunderground.com) (accessed 4/2/2020).

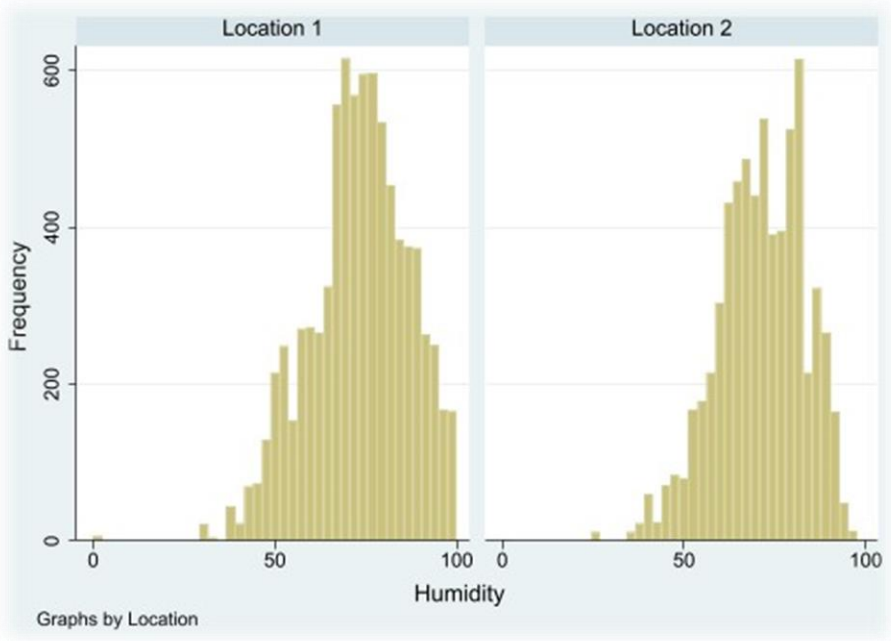

Figure 3. Average relative humidity for the two geographical locations from January 2017-December 2019 (www.wunderground.com) (accessed 4/2/2020).

4.1.2 Hot Rehang Salmonella

Descriptive statistics

The study evaluated hot rehang samples by comparing the *Salmonella* prevalence by location, season, use of fans, misters, wetting, hatchery vaccines, and preharvest interventions. A total of 14,268 samples were collected at hot rehang cultured for *Salmonella.* Of those samples, 7,550 were positive for *Salmonella,* resulting in a prevalence of 52.9% (Table 3).

Table 3. The total number and percentage of positive and negative samples for *Salmonella* collected at hot rehang.

Hot rehang			
Locations	Negative	Positive	Total
Location 1	2,820	2, 978	5,798
Percentage	48.64%	51.36%	100%
Location 2	3, 898	4, 572	8,470
Percentage	46.02%	53.98%	100%
Total	6,718 47.08%	7,550 52.92%	14,268

Bivariable logistic regression

Bivariable logistic regression was conducted to assess the association of location, season, use of fans, misters, wetting, hatchery vaccines, and preharvest interventions with *Salmonella* prevalence for hot rehang samples (Table 4). We found that location was significantly associated ($p<0.05$) with *Salmonella* prevalence for hot rehang samples. The odds of *Salmonella* at hot rehang were 1.11 times greater in Location 2 than in Location 1. The season was also significantly associated ($p<0.05$) with the prevalence of *Salmonella* for hot rehang samples, and the odds of *Salmonella* were 2.29 times greater in spring, 1.44 times greater in summer, and 1.17 times greater in winter compared to fall. Fans, misters, and wetting were significantly associated (p, 0.05) with *Salmonella* prevalence. The use of fans, misters, and wetting increased the odds of *Salmonella* by 1.38, 1.47, and 1.37, respectively. Preharvest interventions were significantly

associated ($p<0.05$) with the prevalence of *Salmonella* at hot rehang. The odds of *Salmonella* were 2.38 times greater for birds subjected to feed treatment and 1.79 times greater for litter treatment compared to no treatment. We found that hatchery vaccines were significantly associated ($p<0.05$) with *Salmonella* prevalence at hot rehang. The odds of *Salmonella* were 1.62 times greater for AviPro®Megan® treated birds when compared to birds treated with gentamicin inovo (Merck's Garasol 0.2mg/chick).

Table 4. Odds ratios and confidence intervals from bivariable logistic regression analysis of the prevalence of *Salmonella* for hot rehang samples by location, season, fans, misters, wetting, preharvest intervention strategies, and hatchery vaccines.

	Hot Rehang *Salmonella* Samples		
Independent Variable			
	OR	P-Value	95% CI
Locations			
Location 1	1.06	0.38	1.00-1.11
Location 2	1.11	0.00	1.04-1.19
Seasonality			
Winter	2.46	0.00	1.83-3.32
Spring	5.31	0.00	4.0-7.20
Summer	2.70	0.00	2.0-3.72
Fall	0.52	0.00	0.40-0.68
Preharvest Treatment			
No Treatment	0.74	0.00	0.69-0.79
Water Treatment	-	-	-
Litter Treatment	1.79	0.00	1.61-1.98
Feed Treatment	2.38	0.00	2.02-2.79
Water and Litter Treatment	1.09	0.15	0.97-1.24
Feed and Litter Treatment	1.45	0.00	1.26-1.66
Hatchery Vaccines			
Gentamicin	1.02	0.52	0.97-1.07
AviPro®Megan®	1.62	0.00	1.44-1.82
Gentamicin/AviPro®Megan®	1.12	0.00	1.04-1.20
Fans	1.38	0.00	1.29-1.49
Misters	1.42	0.00	1.37-1.58
Wetting	1.37	0.00	1.27-1.47

Multivariate Logistic Regression

Based on the results from bivariable logistic regression, a two-way full factorial multivariate logistic regression model was explored for season and location at hot rehang. We found a significant difference by location in *Salmonella* prevalence between winter, spring, summer, and fall (Figure 4). Interestingly, the prevalence was higher in the winter and fall for Location 2 and higher in the spring and summer for Location 1 (Figure 4).

The effect of preharvest interventions on *Salmonella* prevalence was explored across seasons using a 2-way full factorial multivariate logistic regression model separately by each location because the preharvest interventions were unique for each location. We found that at Location 1, the prevalence of *Salmonella* was significantly lower in the spring when water and litter treatments were applied compared to no treatment; however, in the summer, the prevalence of *Salmonella* was significantly greater when water and litter treatments were applied (Figure 5). At Location 2, there were no differences in *Salmonella* prevalence across seasons when litter treatment was applied (Figure 6). *Salmonella* prevalence was significantly higher in the spring than in the summer when feed treatment was administered. There was also a significant difference in *Salmonella* prevalence between feed and litter treatment in the spring and summer. (Figure 6). Fans, misters, and wetting typically occur during the year's warmer months, and the use was notated. The effect of fans, misters, and wetting on *Salmonella* prevalence was explored across seasons and locations using a 3-way full factorial multivariate logistic regression model (Figures 7, 8, and 9). We found that *Salmonella* prevalence was significantly higher for Location 1 in the summer when fans were used (Figure 7).

In the fall, *Salmonella* prevalence was significantly higher in Location 2 than in Location 1, but *Salmonella* prevalence did not differ with the use of fans (Figure 7). In the spring, there was also a significant difference in *Salmonella* prevalence between locations, and the use of fans

was associated with a higher *Salmonella* prevalence for Location 2 (Figure 7). We found that the *Salmonella* prevalence was significantly higher when spray misters were used at Location 1 in the spring, summer, and fall. For Location 2, the prevalence of *Salmonella* was significantly higher in the spring when misters were used (Figure 8). Similar to misting, we found that *Salmonella* prevalence was significantly higher when wetting the litter was conducted in the spring and summer at Location 1. For Location 2, the prevalence of *Salmonella* was significantly higher in the spring when wetting occurred (Figure 9). There was no significant effect on *Salmonella* prevalence for wetting in the fall. However, there was a significant difference in *Salmonella* prevalence by location (Figure 9).

Additionally, we explored hatchery vaccines by season and location using a 3-way full factorial model. It is important to note that at Location 1, AviPro®Megan®) was not used alone but combined with gentamicin (Merck's Garasol 0.2mg/chick). In the winter and fall, we found no treatment effect on *Salmonella* prevalence; however, there was a significant difference by location, with a higher prevalence at Location 2 (Figure 10). In the spring, we saw a significant decrease in the *Salmonella* prevalenc*e* for gentamicin treatment at Location 1. In the summer, there was a significant decrease in *Salmonella* prevalence for gentamicin treatment at Location 2 (Figure 10).

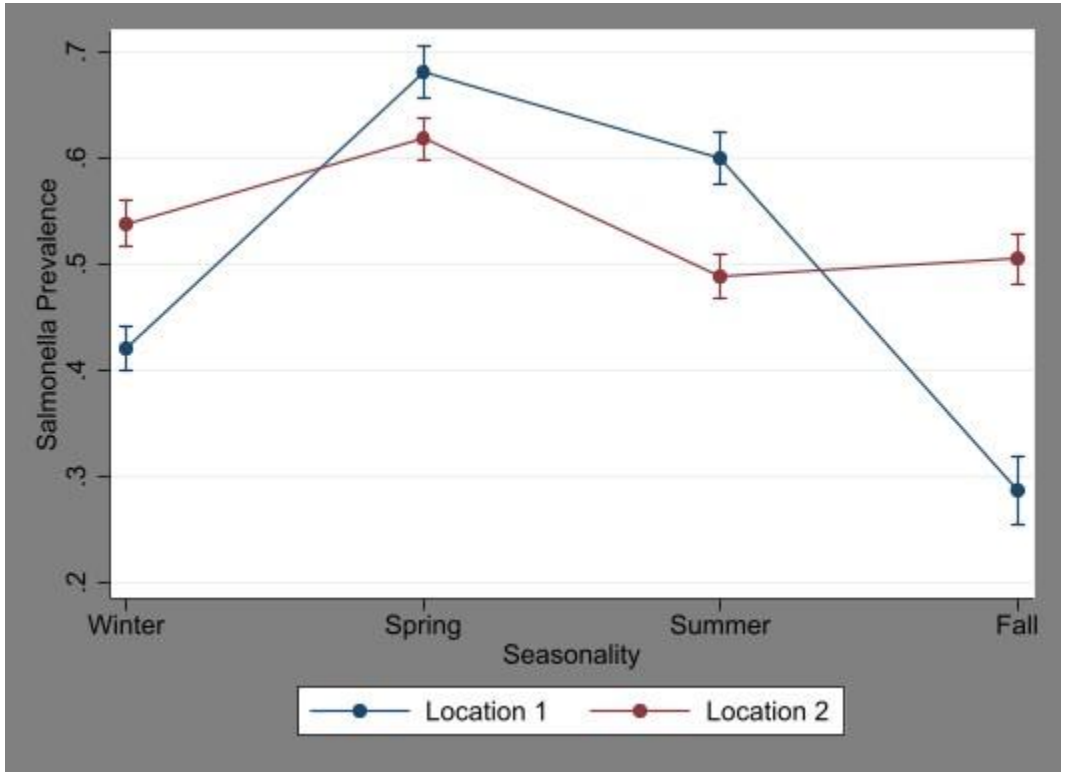

Figure 4. Marginal means graph from a 2-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at hot rehang for season and location.

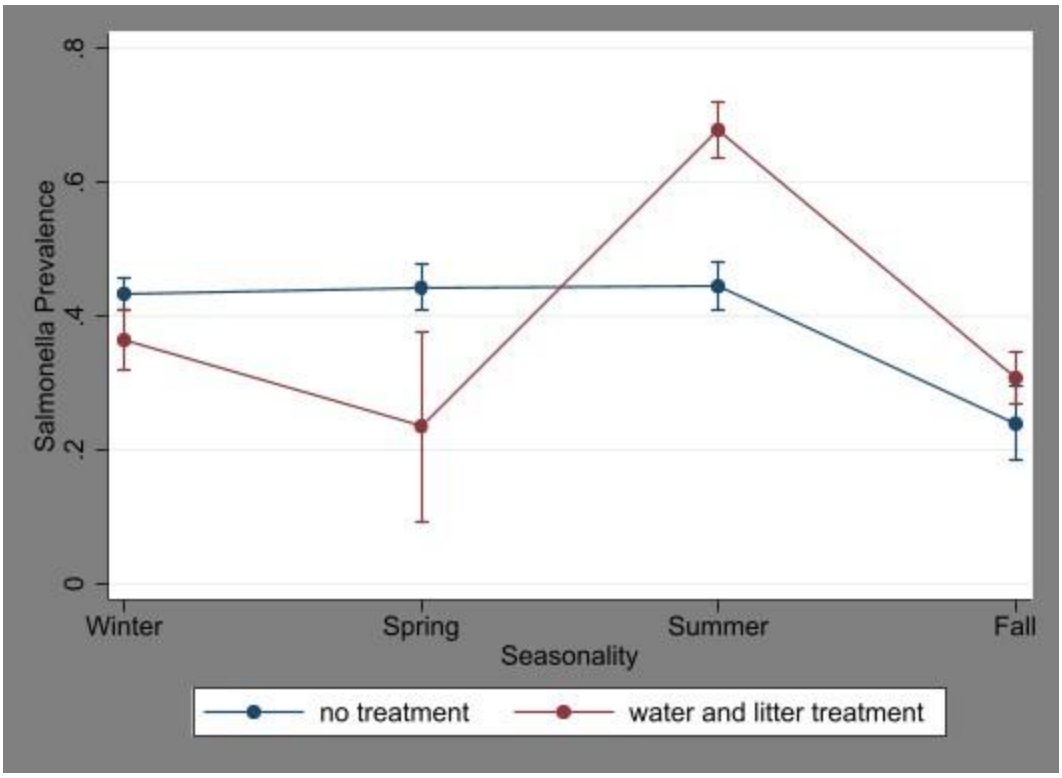

Figure 5. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at hot rehang for Location 1 for preharvest intervention strategies across seasons.

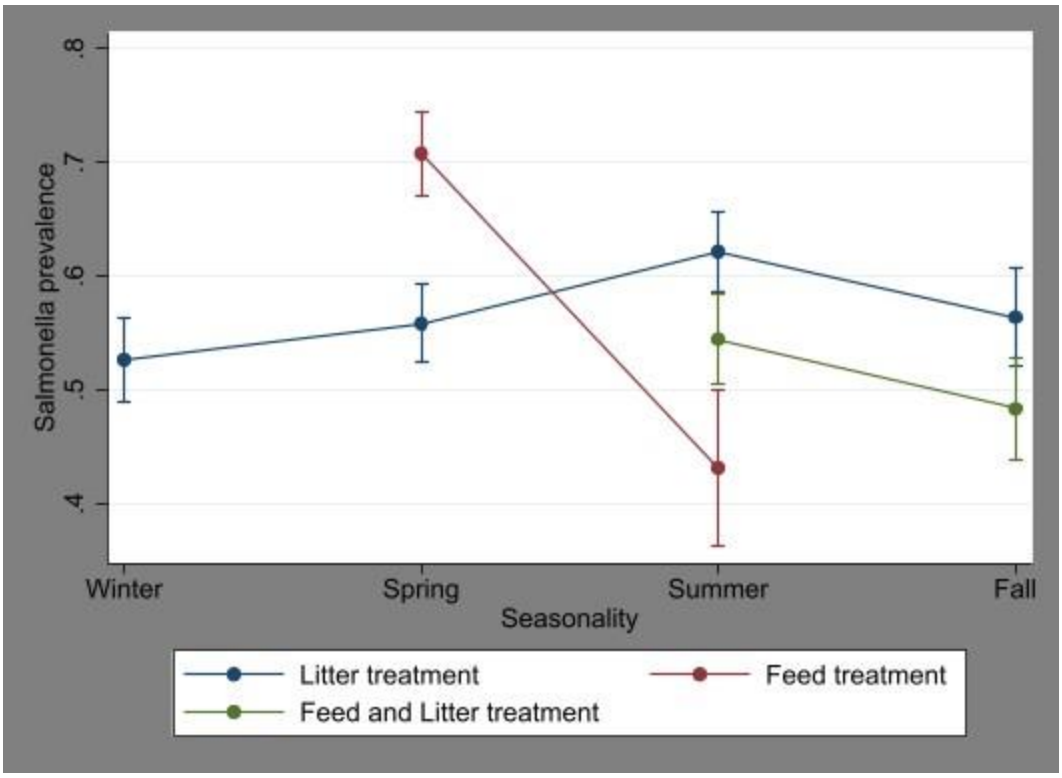

Figure 6. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at hot rehang for Location 2 for preharvest intervention strategies across seasons.

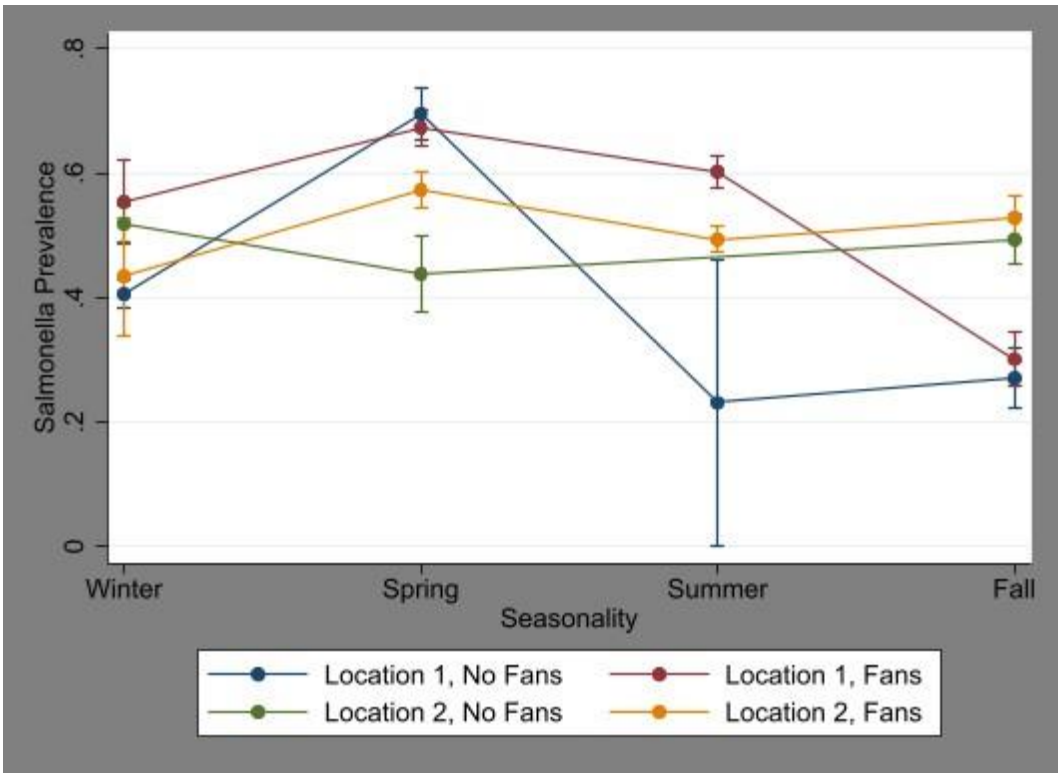

Figure 7. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing the use of fans on *Salmonella* prevalence at hot rehang across seasons by location.

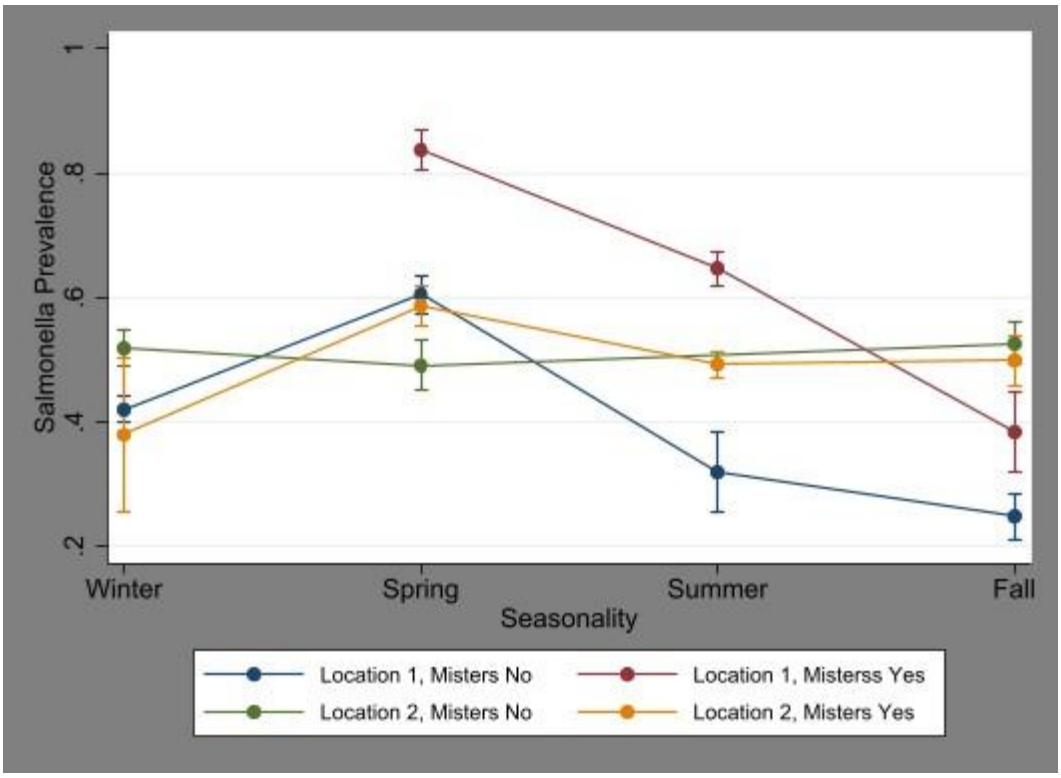

Figure 8. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing the use of misters on *Salmonella* prevalence at hot rehang across seasons by location.

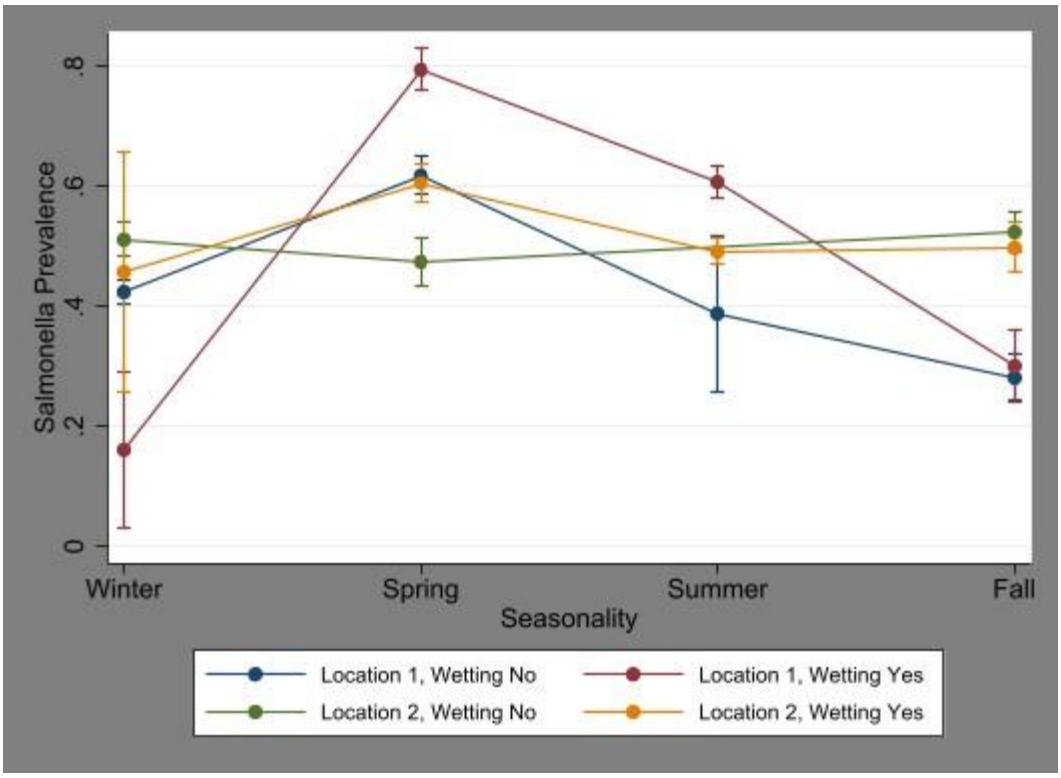

Figure 9. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing the use of wetting on *Salmonella* prevalence at hot rehang across seasons by location.

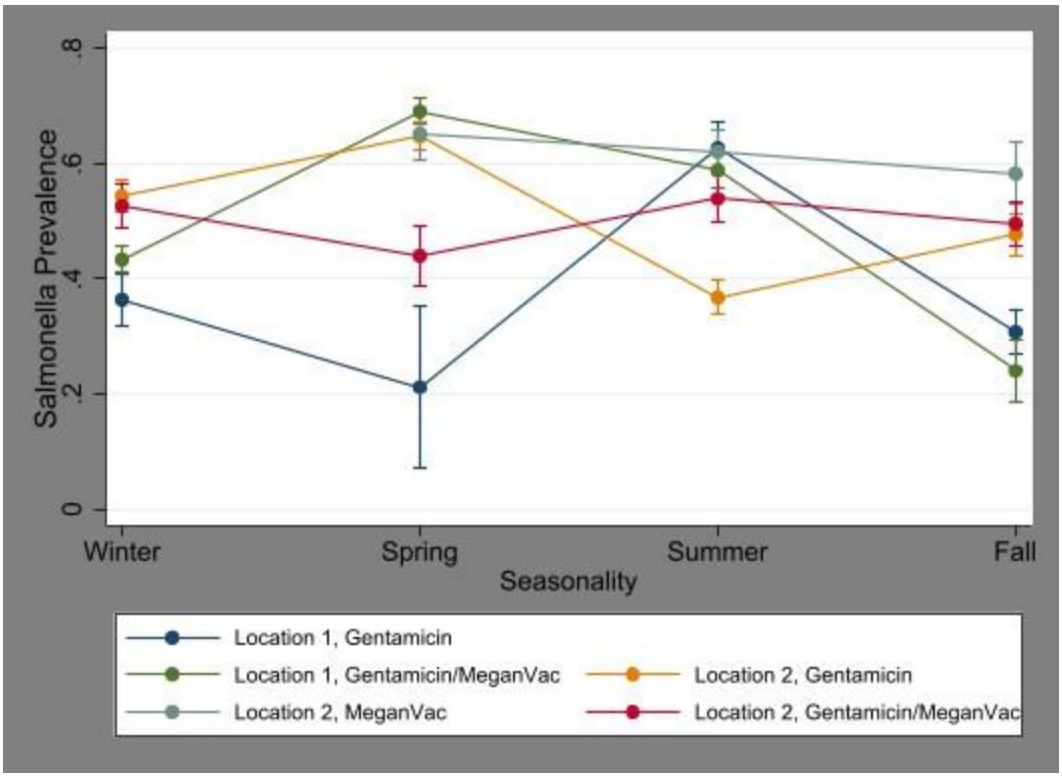

Figure 10. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing administration of hatchery vaccines on *Salmonella* prevalence at hot rehang across seasons by location.

4.1.3 Prechill Salmonella

Descriptive Statistics

There was a total of 14,508 samples collected at prechill. 8,520 samples were negative, and 5,988 were positive for *Salmonella*, with a prevalence of 41.28% (Table 5). Location 1 had a *Salmonella* prevalence of 20.13%, and Location 2 had a *Salmonella* prevalence of 50.02%.

Bivariable Logistic Regression

Bivariable logistic regression analysis was conducted to determine location, seasons, hatchery vaccines, and preharvest interventions with *Salmonella* prevalence for pre-chill samples (Table 6). We found that location was significantly associated ($p<0.05$) with *Salmonella* prevalence for prechill samples and that the odds of *Salmonella* for prechill samples were 2.43 times greater in Location 2 than in Location 1. The season was also significantly associated ($p<0.05$) with *Salmonella* prevalence for prechill samples. The odds of Salmonella at prechill were 1.87 greater in spring and 1.44 times greater in summer and 1.28 times greater in winter compared to the fall. Preharvest interventions were significantly associated ($p<0.05$) with *Salmonella* prevalence for prechill samples. The odds of *Salmonella* for prechill samples were 2.96 times greater for birds subjected to feed treatments, 1.20 times greater for water treatments, and 2.34 times greater for litter treatments compared to no treatments. Hatchery vaccines were significantly associated ($p<0.05$) with *Salmonella* prevalence for prechill samples. The odds of *Salmonella* for prechill samples were 3.96 times greater for birds treated with AviPro®Megan® compared to birds treated with gentamicin (Merck's Garasol 0.2mg/chick).

Table 5. The total number and percentage of positive and negative samples for *Salmonella* collected at prechill.

Pre Chill			
Locations	Negative	Positive	Total
Location1	4, 304	1, 769	6, 073
Location2	4, 216	4, 219	8,435
Total	8,520	5, 988	14,508
Percent	58.72%	41.28%	100.00%

Table 6. Odds ratios and confidence intervals from bivariable logistic regression analysis of the prevalence of *Salmonella* for prechill samples by location, season, fans, misters, wetting, preharvest intervention strategies, and hatchery vaccines.

	Prechill *Salmonella* Samples		
Independent Variable			
	OR	P-Value	95% CI
Locations			
Location 1	0.41	0.00	0.39-0.43
Location 2	2.43	0.00	2.27-2.61
Seasonality			
Winter	1.28	0.00	1.16-1.42
Spring	1.87	0.00	1.68-2.07
Summer	1.44	0.00	1.30-1.60
Fall	0.50	0.00	0.46-0.54
Preharvest Treatment			
No Treatment	0.54	0.00	0.51-0.58
Water Treatment	1.20	0.03	1.02-1.40
Litter Treatment	2.34	0.00	2.11-2.59
Feed Treatment	2.96	0.00	2.51-3.47
Water and Litter Treatment	0.25	0.00	0.21-0.30
Feed and Litter Treatment	2.39	0.00	2.08-2.75
Hatchery Vaccines			
Gentamicin	0.57	0.00	0.54-0.60
AviPro®Megan®	3.96	0.00	3.51-4.47
Gentamicin/AviPro®Megan®	1.15	0.00	1.07-1.23

Multivariate logistic regression

Based on the bivariable logistic regression results, a 2-way full factorial multivariate logistic regression model was explored for the effect of season and location on *Salmonella* prevalence at prechill. *Salmonella* prevalence at prechill for Location 2 was significantly higher than in Location 1 across all four seasons (Figure 11). While at Location 1, the prevalence of *Salmonella* was significantly higher in the spring compared to the summer and fall. For Location

2, the *Salmonella* prevalence was significantly higher in the spring compared to the fall and winter (Figure 11).

The effects of preharvest interventions on *Salmonella* prevalence were examined using a 2-way full factorial multivariate logistic regression model independently by each location due to the preharvest interventions being unique for each location. We found that at Location 1, the prevalence of *Salmonella* was lowest for the combined treatment of water and litter and was significantly lower than the other treatments in the winter, spring, and fall (Figure 12). We also found that the *Salmonella* prevalence was not significantly different across seasons with no treatment, and during the summer, there was no significant difference between treatment groups (Figure 12). For Location 2, there was a significant difference between treatments in the summer, with feed treatment having the lowest *Salmonella* prevalence and litter treatment having the highest prevalence (Figure 13). In the spring, there was also a significant difference between treatment groups; however, the *Salmonella* prevalence was higher for feed treatment than litter treatment (Figure 13).

We observed the effect of hatchery vaccines on *Salmonella* prevalence by seasons and location using a 3-way full factorial model. An important note is that at Location 1, AviPro®Megan® was not used alone but in combination with gentamicin. Across all four seasons, there was an effect of treatment at Location 1, with a lower *Salmonella* prevalence observed for gentamicin treatment compared to the combination of gentamicin (Merck's Garasol 0.2mg/chick) and AviPro®Megan®) treatment (Figure 14). For Location 2, *Salmonella* prevalence was significantly lower for gentamicin use compared to the use of AviPro®Megan®) in spring, summer, and fall (Figure 14). We also saw a significant difference in *Salmonella*

prevalence between locations, with a higher prevalence observed in Location 2 compared to Location 1 for the gentamicin treatment groups (Figure14).

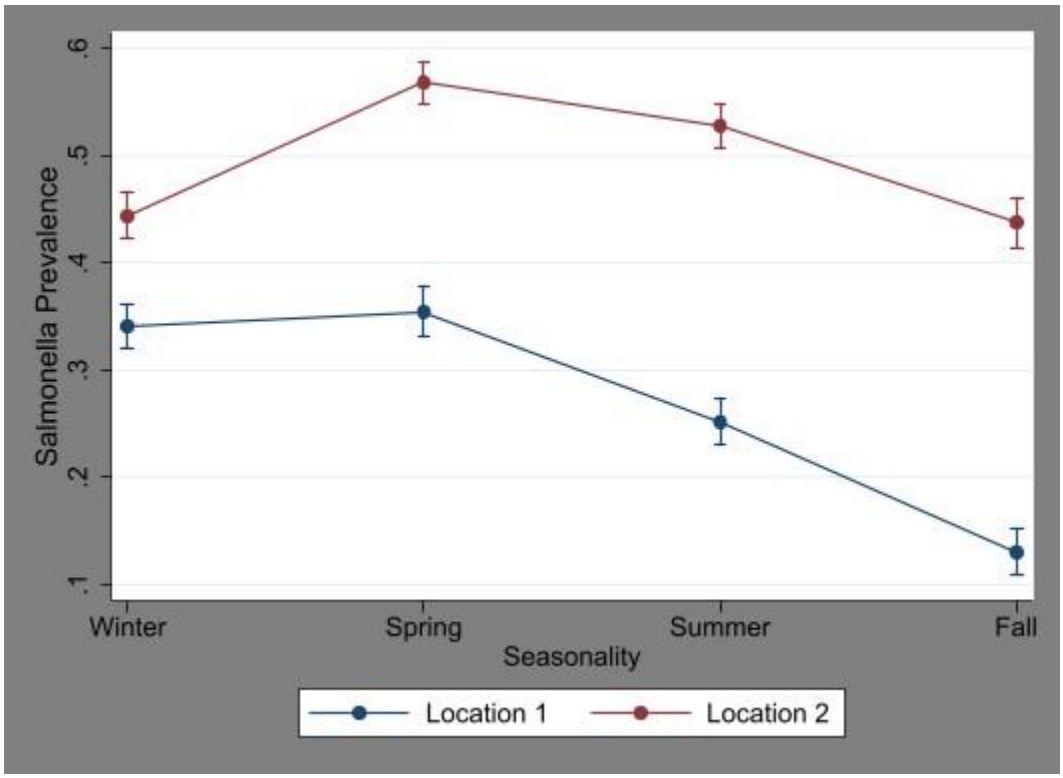

Figure 11. Marginal means graph from a 2-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at prechill across seasons and location.

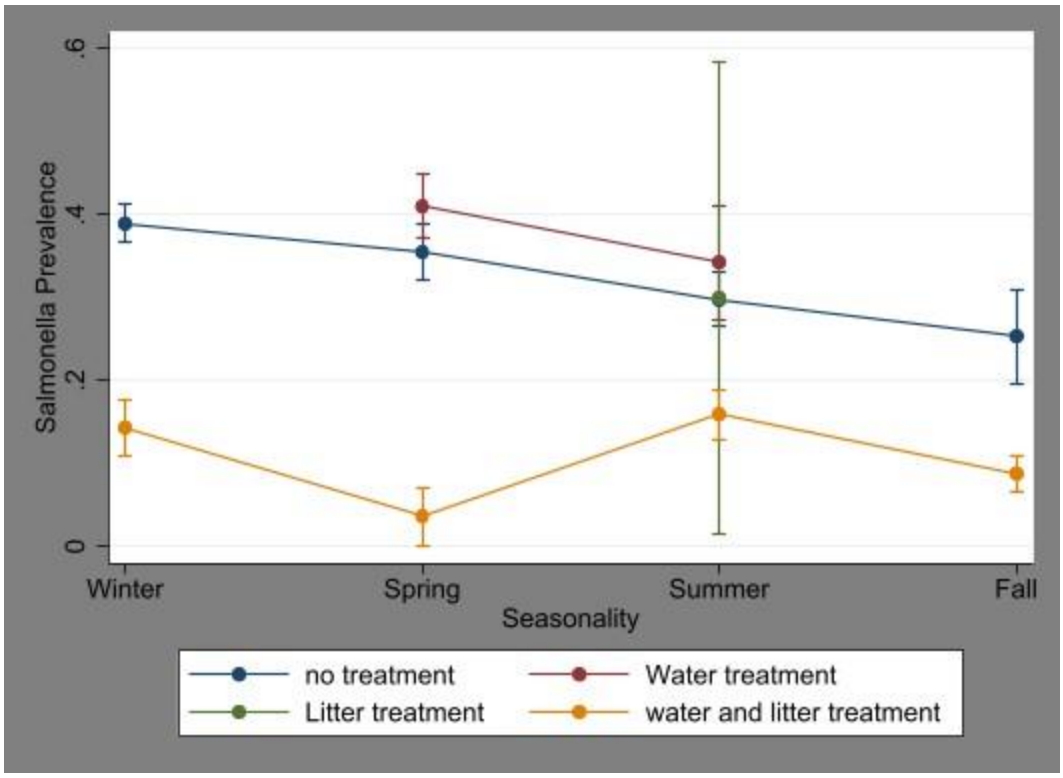

Figure 12. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at prechill for Location 1 for preharvest intervention strategies across seasons.

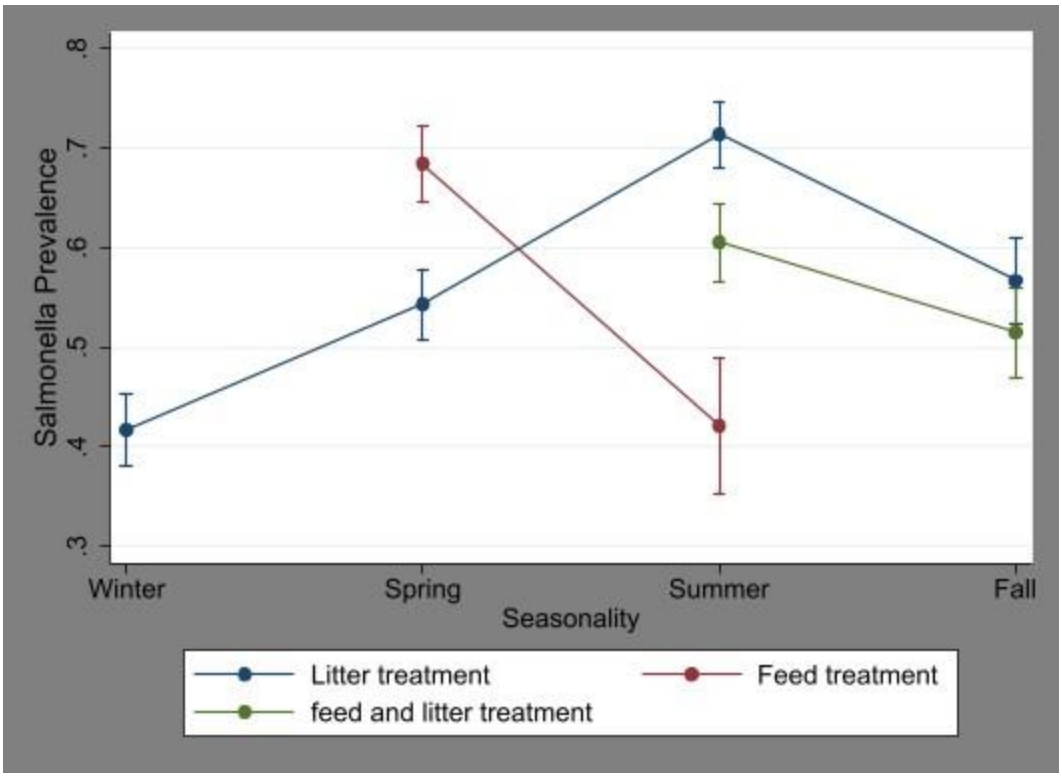

Figure 13. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at prechill for Location 2 for preharvest intervention strategies across seasons.

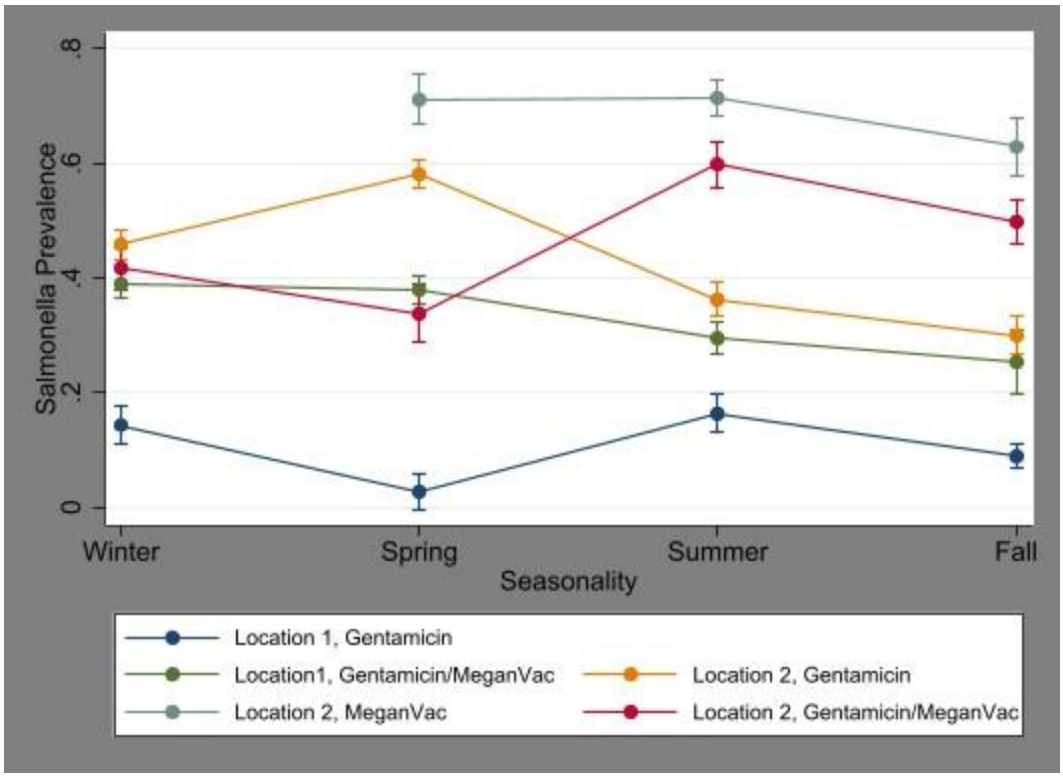

Figure 14. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing administration of hatchery vaccines on *Salmonella* prevalence at prechill across seasons by location.

4.1.4 Post Chill Salmonella

Descriptive Statistics

There were 16,723 samples collected for *Salmonella culturing* at post-chill. There were 16,217 samples collected that were negative, and 506 there were positive for *Salmonella* with a prevalence of 3.03% (Table 8). The *Salmonella prevalence* at Location 1 was 5.34% and 0.90% at Location 2 (Table 8).

Bivariable logistic regression

Bivariable logistic regression analysis was conducted to assess the association of location and season with *Salmonella prevalence* for post chill samples (Table 9). We found that location was significantly associated ($p<0.05$) with *Salmonella prevalence* for post chill samples and that the odds of *Salmonella for* post chill samples were 6.18 times greater in Location 1 than in Location 2. We found that season was also significantly associated ($p<0.05$) with *Salmonella prevalence* for post chill samples. The odds of *Salmonella for* post chill samples were 4.73 times greater in winter, 1.80 times greater in spring, and 1.25 times greater in summer, compared to fall. For this model, preharvest interventions and hatchery vaccines were not analyzed with post chill samples because other interventions are introduced to the process at this step.

Table 7. The total number and percentage of positive and negative samples for *Salmonella* collected at post chill.

Post Chill	Negative	Positive	Total
Location1	7,564	427	7,991
Location2	8, 653	79	8,732
Total	16, 217	506	16, 723
Percent	96.97%	3.03%	100.00%

Table 8. Odds ratios and confidence intervals from bivariable logistic regression analysis of post chill *Salmonella* prevalence by location and seasons.

	Post Chill *Salmonella* Samples		
Independent Variable			
	OR	P-Value	95% CI
Locations			
Location 1	6.18	0.00	4.85-7.88
Location 2	0.01	0.00	0.01-0.01
Seasonality			
Winter	4.73	0.00	3.41-6.57
Spring	1.80	0.00	1.26-2.58
Summer	1.25	0.25	0.86-1.82
Fall	0.01	0.00	0.01-0.02

Multivariate logistic regression

From the bivariable logistic regression results, a 2-way full factorial multivariate logistic regression model was explored to determine the effects of season and location at post chill on

Salmonella prevalence. We found a significant difference in *Salmonella prevalence* between locations across the seasons, with a higher prevalence observed at Location 1 (Figure 15). We also found at Location 1 there was a significantly higher *Salmonella* prevalence in the winter compared to the other seasons (Figure 15).

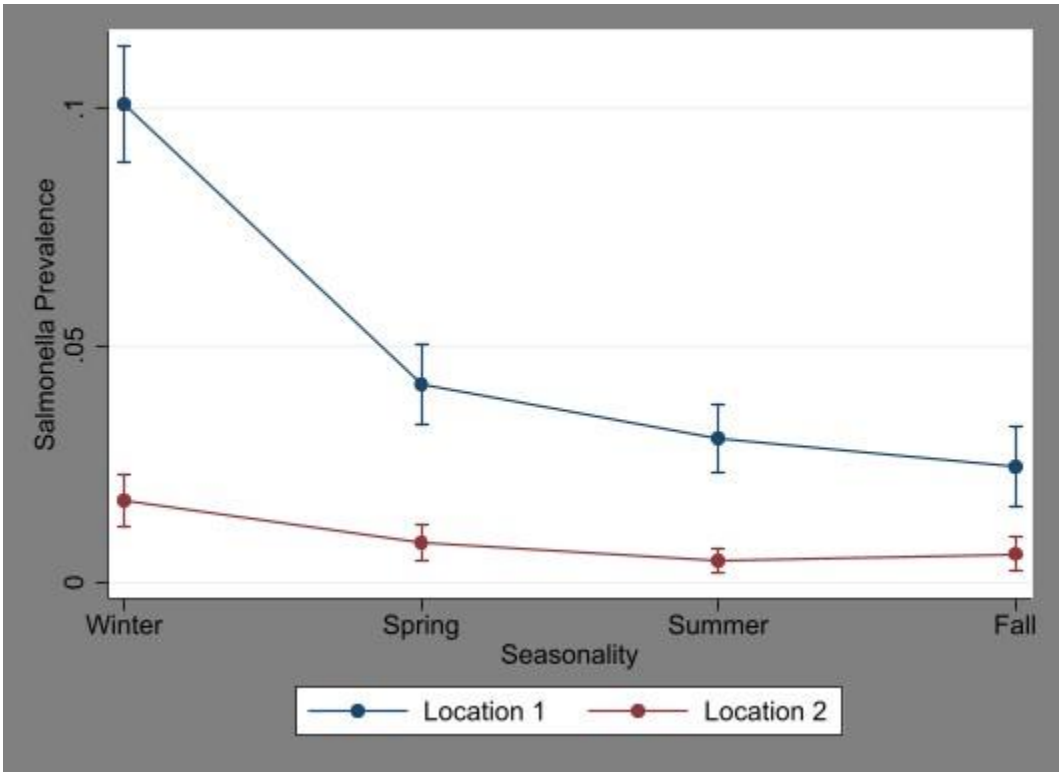

Figure 15. Marginal means graph from a 2-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence at post chill across seasons and location.

4.1.5 Chicken Parts

Descriptive Statistics

There were 8,467 poultry parts collected from January 2017 to December 2019 from the two locations. The parts collected included thighs (718), drumsticks (740), wings (1,724), tenders (1,721), breast (1,678), leg quarters (232), giblets (1,252) (livers, gizzards, and hearts), and necks (402) (Table 9).

Bivariable logistic regression

We found using bivariable logistic regression that the *Salmonella prevalence* of chicken parts was significantly associated ($p<0.05$) with the location. The odds of *Salmonella at* Location 1 were 3.82 times higher than at Location 2. We found that season was also significantly associated ($p<0.05$) with *Salmonella prevalence* in chicken parts. The odds of *Salmonella for* chicken parts were 2.24 times greater in spring, 1.53 greater in winter and 1.12 greater in summer compared to fall (Table 10). Additionally, the chicken parts were categorized according to the FSIS Raw Chicken Parts Sampling Program (USDA, 2016) (Table 9).

Table 9. The total number of chicken part samples collected by location and FSIS testing category.

Locations	Thighs	Drumsticks	Wings	Tenders	Breast	Leg Qtr.	Giblets	Necks
Location 1	438	455	1,151	1,146	1,136	232	851	278
Location 2	280	285	573	575	542	0	401	124
Total	718	740	1,724	1,721	1,678	232	1,252	402
FSIS*	1	1	1	1	1	1	0	0

1=FSIS
0=non-FSIS

Table 10. Odds ratios and confidence intervals from bivariable logistic regression analysis of *Salmonella* prevalence of chicken parts by location, season, and FSIS testing.

	Chicken Parts Samples		
Independent Variable			
	OR	P-Value	95% CI
Locations			
Location 1	3.82	0.00	3.02-4.81
Location 2	0.04	0.00	0.03-0.05
Seasonality			
Winter	1.53	0.00	1.20-1.95
Spring	2.24	0.00	1.79-2.79
Summer	1.12	0.34	0.88-1.43
Fall	0.76	0.00	0.06-0.09
FSIS and non-FSIS			
FSIS	0.05	0.00	0.05-0.07
Non-FSIS	16.73	0.00	14.13-19.82

Multivariate logistic regression

Based on the bivariable logistic regression results, a 2-way full factorial multivariate logistic regression model was explored for the effect of season and location on *Salmonella* prevalence of chicken parts. We found that *Salmonella* prevalence was significantly higher at Location 1 across all four seasons (Figure 16). Figure 17 shows the effect of season on *Salmonella* prevalence for FSIS and non-FSIS tested parts. We found that *Salmonella* prevalence in non-FSIS tested parts is significantly higher than *Salmonella* prevalence in tested FSIS parts across all four seasons. Additionally, a 3-way full factorial multivariate logistic regression model including season, location, FSIS, and the non-FSIS testing category was explored for *Salmonella* prevalence of chicken parts. We found that *Salmonella* prevalence was

significantly higher for non-FSIS tested parts across all seasons for Location 1 and in the spring, summer, and fall for Location 2 (Figure 18). We also found a significant difference in prevalence between locations for FSIS test parts in the spring and summer and for non-FSIS tested parts in the winter, summer, and fall (Figure 18). Finally, we ran a 3-way full factorial multivariate logistic regression model including season, location, and chicken parts category bone-in, boneless, and giblets. We found that *Salmonella* prevalence in giblets was significantly higher than in the other chicken parts in the spring, but there was no significant difference between locations (Figure 19). *Salmonella* prevalence was not significantly different in the other chicken parts (bone-in or boneless) across seasons for either location (Figure 19).

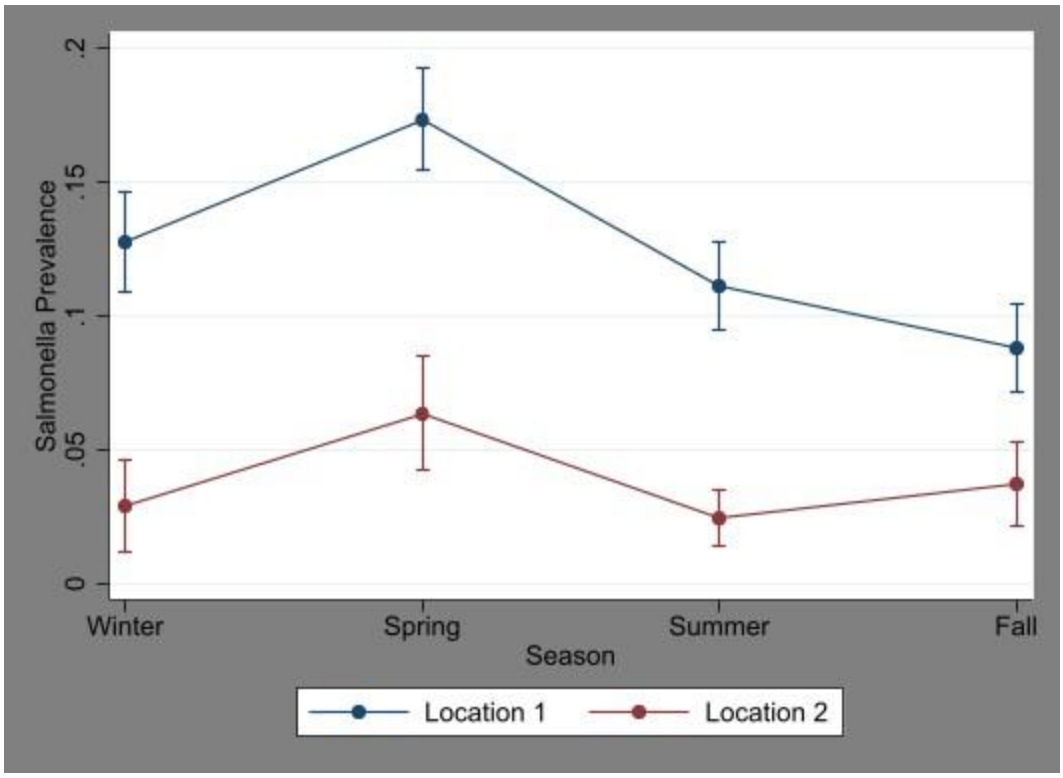

Figure 16. Marginal means graph from a 2-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence for chicken parts across seasons and location.

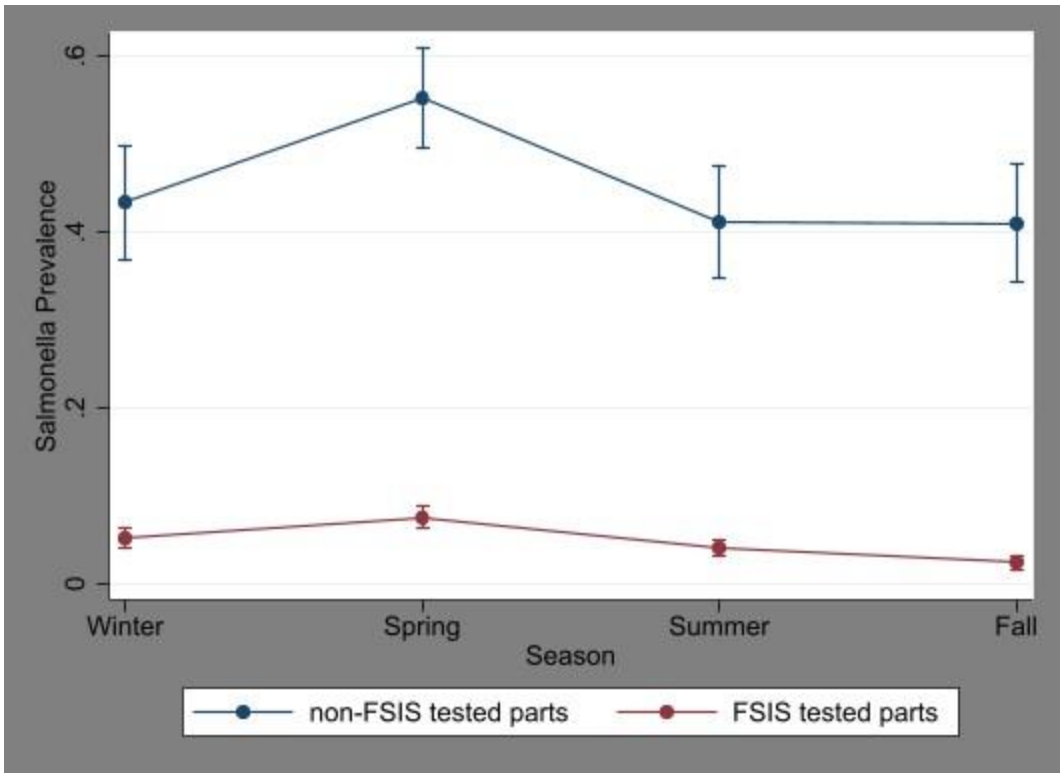

Figure 17. Marginal means graph of *Salmonella* prevalence for FSIS and non-FSIS tested parts across seasons.

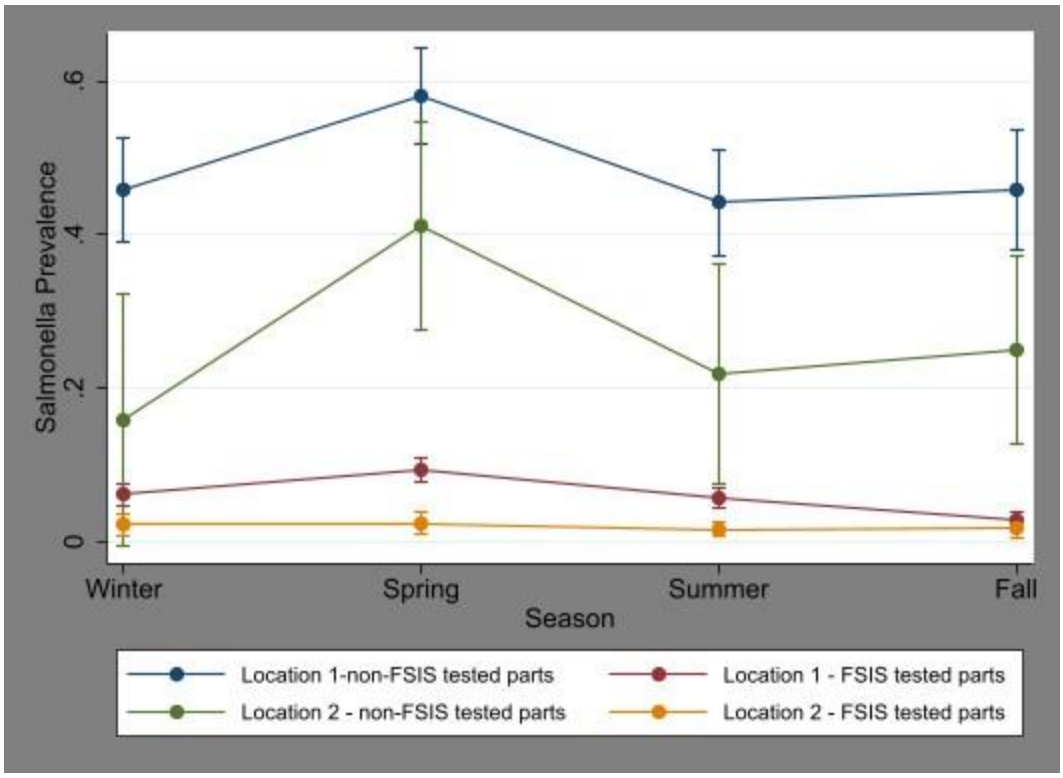

Figure 18. Marginal means graphs from multivariate logistic regression model comparing *Salmonella* prevalence in parts across seasons by location and FSIS and non-FSIS tested parts.

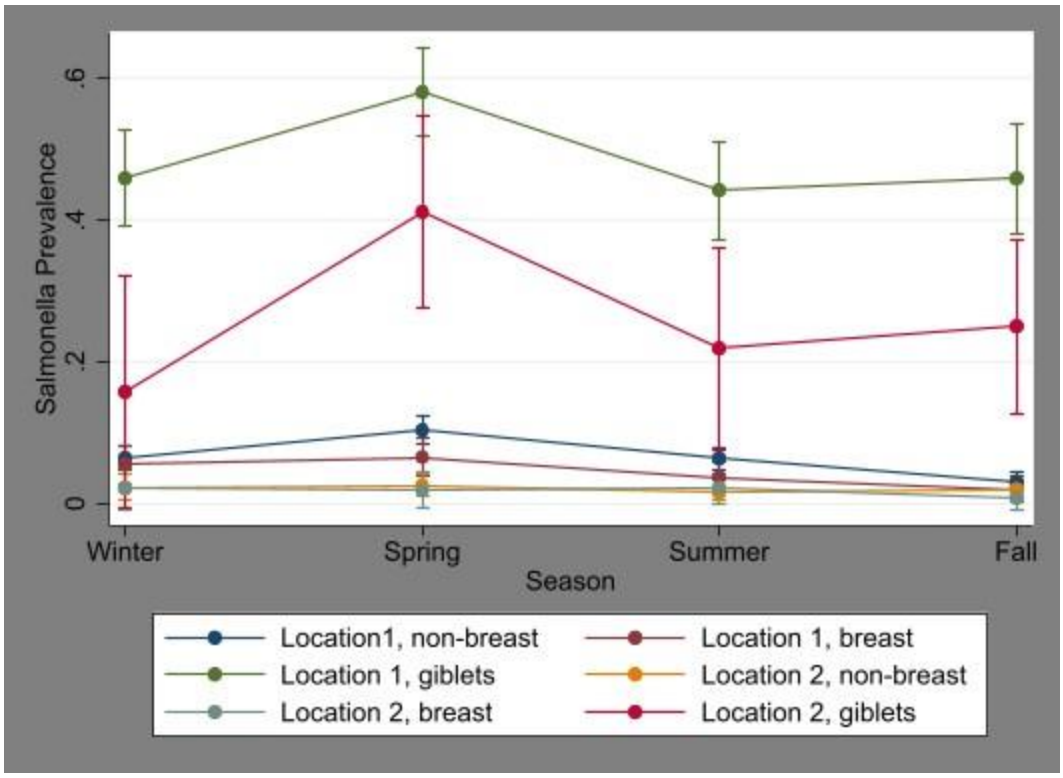

Figure 19. Marginal means graphs from multivariate logistic regression model comparing *Salmonella* prevalence in parts for the season, location, and parts categories.

4.1.6 Boot swabs

Descriptive Statistics

Boot swabs are environmental samples collected to test for pathogens, including *Salmonella.* The boot swabs were collected from various houses within the two locations. There were 3,087 boot swabs samples collected from both Locations 1 and 2. For Location 1, the *Salmonella prevalence* was 68.39%, and in location 2, the *Salmonella prevalence* was 87.75% (Table 11).

Bivariable logistic regression

Bivariable logistic regression was used to determine the effects of location, season, preharvest interventions, and hatchery vaccines on the *Salmonella* prevalence of boot swabs. We found that location was significantly associated ($p<0.05$) with *Salmonella* prevalence of boot swabs samples, and the odds of *Salmonella* at Location 2 were 3.31 times higher than at Location 1. We found that season was also significantly associated ($p<0.05$) with *Salmonella* prevalence of boot swab samples. The odds of *Salmonella* were 6.73 greater in spring, 5.77 greater in winter, and 1.02 greater in summer than fall.

Additionally, bivariable logistic regression was used to determine if *Salmonella* prevalence of boot swabs was associated with the *Salmonella prevalence* at hot rehang and prechill. We found that *Salmonella* positive boot swabs were significantly (p,0.05) associated with Salmonella prevalence at hot rehang and prechill. The odds of *Salmonella* at hot rehang were 1.46 times greater, and the odds of Salmonella at prechill were 1.48 times greater when positive boot swabs were reported.

Table 11. The total number and percentage of positive and negative *Salmonella cultured* from boot swabs.

Boot swabs

Location	Negative	Positive	Total
Location 1	269	582	851
Percentage	31.61%	68.39%	100%
Location 2	274	1,962	2, 236
Percentage	12.25%	87.75%	100%
	543	2, 544	
Total	17.59%	82.41%	3, 087

Table 12. Odds ratios and confidence intervals from bivariable logistic regression analysis of *Salmonella* prevalence in boot swab samples by locations, seasons, hatchery vaccines, and pre-harvest interventions.

Boot swabs *Salmonella* Samples			
Independent Variable			
	OR	P-Value	95% CI
Locations			
Location 1	2.16	0	1.9-2.5
Location 2	3.31	0.00	2.73-4.0
Seasonality			
Winter	5.78	0.00	4.23-7.88
Spring	6.73	0.00	5.0-9.2
Summer	1.02	0.85	0.88-1.35
Fall	1.72	0.00	1.38-2.25
Preharvest Treatment			
Water Treatment	0.64	0.09	0.38-1.08
Litter Treatment	4.90	0.00	2.80-8.68
Feed Treatment	19.67	0.00	10.76-35.95
Water and Litter Treatment	3.76	0.00	2.18-6.49
Feed and Litter Treatment	1.49	0.18	0.83-2.69
Hatchery Vaccines			
Gentamicin	1.91	0.00	1.33-2.72
AviPro® Megan®	4.75	0.00	3.43-6.57
Gentamicin/AviPro®Megan®	0.28	0.00	3.43-6.57

Multivariate logistic regression

Based on the bivariable logistic regression results, a 2-way full factorial multivariate logistic regression model was explored for the effect of season and location on *Salmonella* prevalence for boot swab samples. Location 2 had a significantly higher *Salmonella prevalence* for boot swab samples in winter and spring than Location 1 (Figure 20). The effects of preharvest interventions on *Salmonella* prevalence were examined using a 2-way full factorial multivariate logistic regression model independently by each location due to the preharvest interventions being unique for each location. For Location 1, we found no significant difference in the *Salmonella* prevalence between treatment groups across the seasons (Figure 21). For Location 2, we found that *Salmonella prevalence* was significantly higher for feed treatment than litter treatment in the spring; however, in the summer, *Salmonella* prevalence was significantly higher for litter treatment than the other treatments (feed/litter and feed) (Figure 22). We observed the effect of hatchery vaccines on *Salmonella* prevalence by seasons and location using a 3-way full factorial model. An important note is that at Location 1, AviPro®Megan®) was not used alone but in combination with gentamicin (Merck's Garasol 0.2mg/chick). We found that for Location 2, the prevalence of *Salmonella* was significantly higher for gentamicin treatment in the spring and AviPro®Megan® treatment in the summer (Figure 23).

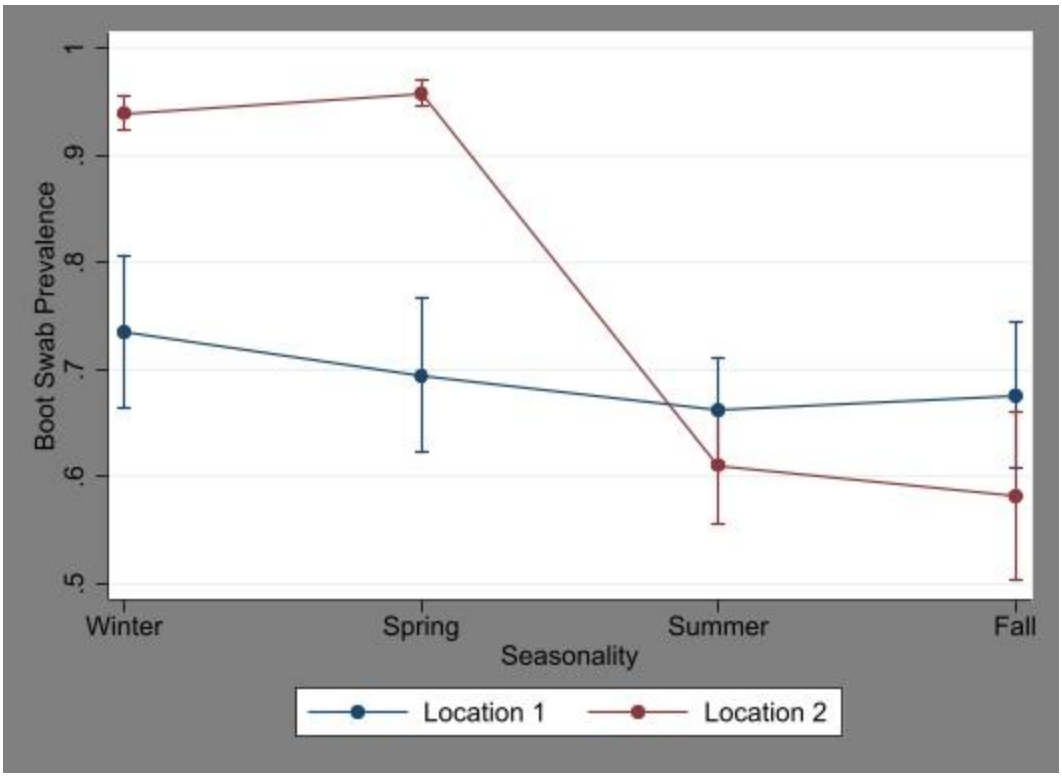

Figure 20. Marginal means graph from a 2-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence of boot swab samples across seasons and location.

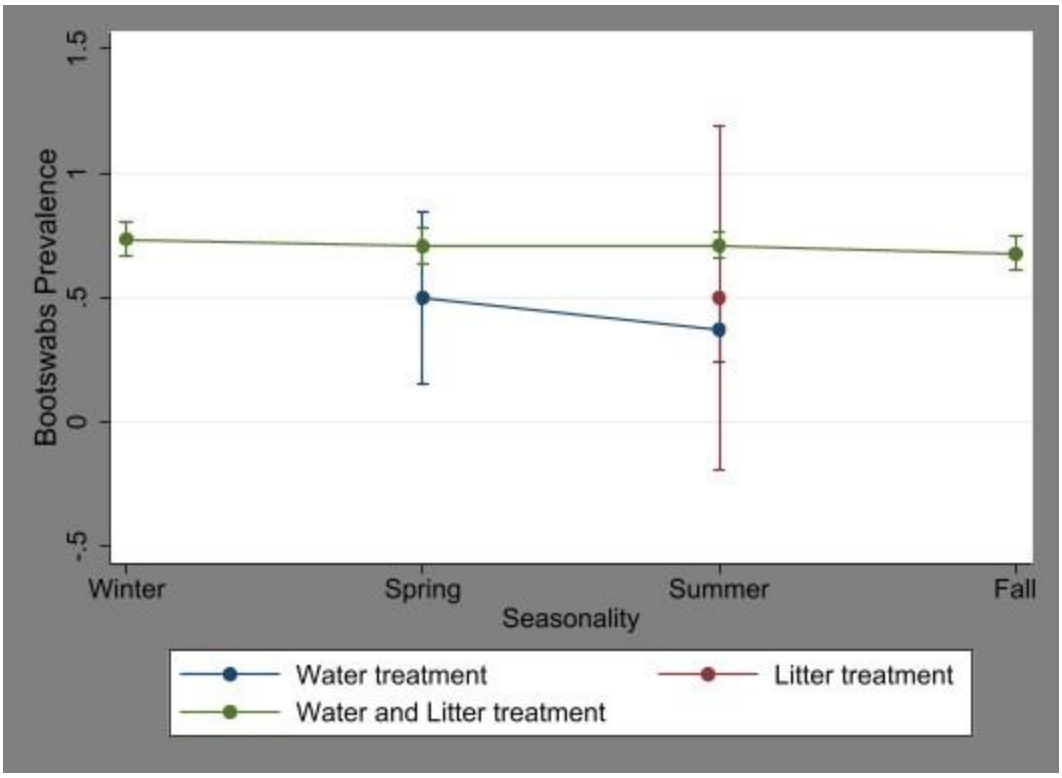

Figure 21. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence of boot swab samples for Location 1 for preharvest intervention strategies across seasons.

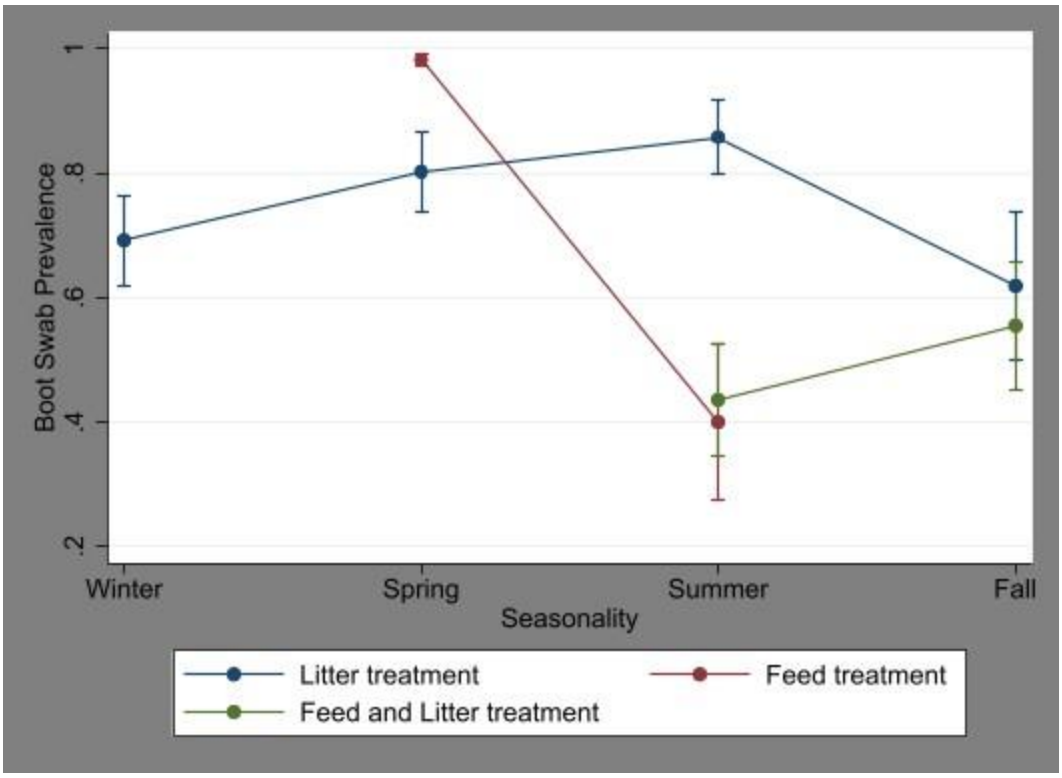

Figure 22. Marginal means graphs from a two-way full factorial multivariate logistic regression model comparing *Salmonella* prevalence of boot swab samples for Location 2 for preharvest intervention strategies across seasons.

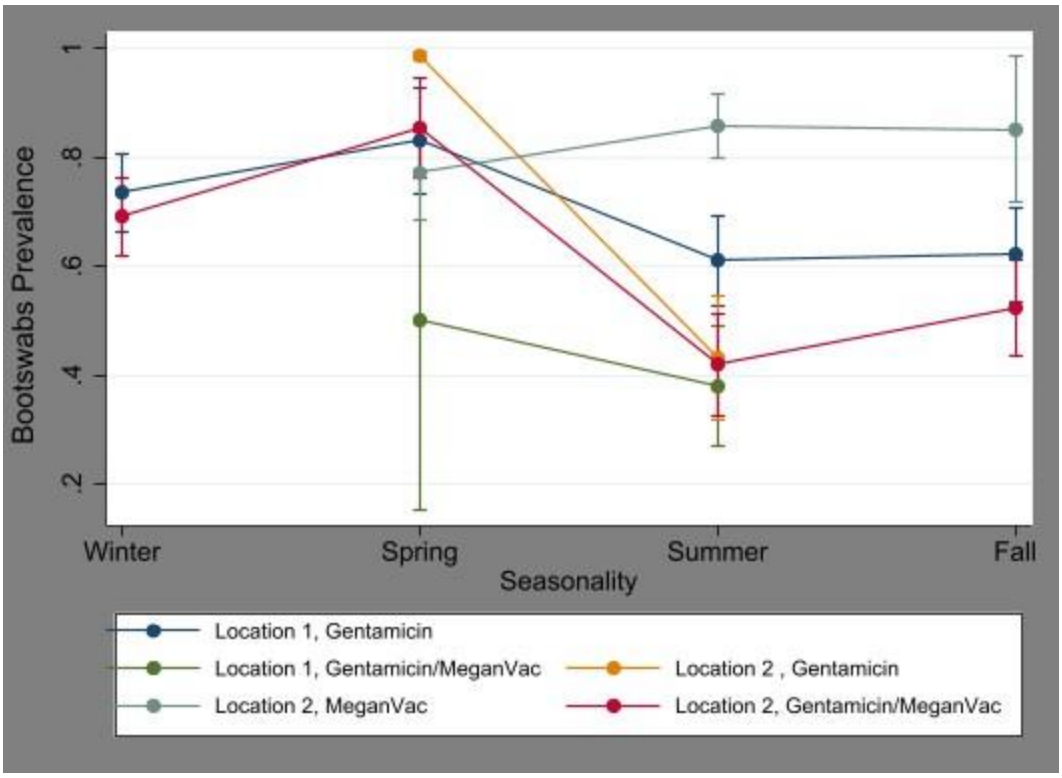

Figure 23. Marginal means graph from a 3-way full factorial multivariate logistic regression model comparing administration of hatchery vaccines on *Salmonella* prevalence of boot swab samples across seasons by location.

CHAPTER V

DISCUSSION

For many years, scientists have been steadily studying the evolution of the bacteria, *Salmonella enterica* subspecies *enterica.* Researchers have spent countless hours developing interventions to reduce the prevalence of *Salmonella* in agricultural animals. This study used two broiler production and processing complexes from different geographical regions to explore the effects of preharvest and post harvest intervention strategies on *Salmonella* prevalence. In this research, samples were collected from boot swab samples, hot rehang, prechill, post chill, and chicken part samples to evaluate interventions used in preharvest and post harvest to determine if there is an impact on the reduction of *Salmonella*. The samples were analyzed by comparing independent variables such as temperatures, humidity, seasons, fans and misters, antibiotics, vaccines, preharvest interventions (feed, litter, and water treatments), and post harvest interventions.

There were many factors in play when analyzing these samples. Locations 1 and 2 were sometimes similar in temperature, but the relative humidity was tough to differentiate. The graph shows that both humidity and temperature are right skewed, indicating a trend towards higher temperatures and relative humidity. We found that location was significantly ($p<0.05$) associated with *Salmonella* prevalence for all sample types collected. The total number of collected samples was not the same between Locations 1 and 2 for the different sample types. There was a 2,672 sample difference between the locations for collecting hot rehang samples. At Location 1, there were 51.36% positive and 53.98% positive at Location 2. The number of positives between both locations was approximately the same. The number of prechill samples collected was 14,508, with a 2 362 difference between both locations. Location 1 had 20.13% positives,

whereas Location 2 had a prevalence of 50.02%. Lastly, there were 16,723 collected samples at post chill with a 741 difference in the collection between the locations. The positive percentage at Location 1 was 5.34%, and 0.90% at Location 2. It was evident with the percent positive there was a significant decrease from hot rehang samples to post chill samples. This evidence proved that throughout the process, there was a decrease in the prevalence of *Salmonella*, which proves that the post harvest interventions in place were adequate.

The study evaluated four seasons: fall, winter, spring, and summer. Each season was evaluated by calendar date during those years of the study. It was observed that as temperatures increase with the seasons, the prevalence of *Salmonella* also increases. The results show within a two year window that *Salmonella* prevalence remains high in the spring but significantly decreases in the fall. As mentioned previously, the results support the study on metrological factors by Hwang and Wales that increasing temperatures impact the prevalence of *Salmonella* (Hwang et al., 2020; Wales et al., 2007). Even though the geographical regions evaluated do not have a "true" four seasons because the temperature remains high for over half the year, it potentially justifies the increase of *Salmonella* seen throughout the seasons (Hwang et al., 2020). In the months such as fall and winter, where temperatures should be cool to cold, the temperatures showed warmer temperatures typically seen in the spring and summer months.

All preharvest interventions, hatchery vaccines, seasons, fans, misters, and wetting were evaluated for hot rehang samples. During this study, hot rehang samples were expected to have high *Salmonella* prevalence because they came directly from the chicken houses, and antimicrobial applications were not applied. At hot rehang during the time of this study, there were no post harvest interventions introduced in the processing facility. Preharvest treatments and hatchery vaccines continued to be evaluated at prechill. Prechill samples were collected

before birds were eviscerated. At this step, it was the first introduction of an antimicrobial at the processing facility.

Studies and research have determined that the gut and ceca are the most prominent areas for *Salmonella* to reside in the bird. These anatomical parts of the bird are an ideal environment for bacterial development. (Ijaz et al., 2021). The microorganisms in the gut are conducive to *Salmonella* and its ability to thrive in that condition. Understanding the dynamics of *Salmonella* colonization in poultry is necessary because it provides targeted areas when developing interventions to reduce *Salmonella* prevalence. The interventions used in this study were Poultry Litter Treatment PLT®, poultry water treatment (PWT®), OPTI-BAC® (Probiotic), gentamicin (Garosol®, Merck Animal Health), and AviPro® Megan® (Vaccine). Preharvest interventions were evaluated at hot rehang, prechill, and boot swab samples. When evaluating preharvest interventions during hot rehang, it was discovered that preharvest interventions were not used simultaneously at each location, nor did the two locations use the same interventions.

PLT® treatment was applied to litter while the birds were in the houses. This treatment was designed to reduce the house's pH or ammonia levels and create an environment that reduces the prevalence of bacteria. This treatment is not uncommon in the industry. Based on this study, hot rehang carcass samples do not show a reduction in *Salmonella* with litter application. When assessing prechill carcass samples, there was a decrease in prevalence at Location 1 when litter and water treatments were used together. At Location 2, there was an increase in prevalence when the litter was used as a single treatment. In boot swabs samples, there were no changes in prevalence when using litter treatment alone or with water and feed.

Nonetheless, when the litter treatment was used with feed treatment at Location 2, there was a slight decrease in the prevalence of *Salmonella* from summer to fall. From the results seen

in this study, it would appear that the use of the litter treatment aided in the reduction of *Salmonella* for some sample types and locations; however, the results were not consistent, and the combination of litter treatment and water treatment resulted in a more significant reduction in *Salmonella* than litter treatment alone. A survey by (Terzich et al., 2000) showed that when samples were collected from poultry litter, and there was a low pH, the bacterial counts were also low. A study on litter treatment in a controlled environment suggested that reducing the litter's pH level will reduce *Salmonella's* prevalence (Payne et al., 2007). The study was not conducted on commercial litter but determined the results could be beneficial. Both studies support that lowering the pH level can potentially reduce the bacterial load in the litter, including *Salmonella.* This concludes that the reduction of *Salmonella* when using the litter treatment was from the lowering of the pH level in the litter to a level of 4 or less (Payne et al., 2007; Williams et al., 2012)

PWT® is a water treatment provided to the birds prior to catching. PWT® was only applied at Location 1. Previous research has shown that the best results from water treatment occur from birds being given PWT® after feed withdrawal (J. Byrd et al., 2003). The treatment comes in many acid forms, including lactic acid, sodium bisulfate, formic acid, and acetic acid. The treatment is designed to neutralize the bird's crop, reducing bacteria and other contamination, including *Salmonella.* The results showed a significant increase in *Salmonella* prevalence at hot rehang from spring to summer when water and litter treatment were used together, but there was a decrease by fall. Also, the prevalence of *Salmonella* was significantly less for water and litter treatment compared to no treatment in the spring. Seasons and temperatures play an intricate part in *Salmonella* prevalence, but these factors can not be controlled. Seasons and temperatures are inevitable. The results from this discovery support

research that shows that as temperatures increase, *Salmonella* prevalence increases (Stephen & Barnett, 2016). This explains the significant increase of *Salmonella* from spring to summer because the temperatures are rising with the seasons. As fall approaches, temperatures decrease, which in theory slows the potential growth of *Salmonella.* Although we observe a decrease in *Salmonella* prevalence for water and litter treatment compared to no treatment in the spring and winter, these interventions are inadequate during higher temperatures in the summer months.

OptiBac® is a feed treatment in the form of a probiotic and was only used at Location 2 during the study. The probiotic is given to the birds to aid with gut health. The probiotic is designed to maintain a healthy gut flora by introducing good bacteria inside the bird. At Location 2, the prevalence of *Salmonella* was significantly higher in the spring when feed treatment was administered than litter treatment. In comparison, *Salmonella* prevalence was significantly lower in the summer when feed treatment was administered with litter treatment or the combination of litter and feed treatment. Information was not collected during this study on when the probiotic was administered. In future research, providing a more definitive time frame to administer the product will be beneficial. It was previously studied that when a probiotic was administered to chicks, specifically *Lactobacillus,* it created an environment that allowed the gut flora to be less prevalent for *Salmonella* (Chen et al., 2020). As with litter treatment, it appeared that feed treatment did affect the *Salmonella* prevalence; however, the effect varied by season.

Gentamicin is an antibiotic used to treat many bacterial infections, including *Salmonella.* In this study, hatchery vaccines were given to all chicks at the day of age. The vaccines consisted of gentamicin (Garasol®) and AviPro® Megan®. AviPro® Megan® is a vaccine designed to reduce *Salmonella* prevalence and specifically targets serovar, Typhimurium. This particular vaccine was a live vaccine, which means the vaccine contains an active form of *S.* Typhimurium.

Using a live vaccine is beneficial when developing immunity against bacteria because it allows the immune system to develop a memory of the bacterial strain in the vaccine (Aehle & Curtiss, 2017). The disadvantage of a live vaccine is the possibility of the bacteria mutating (Rendi-Wagner & Kollaritsch, 2008). Gentamicin usage has been minimized throughout the poultry industry because of its usage in human medicine. AviPro® Megan® was only used alone at Location 2, and at Location 1, AviPro® Megan® was used in combination with gentamicin. When AviPro® Megan® was used by itself, there was no reduction in *Salmonella*. When gentamicin and AviPro® Megan® were used at Location 1, the *Salmonella* prevalence was significantly increased in the summer compared to the use of gentamicin alone; however, there was no significant difference in the *Salmonella* prevalence in the fall between the use of gentamicin and AviPro® Megan® or gentamicin alone.

At Location 2, when gentamicin was used alone, there was a significant decrease in *Salmonella* prevalence in the summer compared to gentamicin and AviPro® Megan®. In the study, it was evident that, alone, AviPro® Megan® was not effective, but when used with gentamicin, it showed a reduction in *Salmonella* prevalence. Due to proprietary information that can not be disclosed, when AviPro® Megan® was applied, *S.* Typhimurium decreased. However, another serovar increased. Therefore, the vaccine may have reduced the targeted serovar, but other serovars not targeted by the vaccine were still detected and contributed to the prevalence. This may be why a decrease or no change in *Salmonella* occurred when using the vaccine. However, the vaccine is reported to not only target *S.* Typhumirum but also *S.* Heidelberg and *S.* Enteritidis.

Lastly, a factor to consider is the vaccine is given to the chicks as a spray. When the spayed is applied, all birds may not receive the dosage. Therefore, it is ideal that the bird's intake

the vaccine by pecking it from other chicks and receive it by oral intake. When the second vaccine dose is given at 14 days of age, it is applied by spray. The spray can potentially contact the litter, which will affect the environment. The vaccine is designed to activate the birds' immune systems to react to the vaccine to develop a response (Washington University, 1998). However, the implications of the vaccine on *Salmonella* in the environment have not been explored. Changes to *Salmonella* in the environment can also affect the host.

Fans, misters, and wetting were only evaluated during hot rehang. Using fans, misters, and wetting was based on a program designed by the complex. Both complexes during this study were on the same program. When temperatures reached 65°F, fans were used. Misters and wetting are used as temperatures increase or based on the birds' comfort. It is evident when fans, misters, and wetting are used that the prevalence of *Salmonella* is significantly increased throughout the seasons. This is because fans, misters, and wetting are highly correlated with the temperatures and seasons. Based on previous research, it is known that the prevalence of *Salmonella* is higher in the hotter months (Stephen & Barnett, 2016). Based on the results, when temperatures did not require fans, misters, or wetting, the prevalence of Salmonella decreased in the summer at Location 1 but remained the same at Location 2. At Location 1, when fans were used, *Salmonella* remained steady with a significant decrease in the fall, but at Location 2, there was no significant difference in the *Salmonella* prevalence. When misters were implemented at Location 1, the *Salmonella* prevalence decreased from spring to summer to fall. At Location 2, there was an increase in *Salmonella* prevalence in the spring, and the *Salmonella* prevalence was significantly higher when misters were used. Observing the prevalence in the spring is important because this is typically the introduction to misters and possibly wetting, depending on the temperature and comfort of the birds.

After the birds were eviscerated, chilled by emersion, and cut up, the post chill and chicken part samples were collected. The birds underwent several intervention points when post chill samples were collected. The post chill sample is the last sample collected before entering the chiller by immersion for approximately 2.5 hours. At this point in the study, the intervention that will impact the prevalence of *Salmonella* are the antimicrobials applied in the plant; therefore, preharvest interventions and hatchery vaccines were not evaluated at post chill. However, seasons and locations were still important factors. The statistical data showed that Location 1 had a significantly higher prevalence of *Salmonella* than Location 2 for post chill samples. The *Salmonella* prevalence at Location 1 was significantly higher in the winter than in other seasons. At Location 2, the *Salmonella* prevalence was low and not significantly different across seasons. There is no research provided that justifies the increase in *Salmonella* prevalence in the winter. A potential hypothesis is that birds are kept in climate controlled houses and therefore *Salmonella* prevalence may not be as affected by the cooler temperatures.

Lastly, chicken parts were evaluated by non-FSIS parts and FSIS parts. Non-FSIS parts included any organs (livers, gizzards) and necks. FSIS parts included drumsticks, leg quarters, breast, bone-in, and bone-out. During statistical analyses, non-FSIS parts had a significantly higher *Salmonella* prevalence than FSIS tested parts. Parts such as bone-in and boneless are low in *Salmonella* prevalence because they have been through multiple intervention steps before sample collection, but the organs have not. Organs parts are not processed through any antimicrobial interventions. Based on the research, the giblets are the organ with the highest *Salmonella* prevalence during the spring.

Boot swabs are samples collected within 12 hours of a farm caught for slaughter. At this phase of analysis, both boot swabs and hot rehang samples represent all interventions used in

preharvest until interventions are applied at the processing Boot swab samples evaluate the effectiveness of all preharvest interventions and hatchery vaccines by season and location. As seen with other sample collections, the same number of boot swabs were not collected at Locations 1 and 2. Location 1 showed that water and litter preharvest interventions had no significant impact on *Salmonella* when used alone or in combination. A study on environmental samples showed that environmental swabs and carcass rinses taken from the same farm on the same day should yield the same *Salmonella* prevalence (Volkova et al., 2010). Even though this information is relevant, no study found correlated preharvest interventions to the reduction of *Salmonella* prevalence in boot swabs. It can be assumed that boot swabs samples may not be a good indicator of *Salmonella* prevalence because of the collection of the samples. The sample collection depends on a person's walking technique in the chicken houses. Moreover, evidence was unavailable during the study to determine if all farms or houses received the same treatment.

Hatchery vaccines at Location 1 showed that gentamicin used alone decreased *Salmonella* prevalence from spring to summer for boot swab samples. When the antibiotic was combined with AviPro® Megan®, *Salmonella* decreased from spring to summer. Location 2 showed that when gentamicin was used alone, there was a significant decrease in *Salmonella*. At location 2, when gentamicin was combined with AviPro® Megan®, it showed a decrease in *Salmonella* prevalence. The season with the most significant effect was spring. Boot swabs showed the most significant *Salmonella* reduction from hatchery vaccines compared to hot rehang samples. The difference in results between boot swabs and hot rehang may result from the processing facility. After the birds arrive at the processing facility, they come into contact with many factors that can contaminate them—shackling of the birds, the scalder with boiling water used to remove feathers from the birds, and the pickers. Since no antimicrobials are used in

the scalder or on equipment before hot rehang samples are taken, it allows for potential pathogen contamination between birds.

Based on this study, preharvest interventions and hatchery vaccines affected *Salmonella* prevalence. Because of the variability of the seasons and interventions, it is hard to determine which factor provided the better response to reduce the prevalence of *Salmonella*. The overall results were consistent between the locations. The increase of *Salmonella* from season to season indicates that the increase in temperature plays a part in *Salmonella* prevalence, which has previously been reported. Seasonality appeared to affect Salmonella prevalence more than preharvest treatments significantly. Additional research is needed to determine the effectiveness of preharvest interventions. Particular interventions may be more effective during particular seasons or at specific locations. When using AviPro® Megan® as a vaccine without gentamicin, there was an increase in *Salmonella* or no change in reduction based on location. Combining the vaccine and gentamicin showed a decrease in *Salmonella* at certain times, but there was no significance. The use of gentamicin alone decreased the prevalence of *Salmonella*; however, this antibiotic is no longer used in the industry for the control of *Salmonella*. The removal of antibiotics is not mandated but voluntary by the industry. As hot rehang samples were evaluated for *Salmonella,* the results were significantly high at the processing facility. However, when samples were collected at prechill and post chill, each processing step showed a significant decrease in *Salmonella*. The decrease in *Salmonella* is positive for the poultry industry because it proves that the postharvest interventions at the processing facility are working effectively. This study shows when post harvest interventions are used, there is a reduction in *Salmonella* prevalence in broilers. There are some promising results from the preharvest interventions and hatchery vaccines; however, further research is needed to determine which interventions are

most effective.

CHAPTER VI

CONCLUSION

Salmonella is a unique bacterium, and it was evident during this study. Several factors were found interesting in this research. From prechill to post chill carcass samples, *Salmonella* prevalence was consistently lower at Location 2 than at Location 1. It was apparent that the postharvest interventions resulted in a low prevalence of *Salmonella*. Cecure (CPC) and PAA were the antimicrobials used in the processing facilities when this study was conducted. At processing, carcass rinses were analyzed, non-FSIS and FSIS tested parts. The initial application of the antimicrobial did not begin until the evisceration process before the collection of prechill carcass samples. Even though an antimicrobial agent was applied, seasons were still analyzed throughout the remaining sample collections. Seasons remained a factor even through post chill carcass samples and chicken parts. The results showed that collecting part samples that included giblets (hearts, livers, gizzards) and necks had a higher *Salmonella* prevalence than other chicken parts (wings, leg quarters, breast, drumsticks). The vaccine gentamicin without the addition of AviPro® Megan® provided a constant *Salmonella* prevalence at an average rate, but the interventions applied with AviPro® Megan® provided a lower prevalence.

Another interesting observation was the increased *Salmonella* prevalence when broilers were applied to fans, misters, and wetting. Ironically these factors are needed to reduce dead on arrival (DOA) while on the farms and staged at the processing facilities, but coincidently aid in increasing *Salmonella* prevalence. This observation was more informative and provided evidence that many industry researchers considered a factor in *Salmonella,* but it is most likely due to season.

Salmonella will continuously be a concern to the food and poultry industry. This research study shows that the combined preharvest interventions may be more effective than single interventions. Further research is needed to determine which interventions significantly reduce *Salmonella* prevalence and would benefit poultry growers. As technology evolves, there will be new interventions and discoveries developed. In the meantime, continuing to apply interventions during preharvest and postharvest processing interventions will help reduce *Salmonella's* prevalence in broilers.

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