Environmental Factors and Management Practices that Influence *Salmonella* and *Listeria* Prevalence at the Sub-Field Level on an Eastern Shore of Virginia Farm

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ABSTRACT

Prior research has shown pathogen prevalence on-farms is not uniformly distributed, instead pathogen prevalence is highly dependent on environmental factors and management practices. A study was performed to determine environmental factors (e.g., landscape features, meteorological events) and management practices (e.g., date of last irrigation, pesticide application) that may impact the prevalence of *Salmonella* spp. and *Listeria* spp. at the sub-field level (0.2 ha grids) on an Eastern Shore of Virginia farm. Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC) farm was used due to the liability of testing for pathogens in commercial produce fields; however, production practices used at the ESAREC farm are similar, if not the same, to production practices used on commercial farms. Fifteen drag swab, one water, and up to five fecal samples were collected every two weeks per sampling occurrence from August 2016 to February 2017 (thus up to 21 samples may be collected during one sampling occurrence. Samples were collected from randomized field plots that were picked during each sampling occurrence. *Salmonella* spp. and *Listeria* spp. were isolated and confirmed using modified versions of the Food and Drug Administration's Bacteriological Analytical Manual. Environmental factors were retrieved by remotely-sensed data for the sample location or date. Management practices were recorded by an observational survey for each sample occurrence.

Two hundred and seventy-four samples (210 drag swab, 50 fecal, and 14 water samples) were collected during the late summer, fall, and winter. *Listeria* spp. and *Salmonella* spp. was detected in 8.3% (23/274) and 1.8% (5/274) of samples, respectively. Neither pathogen was detected in any of the fourteen water samples tested. Findings from this study will support the development of mitigation strategies to reduce pathogen contamination on-farm, with emphasis at the sub-field level. For instance, mitigation strategies include growers electing to not harvest near edges of fields or directly after precipitation events to minimize contamination events. Additionally, management practices were found to be associated with pathogen prevalence; therefore, management practices should be carefully tailored for each unique farm landscape.

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General Audience Abstract

Over the years, fresh produce consumption has been on the rise and the concern with the safety of fresh produce has been the focus of recent studies. Raw produce has been recognized to be a potential source of pathogens that has caused human foodborne illness. Foodborne illnesses are caused by pathogens such as *Listeria monocytogenes* and *Salmonella* spp. just to mention a few. It is essential to minimize potential contamination of products with no kill step including fruits and vegetables during the whole supply chain (farm to fork). A study of environmental factors and management practices was performed to determine the influence that the factors have on pathogen prevalence of *Salmonella* spp. and *Listeria* spp. at the sub field level. As well as develop mitigation strategies to minimize contamination of produce on-farm.

Over the span of 8 months, environmental samples were collected from drag swabs, fecal, and pond water used for irrigation on the farm. Management and environmental factors were recorded during every sampling occasion. Samples where then processed for the selection of *Salmonella* and *Listeria* species. Historically, it has been noted that seasons play a role in pathogen prevalence. Amongst all the samples collected during the study the prevalence for *Salmonella* spp. was 1.8% and *Listeria* spp. was 8.3%. *Salmonella* spp. and *Listeria* spp. recovery was performed by following the

guidelines of the Food and Drug Administration Bacteriological Analytical Manual. Factors such as seasonality, cover crop use, and irrigation use were shown to have an effect on the likelihood of detecting a pathogen positive. Any of the three factors listed were shown to be associated with an increased pathogen prevalence. By identifying these factors, growers may develop targeted mitigation strategies to reduce pathogen contamination in the pre-harvest environment.

To Mom and Dad:

Thanks for always believing in me and your constant support, love, and care in what I decided to do in this one shot at the game called life.

I love you.

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Introduction

Over the past 27 years, consumption of fresh produce has increased (*6*). The rise in produce consumption by consumers can be attributed to a concern for a healthier diet and the fast-paced grab-and-go lifestyle (*6*). Consumers are placing greater value on fresh, nutritious, and convenient products (*1*). Fruits and vegetables generally fit these criteria of fresh, healthy and convenient. "Fresh" is defined as having minimal processing or pre-cooking prior to consumption of the product. Fruits and vegetables are often consumed raw, or with minimal processing which can pose risk for contamination by foodborne pathogens. It is essential to minimize potential contamination of all food products. As it relates to fresh produce, it is especially important due to the susceptibility of produce and produce not having a kill step.

In the United States, it is estimated that approximately 48 million people suffer a foodborne related illness (about 1 in 6 Americans), 128,000 people are hospitalized, and 3,000 people die each (*2*). Most foodborne diseases affect susceptible sectors of the population more severely, including the elderly, children, immunocompromised individuals, and pregnant women. Specifically, illnesses and outbreaks associated with fresh produce (fruits and vegetables) increased from 0.7% to 6% dating over a 20-y span during the years of 1970-1990 alone (*8*). The rise in produce associated illnesses and outbreaks may be attributed to the increase in produce consumption by consumers, as previously stated, or due to advanced detection capabilities by public health organizations. Pathogens of concern in fruits and vegetables include *Escherichia coli*

O157:H7, *Salmonella,* Norovirus, Hepatitis A, *Cryptosporium* and *Listeria* species. *Salmonella* and *L. monocytogenes* account for the largest number of illnesses at 1,027,561 and deaths at 255, respectively, reported each year in the United States (*4*). *Salmonella* illnesses have been associated with the consumption of the following contaminated produce commodities: cantaloupe, cauliflower, chili, cilantro, eggplant, green onions, pepper, salad greens, spinach, and tomato just to name a few (*1*, *6*). *L. monocytogenes* has been associated with such produce as bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushrooms, potatoes, radish, salad vegetables, and tomatoes (*1*). Furthermore, both pathogens have been observed in produce pre-harvest environments (*3*, *9*).

Due to these contamination risks, it is critical to ensure the safety of fresh fruits and vegetables throughout the supply chain. Since most fruits and vegetables do not have a kill step, it is crucial to prevent contamination from occurring (on-farm). Several studies have shown that once produce is contaminated, pathogens may grow and survive during storage, especially during events of storage temperature abuse (*7*). The objectives of this research were to (i) determine the prevalence of *Salmonella* spp. and *Listeria* spp. at the sub-field level on-farm and (ii) to identify environmental factors and management practices that influence the prevalence of *Salmonella* spp. and *Listeria* spp. In this research, sub-field level refers to smaller field plots at Virginia Tech's Eastern Shore Agricultural Research and Extension Center farm. The ultimate goal was to provide recommendations for possible mitigation strategies to minimize the likelihood of contamination events on-farm, with emphasis on the sub-field level (by identification of factors and practices that are associated with an increased pathogen prevalence).

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Literature Review

Foodborne Illness and Outbreaks

Over the past 20 years there has been an increase in foodborne outbreaks that have been associated with fresh produce. It is suggested that this is mainly because of the increase of consumption of ready-to- eat foods and a better surveillance system to track outbreaks and illnesses when they do occur. In the United States (US) since 1990, produce has become a major source for foodborne outbreaks, accounting for 13% of the total outbreaks with a known source. It is estimated that 19,336 annual hospitalizations is due to salmonellosis and 1,455 annual hospitalizations occur due to listeriosis (*38*). According to the Centers for Disease Control and Prevention (CDC), outbreaks in the US associated with contaminated produce have been linked to green salad mix, lettuce, potato, melon, pepper, mango, cucumber, sprout, and tomato, and many others (*9*).

In recent years, outbreaks have had improved surveillance so that the public can remain safe and reactive measures can be implemented expeditiously. According to the CDC, in 2016 there were two multi-state outbreaks associated with *L. monocytogenes*. Specifically looking at fresh produce, an outbreak was linked to packaged salads from a Dole processing facility in Ohio. Nineteen people were hospitalized and one person in Michigan died from the illness (*10*). In 2014, there was another *L. monocytogenes* outbreak associated with sprouts which resulted in five illnesses and two deaths (*17*). Lastly, in 2011, there was an *L. monocytogenes* outbreak linked to cantaloupes from

Jensen Farms in Colorado (US). One hundred forty-seven illnesses, 143 hospitalizations, and 33 deaths were reported across 28 states (*11*).

Salmonella spp. has also been associated with several large-scale outbreaks. In 2016, there were two produce associated *Salmonella* spp. outbreaks, both were linked to alfalfa sprouts. The first outbreak associated with alfalfa sprouts was from a contaminated seed lot in which 26 cases and 8 hospitalizations where reported between 12 different states (*10*). The second *Salmonella* spp.outbreak associated with alfalfa sprouts resulted in 36 cases and 7 hospitalizations across 9 states (*14*). In 2015, there was a large outbreak of *Salmonella* serovar Poona associated with imported cucumbers from Mexico. A total of 907 illnesses, 204 hospitalizations and 6 deaths were reported across 40 states (*13*). In 2014, there was a *Salmonella* serovar Newport outbreak linked to cucumbers which resulted in 275 cases across 29 states (*15*). In 2013, there was an outbreak associated with *Salmonella* serovar Saintpaul, also linked to cucumbers, which resulted in 84 illness and 17 hospitalizations across 18 states (*16*). Two other large *Salmonella* spp. outbreaks associated cantaloupes and mangoes occurred in 2012. During the cantaloupe-borne outbreak it was determined two different *Salmonella* strains were associated with outbreak. The two serovars of *Salmonella* were Typhimurium and Newport. The 2012 cantaloupe-borne outbreak resulted in 261 illnesses, 94 hospitalizations, and 3 deaths in 24 states (*6*). The mango-borne *Salmonella* outbreak resulted in 127 illnesses and 33 hospitalizations in 15 states (*5*). These are just some of the most recent examples of *L. monocytogenes* and *Salmonella* outbreaks.

Foodborne Illness Traced to Produce Grown Virginia

Virginia annually ranks in the top ten of fresh market tomato production in the US to date (*3*). Historically, tomato farms have been implicated in *Salmonella* outbreaks, specifically the serovar Newport (*3*). Multi-state outbreaks have occurred in 2002, 2005, 2006, 2007, and 2010 all resulted from contaminated tomato (as the vehicle) harvested from the Eastern Shore of Virginia. In more than 10 years, no clear source of the contamination has been identified, despite strong efforts from many entities (*28*). Previous studies have shown that *Salmonella* spp. can colonize or proliferate on the surface of tomato depending on the serotype and environmental conditions (*28*). Research to identify sources/reservoirs of *Salmonella* are important for protecting the safety of produce that is grown on the Eastern Shore of Virginia.

Characteristics of Selected Foodborne Pathogens

Listeria spp.

Listeria spp. is part of the genus *Listeria* and family Listeriaceae. First discovered dating back to 1891 from patients that died from the disease named listeriosis (*25*). During the 1920's it was officially described as a human pathogen. Symptoms may appear mild or more severe depending on the immune system of the individual infected. Symptoms include the following: fever, muscle ache, nausea, vomiting, meningitis and diarrhea. The infection can spread to the nervous system in more severe cases. Targeted populations are immunocompromised individuals, elderly, and pregnant women (*22*). Pregnant woman who are infected with listeriosis can experience sudden fetal abortions. *L. monocytogenes* is broken down into 13 different serotypes of1/2a, 1/b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7. Commonly associated with foodborne illnesses are types 1/2a, 1/2b, and 4b (*22*). First described by Murray et al, *L.monocytogenes* is a Gram-positive, rod shaped facultative anaerobe that is non-spore forming which has been the leading cause of death related to foodborne illnesses (*22, 24*). *L. monocytogenes* can survive at low pH values and high salt concentrations although its survival is dependent on temperature (*19*). Being able to survive in high salt concentrations may be dangerous for processing reasons since it can survive the salting methods used to reduce pathogen growth and activity. Often animals are carriers of the pathogen which is secreted and carried through fecal matter. Possible routes of contamination to fresh produce may include fecal contamination, through runoff, and manure application to fields as fertilizer. *L. monocytogenes* may also be found in surface water, soil, silage, read-to-eat foods like deli

meats, soft cheeses, and human feces (*21*). In food processing facilities, it has been found in environments such as food contact surfaces and drainage areas (*20*). Despite robust sanitation of food processing plants, *L. monocytogenes* has been able to persist and survive in plants for long periods of time. It has been found that contamination problems occur post processing because the pathogen has the ability to grow at refrigeration temperatures (approximately 4°C) (*33*). Due to the ability to survive, and grow at low temperatures this pathogen is of particular interest.

Salmonella spp.

Salmonella spp. is one of the most commonly isolated pathogens to date and is the leading cause of foodborne bacterial illness in the US. *Salmonella* spp. is a Gram-negative, non-spore forming, facultative anaerobe, motile rod-shaped bacteria that causes 1.3 billion cases of gastroenteritis and 3 million deaths worldwide. This pathogen is a member of the Enterobacteriaceae family, which spends a majority of its lifespan cycling between host and non-host environments (*48*). Discovered in 1885 by Dr. Daniel E. Salmon, *Salmonella* spp. causes one of the most common intestinal infections in the US (*49*). *Salmonella* is divided into two species, *Salmonella enterica* and *bongori*. S. enterica is broken down into six sub-species: *enterica*, *salmae*, *arizonae*, *diarizonae*, *houtenae,* and *indica*. *Salmonella enterica* sub-species *enterica* (the most commonly associated with *Salmonella* illnesses) has over 2,500 serovars (*41*). Serotyping is referenced by the Kaufman- White typing scheme which was first published in 1934 which distinguished

different *Salmonella* based off of their individual antigenic properties (O, H1 and H2 antigens) (*22*).

Salmonella spp. can survive and multiply in a multitude of environments outside of its host. Mainly found in animal intestines and shed into the environment through fecal matter, the cycle of surviving outside of its host while percolating to another host is constant. The temperature range for most *Salmonella* spp. is between 5 – 47° C with the optimum temperature growth range being 35° C +/- 2 *(37)*. It has been noted that some *Salmonella* spp. can grow outside of that range, for examples as low as 2°C and as high as 54° C (*36*). Interestingly, *Salmonella* spp. is very sensitive to heat they are often killed at temperatures of 70°C and higher (*36*). On Hektoen Enteric (HE) and Xylose Lysine Desoxycholate (XLD) media colonies appear to have black centers with clear edges. The optimum growing pH for *Salmonella* spp.is 6.5- 7.5, but some species are able to grow in acidic conditions of 3.7-9.5 (*32*). In extreme conditions, such as a shift in pH levels, limited nutrient availability, osmotic stress, and large variations in temperature, it has been theorized that *Salmonella* spp. enters into a viable, but non-culturable state (*48*). This particular state is when *Salmonella* spp. is dormant or metabolically the cells are active but they just cannot be cultured in laboratory settings (*48*).

The illness that is associated with *Salmonella* spp. is salmonellosis. In 2015, there were 7,728 cases of salmonellosis, 27% were hospitalized and 0.4% of the hospitalizations ended in death (*30*). Salmonellosis has an overall mortality rate of less than 1%, but symptoms caused from the disease include nausea, vomiting, abdominal cramps, diarrhea, fever, and headache (*22*). Foods that have been associated with

Salmonella spp. have been meats, eggs, poultry, dairy products, tomatoes, peppers, cantaloupes, peanut butter, cream filled desserts containing raw eggs, seafood, and spices just to name a few (*22*). Target population for *Salmonella* spp. infections are immunocompromised individuals, elderly, and very young children (*22*).

Produce Safety Overview

Good Agriculture Practices (GAP's)

The FDA developed guidelines to minimize microbial hazards that are associated with fresh produce in 1998. These guidelines were centered on reducing microbial hazards using good agricultural practices (*34*). During 2004, the FDA released an action plan to effectively reduce foodborne disease associated with fresh produce. This action plan presented new strategies to improve production of fresh produce and make it safer for the consumer. The action plan sought to improve produce safety by meeting the following four objectives: 1.) preventing fresh produce contamination by pathogens; 2.) minimizing the public health impact when contamination of fresh produce occurs; 3). improving communication regarding fresh produce safety with producers, packers, processors, transporters, distributors, preparers, consumers, and other government entities; and 4.) facilitating and supporting research relevant to the contamination of fresh produce (*34*).

To achieve the objectives in the action plan, the development of Good Agriculture Practices (GAPs) were crucial. It was agreed GAP's should include the following aspects: 1.) be economically viable, and environmentally sustainable 2.) inclusive of food safety and quality dimensions, and food production as a main (*35*). The implementation of GAPs on-farm would reduce the likelihood of produce contamination by harmful microorganisms. It is not required by law for growers to be GAP certified, but GAP certification is required by some markets and companies before they will purchase products from farms.

Although it is not mandatory for growers to participate in the program, a huge incentive is that consumers would be interested in buying products from farms that are GAP certified. Consumers are becoming more conscious of the way foods are being handled and grown and therefore looking more into GAP's and which vendors are GAP certified. GAP's focus on food safety at the farm level to reduce risk of produce contamination events. GAPs focus on four sources of contamination - soil, water, hygiene, and surfaces (*45*). For example, by applying safe biological amendments to soil, the risk of exposing produce to harmful pathogens can be reduced as some biological amendments like manure can be a source of pathogens. Another example, implementing a sanitation program, where surfaces are cleaned and sanitized (including transporting bins) will minimalize pathogen contamination risk. Lastly, ensuring workers are always keeping up with hygiene, like proper handwashing, while handling produce minimizes the risk of transferring pathogens to produce (*29*).

Food Safety Modernization Act (FSMA)

It has been estimated that the US suffers a $77 billion dollar loss each year because of food safety related illnesses and deaths (*27*). Over the years there has been a spike in foodborne illnesses and contamination events deemed preventable which has weakened consumer confidence. The Food Safety Modernization Act (FSMA) was signed into law on January 4, 2011 by President Barack Obama amending Title 21 of the United States Code, U.S.C.: Food and Drugs (*40*). This act was the biggest change regarding food laws since the great depression dating over seventy years ago (*40*). This act sought to improve

food safety by focusing on preventive measures to reduce outbreaks associated with foodborne pathogens as opposed to focusing on reactive measures after an outbreak of foodborne illness has occurred (*39*).

The act is broken down into seven rules including: 1. Accredited Third-Party Certification Rule, 2. Foreign Supplier Verification Programs for importers of food for Humans and Animals Rule, 3. Mitigation Strategies to Protect Food Against Intentional Adulteration Rule, 4. Preventive Controls for Food for Animals Rule, 5. Preventive Controls for Human Food Finale Rule, 6. Sanitary Transportation of Human and Animal Food Final Rule, and 7. Standards for Produce Safety Rule (*23*). FSMA heightened the FDA's authority to prevent food problems by giving FDA the power to detect and respond to food safety problems and improve the safety of imported foods. The Produce Safety Rule developed, for the first time, standards for the safe growing, harvesting, packing, and holding of produce for human consumption. The Produce Safety Rule specifically focuses on six major types of contamination: microbial contamination from water, biological soil components, sprouts, domestic or wild, health and hygiene practices of workers, and tools and buildings (*26*). Due to these standards which focus on preventing contamination on-farms before it occurs, the expectation is that, at minimum, a 330,000 reduction should be seen of foodborne illness (*26*).

Sources of Contamination in the Produce Production Environment

There are various sources for contamination in the produce production environment in both the pre-and post-harvest periods. For example, in the pre-harvest environment, water sources, such as surface water (i.e. pond, creeks, and ditches); (*42*) may serve as a vehicle for potential contamination of produce. During one study, *Listeria* spp. prevalence was higher in surface water than other sources (*18*). The internalization of pathogens may also pose a risk to produce while in the fields. It was found that produce contaminated by pathogens may lead to a pathogen becoming internalized in the leaf tissue (*4*). Meteorological factors like temperature and recent precipitation occurrence play a role in pre-harvest contamination events. Along with meteorological factors, inadequate application of manure or bio-solids also play a role. Vegetables may become contaminated by manure or bio-solids used as fertilizer which can put produce at a higher risk of being contaminated (*20*). Insects, birds, deer, and other animals are sources and carriers of pathogens. Insects like flies can come in contact with pathogens and spread pathogens to produce through direct contact (*36*). Birds have also been associated with harboring *Salmonella* spp., for instance bird droppings can contaminate produce (*36*).

Previous Field Studies in Selected States/Produce Growing Regions

A study done in New York by Strawn et al evaluated risk factors associated with *Salmonella* spp. and *Listeria monocytogenes* contamination in 21 New York produce farms over a time period of 5 weeks (*43*). Three of eleven field management practices such as manure application, buffer zone, and soil cultivation were identified as risk factors associated with *Salmonella* spp. contamination. Six out of the eleven management practices such as manure application, wildlife presence, worker activity, irrigation, soil cultivation, and reporting of buffer zone were risk factors that were associated with *L. monocytogenes* contamination in the produce fields (*43*). Another New York study found other geographical and meteorological factors to include soil moisture, proximity to water, and proximity to pastures as being strongly associated with contamination risk of *L. monocytogenes* (*18*). The study also found that *L. monocytogenes* was higher in production environments than natural environments (*18*). Distributions of *Salmonella* spp. in New York produce growing regions and South Florida produce growing regions were studied. This study was important to the understanding if isolates from different regions would differ from each other. There was a difference between *Salmonella* spp. isolates distribution in the different regions (*41*). Out of the 112 isolates, only three shared the same serovars. This study demonstrated certain environments may harbor specific pathogens (*41*). Furthermore, another study conducted by Strawn et al developed a geospatial model that predicted the prevalence of *L. monocytogenes* and *Salmonella* spp. in New York. This study found that meteorological factors could have an effect on pathogen prevalence as well as soil activity (*42*).

In Maryland, a study was performed to investigate the association between long-term adjustments in extreme temperature and precipitation events, and the frequency of salmonellosis in a Maryland area (*31*) . It was found that extreme temperatures and extreme precipitation like flooding all play a role in increased chances of salmonellosis. It had been proven in previous studies due to the changes in climate; the different extreme weather patterns would continue effect the prevalence of *Salmonella* in coastal communities (*31*). A study was done on the Eastern Shore of Virginia to determine sources and reservoirs of potential *Salmonella* spp. contamination in tomato fields. The study was conducted at the Virginia Tech Eastern Shore Agricultural Research and Extension Center which is in proximity to Virginia's largest tomato production area (*3*). The ESAREC is used in studies as a model for commercial growing fields. The study was needed due to the frequencies in outbreaks associated with contaminated tomato. It was found that water and sediments could serve as reservoirs for *Salmonella* spp. including *S. Newport*. Similarly, a study was conducted to see the distribution of *Salmonella* spp. upon the Suwannee River, which spans from the Florida/Georgia border to the Gulf of Mexico and is used for irrigation on produce farms. This study hypothesized the survival of *Salmonella* spp. was due to reintroduction events in the Suwannee watershed irrigation ponds and it was concluded that the persistence of the pathogen in the water was due to numerous reintroduction events associated with a variety of environmental host (*2*). Furthermore, a study performed in Georgia observed the presence and concentration of *Salmonella* spp. in irrigation water from distribution systems in produce production regions in southern Georgia. It was found that *Salmonella* spp.could move through irrigation systems (likely originating from contaminated irrigation water) and thus should

be managed to prevent possible contamination of produce (*1*). Furthermore, during 2013, a study in Georgia looked at the prevalence of *Salmonella* spp. in stream networks of the Satilla River Basin to study the relationship between land use, the presence of poultry houses, and waste water treatment plant discharge *(48)*. It was observed that increasing environmental exposures of the two pathogens from poultry houses and the wastewater treatment plant runoff could expose pathogens to drinking and surface waters, which may lead to non-outbreak associated illnesses (*46*).

In 2010, a survey was performed in Monterey County, California to determine the prevalence of *S. enterica* in and around the environment. It was found that matching or related strains were found isolated from water and wildlife fecal samples which further proposed that these are two reservoirs which can be identified as sources for the propagation of the pathogen in the region (*24*). Since, *Salmonella* spp. has been proven to persist in the environment for extended periods of time, propagation of the pathogen is likely returned to the environment (*24*). Another study performed on an almond orchard found long-term persistence of *Salmonella Enteritidis* phage type 30 on one of the orchard farms that was associated with outbreak (*44*). The study was performed over a 5-year period and determined that *Salmonella* spp. can remain in the environment for long periods of time due to its cyclic environmental habits. Further studies of *Salmonella* spp. on the Californian coastal waterways showed that diversity of *Salmonella* spp. strains can be based upon dispersal limitations in the environment. This brings some confirmation to the theory that certain strains in regions and areas are dependent on the environment (*47*).

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Materials and Methods

1. Sampling

1.1 Sampling Sites

Environmental samples were taken from the Virginia Tech's Eastern Shore Agricultural Research and Extension Center (ESAREC) farm. The ESAREC farm is a total of 226 acres and grows a variety of vegetables for the purpose of research and extension. Crops that are grown on the farm include (but not limited to sweet/field corn, soybean, snap bean, basil, parsley, cilantro, tomato, cucumber, cantaloupe, eggplant, peppers, sweet potato, broccoli, summer squash, leafy greens and more. Additionally, cover crop and chemical trials are also conducted at this site to be able to further research that could be used in commercial agriculture production.

Twenty-one samples were targeted for collection at every sampling occasion, which totaled 14 occasions, consisting of fifteen drag swabs, one pond, and five fecal sample. Drag swabs were used to collect soil samples and the pond sample was used as the water sample. The sampling sites were chosen based on a random number generator which produced numbers from one to one hundred-twenty-six. One hundred- twenty- six was used because that is the number of sub-fields that are testable on the farm. The numbers correlated to the grid numbers on the farm at the ESAREC. Grid sizes were roughly 150-200 square feet per plot. Drag swabs were collected from 15 randomly selected plots during each of the 14 sampling occasions. Drag swabs were then held by the string with sterilized glozes and walked through each of the plots.

Animal fecal samples were opportunistically acquired during the sampling inside of the grid if observed (no more than five samples collected during any one sampling visit). Pond water samples were collected from the one on-farm pond. An observational sheet was made and used during each sampling time to collect data on environmental factors and farm management practices taking place during each of the sampling occasions. The observational sheet covered the following categories: temperature of the day, presence of irrigation, state of the field (if the field was actively growing something or not or if research trials were taking place in the particular plot), crops present, pesticide use, wild life intrusion, soil moisture, last rain fall, plot surroundings, and weather of the sampling day (appendix A).

1.2 Sample Collection

Latex gloves (VWR Nitrile 200 Examination Gloves, Radnor, Pennsylvania) were used and changed for every sample collection during the sampling occasion. To collect the top soil samples, drag swabs were hand made with cotton twine string tied to a folded Fisher-brand 4x4 non-woven Gauze patch, and autoclaved. Drags swabs were then moisturized/wetted by being placed into separate no-filter Whirl-Pack bags (Nasco, Fort Atkinson, WI) containing 10ml of Lactose Broth (Criterion, Santa Maria, CA) for *Salmonella* spp. and 10ml of 0.1% Peptone (Fisher Scientific, Fair Lawn, NJ) for the *Listeria* spp. sampling bags. Swabs were then dragged through the selected plot for 8- 10 minutes, making sure to cover the entire area of the grid. Fecal samples were collected

using latex gloves and a sterile scoop (Nasco, Fort Atkinson, WI) into separate individual filtered Whirl-Pak bags. Surface water samples were collected from the on farm pond in sterile 1 liter containers.

1.3. Pathogen Cultivation

1.3.1. Isolation and Detection of Salmonella spp.

A modified version of the Food and Drug Administration's Bacteriological Analytical Manual (BAM) protocol was used for pathogen detection and isolation. *Salmonella* spp. samples were prepared starting with Lactose Broth enrichment. After sampling, drag swab and fecal samples were prepared by adding a 1:10 Lactose Broth dilution into each bag. One 10ml of the 1 liter collected pond water was aliquoted into Whirl-Pack bags and tested for pathogen prevalence per sampling. Each sample type was stomached for 30 sec in a Stomacher 400 circulator (Seward, Worthington, UK) to become homogenized. Bags were then incubated for 35+/-2 °C for 24h. Following incubation, 1.0 mL from each bag was added to 9.0ml of Tetrathionate (TT) broth (BD Difco, Sparks, MD) and 0.1 ml was taken from the sample bags and added to 9.9 ml of Rappaport-Vassiliadias (RV) broth (BD Difco, Sparks, MD). These two broths are used for selectivity and enrichment of *Salmonella* spp. Both RV and TT enrichments were incubated at 42°C for 24h. After the tubes incubated, 50 μl was aliquoted from both TT and RV broths and T-streaked onto Xylose Lysine Desoxycholate Agar (XLD) (BD

Difco, Sparks, MD) and Hektoen Enteric (HE) Agar (Criterion, Santa Maria, CA) plates. Plates were then incubated for 24 hours at 35°C. Presumptively positive colonies were then sub streaked onto Brain Heart Infusion (BHI) agar (Thermo Fisher Scientific Remel, Inc.) and incubated for 24h at 37 +/-2°C. Presumptive *Salmonella* spp. colonies were confirmed by a Polymerase Chain Reaction (PCR) assay that detects the gene, *invA (9).*

***1.3.2 Isolation and Detection of Listeria* spp.**

Environmental samples were prepared by performing a 1:10 dilution of Buffered *Listeria* Enrichment Broth (BLEB) (BD Difco, Sparks, MD) and stomached for 30 seconds by a Stomacher 400 circulator (Seward, Worthington, UK). Sample bags were then placed in the incubator at 30 +/-2 °C for 24 hours. After 4 hours, an Oxoid supplement (BD Difco, Sparks, MD)was added to the enriched bags then incubation continued. A 50 µl of the enrichment was streaked after the 24-hour BLEB incubation period onto Modified Oxford Agar (MOX) (BD Difco, Sparks,MD) plates and *Listeria monocytogenes* Plating Medium (R&F Laboratories, Grove, IL). After 48 hours, another 50 µl was taken from the enrichment sample bags and plated onto LMPM and MOX agar plates again. LMPM plates were incubated at 35+/-2°C and MOX plates were incubated at 30+/-2 °C. Both MOX and LMPM plates were each incubated for a total of 48 hours respectively. After the 48 hour incubation period, plates were read for presumptive positive colonies. For identification of presumptive positive colonies, colonies were sub streaked onto BHI plates and incubated at 37°C for 24 hours. Presumptive *Listeria*

spp. colonies were confirmed by PCR amplification and sequencing of the partial *sigB* gene *(3,4,10)*. During the second session of sampling the LMPM plates were no longer used because of improper readings that resulted from damaged media.

2. Data Collection

2.1 Environmental Data

Proximity data was assessed with using Google Maps to determine how far tested grids were from closest woods, water sources, roadways and the main extension station building on-site. Soil characteristics were obtained by using the U.S. Geological survey. Evidence of animal presence such as fecal, footprints, and animal sighting was recorded when observed and was compared to grids where samples were tested as positive for each pathogen.

2.2 Meteorological Data

Meteorological data was obtained from the weather station located on the ESAREC's premises. Weather data including temperature and rainfall was collected for the day of and the preceding seven days prior to the sampling date. The average temperature and rainfall was recording respectively. On the observational sheet, it was recorded whether or not on the day of sampling if it was windy and if the weather was sunny, cloudy, rainy, or partially cloudy. Other meteorological data such as temperature on the day of, temperature up to -7 days prior, soil moisture, most recent rainfall up to -7 days of the sampling date, and wind characteristics was noted during sampling, was derived from the observational sheet and tested accordingly. During this study hypothesis

were examined as related to temperature. The null hypothesis being temperature will not be a factor, which influences (increases or decreases) *Salmonella* spp. and *Listeria* spp. prevalence. The alternative hypothesis being temperature will be a factor, which influences (increases or decreases) *Salmonella* spp. and *Listeria* spp. prevalence.

2.3 Management Practices

Data on management practices were acquired by using the observational survey in which field-level practices were of focus. Practices were evaluated for pre-harvest possible contamination. Management practices such as: irrigation presence and time of usage, field trials, manure application, chemical use, and crops being grown in the field at the time of sampling were all evaluated. All practices were confirmed by interviewing the farm manager on-site and respective management staff. During this study hypothesis related to management practices were examined. The null hypothesis being that the prevalence of *Salmonella* spp. would be the same in the presence or absence of irrigation. The alternative hypothesis being that the prevalence of *Salmonella* spp. would not be the same in the presence or absence of irrigation.

2.4 Data Analysis

To identify environmental factors and management practices that influence the prevalence of *Salmonella* spp. and *Listeria* spp., a univariate analysis was used to identify the association between the factors and management practices that were present. The univariate analysis that was used was a Fishers Exact Test due to the small count of

pathogen prevalence. The statistical program JMP 13 Pro created by SAS was used to run the statistical models. A multivariable logistic regression was used to create the final model where the p-value was set to 0.05. Odds ratios were calculated and a chi-square test was also used to see if there was a dependency between pathogen prevalence and factors.

Results

The prevalence of both pathogens was relatively low. In total, there were 275 samples collected over the seven-month duration of this project. Table 1, exhibits the seasonal increase and decrease between the two pathogen types. Table 2 and Table 3 illustrate the breakdown of sample type with respect to pathogen type.

From this particular study, it was found that the frequency of *Salmonella* spp. overall was 1.8% out of a total of 274 samples. *Salmonella* spp. positive samples were observed to be more prevalent during the warmer sampling months in which irrigation was present in the fields. The prevalence among drag swab samples was found to be 1.9% out of a total of 210 swabs. The prevalence amongst fecal samples collected was 2% out of 50 fecal samples collected; 0 % among 14 pond samples collected. Irrigation and *Salmonella* spp. prevalence were dependent on each other (p-value was 0.0002 by a Pearson Chi-Square Test. The probability of *Salmonella* spp. was greater when in the presence of an irrigation system (p-value of 0.0192; Appendix). The type of irrigation system that was used during this study was drip irrigation. This rejects the null hypothesis that the prevalence of *Salmonella* spp. would be the same in the presence or absence of irrigation systems. Table 4 exhibits sample prevalence reviewed over the course of this study.

Listeria spp. was recovered in samples predominately during the months of December, January and February. Over 274 samples in total, the prevalence was 8.3%. Approximately, 10% of the positive samples were discovered in drag swab samples,

while 3.9% of the samples were found in fecal and 0% in pond samples. After running a generalized model fit with a binomial distribution, the data found that cover crop (under management practices) and temperature on day of collection (under meteorological factors) significantly influenced the presence of *Listeria* spp. with p-values of 0.0060 and 0.0019, respectively, which are less than 0.05, validating the significance (Appendix). This accepts the alternative hypothesis that temperature is a factor that influences pathogen prevalence as related to *Listeria* spp. To interpret the temperature variable, cover crop was held constant signifying that as temperature increases the probability of *Listeria* spp. being present decreases. As seen in Table 1, *Listeria* spp. prevalence increased as the temperature dropped and the season changed to winter. While interpreting the meaning of cover crop, the temperature variable was held constant during the model. Variables are held constant in models to be able to understand what the variable being tested is doing compared to the variable in the background.

TABLE 1. Seasonal effect (sample collection number, season, and sample type) on frequency of positive *Listeria* spp. and *Salmonella* spp. samples found in sub-field level.

Sample Number (No. of samples)		Frequency (Percent %) of positives	
		Salmonella spp.	*Listeria* spp.
[α] Sampling No.			
	1(21)[b]	4.7 %	0 %
	2 (20)	5 %	5 %
	3 (19)	0 %	0 %
	4 (21)	0 %	0 %
	5 (19)	10 %	0 %
	6 (21)	0 %	0 %
	7 (17)	0 %	0 %
	8 (20)	5 %	0 %
	9 (21)	0 %	0 %
	10 (19)*	0 %	15.7 %
	11 (20)*	0 %	30 %
	12 (20)*	0 %	30 %
	13 (17)*	0%	23.5%
	14 (19)*	0 %	15.7 %
Season			
	Summer (41)[c]	4.8%	2.4%
	Fall (138)	2.8%	0%
	Winter (95)*	0%	23.1%
Sample Type			
	Drag Swab (210)	1.9%	10%
	Fecal (50)	2%	4%
	Water (14)	0%	0%

[α] Sampling occurrence number which took place at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia.
[b] Sampling occurrences and how many samples were collected during the sampling period. Farm samplings were arbitrary and do not imply spatial proximity.
[c] Number of samples collected during the respected season. Summer consisted of June-Aug, Fall Sept- Nov, Winter Dec-Feb, of year 2016-2017.

TABLE 2. *Listeria* spp. prevalence for each sampling date and sampling type during the study at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia.

Year	Sampling Date	Drag swab[α]	Fecal Sample [b]	Pond [c]	*Listeria* spp.
2016					
	8/8	0/15 (0) [d]	0/5 (0)	0/1 (0)	0/21 (0)
	8/22	0/15 (0)	1/4 (25)	0/1 (0)	1/20 (5)
	9/6	0/15 (0)	0/3 (0)	0/1 (0)	0/19 (0)
	9/19	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	10/3	0/15 (0)	0/3 (0)	0/1 (0)	0/19 (0)
	10/17	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	10/31	0/15 (0)	0/1 (0)	0/1(0)	0/17 (0)
	11/15	0/15 (0)	0/4 (0)	0/1(0)	0/20 (0)
	11/29	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	12/13	3/15 (20)	0/3 (0)	0/1 (0)	3/19 (15.7)
2017					
	1/17	6/15 (40)	0/4 (0)	0/1 (0)	6/20 (30)
	1/30	6/15 (40)	0/4 (0)	0/1 (0)	6/20 (30)
	2/13	3/15 (20)	1/1 (100)	0/1(0)	4/17 (23.5)
	2/27	3/15 (20)	0/3 (0)	0/1 (0)	3/19 (15.7)
	Frequency (%)	10 %	4%	0%	8.3 %

[α] Pre-moistened swabs used for the evaluation of top soil in 0.5 acre plots for each sampling.
[b] Fecal samples that were opportunistically collected in each sample plot by sterile scoops.
[c] Water collected in one liter increments from the pond located on site used for irrigation, in which 10ml were tested for presence of *Salmonella* spp. or *Listeria* spp.
[d] Percentage of positive samples detected during the sampling date from each sample type.

TABLE 3. *Salmonella* spp. prevalence for each sampling date and sampling type during the study at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia.

Year	Sampling Date	Drag swab[α]	Fecal Sample[b]	Pond[c]	*Salmonella* spp.
2016					
	8/08	1/15 (6)[d]	0/5 (0)	0/1(0)	1/21 (4.7)
	8/22	1/15 (6)	0/4 (0)	0/1 (0)	1/20 (5)
	9/6	0/15 (0)	0/3 (0)	0/1 (0)	0/19 (0)
	9/19	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	10/3	2/15 (13.3)	0/3 (0)	0/1 (0)	2/19 (10)
	10/17	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	10/31	0/15 (0)	0/1 (0)	0/1 (0)	0/17 (0)
	11/15	0/15 (0)	1/4 (25)	0/1 (0)	1/20 (5)
	11/29	0/15 (0)	0/5 (0)	0/1 (0)	0/21 (0)
	12/13	0/15 (0)	0/3 (0)	0/1 (0)	0/19 (0)
2017					
	1/17	0/15 (0)	0/4 (0)	0/1 (0)	0/20 (0)
	1/30	0/15 (0)	0/4 (0)	0/1 (0)	0/20 (0)
	2/13	0/15 (0)	0/1 (0)	0/1 (0)	0/17 (0)
	2/27	0/15 (0)	0/3 (0)	0/1 (0)	0/19 (0)
	Frequency (%)	1.9 %	2 %	0%	1.8 %

[α] Pre-moistened swabs used for the evaluation of top soil in 0.5 acre plots for each sampling.
[b] Fecal samples that were opportunistically collected in each sample plot by sterile scoops.
[c] Water collected in one liter increments from the pond located on site used for irrigation, in which 10ml were tested for presence of *Salmonella* spp. or *Listeria* spp.
[d] Percentage of positive samples detected during the sampling date from each sample type.

TABLE 4. Number of samples collected and tested positive for *Salmonella* spp. and *Listeria* spp. over the sampling months.

Month [α]	No. of samples tested	No. of samples positive for *Salmonella* spp.	No. of samples positive for *Listeria* spp.	% Positive *Salmonella* spp.	% Positive *Listeria* spp.
August 2016	41	2	1	4.8	2.4
September 2016	40	0	0	0	0
October 2016	57	2	0	3.50	0
November 2016	41	1	0	2.4	0
December 2016	19	0	3	0	15.7
January 2017	40	0	12	0	30
February 2017	36	0	7	0	19.4

[α] The specific months in which sampling took place at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia. Only one sampling occurrence took place in December.

TABLE 5

Year	Date	Weather[d]	Sample Type [c]	Num. Days Prior Rain	Plot [a]	Crop	Proximity To [b]: Animal	Chemical	Water	Forest	Road
2016											
	08/08	84	D	1	117	Soy Beans	1	1	5	2	3
	08/22	81	F	6	37	Pumpkin	1	0	5	2	2
		81	D	6	70	None	0	1	2	1	3
	10/3	74	D	2	25	None	1	1	4	2	2
		74	D	2	72	None	0	0	2	2	3
	11/15	57	F	1	94	None	1	0	4	2	3
	12/13	46	D	1	7	None	0		2	2	2
		46	D	1	29	Wheat Harvested	0	1	4	2	2
		46	D	1	82	None	1	0	3	2	3
2017											
	01/17	47	D	2	3	None	0	0	2	1	1
		47	D	2	5	None	0	0	2	1	1
		47	D	2	12	None	0	0	3	2	1
		51	D	2	18	None	1	1	3	2	2
		51	D	2	20	Soybean	0	1	3	2	2
		51	D	2	67	None	0	0	3	2	2
	01/30	35	D	4	22	Soybean	0	0	4	1	1
		35	D	4	57	Wheat	1	0	6	1	1
		35	D	4	83	Wheat	1	0	3	2	3
		35	D	4	106	Wheat	1	0	5	2	3
		35	D	4	119	Wheat	1	0	5	2	3
		35	D	4	126	Wheat	1	0	6	1	3
	02/13	56	D	8	53	Wheat	0	1	5	1	2
		56	D	8	104	Wheat	0	0	5	2	2
		56	D	8	127	Wheat	0	0	6	1	3
		56	F	8	Fecal	None	1	0	0	0	0
	02/27	55	D	1	55	Mixed	1	0	6	1	1
		56	D	1	56	Wheat	0	1	6	1	1
		56	D	1	95	Wheat	0	0	4	2	4
	Total	28									

Table 5 (above). Factors that related to the prevalence of both *Salmonella* spp and *Listeria* spp. over the course of the fourteen sampling occurrences which took place at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, Virginia.

[α] Plots that were shown positive for *Salmonella* spp. and/or *Listeria* spp.

[b] Represents the proximity in meters to the on-farm Pond Water Source, Forrest, and Main Road. Ranking from 1-5 as follows:

1= <100
2=101-300
3=301-500
4=501-700
5=701-900
6=901-1,100
7=1,101<

[c] Represents sample type being drag swab, fecal, or pond.

[d] Temperature on the day the sample was collected in °F.

Discussion

Objective 1: To determine the prevalence of *Salmonella* spp. and *Listeria* spp. in drag swab, fecal, and pond water samples collected on the ESAREC farm.

1. Pathogen detection

1.1 *Salmonella* spp. Prevalence

Sampling occurred over the course of seven months, covering the following seasons: late summer, fall, and winter. As the seasons changed, *Salmonella* spp. prevalence seemed to decrease. Samples that were detected as positive for *Salmonella* spp. were identified during the summer and late fall months, although statistically, season and temperature did not have an influence on *Salmonella* spp. presence. During the period of warmer temperatures, more crops were being grown which, in turn, meant the field was more active; there was more worker traffic, and irrigation systems were present. The irrigation type that was used during sampling was drip, not overhead irrigation. A total of 4 out of the 9 samplings that occurred during the late summer/fall season were positive for *Salmonella* spp. During that time of sampling, it was still irrigation months. Only one of the 4 samplings that tested positive was collected from a fecal sample.

The three other samples out of the four that were shown to be positive were found in drag swab samples collected from top soil, but no sample that was positive were retrieved from any of the pond collections. Rudolfs et al reported that basic survival for *Salmonella* spp. depends on environmental conditions (i.e. rain and environmental temperatures) and soil characteristics (*12*). The pond collection was carried out using the pond that was present on site for irrigation. A study by Elizabeth et al suggested that even though at low concentrations, the pathogen moves through irrigation systems through which water is supplied by the on farm pond (*1*). During their study, a higher volume of pond water was collected. This factor could account for the reason the *Salmonella* spp. prevalence in water samples was higher in their study, compared to this particular study (*1*). Note: Surface water is also known and recognized to pose a higher risk of contaminating crops during the pre-harvest stage.

A review published by Winfield et al suggested that certain bacteria, such as *Salmonella* spp. can survive stressful conditions by entering a dormant state (*20*). Stressful conditions include: change in temperature, decrease in nutrient availability, change in pH, osmotic changes etc. The dormant stage is called a viable but non-culturable state (VBNC) (*20*). When in this state, metabolic processes are still occurring within the bacteria, but the bacteria is not usually able to be cultivated by laboratory techniques during this state (*20*). This may also account for why decreases in positive samples were observed during the winter months, largely due to the fact that during winter, temperature conditions can be harsh. It was also noticed that during this particular winter season, the temperature varied from extreme cold to warm in the beginning (less *Salmonella* positives were detected at the end of the study).

1.2 *Listeria* spp. Prevalence

Listeria spp. was recovered in samples predominately during the winter months in this study, supporting the indication that seasonal changes due to temperature have an effect on the prevalence of pathogens in the environment. During the late summer and fall months only one sample was discovered to be positive for any species of *Listeria* (detected in a fecal sample). During the winter months of cool/cold conditions, *Listeria* was found 5 of 5 sampling occurrences. In the samples that tested positive for *Listeria* spp., only one tested positive for *L. monocytogenes* (a fecal sample). The other positive samples were detected in drag swabs. Moreover, all pond samples that were collected were negative, as the same with the *Salmonella* spp. study results.

Listeria spp. is known for the optimal temperatures to be cooler than other foodborne pathogens. Optimal growth temperatures range from 30℃ -37℃ and surviving in as low as 4℃ . For this reason, during the winter season the pathogen can survive without much damage. During the winter, field traffic had decreased considerably with exception of the occasional maintenance from the farm crew. There were minimal crops growing on the farm during this period. One study found that the likelihood of finding *Listeria* spp. in the field increased more when the field had been cultivated in the recent days (7 day period before sampling) (*6*). However, contrary to the observations found in that study, this study found that even without the field being cultivated, *Listeria* spp. was still detected (no significant difference).

Another study conducted by Weller et al found that irrigation as recent as 3 days before sample collection could increase the chance of isolation of *Listeria* spp. It was also uncovered that more positive samples were found from fecal samples during that particular study (*17*). In comparison to the current study conducted, prevalence was low in fecal samples and prevalence was higher in drag swab samples. During the Weller et al study, only 11 fecal samples were collected in which five were positive for *Listeria* spp. (*17*). During this study, a total of 51 fecal samples were collected in which only two of the fecal samples tested positive for *Listeria* spp. This points to the fact that pathogen detection is based on environment conditions and often sporadically distributed in the environment.

Objective 2: To identify environmental factors and management practices that influence the prevalence of *Salmonella* spp. and *Listeria* spp. in samples collected on the ESAREC farm.

2. Factors that Influenced Pathogen Prevalence

2.1 Environmental Influences: Animal and Proximity to Surrounding Factors

Environmental influences such as soil moisture and animal presence were found not to be significant factors that influenced either pathogen. Prevalence in fecal samples was relatively low for both pathogens, as well as animal sighting at the time of the sampling occasion. Pathogen prevalence in wildlife fecal samples were observed to be 2% for *Salmonella* spp. and 4% for *Listeria* spp. Studies suggest that wildlife may contribute to contamination of fields from animal feces and propagate into the environmental waters (i.e. pond waters) which can lead to contamination of produce (*14*).

Proximity to water sources, side roads, and to the ESAREC main station building were all recorded in meters by using the Google Map measure distance application. For positive samples which were at plots/grids: 3, 5, 7, 12, 18, 20, 22, 25, 29, 53, 55, 56, 67, 70, 72, 82, 83, 95, 104, 117, 119, 126, and 127. For the edge, positive plots that were in close proximity to woodlands in which animals (i.e. deer's, fox's, rabbits, frogs, and

geese) could leave fecal droppings and/ or introduce possible pathogens was noted. The next closest pond besides the on-site pond used for irrigation was Duer Pond, which is located between 300-700 meters from the sampled farm. Proximity to any of the surrounding factors listed where not significant after statistical analysis was performed. This further supports the notion that pathogen prevalence is not uniformly distributed. In previous studies, it has been concluded that proximity to water, roads, forest and grassland, pastures, scrublands, urban development, and wetlands all influence the likelihood of detecting *Listeria* spp. despite the results from the current study (*5*, *14*, *15*, *16*, *17*, *18*).

2.2. Meteorological Influences

Meteorological influences, such as temperature and rainfall, up to seven days prior were collected and analyzed. Statistical data showed temperature on day of collection did have a significant influence on *Listeria* spp. prevalence. This can be linked to seasonal effects because with the seasons the temperatures change and transition. While statistical data also determined that neither rainfall, nor wind characteristics influenced pathogen prevalence; those factors may still be modes of transportation for the pathogen. Statistical data showed that temperature had an effect on *Listeria* spp. prevalence, but not *Salmonella* spp. *Listeria* spp. were detected more during the winter as also seen in a study performed by Strawn et al (*14*). Two previous studies found that seasons are important factors which influence pathogen prevalence (*13*, *19*) . One of the two studies determined that not only winter, but also spring along with winter would be

more favorable for the detection of *Listeria* spp. (*19*). In the current study, spring was not included in the study so it could not be examined. Another possible reason for the higher prevalence of *Listeria* spp. during the winter season may have been due to the freeze-thaw cycles. Ivanek et al determined that *Listeria* spp. isolation was associated with fewer freeze- thaw cycles during a period of time (*8*).

Chapin et al found that during the summer prevalence was higher compared to the later seasons like fall for *Listeria* spp. (*5*). Which is opposite of the findings produced from the current study, in which prevalence was low during the summer and fall seasons. Although season/temperature did not have an effect on *Salmonella* spp. prevalence, Haley et al suggest that seasonal patterns in surface water (i.e. pond water used for irrigation) in conjunction with elevated temperatures and rain fall patterns may increase *Salmonella* spp. prevalence during the summer months (*7*). Even though it was not determined in this specific study, Weller et al suggest that heavy rain, melting snow, wind, flooding, and human activity may act as mechanisms for the spread of foodborne pathogens from ditches and waterways to produce fields (*18*).

2.3. Management Practice Influences

Management practices for this study included the following listed variables: if irrigation was present; whether or not the plot being tested was active with growing produce/cover crop test or not; if the plot was practicing organic methods or not, chemical application, compost or manure application, and staff employees that were

present during the day-to-day activities around the sampling time. Irrigation was present in fields for the first eight sampling occasions. During that time, *Salmonella* spp. was detected in three of the samplings and four positives where collected. Manure was not present during this study, accordingly, that had no influence on the pathogen prevalence. A nominal logistic analysis showed that irrigation had an effect on the *Salmonella* spp. prevalence in this study. Additionally, cover crop was also recorded as having an effect on *Listeria* spp. prevalence, but not *Salmonella* spp. prevalence. When performing an odds ratio statistical analysis three temperature points where tested in relation to cover crop presence and its effect on *Listeria* spp. The three temperature points were : 74°F, 57°F, 37°F (which were all collected from sampling day temperatures from the study). During all three tested points, fields with no cover crop present were two times more likely to be free (not detected) of *Listeria* spp. than those with cover crop present (Appendix).

Salmonella spp. has been known to be associated with irrigation presence from previous studies. Antaki et al suggest that *Salmonella* spp. will move through irrigation systems even when in low concentrations. Irrigating with surface water has to be closely monitored for this reason as it can pose a high risk for pre-harvest contamination as stated previously (*1*). Baoguang et al also found that irrigation could be a potential vector for *Salmonella* spp. contamination in fresh produce during the pre-harvest stage (*2*). Reed-Jones et al and Shutter et al found that cover crop may play a role in influencing microbial pathogen existence on the farm, but cover crop is not the main driving force for pathogen prevalence. In the current study cover crop had an effect on *Listeria* spp.

prevalence, but that does not necessarily suggest that cover crop being present o absent will affect pathogen prevalence on all farms (*11*, *13*), as farms can be very different.

Conclusion

Pathogen prevention and control are very important during production, from pre-harvest through consumer purchase. This study was performed to examine management, meteorological, and environmental factors which may have an effect on the overall prevalence of *Salmonella* spp. and *Listeria* spp. During this study, the strain variability or species of the pathogen was not examined. Nevertheless, the presence of *Listeria* species (includes majority non-pathogenic species of *Listeria*) can be used as an indicator organism for *L. monocytogenes*, suggesting possible contamination. The United States has a zero-tolerance policy for *L. monocytogenes* in foods to be distributed to consumers. If farmers tested for *L. monocytogenes* in fields or produce samples they may be concerned about harvesting fields or shipping produce due to the food safety risk. However, by the use of indicator organisms, farmers can monitor areas on the farm of high risk. For example, high *Listeria* spp. areas may be associated with a higher likelihood for *L. monocytogenes* contamination; and mitigation practices might be focused on these areas.

Over the course of this study, it was determined that seasonal changes in temperature, irrigation, and cover crop presence had an effect on pathogen prevalence. While pathogen prevalence was not uniformly found in every plot, the study shows that there were definite factors that increased the likelihood of pathogen prevalence. Surprisingly, areas with fecal matter present and or recent rainfall did not seem to have much of an influence on pathogen prevalence. Moreover, the hypothesis that suggested

temperature would have an effect on pathogen prevalence was accepted (results showed high *Listeria* spp. positive samples during the cooler temperatures).

Current practices such as following Good Agriculture Practices (GAP's) will help prevent possible contamination when performed routinely. For example, sanitation will help reduce on-farm contamination events (clean and sanitize areas frequently to inhibit possible accumulation of bacteria on surfaces). In addition, assessing produce safety risks and implementing practices to control those risks, such as monitoring practices to spot contamination incidents/events. As for water safety, frequent water test are always recommended to make sure the water sources near and on farm are of safe quality (for its intended use). Nevertheless, results from this study highlight possible factors and practices on-farm that may lead to contamination events. This information will continue to assist in the development of mitigation strategies that aim to reduce pathogen contamination on-farm.

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Appendix

Figure 1. Observational sheet used in this study.

Observational Sheet

Date: ____________ Name of collector: ______________________

Time of collection: ___________

Block Number: ____________

Irrigation present: Yes / No

Type of irrigation (If yes): ____________________ Last date of irrigation: ____________

State of field: Fallow / cover crop / active : ____________________ (circle one)

Crop type(s) in block: ________________________ Block size: ______________

Type of cover crop if present __________________

Block surroundings (what's around the block, what other crops are being grown etc…): _____

____

Weather: ____ **Temperature**: ________

Windy: _____ Yes / No ___

Last rain fall before collection: _________________________

Pesticides or chemicals in use in block: Yes / No __

Type of pesticides or chemical being used (if yes): ____________________

Last date of pesticide application: ____________________

Wild life intrusion evidence: Yes / No ___ If yes, details: ______________________

____

Other observations in the block: ____

____

Soil moisture: _______________

Photo available: ___ Yes / No __

Figure 2. ESAREC farm plot/grid numbers used to identify fields in this study.

Figure 3-7. Active sampling and plots.

Figure 8-11. ***Salmonella*** **spp. processing and plate results.**

Figure 12-14. ***Listeria spp.*** **plate readings.**

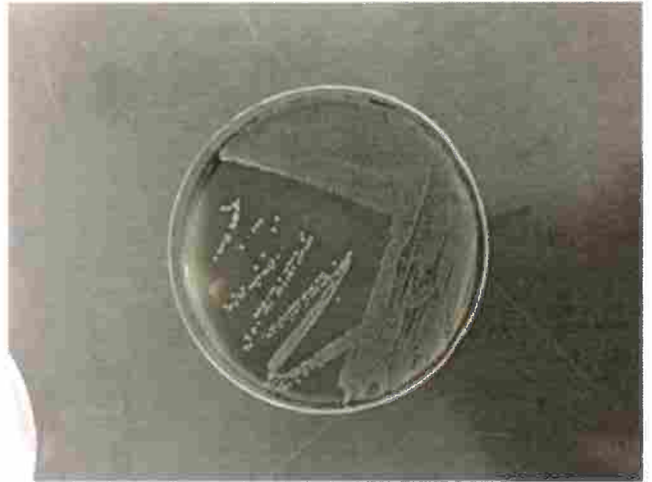


Figure 15. 2 Odds Ratio examples worked for *Listeria* spp. (cover crop and temperature).

At 74°F

When there is present of CC : Y= (b0 + b1x1)+ (b2x2)

$$Y = -0.33 + 0.04339(\mathbf{74}) = 2.8809$$

$$e^{(2.8809)} = 17.8296$$

When NO cover crop present:

$$Y = -0.33 + 0.04339(\mathbf{74}) + 0.6912 = 3.5721$$

$$e^{(3.5721)} = 35.5898$$

$$35.5898/17.8296 = 1.9961$$

**The odds are 2 times more likely for plots with no cover crop to be free of *Listeria* spp. than those with cover crop, at 74° F degrees.

At 57

When there is present of CC : Y= (b0 + b1x1)+ (b2x2)

$$Y = -0.33 + 0.04339(57) = 2.1432$$

$$e^{(2.1432)} = 8.5269$$

When NO cover crop present:

$$Y = -0.33 + 0.04339(\mathbf{57}) + 0.6912 = 2.8344$$

$$e^{(2.8344)} = 17.0207$$

$$17.0207/8.5269 = 2.1432$$

** The odds are 2 times more likely for plots with no cover crop to be free of *Listeria* spp. than those with cover crop, at 57 ° F degrees.

Figure 16. *Listeria* spp. statistical model.

Generalized Linear Model Fit

Response: Listeria monocytogenes
Modeling P(Listeria monocytogenes=0)
Distribution: Binomial
Link: Logit
Estimation Method: Maximum Likelihood
Observations (or Sum Wgts) = 251

Whole Model Test

Model	-LogLikelihood	L-R ChiSquare	DF	Prob>ChiSq
Difference	11.2967262	22.5935	2	<.0001*
Full	63.2666887			
Reduced	74.5634149			

Goodness Of Fit Statistic	ChiSquare	DF	Prob>ChiSq
Pearson	213.5977	248	0.9443
Deviance	126.5334	248	1.0000

AICc
132.6305

Effect Summary

Source	LogWorth	PValue
Temperature on Day of collection	2.714	0.00193
Cover Crop Present (0,1)	2.224	0.00597

Effect Tests

Source	DF	L-R ChiSquare	Prob>ChiSq
Cover Crop Present (0,1)	1	7.5596172	0.0060*
Temperature on Day of collection	1	9.6121462	0.0019*

Parameter Estimates

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq	Lower CL	Upper CL
Intercept	-0.33434	0.7120663	0.2184581	0.6402	-1.743306	1.1107852
Cover Crop Present (0,1)[0]	0.6911199	0.2441083	7.5596172	0.0060*	0.20454	1.1703796
Temperature on Day of collection	0.0433944	0.0138006	9.6121462	0.0019*	0.0163473	0.0715289

Studentized Deviance Residual by Predicted

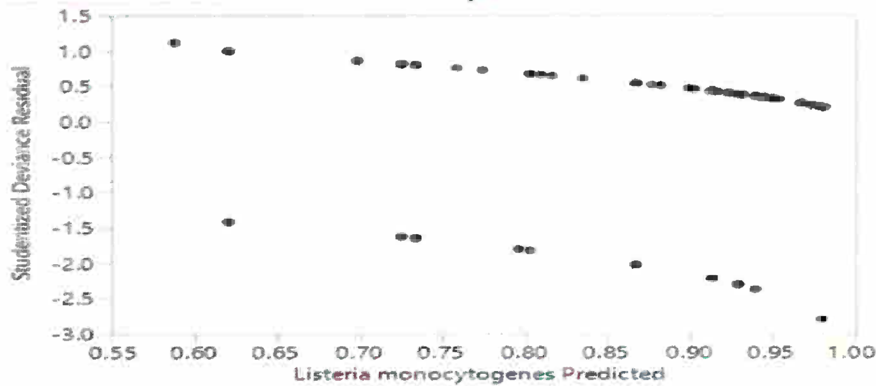
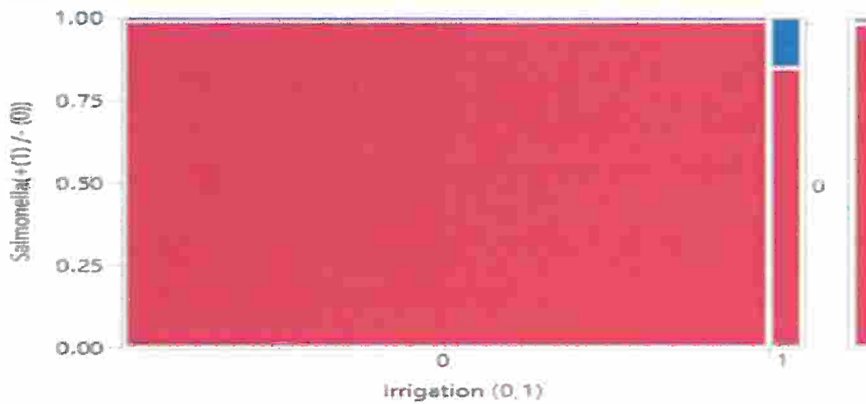


Figure 17. ***Salmonella*** **spp. statistical model.**

Contingency Analysis of Salmonella(+(1) /- (0)) By Irrigation (0,1)

Mosaic Plot



Contingency Table

Salmonella(+(1) /- (0)) by Irrigation (0,1). Each cell lists: Count / Total % / Col % / Row %

Irrigation (0,1)	0	1	Total
0	258 94.16 95.91 98.85	3 1.09 60.00 1.15	261 95.26
1	11 4.01 4.09 84.62	2 0.73 40.00 15.38	13 4.74
Total	269 98.18	5 1.82	274

Tests

N	DF	-LogLike	RSquare (U)
274	1	3.0109347	0.1206

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	6.022	0.0141*
Pearson	14.007	0.0002*

Fisher's Exact Test	Prob	Alternative Hypothesis
Left	0.9992	Prob(Salmonella(+(1) /- (0))=1) is greater for Irrigation (0,1)=0 than 1
Right	0.0192*	Prob(Salmonella(+(1) /- (0))=1) is greater for Irrigation (0,1)=1 than 0
2-Tail	0.0192*	Prob(Salmonella(+(1) /- (0))=1) is different across Irrigation (0,1)