Investigating the Prevalence, Persistence, and Diversity of *Listeria monocytogenes* and *Listeria* species in Produce Packinghouses

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Investigating the Prevalence, Persistence, and Diversity of *Listeria monocytogenes* and *Listeria* species in Produce Packinghouses submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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ABSTRACT

Listeria monocytogenes has emerged as a food safety concern for a number of produce commodities. While *L. monocytogenes* contamination can occur throughout the supply chain, contamination from the packinghouse environment represents a particular challenge and has been linked to recalls. This study aimed to investigate the prevalence, persistence, and diversity of *Listeria monocytogenes* (LM) and other *Listeria* species (LS) in produce packinghouses. A longitudinal study was performed in 11 packinghouses (commodities included micro-green, peach, apple, tomato, broccoli, cauliflower, and cucumber) in three US states. In each packinghouse, 34 to 46 sites representing zones 2-4 were selected and swabbed. Packinghouses were visited 4 times and samples were processed for *Listeria* by US Food and Drug Administration's Bacteriological Analytical Manual methods. Presumptive *Listeria*-positive isolates were confirmed by PCR. Species and allelic type (AT) were identified by *sigB* sequencing. Among the 1,584 samples tested, 3.2%, 2.7%, and 0.6% of the samples were positive for LM, LS, and both LM and LS, respectively. Five different species of *Listeria* were identified with *L. monocytogenes* being the most prevalent species. A high AT diversity (0.95 Simpson's Diversity Index) was observed amongst *Listeria* isolates. There were 15 instances of *Listeria* repeated isolation (site testing positive ≥2 times). Upon analysis of subtype data, only 3 sites tested positive for the same *Listeria* AT >2 times. Data showed in this longitudinal study that *Listeria* prevalence and persistence in packinghouses was low (e.g., <4% prevalence). Therefore, sanitation program development and implementation in packinghouses are critical to limit *Listeria* harborage and residence.

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GENERAL AUDIENCE ABSTRACT

Listeria monocytogenes is one of the deadliest foodborne pathogens, accounting for about 20% of the deaths caused by foodborne illnesses in the US. Historically, *L. monocytogenes* has been a big concern for Ready-to-Eat products (ice cream, deli meats, etc.), but in the last decade, there have been several listeriosis outbreaks associated with fresh produce (e.g. cantaloupes, apples, celery, packaged salad) becoming a produce safety concern. Some of these outbreaks have been traced back to the produce farm (pre-harvest) and the operations after harvesting (post-harvest). Though there is research focusing on the prevalence of *Listeria* in the pre-harvest environment, there is a need for studies investigating *Listeria* at the post-harvest level. This research project, focused on gaining a better understanding of the prevalence, persistence, and diversity of *Listeria* (including *L. monocytogenes*) in produce packinghouses. 11 packinghouses facilities were sampled four times during the packing season. The samples were obtained from different stationary (e.g. walls, drains, floors) and moving (e.g. bins, forklifts, pallets) non-food contact surfaces and equipment during operation hours. Isolates were processed to detect and isolate *Listeria* species (including *L. monocytogenes*). *Listeria* isolates were confirmed and fingerprinted. *Listeria* prevalence in these packinghouses was low (6.4%), and it varied among packinghouses. Drains, cold storages, and wet non-food contact surfaces were the sites with the highest *Listeria* prevalence. There were 3 cases of *Listeria* repeated isolation (same *Listeria* detected in the same site in at least 2 of the 4 visits). The diversity of *Listeria* in these packinghouses was high. The information gathered through this research provides a better understanding of where and what species of *Listeria* can be found in a produce packinghouse

facility. This knowledge may be used to develop and implement mitigation strategies and interventions to control and/or reduce the risk of *Listeria* contamination in produce packinghouses

DEDICATION

Dedicated To My Family Who Encouraged Me To Pursue My Dreams.

Pa, Ma, Paty, and Luis

And In Loving Memory of My Aunt, Daria Martinez.

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ATTRIBUTIONS

Several colleagues contributed to Chapter 2 of this thesis. A short description of their contributions is described below.

Chapter 2: Prevalence, Persistence and Diversity of *Listeria monocytogenes* and *Listeria* spp. in Produce Packinghouses in Three U.S. States.

Alexis M. Hamilton, a formal Master's student in the Department of Food Science at The University of Tennessee assisted with environmental sample collection from packinghouses located in Tennessee.

Genevieve B. Sullivan, a current Ph.D. student in the Department of Food Science and Technology at Cornell's University assisted with building a phylogenetic three and allelic typing.

Faith J. Critzer, PhD., a current faculty member in the Department of Food Science and Technology at the Washington State University assisted with environmental sample collection from packinghouses located in Tennessee.

Martin Wiedmann, PhD., a current faculty member in the Department of Food Science and Technology at Cornell's University assisted with the study design.

Laura. K. Strawn, PhD., a current faculty member in the Department of Food Science and Technology at Virginia Polytechnic Institute and State University assisted in the study design, sample collection, data interpretation, compilation and completion of the manuscript.

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

According to the Centers for Disease Control and Prevention (CDC), 48 million people experience a foodborne illness every year in the United States. Foodborne illnesses are caused by the consumption of contaminated food with microbial pathogens; bacteria, viruses, parasites, or by the ingestion of microbial chemicals and toxins (*74*).

Food illnesses are known for causing gastrointestinal problems such as diarrhea, nausea, and vomiting. However, the severity of these symptoms depends on the individual's health and on the microorganism/toxin ingested. Older adults, younger children, pregnant women, and people with a compromised immune systems are more vulnerable to develop a foodborne illness (*74*). Some foodborne infections can progress to life-threating conditions and lead to death. The CDC estimates that every year, 128,000 people are hospitalized and 3,000 die from food illnesses in the USA (*74*).

Listeria monocytogenes is a human pathogen causing a small number of foodborne infections when comparing to other organisms (*74*). However, it is estimated that every year, *L. monocytogenes* is responsible for 255 (20%) deaths caused by food illnesses in the USA (*19*).

L. monocytogenes can resist and adapt to a wide range of environments: high salt concentration, cold temperatures, and different pH values. This adaptation has allowed *Listeria monocytogenes* to populate a wide range of environments such as soils, sewage, water, and house and processing environments including equipment (*17*). Cross-contamination between processing equipment and produce has raised concerns, especially after the several listeriosis outbreaks documented in the last few years. In 2011, a multistate listeriosis outbreak with 147 cases and 33 deaths was caused by the consumption of contaminated cantaloupes (*41*). In the past 7 years, caramel apples and celery contaminated with *Listeria monocytogenes* led to at least

40 cases and 12 deaths (*19*, *21*). The origin of these outbreaks suggests that post-harvesting environments represent a considerable risk for *Listeria* cross-contamination.

However, there is a knowledge gap in understanding *Listeria* in the post-harvest environment. Thus, the development of more specific and effective guidelines for the effective control and reduction of *Listeria monocytogenes* targeting such environments is limited. This project aimed to study the prevalence, persistence, and diversity of *Listeria species* (including *Listeria monocytogenes*) in produce packinghouses. This project's findings provide science-based information that may be utilized to implement effective environmental monitoring programs, control and intervention strategies to eliminate and reduce *Listeria monocytogenes* in produce packinghouses.

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CHAPTER 2: LITERATURE REVIEW

Introduction to Foodborne Illness (Produce Safety)

The Centers for Disease Control and Prevention (CDC) estimates that each year, in the United States, 48 million people experience a foodborne illness, 128,000 are hospitalized, and 3,000 people die as a consequence of foodborne illnesses. The symptoms of foodborne illnesses vary depending on the pathogen or pathogen's toxin that is ingested, but the most common symptoms are diarrhea, vomiting, cramps, and fever. Most symptoms disappear within a couple days or weeks. However, occasionally food illnesses can develop into a severe condition requiring medical assistance, chronic health problems, and even death (*74*). The CDC recognizes 31 main pathogens causing food illnesses in the US. Nontyphoidal *Salmonella, Toxoplasma gondii, Listeria monocytogenes*, Norovirus, and *Campylobacter spp*. represent the main concern since they caused the most deaths (*72*).

In the last 3 decades, the consumption of fruits and vegetables has increased and commensurately, produce associated outbreaks have increased. From 1970 to 1990, fresh produce outbreaks increased by 5.3% (*57*) and between 1996 and 2010, 14,350 illnesses and 34 deaths resulted from 131 produce-related outbreaks (*46*). In 2002-2011, the Center for Science in the public interested stated that fresh produce caused the major number of outbreaks during 2002-2011 (*29*).

Due to its high mortality rate and its association with several produce-associated outbreaks, *Listeria monocytogenes* has become a major pathogen of interested.

Overview of *Listeria* species

Listeria is a genus rod-shaped, flagellated, gram-positive, facultative anaerobe bacteria. The genus *Listeria* contains 17 species and is mainly classified into two different groups: *Listeria* sensu strictu and *Listeria* sensu lato. These two groups were separated based on the resemblance of the different species to *Listeria monocytogenes* (*48*). *Listeria* sensu lato contains eleven species, all of which possess similar characteristics: they are all catalase positive, non-motile (except *L. grayi*), capable of reducing nitrate (except *L. floridensis*), and non-spore forming. Species in this group include *L. grayi, L. fleischmannii, L. floridensis, L. aquatica, L. newyorkensis, L. cornellensis, L. rocourtiae, L. weihenstephanensis, L. grandensis, L. riparia, and L. booriae.* None of these species have been found to be a threat to human health (*48*).

Listeria sensu strictu group contains six *Listeria* species: *L. monocytogenes, L. seeligeri, L. marthii, L. ivanovii, L. welshimeri, and L. innocua* (*13*). All these species are motile, cannot reduce nitrate, and can grow under cold temperatures. *L. monocytogenes* and *L. ivanovii* are considered the only pathogenic species. However, *L. ivanovii* is better known to cause animal (sheep) disease and not a human disease (*48*).

Listeria monocytogenes.

Out of the 17 species, *Listeria monocytogenes* is the main species of concern because it is one of the leading causes of death from foodborne illnesses in the USA. *Listeria monocytogenes* has been isolated from different environments all over the world, including Europe, Oceania, Asia, Africa, North, Central, and South America (*12*).

Listeriosis is the illness caused by the consumption of *Listeria monocytogenes*. Listeriosis has been documented in all the continents (*48*). The infective dose is unknown; however, it is thought that the dose varies depending on the strain and the susceptibility of the host (*24*).

Listeriosis symptoms among the general population range from cramps, nausea, vomiting, fever, and diarrhea. However, in susceptible individuals such as the elderly, newborns, pregnant woman, and the immunocompromised population, listeriosis can be more severe, leading to life-threating conditions such as meningitis, septicemia, and even death (*76*). The CDC estimates that in the United States, there are 1,600 cases of listeriosis and 260 deaths per year.

Listeria monocytogenes is a widely spread pathogen that has been found in different environments with different conditions, including but not limited to moist environments, soil, waters, and refrigerators (*48*). *Listeria monocytogenes* has been found in food-manufacturing environments and its presence in these environments has been linked to several food outbreaks (*19*, *21*, *41*). A food outbreak is defined as the event when more than one person develops the same illness from the same source.

In the last decade, *Listeria monocytogenes* has emerged as a major concern due to the several outbreaks it has caused. In 2010, *Listeria*-contaminated diced celery caused 10 cases of listeriosis where 50% of patients died due to its complications (*19*). In 2011, the consumption of contaminated cantaloupe led to 147 cases of listeriosis in 28 states, and 33 (22%) of the patients died (*41*). In 2014, contaminated caramelized apples caused a multistate outbreak which caused 7 death and 44 hospitalizations (*21*). In the same year (2014), a bean sprout outbreak caused 5 people to become ill in two states. All were hospitalized and two died. *Listeria monocytogenes* was found in environmental samples from the facility, such samples were highly related to the isolates from the ill patients (*6*). In 2015, 19 people became ill in nine states due to the consumption of *Listeria*-contaminated packaged salad. 100% of the patients were hospitalized and one patient died (*7*). All these *Listeria* outbreaks were linked back to the processing environment through swabbing samples.

Currently, the US follows a zero-tolerance policy when it comes to *L. monocytogenes*. If the presence of *Listeria monocytogenes* in the final product is suspected, the industry may recall their product. Food recalls may occur when a manufacturer, a producer, or a government agency (FDA or USDA) suspect the presence of a pathogen in a food product, an allergen, or a labeling mistake (*18*). In 2015, Dole facility made a voluntary recall of packaged salad after 19 people became ill due to *Listeria* contamination (*47*). In the same year, some companies voluntarily recalled caramel apples due to *Listeria* contamination concern (*5*). It is estimated that about 200 recalls were caused by *Listeria* contamination, 30 of the recalls were fruits and vegetables (*39*). In the past 2 months, January to February 2019, 3 fresh produce have been recalled due to concerns of *Listeria* contamination. Some of these produce included bean sprouts, green beans, butternut squash, peaches, nectarines and plums (*1*).

In the past 5 years, food recalls have increased by 4-fold. Food recalls have a significant negative impact on food companies. It is estimated that the average cost of a recall is about $10 million. A company's reputation is also affected by recalls, which often causes consumers to stop buying products from the companies who have recalled food (*78*). Thus, it is important that companies develop and follow adequate sanitary programs to reduce the cross-contamination of pathogens originating and being carried into the post-harvest facilities.

Listeria Prevalence in the Supply Chain

Overview

Listeria monocytogenes is a facultative intracellular pathogen. *L. monocytogenes* can live inside or outside of a host and can survive at a wide range of pH values (4.7-9.2), temperatures and salt concentrations (up to 10%, wt/vol) (*17*). These characteristics allow *Listeria* to exist in different environments such as soil, manure, stream water, decaying vegetation, processing

equipment, floors, and drains (*3*). *Listeria monocytogenes* has been isolated from different mammals (cows, deer, fox), birds, and humans. *Listeria* is considered a ubiquitous pathogen due to its survival rate and prevalence in different environments. *Listeria monocytogenes* and *Listeria* spp. cannot be completely eliminated from the food supply chain but its presence can be reduced in order to minimize *Listeria* food contamination (*3*).

Listeria monocytogenes and Listeria spp. contamination can happen in all of the different levels of the supply chain. In the case of produce, contamination may happen in the production field (pre-harvest level), processing, retail, and domestic preparation and storage (post-harvest level). Monitoring and eliminating *Listeria* contamination sources in the produce and Ready-To-Eat industry are pivotal, especially since these foods are often consumed without being subjected to thermal treatments, and thus, deactivation of *L. monocytogenes* (and other pathogens) does not happen.

Listeria Prevalence at the Pre-harvest Level

At the pre-harvest level, contaminated irrigation water, composts, organic fertilizers, and agriculture soil could potentially serve as a contamination source for produce (*3*). *Listeria spp*. and *Listeria monocytogenes* have been previously isolated from all those environments. There are few studies that investigated the prevalence of *Listeria* in produce production fields and farms (*9*, *58*, *69*). These studies have obtained environmental samples from the pre-harvest environment and reported *Listeria prevalence.* Also, it is known that harvesting equipment could potentially serve as a cross-contamination medium (*41*). Thus, adequate cleaning and sanitation of equipment are crucial processes in decreasing the risk of contamination from the field to the food products. The presence of *Listeria monocytogenes* at the pre-harvest level can cause the introduction of this pathogen to the post-harvest environment (*3*)

Listeria Prevalence at the Post-harvest Level

Contamination of foods at the production level has been linked to several food outbreaks. Post-harvest environment (packinghouses and processing plants) can be contaminated by the introduction of *Listeria* via incoming raw materials, including produce themselves, air, workers, and equipment (*3*).

Surrounding environment plays an important role in the introduction of *Listeria* to a facility. Standing water and vegetation around the facility could support the growth and the harborage of *Listeria. Listeria* could then be introduced to the facility by rolling equipment, forklifts tires, or employees' shoes. (*3*). Thus, these potential harborage sites should be carefully monitored for the presence of *Listeria*. Once *Listeria* has been introduced, it tends to populate moist, undisturbed environments such as cracked floors, drains, cooling units, and difficult-to-clean/difficult-to-access pieces of equipment and sites (*3*).

Equipment design and maintenance is important to decrease cross contamination risk. Equipment should be designed with sanitary materials and should be easily cleanable with no sharp edges that could potentially cut into the food causing an internalization of *Listeria* (*3*). Conveyor rollers are a potential source of contamination since these surfaces come in direct contact with foods. In general, all equipment or surfaces with any cracks larger than 0.001 inches could potentially become a *Listeria* harborage site (*3*).

Drains are ideal sites for *Listeria* harborage. Drain design and cleanliness in a facility is key for the reduction of *Listeria*. Minimizing traffic flow through drains is a way to reduce potential *Listeria* proliferation to other facility floors and sites (*3*). The use of pressure hoses could potentially aerosolize *Listeria* in the drains which could land on the product or equipment surfaces that come in direct contact with the food products (*3*).

Although unusual, *Listeria spp.* and *Listeria monocytogenes* can be carried into and around the facility through the air. Air filters and air pressure units are often used to reduce *Listeria* cells and other airborne contaminants from the air (*3*). Workers themselves can be a source of contamination. An employee could be a *L. monocytogenes* carrier even if they do not present any symptoms. Gloves and employees' hands have been recognized as a potential source of contamination (*17*).

Some processes themselves are more prone to cause food contamination. For example, using recirculating water to wash the fresh produce could introduce and disseminate pathogens in food (*20*). If a batch of the produce is contaminated with *Listeria monocytogenes* then the wash water will become contaminated and if the disinfection of water is not done properly, the contaminated water will be used to wash other batch products. The process of cutting, dicing, and sheering can cause contamination because food products come in direct contact with the equipment. Often, the equipment is complex and hard to clean, which can be used a harborage for *Listeria* and other organisms (*24*).

Table 2.1. List of journal articles focused on studying *Listeria* prevalence in different pre-harvest and post-harvest environments.

Paper Title	Reference	Environment Type	Sample Type	Sites sampled	Prevalence of *L. monocytogenes*	Prevalence of *L.* spp[a]	Total *Listeria* genus	Co-isolation
Prevalence and Molecular Diversity of *Listeria monocytogenes* in Retail Establishments	D. Sauders et al, "Prevalence and Molecular Diversity of *Listeria monocytogenes* in Retail Establishments," J. Food Prot., vol. 72, (11), pp. 2337-49, 2009.	Retail Food Establishments (n=121)	Sponges	FCS and NFCS	Total 13.0% (151/1161). 125 (16.7%) NFCS and 26 (6.3%) FCS	-	-	-
Molecular Studies on the Ecology of *Listeria monocytogenes* in the Smoked Fish Processing Industry	Norton, D. M., M. A. Mccamey, K. L. Gall, J. M. Scarlett, K. J. Boor, and M. Wiedmann. 2001. Molecular Studies on the Ecology of *Listeria monocytogenes* in the Smoked Fish Processing Industry Downloaded from. Appl. Environ. Microbiol. 67:198–205.	Smoked Fish Processing Facilities (n= 5)	Food and environmental swabs	Environmental swabs: Drains, cooler floors, and equipment surfaces (n=206).	Total = 17.8% (n=531), environmental, in-process and finish product and raw material. Environmental =27.7% (n=206)	-	-	-
Listeria monocytogenes and *Listeria* spp. Contamination Patterns in Retail Delicatessen Establishments in Three U.S. States	Simmons, C., M. J. Stasiewicz, E. Wright, S. Warchocki, S. Roof, J. R. Kause, N. Bauer, S. Ibrahim, M. Wiedmann, and H. F. Oliver. 2014. *Listeria monocytogenes* and *Listeria* spp. Contamination Patterns in Retail Delicatessen Establishments in Three U.S. States. *J. Food Prot.* 77:1929–1939.	Retail deli Establishments (Phase I n=15, Phase II n=30)	Sponges	Phase I = 6 NFCS and 1 FCS. Phase II = 10 FCS, 15 NFCS, 3 TP	Phase I = 6.8% (n=314). Phase II = 9.5% (4,503). NFC and TP = 14.2 and 3.3%	Phase I = 3.8% (n=314) Phase II = 5.3% (n=4,503)	Phase I = 10.6% Phase II =9.8%	-
Molecular Ecology of *Listeria monocytogenes* and Other *Listeria* Species in Small and Very Small Ready-to-Eat Meat Processing	S. K. Williams et al, "Molecular Ecology of *Listeria monocytogenes* and Other *Listeria* Species in Small and Very Small Ready-to-Eat Meat Processing Plants," J. Food	Small meat processing plants (n=6)	Sponges	NFCS (walls, doors, sinks, cart wheels and equipment surfaces)	6.1% (n=688)	9.5 % (n=688)	15.55 % (107/688)	-

Plants	Prot., vol. 74, (1), pp. 63-77, 2011.							
Longitudinal Monitoring of *Listeria monocytogenes* Contamination Patterns in a Farmstead Dairy Processing Facility	Ho, A. J., V. R. Lappi, and M. Wiedmann. 2007. Longitudinal Monitoring of *Listeria monocytogenes* Contamination Patterns in a Farmstead Dairy Processing Facility. J. Dairy Sci. Elsevier 90:2517–2524.	Dairy production on farmstead operation (n=1)	Sponges Farm and processing environment	NFCS (drains, floor areas, equipment surfaces, walls and door-ways)	Farm 9.4 % (n=85) and Dairy production 2.7% (n=674). Overall = 29 / 759 (3.4 %)	Procesing Facility = 13.9% (n=674). Farm =21.2% (n=85) Over all = 14.8% (112/759)	112 + 26 =138 Positive samples n=759. 18.2 %	-
Longitudinal Studies on *Listeria* in Smoked Fish Plants: Impact of Intervention Strategies on Contamination Patterns	Lappi, V. R., J. Thimothe, K. K. Nightingale, K. Gall, V. N. Scott, and M. Wiedmann. 2004. Longitudinal Studies on *Listeria* in Smoked Fish Plants: Impact of Intervention Strategies on Contamination Patterns. Journal of Food Protection. Lappi VR, Thimothe J, Nightingale KK, Gall K, Scott VN, Wiedmann M. Longitudinal studies on Listeria in smoked fish plants: impact of intervention strategies on contamination patterns. J Food Prot. 2004 Nov;67(11):2500-14.	Fish plants (n=4)	Food and Environmental (sponges)	NFCS (Drains, floors, rolling carts, door handles, aprons) and FCS (slicing machines, skinning machines)	Overall = 7.9% (including food samples)	Before intervention strategies= 26.1% (n=617). During and After =19.5% (n=527). Overall (including food samples) - 18.7%	-	-
Incidence of *Listeria monocytogenes* and *Listeria* spp. in a Small-Scale Mushroom Production Facility	Viswanath P, Murugesan L, Knabel SJ, Verghese B, Chikthimmah N, Laborde LF. Incidence of *Listeria monocytogenes* and *Listeria* spp. in a small-scale mushroom. production facility. J Food Prot. 2013 Apr;76(4):608-15	Small mushrooms production facility	Environmental (sponges)	Conveyors, shovels, hoses, drains, doors, harvesting knives	1.60%	14.20%	15.8% (29/184)	

Monitoring occurrence and persistence of *Listeria monocytogenes* in foods and food processing environments in the Republic of Ireland	Alvarez-Ordóñez A, Leong D, Morgan CA, Hill C, Gahan CG, Jordan K. Occurrence, Persistence, and Virulence Potential of *Listeria* ivanovii in Foods and Food Processing Environments in the Republic of Ireland. Biomed Res Int. 2015;2015:350526.	Food processing facilities (n=48)	Environmental processing areas	FCS and NFCS	4.4% (n=1574)	-	-	
Prevalence and Persistence of *Listeria monocytogenes* in Ready-to-Eat Tilapia Sashimi Processing Plants	. Chen BY, Wang CY, Wang CL, Fan YC, Weng IT, Chou CH. Prevalence and Persistence of *Listeria monocytogenes* in Ready-to-Eat Tilapia Sashimi Processing Plants. J Food Prot. 2016 Nov;79(11):1898-1903.	Sashimi processing plants (n=2)	Environmental	surfaces in processing areas and final product (CONFUSING RESULTS)	3.92% (79/2016) (Surface areas and final product)	-	-	-
Listeria monocytogenes Contamination Patterns for the Smoked Fish Processing Environment and for Raw Fish	Hoffman AD, Gall KL, Norton DM, Wiedmann M. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. J Food Prot. 2003 Jan;66(1):52-60.	Smocked fish processing facility (N=2)	Environmental sponges	Drains, floors, door handles equipment, plastic crates	22.46% (n=512)	-	-	-
Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR.	Lawrence, L. M., and A. Gilmour. 1994. Incidence of *Listeria* spp. *and Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. Appl. Environ. Microbiol. American Society for Microbiology (ASM) 60:4600–4.	Raw and cooked poultry processing environment (n=1)	Environmental (Swabs)	-	Raw poultry processing environment 26% (21/79) and Cooked PE 15% (26/173)	RPPE = 46% (15/79).CPE = 29% (25/173)	RPE = 46%. CPE= 29%	-

Geographical and Meteorological Factors Associated with Isolation of *Listeria* Species in New York State Produce Production and Natural Environments	Chapin TK, Nightingale KK, Worobo RW, Wiedmann M, Strawn LK. Geographical and meteorological factors associated with isolation of *Listeria* species in New York State produce production and natural environments. J Food Prot. 2014	Produce production (pp) and natural environments (ne)	Environmental	Soil, drag swab and water	PP= 14.96% (88/588) and NE= 3.8% (59/734)	PP = 31.6% (186/588) and NE= 35.3% % (259/734)	PE= 34% (201/588). NE= 33.4% (245/734)	NE= 3% and 9% PE
Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets	Chen BY, Pyla R, Kim TJ, Silva JL, Jung YS. Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food Microbiol. 2010 Aug;27(5):645-52.	Catfish processing environment	Environmental swab	Outside (catfish holding tank and water) and inside (processing surfaces, floor and drains)	21.6 % (68/315)	42.5%(134/315)	64%	
Irrigation Is Significantly Associated with an Increased Prevalence of Listeria monocytogenes in Produce Production Environments in New York State.	Weller D, Wiedmann M, Strawn LK. Irrigation Is Significantly Associated with an Increased Prevalence of Listeria monocytogenes in Produce Production Environments in New York State. J Food Prot. 2015 Jun;78(6):1132-41.	Produce farms (n=10)	Environmental	Water, terrestrial samples and fecal	16% terrestrial environment and 30% water samples and 45% fecal. Overall= 23% (28/124)	19.30%	Total overall 42% (52/124)	-
Risk Factors Associated with Salmonella and *Listeria monocytogenes* Contamination of Produce Fields	Strawn LK, Gröhn YT, Warchocki S, Worobo RW, Bihn EA, Wiedmann M. Risk factors associated with Salmonella and *Listeria monocytogenes* contamination of produce fields. Appl Environ Microbiol. 2013 Dec;79(24):7618-27	Produce farms (n=21)	Environmental	Soil, drag swab and water	9.7% Terrestrial (51/526) 30% of water samples (20/51) .	-	-	-

Landscape and Meteorological Factors Affecting Prevalence of Three Food-Borne Pathogens in Fruit and Vegetable Farms.	Strawn LK, Fortes ED, Bihn EA, Nightingale KK, Gröhn YT, Worobo RW, Wiedmann M, Bergholz PW. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl Environ Microbiol. 2013 Jan;79(2):588-600.	Produce Farms (n=5)	Environmental	Soil, drag swab and water	15.00%	-	-	-
Molecular Characterization of *Listeria monocytogenes* from Natural and Urban Environments	Sauders BD, Durak MZ, Fortes E, Windham K, Schukken Y, Lembo AJ Jr, Akey B, Nightingale KK, Wiedmann M. Molecular characterization of *Listeria* monocytogenes from natural and urban environments. J Food Prot. 2006 Jan;69(1):93-105.	Urban and natural environments	Environmental	N = soil, vegetation, and surface water. U= floors. Sidewalks and human contact surfaces.	U=7.5 and N=1.4%,	-	-	-

[a] *Listeria* spp. represents all *Listeria* species except *Listeria monocytogenes*.

Listeria Prevalence in Domestic Environments and in Retail Surfaces.

Similarly to the processing environment, *Listeria spp.* and *Listeria monocytogenes* have been isolated from many retail surfaces and equipment, including food store refrigerators (*55*). *Listeria* spp. and *Listeria monocytogenes* have also been isolated from domestic kitchens (*17*) and the incorrect preparation handling and storage of food can lead to the contamination of food. *Listeria monocytogenes* is able to survive and attach to different surfaces such as cutting boards and countertops (*15*). *Listeria monocytogenes* and *Listeria spp.* have been isolated from different surfaces in domestic environments such as refrigerators, salad compartments, and dishcloths (*15*). Cross-contamination from these surfaces to ready to eat foods and produce might be a risk hazard for consumers, especially for those who are more vulnerable to acquire listeriosis. However, there is a lack of studies that evaluate the impact of *Listeria* cross-contamination in the domestic environment. More studies analyzing the impact of the presence of *Listeria* in domestic environments may help clarify the relative importance of these domestic sources in contributing to outbreaks of listeriosis.

Cross-contamination and Survival of *Listeria monocytogenes* in Produce Packinghouses

Overview

Contamination of *Listeria monocytogenes* can occur at any point in the supply chain from pre-harvesting of the fresh produce to preparing them in a domestic kitchen. Recent *Listeria monocytogenes* outbreaks have been traced back to the packinghouse environment. One of the greatest examples is the Jensen farms *L. monocytogenes* outbreak in 2011. During this outbreak, cantaloupes were contaminated with *L. monocytogenes* leading to a multistate outbreak causing more than 147 listeriosis cases and 33 deaths (*41*). The FDA investigation of this outbreak concluded that 13 environmental samples from the Jensen

Farms were positive for *L. monocytogenes.* Positive samples came from equipment, standing water in the facility, and cantaloupe conveyor belts (*75*).

Listeria Introduction, Growth Niches, and Transfer Sites

In a packinghouse, *Listeria* may enter the facility using the raw material, equipment (forklift tires), and personnel as a transportation vehicle. Once *Listeria* is introduced, it can populate different sites in the facility. There are many conditions within a packinghouse that could allow the growth and persistence of *Listeria,* even after routine sanitation practices have been completed (*3*). These sites are referred to as growth niches. There are several factors that play a role in the development of these growth niches, including poor equipment design such as hollow areas, hard to clean areas, and gaps between equipment piece connections (*79*). If a growth niche is established, it can become a potential source for the transfer of *Listeria* within the facility.

Food contact surfaces like trays and worktops tend to be more likely to transfer sites than growth niches. Other transfer sites include door handles, raw materials, and floors in high traffic areas. Some equipment can become both a growth niche and a transfer site. A transfer site does not necessarily allow for the growth of *Listeria* but can facilitate the dissemination of *Listeria*. Examples of a transfer site are forklift tires, product handler's gloved hands, door handles, and food contact surfaces (*3*).

Post-harvest water could also potentially allow the contamination of the product. Post-harvest water is any water that comes in contact with the fresh produce after harvest. In the packinghouse facility, post-harvest water is used for rinsing, washing, and moving produce water (*77*). Often, post-harvest washing system involves the recirculation of water

and this practice increases the risk for cross-contamination. To reduce this risk, water sanitizer is used for maintaining the quality of the water throughout the operation time (*77*).

In summary, *L. monocytogenes* could reside in different sites in the packinghouse environment. Some of these sites are growth niches and others are transfer sites. Detecting and eliminating growth niches is pivotal for the reduction of *L. monocytogenes* persistence and distribution in the packinghouse environment.

Strategies to Limit or Reduce *Listeria* in Produce Packinghouses and to Prevent Contamination to Foods.

Understanding the environmental conditions preferred by *Listeria* is an important point to reduce and limit *L. monocytogenes* in a facility. *Listeria monocytogenes,* unlike other pathogens, has the ability to grow under cool and moist conditions. These conditions are often found in packinghouses facilities; therefore, monitoring sites and controlling the introduction of *Listeria monocytogenes* into the facility are some of the most important strategies to reduce the presence of this pathogen in the packinghouse environment. However, *Listeria monocytogenes* is considered to be ubiquitous and the introduction of this pathogen is often inevitable, even when preventive measures have been put in place (*60*).

Cleaning and Sanitizing

Implementing an efficient cleaning and sanitizing program can potentially reduce *L. monocytogenes* incidence and decrease the risk of *L. monocytogenes* to become a resident pathogen in the facility (See section 6.4 for resident definition). It is important to understand the difference between cleaning and sanitizing when developing a sanitation program. Cleaning refers to the removal of food, organic matter, and filth of a surface. Sanitizing is the action of

reducing the number of microorganisms on a clean surface. Sanitation should not be done if the surfaces have not been cleaned. Cleaning often includes detergents, solvent, acid, and abrasive cleaners. All of these remove food, soil, rust stains, minerals, and other deposits (*73*).

Because all facilities are different, it is important to develop a cleaning and sanitizing program targeting a particular facility. Similarly, procedures to monitor the effectiveness of cleaning and sanitizing practices should be focused on individual operations (*60*). Monitoring programs are a common practice to evaluate sanitation procedures. Testing for the presence of *L.* spp. allows for the establishment of a baseline of the efficiency of sanitation procedures. *L.* spp. has been the indicator organism for *L. monocytogenes* – the presence of *L.* spp. indicates the potential presence of *L. monocytogenes* (*60*)

Sites sampled, frequency, and time of testing should be tailored to a particular facility needs. Sampling site selection is recommended for the areas that support *Listeria monocytogenes* growth (e.g. drains, floors, cold storages) and for areas where cross-contamination can happen (e.g. conveyors, slicers, dicers, filling and packaging equipment) (*60*). Positive *Listeria* samples could help determine contamination patterns as well as to evaluate sanitation procedures. When finding a positive *Listeria* sample in a food contact surface (any surface that touches the food such as conveyors, dicers, etc.), it is recommended that the product is contained within the facility until specific species of the positive *Listeria* sample is determined. Corrective actions regarding cleaning and sanitation practices, equipment design, and retraining of the personnel should be executed to prevent future contamination (*60*).

Environmental Monitoring Programs

Overview

Environmental monitoring refers to the practices to assess the quality and characteristics of the environment. In food manufacturing and processing, environmental monitoring programs (EMP) are designed to test for contaminants while evaluating and monitoring the hygiene of the processing environment (*8*). EMPs do not minimize the presence of microorganisms, but they provide information about the efficiency of sanitation and cleaning programs by revealing the presence of a microorganism that could potentially create a food safety concern (*8*). Therefore, developing an effective EMP is pivotal for the identification, minimization, and prevention of food contamination in the food processing environment (*3*).

Environmental monitoring programs can provide invaluable information about the overall microbial occurrence in the processing environment. EMPs can detect the presence and harborage of undesired microorganisms and their niches, and the preferred locations and sources that can potentially cause microbial contamination (*3*). Therefore, environmental monitoring programs can be used as a warning system to minimize food contamination and avoid costly and severe consequences such as food recalls and foodborne outbreaks.

Implementing an Environmental Monitoring Program

To effectively implement and design an environmental monitoring program, it is necessary to gather individuals who are familiar with the facility who can help detect potential areas of risk. Also, it is important to have a person with experience in developing and implementing EMPs such as a consultant or a microbiologist. The team should evaluate potential risk areas and define environmental zones (*30*). In the food industry, the facility is typically divided into 4 categories. Zone 1 refers to the surfaces that come in direct contact with food such as work tables,

conveyors, and product contact utensils (*3*). Surfaces that are hard to clean and disinfect are the most appropriate for sampling. Zone 2 refers to the surfaces and areas that do not necessarily come in contact with the food but are in close proximity to the food. Areas and surfaces that are often touched by produce handlers such as the exterior and framework of equipment, switches, and equipment housing fall into zone 2 (*3*). Zone 3 includes surfaces that do not come in contact or are directly associated with (or close from) food. This zone includes walls, forklifts, drains, and floor. (*3*). Lastly, Zone 4 refers to the areas outside the processing environment such as lunchrooms, hallways, office areas, etc.

Sampling from all the four zones is important to locate the niches and potential contamination points. The testing frequency should be based on environmental sampling results. It is important to remember that the design and implementation of the EMPs depend on the specific facility, individual facility operations, and the set goals for the EMP (*8*).

Listeria Environmental Monitoring

Pathogen environmental monitoring (PEM) is extremely important for the detection of microbial pathogens. PEM is vital in fresh produce facilities since these food types are often consumed raw. Current foodborne pathogens of main concern are *Listeria*, *E. coli*, and *Salmonella* (*30*). EMP targeting *Listeria* detection assess *Listeria* control practices, prevent transient *Listeria* from becoming entrenched (persistent), and indicate entrenched *Listeria* and niche areas in the processing facility (*3*). EMP for *Listeria* determine the timing and the type of corrective action to minimize *Listeria* contamination (*3*). *Listeria monocytogenes* environmental monitoring uses *Listeria spp*. as the indicator microorganism. It is believed that the detection of *Listeria ssp*. indicates the potential presence of *L. monocytogenes*. Test results for *Listeria spp*.

are generated faster and since *Listeria ssp.* is more prevalent in the environment than *L. monocytogenes* and *Listeria spp.*, it is easier to detect.

Transient versus resident *Listeria monocytogenes*

Positive *Listeria* results (*Listeria* isolates) can be classified into two different categories: transient isolate or resident (or persistent) isolate. Transient isolate refers to an isolate that has not been previously detected via swabbing (*3*). A persistent isolate refers to the isolate whose presence has been repeatedly detected over time. Identification of persistent isolates likely indicates the growth and survival of *Listeria* in the facility (*17*). The differentiation between a transient and resident isolate is challenging. Repeated detection of a strain may be caused by the re-introduction of the same strain and do not necessarily reflect true persistence of a Listeria subtype (*17*).

A transient isolate can become resident if the isolate develops physical adaptation and tolerance to environmental condition and processing factors (disinfectants and sanitizers). It is thought that a key characteristic of *Listeria* that contributes to its persistence is its ability to attach to diverse surfaces like glass, stainless steel, and others, and the formation of biofilms (*17*). Biofilms are an organized complex structure that provides protection against harsh condition and it also provides nutrients to the *Listeria* forming the biofilm. The persistence of *Listeria monocytogenes* strains is not clear. Some persistent *L. monocytogenes* strains were isolated after the disinfection process, possibly explaining the resistance of persistent strains. Other studies suggest that persistence of *Listeria monocytogenes* is due to the formation of biofilms (*17*). Presence of biofilms and resident *Listeria* is a major concern of the food industry since these can act as sources for *Listeria* contamination.

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CHAPTER 3: PREVALENCE, PERSISTENCE AND DIVERSITY OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP. IN PRODUCE PACKINGHOUSES IN THREE U.S. STATES

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Abstract

Listeria monocytogenes has emerged as a food safety concern for a number of produce commodities. While *L. monocytogenes* contamination can occur throughout the supply chain, contamination from the packinghouse environment represents a particular challenge and has been linked to recalls. This study aimed to investigate the prevalence, persistence, and diversity of *Listeria monocytogenes* (LM) and *Listeria* species (LS) in produce packinghouses. A longitudinal study was performed in 11 packinghouses (commodities included micro-green, peach, apple, tomato, broccoli, cauliflower, and cucumber) in three US states. In each packinghouse, 34 to 46 sites representing zones 2-4 were selected and swabbed. Packinghouses were visited 4 times and samples were processed for *Listeria* by US Food and Drug Administration's Bacteriological Analytical Manual methods. Presumptive *Listeria*-positive isolates were confirmed by PCR. Species and allelic type (AT) were identified by *sigB* sequencing for up to four isolates per sample. Among 1,584 samples tested, 50 (3.2%), 42 (2.7%), and 10 (0.6%) samples were positive for LM, LS (excluding *L. monocytogenes*), and both LM and LS, respectively. Five different species of *Listeria* (*monocytogenes*, *innocua*, *seeligeri*, *welshimeri*, and *marthii*) were identified with *L. monocytogenes* being the most prevalent species. The 102 *Listeria*-positive samples yielded 128 representative isolates. Representative isolates were defined as having a unique subtype (AT). Approximately 21% (21/102) of those *Listeria*-positive samples contained two or more different subtypes. A high AT

diversity (0.95 Simpson's Diversity Index) was observed amongst *Listeria* isolates. There were 15 instances of LM or LS repeat isolation (site testing positive >2 times). Upon analysis of subtype data, only 3 sites tested positive for the same *Listeria* AT >2 times. Data showed in this longitudinal study that *Listeria* prevalence and persistence in packinghouses was low (e.g., <4% prevalence). Therefore, sanitation program development and implementation in packinghouses are critical to limit *Listeria* harborage and residence.

Introduction

Listeria monocytogenes is a widely distributed foodborne pathogen accounting for about 20% of the deaths caused by reported cases of foodborne disease in the United States (US) (*46*). In the last ten years, several *L. monocytogenes* outbreaks have been associated with consumption of fresh produce. While *L. monocytogenes* contamination can occur at several stages along the produce supply chain, several listeriosis outbreaks and recalls have been attributed to contamination in the packinghouse (*4*, *5*, *12*, *28*, *43*, *44*, *51*). For example, a cantaloupe-borne outbreak in 2011 caused 147 cases of listeriosis and 33 deaths in 28 US states (*28*). In 2014, a multi-state *L. monocytogenes* outbreak was associated with caramel apples, which resulted in 34 hospitalizations and 7 deaths (*4*). In the same year, another *L. monocytogenes* outbreak occurred due to bean sprout consumption and resulted in five illnesses and two deaths across two US states (*5*). In 2016, *Listeria*-contaminated packaged salads caused 19 cases of listeriosis (*51*).

Prior to these outbreaks, *L. monocytogenes* was not a primary target pathogen of concern in fresh produce, such as *Salmonella* and *Escherichia coli* O157:H7 (*26*). In fact, there are minimal published studies on *Listeria* contamination in produce (*1*, *14*, *23*, *34*) and produce-associated environments with most focused on the pre-harvest environment (*6*, *32*, *39*, *40*, *49*). These studies have also investigated the prevalence, persistence, and diversity of *L. monocytogenes* and other species of *Listeria* in produce pre-harvest environments (e.g., produce production fields). One cross-sectional study (*40*) observed a *L. monocytogenes* prevalence of 9.7 (51/526) and 9.0% (2/23) in terrestrial (combined sub- and top-soil) and aquatic (irrigation water) samples collected across 21 produce farms in western, central and eastern New York. A similar study (*49*), also sampling farms (n=10) in four New York state regions (central, Finger Lakes, southern tier, and western), reported a 16 (n=80) and 30% (n=33) *L. monocytogenes*

prevalence in terrestrial (top and sub-soil) and aquatic (pond, creek water) samples, respectively. A study (*39*) performed by Strawn and colleagues identified some instances of *L. monocytogenes* persistence (defined as the same subtype being isolated repeatedly from the same sample location), with most instances from water sources. Additionally, this same study found a high diversity of *L. monocytogenes* subtypes across terrestrial, aquatic, and fecal samples collected from five produce farms across multiple seasons (2009-2011). These studies (*6*, *32*, *39*, *40*, *49*) suggest *L. monocytogenes* can be observed in produce pre-harvest environments; specifically, environmental factors and management practices associated with soil and water can influence the risk of *L. monocytogenes* contamination on-farm. If the product, equipment (e.g., trucks, harvesters), tools (knives, harvest bins), and or other miscellaneous items (e.g., worker boots) become contaminated in the field (e.g., by contaminated soil, water, feces), it is possible for contamination to be introduced in post-harvest environments (*51*).

Species of *Listeria* have been isolated from a wide variety of post-harvest food environments, excluding produce. Several studies (*7*, *8*, *16*, *17*, *21*, *31*, *36*, *38*, *45*, *50*) have investigated the prevalence of *Listeria* spp. (5.3 to 46%) and *L. monocytogenes* (1.6 to 27.7%) in non-produce food environments including fish, meat, dairy, mushroom, and poultry processing facilities, and deli retail environments. For example, a study at the Mushroom Test Demonstration Facility at Pennsylvania State University found that 1.6% of the environmental samples collected (n= 184) were positive for *Listeria monocytogenes.* Another study (*50*) in ready-to-eat meat processing environments reported an *L. monocytogenes* prevalence of 6.1% (based on 688 samples collected in 6 plants located in Colorado, Kansas, and Nebraska). Environmental contamination of *L. monocytogenes* can be challenging as several food associated environments maintain temperatures and relative humidity that may promote *L. monocytogenes*

growth and survival (*3*). Furthermore, *L. monocytogenes* has also been detected from retail deli environments, as well as study (*38*) authors observed an increase in *L. monocytogenes* prevalence during deli operation (9.5%, n=4,503), compared to before initiation of deli operation (6.8%, n=314). This finding suggests that *L. monocytogenes* prevalence may be associated with increased activity or traffic (e.g., product movement). Overall, prior studies have observed *L. monocytogenes* in several non-produce food associated environments; however, published studies are lacking on *L. monocytogenes* and other *L*. spp. prevalence, persistence, and diversity in post-harvest produce environments, specifically produce packinghouses dealing with raw agricultural commodities. Data are needed to evaluate contamination risks in produce packinghouses and also effectively develop control measures to minimize produce contamination (e.g., limit cross-contamination, harborage, and transfer sites).

Therefore, the goal of this study was to determine the prevalence of *L. monocytogenes* and other *Listeria* spp. in produce packinghouses over a full packing season. Additionally, *Listeria* isolates were subtyped to investigate the persistence and diversity within and across packinghouses, as well as compare harborage sites in these facilities. By obtaining data on *L. monocytogenes* and other species of *Listeria* in produce packinghouse environments, we seek to provide the produce industry with best practices to reduce and or control the risk of produce contamination events in the packinghouse.

Materials and Methods

Study design. A longitudinal study was performed on 11 produce packinghouses handling and packing raw agricultural commodities in three U.S. States: Maryland, Tennessee, and Virginia. Commodities included micro-green, peach, apple, tomato, broccoli, cauliflower, and cucumber. The distance between packinghouses ranged from approximately 30 to 1,050 km. Further

location details are not provided due to confidentiality. Packinghouses were selected based on the willingness of packers to participate; however, all packinghouses had completed successful third-party audits and are covered by the Food Safety Modernization Act Produce Safety Rule. Packinghouses were sampled four times during the growing season from July 2017 to March 2018. Additionally, samples were collected 3-4 hours into packinghouse operation to capture where contamination events would more likely be identified (heaviest product flow and worker/equipment traffic; prior to sanitation).

Sample sites. To select sampling sites, authors met with owners/food safety managers to discuss how fruits and or vegetables were handled and packed in the packinghouse. No food contact surface (FCS) sites were selected. Between 34 to 46 non-food contact surface (NFCS) sites were sampled based on the United Fresh *Guidance on Environmental Monitoring and Control of Listeria for the Fresh Produce Industry* (*2*). Briefly, NFCS sites for each packinghouse were split into zones 2 (located adjacent to FCS), 3 (located in main handling and packing areas), and 4 (located outside of main handling and packing areas), where most of the samples selected were from zones 2 and 3. Site examples included forklift wheels, drains, dump tank legs, cold room floors, and squeegees, among other sites (S1). Sites from each packinghouse were mapped on building schematics, photographed and location details described to ensure sites were re-sampled upon each packinghouse visit. Sites were further grouped into 6 sub-categories to assist in identification of *Listeria* niches; cold storage (CS), drain (D), wet non-food contact surface (WNFCS), dry non-food contact surface (DNFCS), mobile non-food contact surface (MNFCS) and outside the packinghouse main handling and packing area (OP).

Sample collection. Samples were collected by the authors (EE, AH, and LS). Latex gloves (Nasco, Fort Atkinson, WI) were worn for sample collection. Gloves were changed between each

sample and disinfected with 70% ethanol prior to sample collection. Samples were collected using 3M^{TM} Sponge-sticks with 10 mL of D/E (Dey Engley) Neutralizing broth (3M, Maplewood, MN). For each sample, the sponge-stick was aseptically removed from the labeled bag, used to sample the selected site, and immediately returned to the labeled bag. For selected sites with large surface areas, such as cold room floors, dump tank legs or forklift wheels, approximately 645 cm^2 (100 in^2) was swabbed. For selected sites with surfaces areas less than 645 cm^2, the whole available surface was swabbed (e.g., squeegee, cart handle). All samples were transported on ice, stored at 4±2°C and processed within 24 hours of collection.

***L. monocytogenes* and other *Listeria* spp. detection and isolation.** Each sponge-stick sample was tested to detect and isolate *Listeria* spp. using a modified version of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* procedure (*15*, *16*, *38*). Briefly, sticks were aseptically removed leaving only the sponge in the labeled bag. A 90 mL volume of buffered *Listeria* enrichment broth (Difco, BD, Sparks, MD) was added to each sample bag, homogenized in a Stomacher 400 Circulator at 230 rpm for 1 minute, and incubated at 30°C. After a 4 h incubation period, 360 µL of *Listeria* selective enrichment supplement (Oxoid, Cambridge, UK) was added to each sample bag and returned to the incubator (30°C). At both 24 and 48 h post-incubation, 50 µL of enrichment from each sample bag was streaked to *Listeria monocytogenes* plating medium (LMPM; R&F Laboratories, Downers Grove, IL) and modified Oxford medium (MOX; Difco, BD) agars. LMPM and MOX agar plates were incubated at 35 and 30°C for 48 h, respectively. Colonies representing *Listeria* growth were sub-streaked, if needed, for isolation in preparation for identification. Presumptive *Listeria*-positive colonies were confirmed by polymerase chain reaction (PCR) for the partial *sig*B gene using previously described methods (*30*, *38*). For each sample, up to eight confirmed *Listeria*-positive isolates (up to four putative *L.*

monocytogenes and up to four putative *Listeria* spp. other than *L. monocytogenes*; selecting equally, if possible, from both the 24 and 48 h enrichment platings) were sub-streaked on brain heart infusion agar (BHI; Difco, BD), incubated at 35°C for 18 hours, and frozen at -80°C in 15% glycerol.

Identification of *Listeria* species and allelic type. *Listeria* isolates were streaked from frozen culture to BHI and incubated at 37°C for 18 h and a well-isolated colony was selected. Partial *sigB* sequencing is a proven approach (rapid, low cost, and reliable) to identify species of *Listeria* and allelic type (AT) as described by Nightingale et al (*30*). Nucleotide sequences of *sigB* from *Listeria* isolates were obtained by Sanger sequencing performed by the Cornell University Life Sciences Core Laboratories Center (Ithaca, NY) and compared with those in the Food Microbe Tracker database (http://www.foodmicrobetracker.com) to assign species and AT, as defined by a unique combination of polymorphisms as previously described (*6*, *30*, *38*, *39*, *49*).

Categorical Analysis. Categorical analysis was performed as previously described (*6*). Briefly, univariate associations between *Listeria* positive samples and packinghouses were performed by a chi-square test. Confidence intervals (95%) were calculated for each variable assuming a binomial distribution. Subtype diversity using *sig*B allelic type (AT) within and across packinghouses was quantified using Simpson's Index of Diversity (SID), which was calculated as previously described (*18*, *30*). All categorical analyses were performed using Microsoft Excel (2007).

Results and Discussion

In the study reported here, there are three ways *Listeria* is reported and discussed: (i) *Listeria*, (ii) *Listeria* spp. and (iii) *L. monocytogenes*. *Listeria* refers to the genus level and includes all species of *Listeria*. *Listeria* spp., refers to all species of *Listeria*, excluding *L. monocytogenes*. *L. monocytogenes* refers only to the species of *Listeria*: *L. monocytogenes* (the human pathogen responsible for several produce associated outbreaks and recalls). A total of 1,584 samples were collected from 397 sites during the study.

While *Listeria* prevalence was low, approximately half of the total *Listeria* isolates were identified as *L. monocytogenes*. Of the 1,584 samples collected, 102 samples were positive for the genus *Listeria* (6.4%). *Listeria* prevalence varied among packinghouse from 0.0 to 17.1%. Of the 11 packinghouses sampled, one packinghouse (K) showed zero *Listeria* prevalence, two packinghouses (I and J) showed very low *Listeria* prevalence (<1%), five packinghouses (D, E, F, G, and H) showed a *Listeria* prevalence of 1-10% and the remaining three packinghouses (A, B, and C) showed a *Listeria* prevalence of over 10% (Table 3.1). The *Listeria* prevalence (6.4%) reported in this study was lower, compared to the *Listeria* prevalence reported in other food environments (*7*, *16*, *21*, *38*, *45*, *50*). For example, one study (*50*) observed approximately 15.6% (107/688) of samples collected from non-food contact surfaces were positive for *Listeria* in six small ready to eat (RTE) meat processing plants. Similarly, another study (*45*) reported a *Listeria* prevalence of 15.8% (29/184) from samples (conveyors, shovels, hoses, drains, doors, and harvesting knives) collected in a small-scale mushroom production facility. Among the 102 *Listeria* positive samples, 60 (3.8%) and 52 (3.3%) were positive for *L. monocytogenes* and *Listeria* spp. where 10 (0.6%) of those samples were positive for both *L. monocytogenes* and *Listeria* spp. (co-isolation), respectively (Table 3.1). The *L.* monocytogenes prevalence in a given packinghouse ranged from 0.0 to 11.4%. Only two of the packinghouses (H and K)

sampled had no *L. monocytogenes* positive samples detected during the study (n=136 and 140, respectively), while the remaining nine packinghouses had at least one *L. monocytogenes* positive sample during the study. *L. monocytogenes* prevalence was highest in packinghouses A (11.4%), C (11.4%), and D (7.1%) (Table 3.1). Other food associated environments have observed the range of *L. monocytogenes* prevalence between 1.6 to 26% (*7*, *16*, *17*, *21*, *22*, *36*, *38*, *45*, *50*). *L. monocytogenes* was found in 1.6% of environmental samples from a mushroom production facility, compared to 26% of environmental samples from a raw poultry processing facility (*21*, *45*). The *Listeria* spp. prevalence in a given packinghouse ranged from 0.0 to 11.4%. Three packinghouses (I, J, and K) had no samples test positive for *Listeria* spp. (n=144, 184, and 140, respectively). *Listeria* spp. prevalence was highest in packinghouses B (11.4%), A (10.7%), and E (5.0%) (Table 1). Interestingly, in the study reported here, only packinghouse A shared a high prevalence of *L. monocytogenes* and *Listeria* spp. (ranked in the top 3 for *L. monocytogenes* and *Listeria* spp. positive samples amongst the 11 packinghouses). *Listeria*, *Listeria* spp. and *L. monocytogenes* prevalence was highly based on individual packinghouse (range 0.0 to 17.1%). Several of the published studies are based on sampling less than five operations, except the retail studies (*36*, *38*); therefore, prevalence may be driven higher or lower based on enrolled operations. In the study reported here, 11 produce packinghouses were sampled. More studies are needed in produce packinghouses across the country to determine if these findings are applicable to other produce packinghouse environments, as well as studies need to include larger numbers of operations to capture the diversity and variability across and within operation.

Over half of the *Listeria* positive samples detected in this study were identified to be *L. monocytogenes* (60/102). In fact, several packinghouses (6/11) in this study had a higher *L. monocytogenes* prevalence, compared to *Listeria* spp. prevalence (4/11) (Table 3.1). One

packinghouse had no *Listeria* positive samples. Other studies (*7*, *16*, *21*, *45*, *50*) from meat, dairy, fish and mushroom food environments have observed other species of *Listeria* are more prevalent than *L. monocytogenes* suggesting produce associated environments pose unique challenges, compared to other food environments. Prior studies (*6*, *39*, *40*, *49*) in produce production environments in New York State have observed *L. monocytogenes* prevalence from 9 to 51% in samples collected from field soil to agricultural water, suggesting *L. monocytogenes* may be introduced in produce packinghouses, dependent on harvest and handling practices. One study (*38*) performed in the retail environment observed higher *L. monocytogenes* prevalence in environmental samples collected from 15 stores (6 NFCS, 1 FCS sites in the retail deli), compared to *Listeria* spp. prevalence (6.8 and 3.8%, n=314 in Phase I of project). Based on the study findings reported here, *Listeria* positive samples detected in the produce packinghouse environmental should trigger rapid and robust corrective measures to remove the contamination issue. Approximately <1% (10/102) of *Listeria* positive samples yielded *L. monocytogenes* and one other species of *Listeria* (co-isolation). Only three of the 11 packinghouses (A, B, and G) had co-isolation of *L. monocytogenes* and one other species of *Listeria* (7, 2, and 1 sample(s) in packinghouse A, B and G, respectively). Findings suggest that co-isolation of *L. monocytogenes* and another species of *Listeria* from the same sample wasn't common. Instead, samples were more likely to be positive for one species of *Listeria*. In the study reported here, up to four putative *L. monocytogenes* and four *Listeria* spp. isolates were selected, equally from the 24 and 48 h enrichments, for confirmation and further subtyping analyses. Thus, the methodology for *Listeria* detection and isolation from samples should not be a limitation to capture cases of *Listeria* co-isolation in this study. Most studies do not report co-isolation of *L. monocytogenes* and *Listeria* spp.; however, one study (*6*) observed the co-isolation of *L. monocytogenes* and at

least one other species of *Listeria* in a given sample was 9% in produce production environments.

While the *Listeria* prevalence in produce packinghouses was lower compared to other food associated environments, it is difficult to compare across studies, due to variable experimental designs and sampling methodologies. The study reported here included 10 of 11 packinghouses that were considered large (>$500,000) under the Food Safety Modernization Act Produce Safety Rule (*42*) and 11 of 11 packinghouses that had successfully passed a Good Agricultural Practices audit within the year. However, the packinghouses varied tremendously in sanitation program from lack of any visual inspection, cleaning and removing accumulated organic matter (debris) to daily cleaning and sanitizing of FCS and adjacent areas by a separate group of individuals. In future studies, formal questionnaires or surveys with management personnel assessing sanitation regimes and practices should be paired with environmental samples to identify trends and associations between pathogen positive and sanitation activity.

***L. monocytogenes* and *Listeria* spp. were most prevalent in samples collected from Drain (D), Cold Storage (CS) and Wet Non-Food Contact Surface (WNFCS) Sites.** Of the 396 sites sampled, 77 sites were positive for the genus *Listeria* (19.4%). Among the 77 *Listeria* positive sites, 49 (12.4%) and 43 (10.9%) were positive for *L. monocytogenes* and *Listeria* spp., where 15 (3.8%) of those sites were positive for both *L. monocytogenes* and *Listeria* spp. either simultaneously (co-isolation) or upon one of the four visits over the course of the study (Table 3.2). Similar to above, *L. monocytogenes* and *Listeria* spp. prevalence in sites varied across packinghouses. The range of *L. monocytogenes* and *Listeria* spp. prevalence in samples collected from sites was 0.0 to 34. 3% and 0.0-37.1%, respectively. Packinghouse A (34.3%), C (34.3%), and D (22.9%) had the highest number of sites positive for *L. monocytogenes*, while

packinghouse A (37.1%), B (34.3%), F (14.3%) and E (14.3%) had the highest number of sites positive for *Listeria* spp. in the study (n=35 sites for each packinghouse). Five of the 11 packinghouses (A, B, C, F, and G) had sites test positive for *L. monocytogenes* and *Listeria* spp. during the same visit (co-isolation within sample from site), or upon one of the four visits over the course of the study (Table 3.2).

The 396 sites represented zones 2 (adjacent to FCS), 3 (in the main packinghouse handling and packing area) and 4 (outside the main packinghouse handling and packing area) according to traditional environmental monitoring programs (*27*). To further elucidate trends and potential harborage niches within the packinghouse, samples from sites were sub-grouped into the following categories: drain (D, n=36), cold storage (CS, n=47), wet non-food contact surface (WNFCS, n=71), dry non-food contact surface (DNFCS, n=138), mobile non-food contact surface (MNFCS, n=83) and outside the main packinghouse handling and packing area (OP, n=22) (A1). The *L. monocytogenes* and *Listeria* spp. prevalence ranged from 4.3 to 36.1% and 7.2 to 30.6% across the sub-groups, respectively (Table 3.3). The sub-groups D, CS and WNFCS had the most samples positive for *L. monocytogenes* (36.1, 25.5, and 23.9%, respectively) and *Listeria* spp. (30.6, 23.4, and 14.1%, respectively). Interestingly, the categories D and CS represented 7 of 10 samples that were positive for both *L. monocytogenes* and *Listeria* spp. during the same visit (co-isolation). Previous studies (*16*, *20*, *36*, *38*, *46*) have also shown high *L. monocytogenes* and *Listeria* spp. prevalence in similar sites as the study reported here. For example, a study (*16*) in a farmstead dairy production environment observed samples collected from drain sites accounted for the largest prevalence of *L. monocytogenes*, compared to samples collected from floor, equipment, wall and, doorway sites. A longitudinal study (*20*) in smoked fish processing plants also observed samples collected from drain sites represented the largest

prevalence of *Listeria*. Furthermore, Simmons et al. (*38*) found that in deli retail establishments, samples collected from cold room sites were among the sites with the highest *L. monocytogenes* prevalence; however, drains in cold rooms, along with other sites including floors and walls were also included in this category. Specifically, Simmons et al. (*38*) detected *L. monocytogenes* in 34.5 (41/119) and 20.5% (36/176) of samples from drain and floor sites, respectively. In the study reported here, all samples from drain sites were grouped together, regardless of location, to minimize *Listeria*-positive samples from drains confounding other sub-grouping results. WNFCS sites were also associated with a high *L. monocytogenes* and *Listeria* spp. prevalence in the study. Prior studies (*19*, *48*, *50*) have identified *L. monocytogenes* to persistent, and even grow, in environments that are cold and wet, as those environments may allow *L. monocytogenes* to outcompete other microorganisms. The three site categories (D, CS, and WNFCS) associated with the highest *L. monocytogenes* and *Listeria* spp. prevalence would be characterized as both wet and cold, which may have increased the likelihood of *L. monocytogenes* detection. The sub-group DNFCS had the lowest prevalence of *L. monocytogenes* and *Listeria* spp. over the study period. Dry environments have historically not been associated with *L. monocytogenes*, and maintaining a dry environment has been a *Listeria* control strategy (*33*). Findings from this study suggest packinghouse operators should develop and implement targeted interventions at D, CS, and WNFCS sites in produce packinghouses as those three sites were identified as potential *Listeria* niches (harborage areas). Niches are referred to as sites in the environment that may protect and allow *Listeria* persistence and potential replication (*11*).

Cases of *L. monocytogenes* and *Listeria* spp. repeated isolation were rare upon subtyping. Similar to cases of co-isolation (*L. monocytogenes* and another species of *Listeria* detected in the same sample), cases of *Listeria* repeated isolation were rare. Initially, if a sample from a site

tested positive for *Listeria* on at least two of the four sample collection visits during the study, it was considered repeated isolation. At this phase in the project, *Listeria* was confirmed by *sig*B PCR, and presumptive species identification was based on differential agar results (where blue colonies represent *L. monocytogenes* and while colonies represent *Listeria* spp. on LMPM agar). Under that definition, there were 15 cases of *Listeria* repeated isolation from 5 of the 11 packinghouses (A, B, C, D, and F) (Table 3.4). Packinghouse A yielded the highest number of repeated isolation cases (5), followed by packinghouse C (4), B (3), D (2), and E (1). Of the 15 cases of *Listeria* repeated isolation, 9 were *L. monocytogenes*, 5 were *Listeria* spp. and 1 was both *L. monocytogenes* and *Listeria* spp. (co-isolation). Approximately 40% of the *Listeria* repeated isolation cases were from WNFCS sites, followed by CS (3 cases), DNFCS (3 cases), D (2 cases), MNFCS (1 case) and OP (1 case) sites. Differentiation between *Listeria* re-introduction and true persistence (survival of *Listeria* subtype in a location) in a specific environment is particularly challenging (*11*). For all 11 produce packinghouses, the packinghouses were not closed to the environment, instead, raw product is continuously transported from fields to packinghouse, with lots of activity from fork-lifts and employees moving in and out of the packinghouses. Traffic flow of equipment, tools, and people may facilitate the initial introduction and re-introduction of *L. monocytogenes* to the packinghouse.

Subtyping was performed to discriminate the 15 cases of *Listeria* repeated isolation. Upon subtyping using partial sequencing of the *sig*B gene, only three sites of repeated isolation remained; now defined as the same *Listeria* allelic type (AT) detected and isolated in a sample from a site on at least two of the four sample collection visits during the study. Partial *sig*B gene sequencing has been shown to reliably discriminate *Listeria* isolates for environmental studies (*24*, *29*, *30*) similar to this; however, it may be hypothesized even less cases of repeated isolation

would be identified upon use of more discriminatory subtyping methods, such as Pulsed Field Gel Electrophoresis. The three cases of repeated isolation were from three different packinghouses (A, B, and C) and were from three different site locations (CS, WNFCS, and DNFCS) (Table 3.4). In the first case of repeated isolation, *L. monocytogenes* AT 64 was detected and isolated from samples collected from a CS site in packinghouse A during July and August 2017. The second case of repeated isolation, *Listeria* spp. AT 6 was detected and isolated from samples collected from a DNFCS site in packinghouse B during July 2017 and March 2018. The third case of repeated isolation, *Listeria monocytogenes* AT 260 was detected and isolated from samples collected from a WNFCS site in packinghouse C during September 2017 and February 2018. Prior studies in New York state and Canada (*6, 9, 39, 49*) have observed *L. monocytogenes* and *Listeria* spp. are prevalent in produce production environments including agricultural soil and water, which may contribute to *Listeria* introduction into produce packinghouses. Additionally, *L. monocytogenes* repeated isolation cases have been observed in agricultural water adjacent to produce fields and top soil from agricultural fields (*39*). Unfortunately, due to the sampling limitations of this study (four visits spread out over the packing season), it was not possible to determine whether the cases of repeated isolation (same *Listeria* ATs detected and isolated in samples from the same sites) are a result of re-introduction or true persistence of *Listeria*. Increasing sampling frequency (before, during, and after operation; as well as before and after sanitation practices) would assist in identification of *Listeria* persistence.

Diversity of *L. monocytogenes* and *Listeria* spp. allelic types (AT) was high. Of the 102 *Listeria*-positive samples, 218 isolates were prepared for characterization by partial *sig*B gene sequencing. Of the 218 isolates, 128 were classified as representative isolates (A2).

Representative isolates were defined as having a unique allelic type (AT) per sample. Five different species of *Listeria* were identified: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, and *L. marthii* (Table 3.5). Approximately 21% (21/102) of the *Listeria*-positive samples contained two or more different ATs (A2). Representative isolates from the study reported here, yielded a total of 40 different *Listeria* ATs (15, 12, 7, 4 and 2 ATs for *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. marthii*, and *L. seeligeri*) (Table 3.5). The most prevalent *L. monocytogenes* ATs were 57, 79, and 112, while the most prevalent *Listeria* spp. ATs were 12 (*L. seeligeri*), 6 (L. *innocua*), 23 (*L. innocua*), 24 (*L. seeligeri*), and 31 (*L. innocua*) (Table 3.5). Similarly, in prior studies (*6*, *37*, *38*, *39*, *40*, *47*), *L. monocytogenes* and *L. innocua* have been the most prevalent species of *Listeria* documented in other food associated environments, produce production/pre-harvest environments, natural and urban environments and in ready to eat products. For example, Chapin et al. (*6*) reported that *L. monocytogenes* and *L. innocua* were the most prevalent *Listeria* identified in soil, water, and fecal samples from produce production environments. Moreover, Simmons et al. (*38*) also found that *L. monocytogenes* and *L. innocua* were the most prevalent species detected and isolated from retail deli environments. Additionally, some of the *L. monocytogenes* ATs identified in the study reported here (AT 57, 58, and 61) were also isolated from natural, urban and retail environments previously described (*37*, *38*), suggesting a wide distribution of these *Listeria* ATs. However, the frequency of shared *Listeria* ATs between environments were not similar, as *L. monocytogenes* AT 57 was isolated from 5 of 11 produce packinghouses and only 6 of 30 retail deli establishments (*38*). Interestingly, prior studies (*6*, *13*) have stated that *L. marthii* has only been isolated from a particular area in New York State (US). However, in the study reported here, *L. marthii* (AT 261, 263, 264, and 265) was detected and isolated from samples collected in two

packinghouses (A and C). These *L. marthii* isolates belong to 4 of the 14 new ATs identified in this study (A3). A new allelic type was assigned to an isolate if the partial *sigB* gene nucleotide sequence (approximately 660 bp) was different from *Listeria* isolates in the Food Microbe Tracker database housed at Cornell University (which has approximately > 4,000 partial *sig*B sequences from dairy, meat, seafood, urban environments and among others). The majority Listeria monocytogenes AT identified in this study (10/15) belonged to lineages I, II and IIIA (A6). The lineage of the rest for the 5 reminding ATs isolated could not be determine because they were classified as new ATs.

While diversity across packinghouses was high (Simpson's Index of Diversity =0.95), each packinghouse did have a unique distribution of *L. monocytogenes* and *Listeria* spp. ATs. Six of the 40 *Listeria* ATs (*L. monocytogenes* AT 57, 59, and 79, *L. innocua* AT 23 and 31, and *L. seeligeri* AT 12) were isolated from at least three packinghouses (Table 3.5), while 29 of the 40 *Listeria* ATs were isolated from a specific packinghouse. Packinghouses A, B, and C had the highest occurrence of packinghouse specific ATs, with each packinghouse yielding at least five unique ATs (shared by no other packinghouse). These same packinghouses (A, B, and C) also showed the highest diversity of *Listeria* ATs within packinghouses (SID = 0.91 ± .015, 0.90 ± .02, 0.87 ± .02, respectively). There are limited published studies quantifying *Listeria* AT diversity in other environments. One study (33) observed the diversity in urban (SID=0.64) and natural (SID=0.29) environments to be much lower than the diversity reported in this study (SID=0.95) using the same subtyping method (AT) and diversity calculation (SID). Findings reported here, suggest each packinghouse has its own ecological niches and contamination routes that allow certain *Listeria* to survive in specific packinghouses. It has been documented that the species of *Listeria* isolated from an environment are dependent on the characteristics of a

specific environment (*6*, *25*, *35*). All the packinghouses in this study were slightly different; therefore, variables including building layout and age, equipment design, and traffic patterns may impact the distribution and diversity of *Listeria* in produce packinghouse environments. Environmental monitoring results need to be paired with observational data (e.g., handling practices, traffic patterns) to identify factors that affect the likelihood of *L. monocytogenes* and *Listeria* spp. in produce packinghouses.

Both the 24 and 48 h enrichments for *Listeria* detection and isolation are key to capture *Listeria* diversity. A higher number of *Listeria* isolates were recovered after the 48 h enrichment (n=99 isolates), compared to the 24 h enrichment (n=70 isolates). Furthermore, a higher number of *Listeria monocytogenes* was isolated from the 48 h enrichment (n=58) compared to the 24 h enrichment (n=43). Similarly, more *Listeria* spp. isolates (e.g., *L. innocua, L. welshimeri*) were recovered from the 48 h enrichment (n=41 isolates), compared to the 24 h enrichment (n=27 isolates). Previous studies have found similar results that reported the 48 h enrichment recovered a higher number of *Listeria* spp. and *L. monocytogenes* isolates (*10*, *41*). For example, a study (*10*) was able to recover 24 of 26 *L. monocytogenes* cultures after a 24 h enrichment; however, all 26 *L. monocytogenes* cultures were recovered after a 48 h enrichment.

Of the 60 *L. monocytogenes* positive samples, 31 samples yielded *L. monocytogenes* isolates from only the 24 h (n=12) or the 48 h (n=19) enrichments. Less than half (19/60) of the samples yielded the same *L. monocytogenes* subtype (based on AT) at both the 24 and 48 h enrichments. The remaining 10 samples yielded a different *L. monocytogenes* isolate (based on AT) from the 24 and 48 h enrichment. Additionally, 12 different *L. monocytogenes* ATs were identified from the 24 h enrichment, while 18 different ATs were identified from the 48 h enrichment (A4). Similar results were observed when analyzing the distribution of *Listeria* spp.

positive samples detected from the 24 and 48 h enrichments (A4). To our knowledge, no studies compare the *L. monocytogenes* and *Listeria* spp. detection, isolation and diversity after the 24 and 48 h enrichments, or even in the absence of culture-based methods. However, the study reported here supports the use of culture-based methods and both a 24 and 48 h enrichment to fully capture *Listeria*-positive samples, as well as *Listeria* diversity in produce packinghouses.

Conclusion

The prevalence of *L. monocytogenes* and *Listeria* spp. in produce packinghouses was lower, compared to the *L. monocytogenes* and *Listeria* spp. prevalence in other food associated environments (*16*, *17*, *21*, *31*, *36*, *38*, *45*, *50*). The data reported in this study suggest over half of the *Listeria* detected and isolated were *L. monocytogenes*. This study showed that the prevalence of *L. monocytogenes* and *Listeria* spp. varied among the produce packinghouses and within site sub-groups (e.g., D, WNFCS, DNFCS). Sites that were cold and or wet presented the highest *L. monocytogenes* and *Listeria* spp. risk for harborage. These results suggest that it is critical that *Listeria* control programs are developed for specific operations and targeted on sites or areas that may serve as niches for *Listeria* rather than using a balanced, general approach. *Listeria* repeated isolation cases in the sites tested was low; however, further studies are needed to fully address potential *Listeria* persistence with increased sampling frequencies. Further studies aimed to investigate routes of *Listeria* introduction, contamination patterns, persistence, and diversity in produce packinghouses across the US and abroad, especially in other produce packing regions, are necessary to fully understand the risk *L. monocytogenes* poses to product contamination in produce packinghouses.

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Research Tables

Table 3.1. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained from produce packinghouses (n=11)

Packinghouse	No. of Samples	No. of positive samples (% prevalence)			
		Listeria[a]	*L. monocytogenes*[b]	*Listeria* spp.[c]	Co-isolation of *L. monocytogenes* and another species of *Listeria*[e]
A	140	24 (17.1) A[e]	16 (11.4) A	15 (10.7) A	7 (5.0)
B	140	21 (15.0) A	7 (5.0) A	16 (11.4) A	2 (1.4)
C	140	19 (13.6) A	16 (11.4) A	3 (2.1) B	0 (0.0)
D	140	11 (7.9) A	10 (7.1) A	1 (0.7) C	0 (0.0)
E	140	10 (7.1) A	3 (2.1) B	7 (5.0) ABC	0 (0.0)
F	140	7 (5.0) A	2 (1.4) B	5 (3.6) ABC	0 (0.0)
G	140	4 (2.9) B	4 (2.9) AB	1 (0.7) C	1 (0.7)
H	136	4 (2.9) B	0 (0.0) B	4 (2.9) ABC	0 (0.0)
I	144	1 (0.7) B	1 (0.7) B	0 (0.0) BC	0 (0.0)
J	184	1 (0.5) B	1 (0.5) B	0 (0.0) BC	0 (0.0)
K	140	0 (0.0) B	0 (0.0) B	0 (0.0) BC	0 (0.0)
Total	1584	102 (6.4)	60 (3.8)	52 (3.3)	10 (0.6)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria*: *L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria*, excluding *L. monocytogenes.*

[d] Co-isolation referred to samples positive for both *L. monocytogenes* and *Listeria* spp. simultaneously.

[e] Values with the same letter are not significantly different according to chi-square or Fisher's exact test ($P<0.05$). Only *Listeria*, *L. monocytogenes* and *Listeria* spp. samples amongst packinghouses were compared.

Note that adding the number of positive number of samples for *L. monocytogenes* and *L*. spp. does not add to the total of *Listeria* positive samples. *L. monocytogenes* and *L*. spp. positive samples include co-isolation samples. Thus to obtain the total number of *Listeria* positive samples instances of co-isolation must be subtracted from *L. monocytogenes* and *L*. spp. samples and accounted as their own individual positive samples. For example, packinghouse A had 9 positive samples for *L. monocytogenes* only, 8 positive samples for other *L*. spp. only, and 7 samples that were positive for both *L. monocytogenes* and *L*. spp. (9 + 8 + 7 = 24). Total *L. monocytogenes* samples; 9 + 7 = 16. Total *L*. spp. samples 8 +7 = 15.

Table 3.2. Frequency of environmental sites positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained from produce packinghouses (n=11)

		No. of positive sites (% prevalence)			
Packinghouse	Sites[a]	*Listeria*[b]	*L. monocytogenes*[c]	*L*isteria spp.[d]	Isolation of *L. monocytogenes* and another species of *Listeria*[e]
A	35	15 (42.9)	12 (34.3)	13 (37.1)	10 (28.6)
B	35	16 (45.7)	6 (17.1)	12 (34.3)	2 (5.7)
C	35	13 (37.1)	12 (34.3)	2 (5.7)	1 (2.9)
D	35	9 (25.7)	8 (22.9)	1 (2.9)	0 (0.0)
E	35	8 (22.9)	3 (8.6)	5 (14.3)	0 (0.0)
F	35	6 (17.1)	2 (5.7)	5 (14.3)	1 (2.9)
G	35	4 (11.4)	4 (11.4)	1 (2.9)	1 (2.9)
H	34	4 (11.8)	0 (0.0)	4 (11.8)	0 (0.0)
I	36	1 (2.8)	1 (2.8)	0 (0.0)	0 (0.0)
J	46	1 (2.2)	1 (2.2)	0 (0.0)	0 (0.0)
K	35	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	396	77 (19.4)	49 (12.4)	43 (10.9)	15 (3.8)

[a] Sites were re-sampled four times over the duration of the packing season

[a] *Listeria* referred to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria*: *L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria*, excluding *L. monocytogenes.*

[e] Isolation referred to sites positive for *L. monocytogenes* and *Listeria* spp.

Note that adding the number of positive number of sites for *L. monocytogenes* and *L*. spp. does not add to the total of *Listeria* positive sites. *L. monocytogenes* and *L*. spp. positive sites include positive sites for both L. monocytogenes and other L. spp. Thus to obtain the total number of *Listeria* positive sites, sites for both L. monocytogenes and other L. spp. must be subtracted from *L. monocytogenes* and *L*. spp. and accounted as their own individual positive sites. For example, packinghouse A had 2 positive sites for *L. monocytogenes* only, 3 positive sites for other *L.* spp. only, and 10 sites that were positive for both *L. monocytogenes* and *L*. spp. (2 + 3 + 10 = 15). Total *L. monocytogenes* sites; 2 + 10 = 12. Total *L*. spp. samples 3 + 10= 13.

Table 3.3. Frequency of environmental sites positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. by category obtained from produce packinghouses (n=11

			No. of positive sites (% prevalence)			
Site category[a]	Site description	Total[b]	*Listeria*[c]	*L. monocytogenes*[d]	*Listeria* spp.[e]	Co-isolation of *L. monocytogenes* and another species of *Listeria*[f]
D	Drains	36	21 (58.3)	13 (36.1)	11 (30.6)	3 (8.3)
CS	Non-food contact surfaces from cold storage areas (excluding drains)	47	19 (40.4)	12 (25.5)	11 (23.4)	4 (8.5)
WNFCS	Stationary non-food contact surfaces that get wet	71	25 (35.2)	17 (23.9)	10 (14.1)	2 (2.8)
OP	Outside of the main packing and handling area	22	5 (22.7)	2 (9.1)	3 (13.6)	0 (0.0)
MNFCS	Moveable non-food contact surfaces	83	16 (19.3)	10 (12.0)	7 (8.4)	1 (1.2)
DNFCS	Stationary non-food contact surfaces that stay dry	138	16 (11.6)	6 (4.3)	10 (7.2)	0 (0.0)
-	-	397	102 (27.8)	60 (16.3)	42 (10.6)	10 (2.5)

[a] Sites were divided in six categories; DNFCS: Dry Non-food Contact Surfaces, MNFCS: Moveable Non-Food Surfaces, WNFCS: Wet Non-food Contact Surfaces, CS: Cold Storage areas, D: Drains, and OP Outside main packing and handling area.

[b] Sites were each sampled four times (total sites calculated by no. of sites x 4)

[c] *Listeria* refers to the genus level and includes all species of *Listeria.*

[d] *L. monocytogenes* referred only to the species of *Listeria*: *L. monocytogenes.*

[e] *Listeria* spp., referred to all species of *Listeria*, excluding *L. monocytogenes.*

[f] Co-isolation referred to samples positive for both *L. monocytogenes* and *Listeria* spp. simultaneously.

Note that adding the number of positive number of samples for *L. monocytogenes* and *L*. spp. does not add to the total of *Listeria* positive samples. *L. monocytogenes* and *L*. spp. positive samples include co-isolation samples. Thus to obtain the total number of *Listeria* positive samples instances of co-isolation must be subtracted from *L. monocytogenes* and *L*. spp. samples and accounted as their own individual positive samples. For example, 10 drains were positive for *L. monocytogenes* only, 8 positive samples for other *L*. spp. only, and 3 samples that were positive for both *L. monocytogenes* and *L*. spp. (10 + 8 + 3 = 21). Total drains positive for *L. monocytogenes*; 10 + 3 = 13. Total drains positive for *L*. spp. samples 8 + 3 = 11.

Table 3.4. *L. monocytogenes* and *Listeria* spp. repeated isolation cases obtained from produce packinghouses (n=11)

Packinghouse	Site category[a]	No. of visits[b]	*L. monocytogenes* AT[c]				*Listeria* spp. AT			
			V1[d]	V2	V3	V4	V1	V2	V3	V4
A	CS 1	3	57	64	-	-	-	-	-	-
A	CS 2	2	64	57, 64	-	-	-	-	-	-
A	WNCFS 1	2	57	61, 112	-	-	-	-	-	-
A	WNCFS 2	2	-	-	-	-	-	-	12	47
A	OP	3	112	96	-	-	-	-	-	-
B	D	2	-	-	-	-	-	12	-	6
B	DNFCS 1	3	-	-	59	57	-	6,24	134	-
B	DNFCS 2	3	-	-	-	-	6	53, 219	-	6
C	CS	3	-	-	-	-	-	261	31	-
C	WNCFS 1	3	-	112, 260	64	260	-	-	-	-
C	WNCFS 2	2	-	64	-	262	-	-	-	-
C	WNCFS 3	2	-	260	-	262	-	-	-	-
D	D	2	-	-	112	59	-	-	-	-
D	MNFCS	2	260	-	-	79	-	-	-	
E	DNFCS	3	-	-	-	-	-	37	31	23

[a] Sites were divided in six categories; DNFCS: Dry Non-food Contact Surfaces, MNFCS: Moveable Non-Food Surfaces, WNFCS: Wet Non-food Contact Surfaces, CS: Cold Storage areas, D: Drains, and OP Outside main packing and handling area.

[b] Each packinghouse was sampled four times (max four visits)

[c] Allelic Type (AT) based on partial *sig*B sequencing.

[d] Visit (V) 1-4 (time packinghouse was visited and sampled

Table 3.5. *Listeria sig*B allelic types yielded from produce packinghouses (n=11)

*sig*B AT[a]	No. of packinghouses[b]	No. of Isolates[c]	No. of Representative Isolates[d]
L. innocua			
6	1	6	5
11	1	2	1
22	1	1	1
23	3	7	5
31	3	11	5
33	1	2	2
37	1	2	2
44	1	2	1
53	1	1	1
109	1	2	2
266	1	1	1
268	1	2	1
L. marthii			
261	1	2	1
263	1	2	1
264	1	1	1
265	1	4	2
L. seeligeri			
12	3	10	7
24	2	5	5
L. welshimeri			
47	1	2	1
55	1	2	1
89	1	3	2
133	1	2	1
134	2	5	3
219	1	1	1
267	1	1	1
L. monocytogenes			
57	5	27	17
58	1	4	1
59	3	9	6
61	2	15	6
64	1	14	8
79	4	20	12
80	1	1	1
81	1	2	1
96	2	4	2
112	5	21	10
119	1	2	1

195	1	2	1
202	1	2	1
260	1	12	5
262	1	4	2

[a] Allelic Type (AT).

[b] Number of packinghouses the AT was detected and isolated in (out of 11)

[c] 218 isolates were obtained from the *Listeria*-positive samples

[d] Representative isolates were defined as having a unique allelic type (AT) per sample

(Of the 218 isolates, 128 were classified as representative isolate

CHAPTER 4: CONCLUSION AND FUTURE WORK

The ultimate goal of this work was to generate science-based knowledge about the prevalence, persistence, and diversity of *Listeria* species including *Listeria monocytogenes* in produce packinghouses. To achieve this goal we utilized several different approaches such as environmental monitoring, culturing and molecular techniques.

Before the multistate listeriosis outbreak linked with contaminated cantaloupes from farms in California, *Listeria monocytogenes* was not a pathogen of concern for the produce industry. However, in the last ten years, the number of *Listeria* outbreaks and recalls have put *Listeria monocytogenes* as one of the primary target pathogens in the fresh produce industry. Several of the recent outbreaks have been traced back to the postharvest environment. The work described in Chapter 3 studied eleven packinghouses to gain an understanding of the overall prevalence and potential niches of *Listeria* in produce packinghouses.

Due to variability in the methodology and study design among studies, it is difficult to compare the *Listeria* prevalence reported and the prevalence obtained in this study. However, by only looking at the percentages of *Listeria* prevalence in other studies and comparing it to the prevalence found in this study, it can be stated that food environments previously studied yielded to *Listeria* prevalence higher than the prevalence found in produce packinghouses. The overall prevalence of *L. monocytogenes* was higher than the prevalence of other *Listeria* species suggesting that testing and implementing interventions for *L. monocytogenes* specifically may be a better strategy to reduce *L. monocytogenes* in produce packinghouses. Future studies should focus on investigating the correlation between *L. monocytogenes* and other *L.* species in the produce packinghouse environment. Consistently to studies on different food processing environments, wet and cold sites indicated the highest *Listeria* prevalence. Monitoring and

eliminating sites with such characteristics should be a priority for the produce industry. Cases of *Listeria* persistence were low, suggesting that the majority of the *Listeria* isolates found in the packinghouses were transient *Listeria*. These data supported that current cleaning and sanitizing practices are controlling the *Listeria* prevalence in these environments. Studies investigating the initial sources of *Listeria* introduction and the contamination patterns may allow the developing of specific strategies to limit *Listeria* introduction, to identify potential *Listeria* points of contamination in produce packinghouses.

Lastly, *Listeria* diversity in packinghouses was unique to each packinghouse, and because of that and confidentiality purposes the study described in chapter 3 did not link *Listeria* prevalence to specific commodities. Though our study was not an observational study, we hypothesized that traffic patterns, facility's layout and workers' training may have an impact in the prevalence of *Listeria* in some of the packinghouses. During visits 1 and 4, packinghouse B had a bidirectional traffic flow (Appendix B). During such those visits the prevalence of *Listeria* was high. After the traffic flow was fixed, the facility was sampled and only 1 of 35 samples collected was positive for *Listeria* (data is not shown in Chapter 3). Similarly, packinghouse A, the packinghouse with the highest prevalence during the study, was one of the oldest facilities tested. Packinghouse A presented structural problems, older drains, deteriorated and hard to clean floors, drains and walls (Appendix B). Overall, the environmental conditions of these sites may support *Listeria* presence. Proper drains away from areas with high traffic flow may reduce the likelihood of *Listeria* transfer to other sites in de facility. Over the duration of the study, some recommendations such as the elimination of areas with standing water, deep-cleaning and sanitation of some of the problem areas (sites testing positive in during different visits) were recommended. The impact of these recommendation has not been evaluated, yet but sampling

facility A after some of the recommendations are implemented may validate the recommendations provided. Lastly, workers spend a lot of time in the facility and they may notice potential *Listeria* niches quicker than the cleaning crew, thus training workers about *Listeria* preferred niches and *Listeria* problems may be allowed workers to become the first line of defense against this pathogen.

While some questions remind unanswered regarding *Listeria monocytogenes* and other Listeria species introduction, persistence, and contamination patterns in the produce packinghouses. The information gathered from this study has increased our understanding of the prevalence, persistence, and diversity of *Listeria monocytogenes* and other *Listeria* species in produce packinghouses. This information may be used to develop produce specific mitigation and intervention strategies that may control and/or reduce the likelihood of *Listeria* in the produce packinghouse environments.

APPENDICES

Appendix A: Supplemental Tables for Chapter 3

Supplemental Table A1. Sites description of all the sites sampled in 11 packinghouse facilities.

Packinghouse	Sample ID[a]	Zone[b]	Site description	Category
A	141	4	Field bin	5
A	142	3	Wall cold storage	1
A	143	3	Wall cold storage	1
A	144	3	Fork lift	5
A	145	3	Post cold storage	1
A	146	3	Pipe air cooling	1
A	147	3	Drain	2
A	148	2	Roller bins	4
A	149	3	Leg/floor flume leg	3
A	150	3	Catwalk floor	4
A	151	2	Waxer	4
A	152	3	Drain	2
A	153	3	Donut peach line	3
A	154	3	Floor mat	5
A	155	3	Leg/floor grading line	4
A	156	2	Cull shoot	4
A	157	3	Control panel	4
A	158	2	RPC bins	5
A	159	2	Pack box strand	4
A	160	3	Leg packing table	4
A	161	3	Floor/wall junction control station	4
A	162	3	Stairs towards grader table	4
A	163	2	Automatic apple bagger	4
A	164	3	Drain	2
A	165	2	Dryer	4
A	166	3	Floor pit	3
A	167	2	Adjacent to optical sorter	4
A	168	3	Floor/wall junction	3
A	169	3	Fork lift	5
A	170	3	Pillar junction finish Product	1
A	171	3	Pallet	5
A	172	3	Flap curtains	4
A	173	4	Break area	6
A	174	4	Break area	6
A	175	4	Lobby	6
B	106	3	AC unit	1
B	107	3	Pipe connection	1

B	108	3	Floor/wall junction	1
B	109	3	Drain	2
B	110	3	Trench drain	2
B	111	3	Floor/wall junction	4
B	112	3	Wall	4
B	113	3	Table leg	4
B	114	3	Utility cart wheel	5
B	115	3	Flap curtains	3
B	116	3	Hose cart	3
B	117	3	Drain	2
B	118	3	Spinner	3
B	119	3	Handle	3
B	120	3	Cart wheels	5
B	121	3	Cart wheels	5
B	122	3	Drain	2
B	123	2	Cart handle	5
B	124	3	Utility chest	5
B	125	3	Floor/trench drain	2
B	126	3	Packet jack	5
B	127	3	Flap curtains	4
B	128	3	Cart wheels	5
B	129	2	Cart handle	5
B	130	3	Squeegee	5
B	131	3	Cart wheels	5
B	132	2	Cart frame	5
B	133	3	Floor/trench drain	2
B	134	2	Sorter frame	4
B	135	3	Cutting table leg	4
B	136	3	Flap curtains	4
B	137	4	Break area	6
B	138	4	Floor (condenser)	3
B	139	2	Product racks	5
B	140	3	Pallet jack	5
C	36	3	Cold storage	1
C	37	3	Cold storage	1
C	38	3	Cold storage hallway	1
C	39	3	Cold storage handle	1
C	40	3	Fork lift	5
C	41	3	Pull cord	4
C	42	3	Outside cold storage - light switch	4
C	43	3	Cold storage	1
C	44	3	Field bin (wood)	5
C	45	3	Field bin (plastic)	5
C	46	3	Cold storage	1
C	47	2	Bin line dumper entry	4

C	48	2	Bin line dumper return	3
C	49	3	Floor	3
C	50	2	Water rinser	3
C	51	3	Rubber floor	5
C	52	3	Water pit	3
C	53	3	Water pit	3
C	54	2	Grading area	4
C	55	2	Sorting area	4
C	56	2	Pack table trays	4
C	57	3	Leg of packing table	4
C	58	3	Box staples	4
C	59	3	Floor mat	5
C	60	3	Floor mat	5
C	61	3	Leg of bagger	4
C	62	3	Drain	2
C	63	3	Drain	2
C	64	3	Floor wash line	3
C	65	3	Leg wash line	3
C	66	4	Break area	6
C	67	3	Hose	3
C	68	3	Cold storage	1
C	69	3	Fork lift	5
C	70	4	Office	6
D	1	4	Field Pack bin (Plastic)	5
D	2	4	Field Pack bin (Wood)	5
D	3	4	Gas pump hose	4
D	4	3	Wall/Light switch	4
D	5	3	Floor	4
D	6	3	Floor (cold storage)	1
D	7	3	Floor (cold storage)	1
D	8	3	Floor (cold storage)	1
D	9	3	Floor (cold storage)	1
D	10	3	Handle (cold storage)	1
D	11	3	Ladder	4
D	12	3	Packing line	4
D	13	2	Dumper	3
D	14	2	Bin line return	3
D	15	3	Catch canner apples	3
D	16	3	Fork lift	5
D	17	3	Drain	2
D	18	3	Fork lift	5
D	19	3	Drain	2
D	20	2	Wash line	3
D	21	2	Sorting area	4
D	22	2	Brush roller	3

D	23	2	Waxing area	3
D	24	2	Drying tunnel	4
D	25	3	Floor	4
D	26	2	Sorting table	4
D	27	3	Fan	5
D	28	2	Loading trays	4
D	29	2	Loading table	4
D	30	3	Break area	6
D	31	4	Office	6
D	32	3	Break area	6
D	33	3	Fork lift	5
D	34	3	Floor	4
D	35	3	Wood pallet	5
E	246	4	Trucking lounge	6
E	247	3	Drain	2
E	248	3	Curtain flaps	3
E	249	3	Drain - cooler	2
E	250	3	Floor/support	3
E	251	3	Floor - cooler	3
E	252	3	Pipes - Air cooler	4
E	253	3	Drain brush	5
E	254	3	Floor/wood -tomato cooler	4
E	255	3	Pillar/floor hydro cooler	3
E	256	3	Drain pipe	2
E	257	3	Leg/floor Hydro-cooler	3
E	258	3	Drainage table	3
E	259	3	Fork lift	5
E	260	3	Fork lift	5
E	261	3	Floor - clam sheller	3
E	262	4	Drain	2
E	263	3	Leg/floor Hydro-cooler	3
E	264	3	Trench drain	2
E	265	3	Floor/leg - ice machine	3
E	266	3	Hose	3
E	267	3	Bobcat	5
E	268	3	Wall/floor - ice room	3
E	269	3	Wall/ledge - ice room	3
E	270	3	Floor - storage area	4
E	271	3	Pack table	4
E	272	2	Wrapping table	4
E	273	3	Hydro-cooler	3
E	274	4	Break area	6
E	275	3	Hydro-cooler	3
E	276	3	Curtain flaps	4
E	277	2	Pack table	4

E	278	3	Squeegee	5
E	279	3	Hand-cart wheels	5
E	280	4	Office	6
F	176	3	Floor/wall Cold room - outside	4
F	177	3	Door seal - Cold room	1
F	178	3	Floor/wall Cold room	1
F	179	3	Floor/wall Cold room	1
F	180	3	Fork lift	5
F	181	3	Shipping docks	4
F	182	4	Break area	6
F	183	3	Drain cap	2
F	184	3	Floor/wall	4
F	185	3	Fork lift	5
F	186	3	Floor/wall	4
F	187	3	Cull bin	5
F	188	3	Floor/wall junction	3
F	189	3	Floor (pooling water)	3
F	190	3	Floor - under trash can	3
F	191	3	Floor mat (flume)	5
F	192	3	Squeegee	5
F	193	3	Trench drain	2
F	194	3	Chill line	3
F	195	2	Cull pan grading table	3
F	196	2	Cull pan grading table	3
F	197	3	Floor - grading table	4
F	198	2	Waxer	4
F	199	3	Panel button	4
F	200	2	Stool grading table	5
F	201	2	Fan	5
F	202	2	Top celling	4
F	203	2	Grading table	4
F	204	3	Trench drain	2
F	205	3	Flume culls	5
F	206	3	Drain	2
F	207	3	Flume leg	3
F	208	3	Metal platform	3
F	209	3	Platform mat	5
F	210	4	Office entry	6
G	71	3	Floor storage	1
G	72	3	Floor storage	1
G	73	3	Wall storage	1
G	74	3	Wall storage	1
G	75	3	Wall storage	1
G	76	3	Fork lift	5
G	77	3	Wall storage	1

G	78	3	Drain	2
G	79	3	Drain	2
G	80	3	Leg/floor table	1
G	81	2	Roller bins	4
G	82	3	Ladder	3
G	83	3	Water hose	3
G	84	3	Walkway	4
G	85	2	Grading area	4
G	86	4	Control panel	4
G	87	3	Drain	2
G	88	3	Drain	2
G	89	3	Table leg	3
G	90	3	Catch drain	1
G	91	3	Leg/floor table	4
G	92	3	Wood pallet	5
G	93	3	Fork lift	5
G	94	3	Rubber floor	5
G	95	3	Leg/floor grader	4
G	96	3	Leg/floor packing area	4
G	97	2	Packing table	4
G	98	3	Catwalk floor	4
G	99	3	Fan base	5
G	100	2	Scale	4
G	101	4	Lobby	6
G	102	4	Field bin	5
G	103	4	Field bin	5
G	104	4	Break area	6
G	105	4	Office	6
H	281	2	Product bin	5
H	282	2	Product bin	5
H	283	2	Product bin	5
H	284	3	Field bin	5
H	285	3	Field bin	5
H	286	2	Packing line	4
H	287	2	Packing line	4
H	288	2	Packing line	4
H	289	3	Wooden pallet	5
H	290	3	Wooden pallet	5
H	291	3	Wooden pallet	5
H	292	3	Drain	2
H	293	3	Drain	2
H	294	3	Drain	2
H	295	3	Drain	2
H	296	3	Floor flap	4
H	297	3	Floor flap	4

H	298	3	Floor - packing area	4
H	299	3	Floor - packing area	4
H	300	3	Floor	4
H	301	3	Floor	4
H	302	3	Floor - cooler	1
H	303	3	Floor - cooler	1
H	304	3	Wall - cooler	1
H	305	3	Fan Box - cooler	5
H	306	3	Fan Box - cooler	5
H	307	3	Floor - cooler	1
H	308	3	Floor - cooler	1
H	309	3	Wall - cooler	1
H	310	3	Fan Box - cooler	5
H	311	3	Fan Box - cooler	5
H	312	4	Lobby	6
H	313	4	Lobby	6
H	314	4	Lobby	6
I	315	2	Dump tank	3
I	316	2	Dump tank	3
I	317	2	Dump tank	3
I	318	3	Dump tank	3
I	319	3	Dump tank	3
I	320	3	Catwalk railing	4
I	321	3	Roller belt	4
I	322	3	Roller belt	4
I	323	3	Roller belt	4
I	324	3	Roller belt	4
I	325	2	Brush rollers	3
I	326	2	Sorting belt	4
I	327	2	Sorting belt	4
I	328	2	Roller	4
I	329	2	Grader belt	4
I	330	2	PVC roller	4
I	331	3	Sizer belt	4
I	332	3	Conveyer box	5
I	333	3	Catwalk	4
I	334	3	Catwalk	4
I	335	3	Roller belt	4
I	336	2	Rolling drum	4
I	337	2	Rolling drum	4
I	338	3	Wooden pallet	5
I	339	3	Wooden pallet	5
I	340	3	Wooden pallet	5
I	341	3	Drain	2
I	342	3	Drain	2

I	343	3	Floor - packing area	4
I	344	3	Floor - packing area	4
I	345	3	Floor - packing area	4
I	346	3	Floor - packing area	4
I	347	3	Floor - packing area	4
I	348	3	Floor - cooler	1
I	349	3	Floor - cooler	1
I	350	3	Wall - cooler	1
J	211	3	Pit	3
J	212	3	Trash Can corner	4
J	213	3	Floor top	3
J	214	3	Floor top	3
J	215	3	Squeegee	5
J	216	3	Leg/floor OPR system	3
J	217	3	Fork lift	5
J	218	3	Floor- Dump area	3
J	219	3	Floor- Dump area	3
J	220	3	Floor - crack	4
J	221	3	Floor/beam	4
J	222	3	Pre-grading area	4
J	223	3	Pre-grading area	4
J	224	2	Tomato elevator	4
J	225	3	Floor/leg	4
J	226	3	Stairs	4
J	227	3	Sorting machine - leg	4
J	228	2	Grading table handle	4
J	229	3	Multi-can catch pan	3
J	230	3	Drain	2
J	231	3	Stair drain	2
J	232	2	Cull shoot area	3
J	233	3	Cull shoot area	3
J	234	3	Floor mats	5
J	235	3	Drain	2
J	236	3	Door way	4
J	237	3	Floor/beam	4
J	238	3	Fork lift	5
J	239	3	Cold room	1
J	240	3	Cold room	1
J	241	3	Door seal	1
J	242	3	Cold room	1
J	243	4	Break area	6
J	244	3	Clam shell machine	4
J	245	4	Office	6
K	351	2	Dump tank	3
K	352	2	Dump tank	3

K	353	2	Dump tank	3
K	354	3	Paddles	3
K	355	3	Fan	5
K	356	3	Fan	5
K	357	3	Roller/fan	5
K	358	3	Roller/fan	5
K	359	3	Roller/brush	3
K	360	3	Roller/brush	3
K	361	3	Roller/sorter	4
K	362	3	Roller/sorter	4
K	363	3	Rollers	4
K	364	3	Rollers	4
K	365	3	Rollers	4
K	366	3	Rollers	4
K	367	3	Rollers	4
K	368	3	Paddles	3
K	369	3	White rollers	4
K	370	3	Roller/fan	5
K	371	3	Rollers	4
K	372	3	Roller/brush	3
K	373	3	Roller/sorter	4
K	374	3	Rollers	4
K	375	3	Sorter stands	4
K	376	3	Rollers	4
K	377	3	Rollers	4
K	378	3	Rollers	4
K	379	3	Rollers	4
K	380	3	Sorter stands	4
K	381	3	Sorter stands	4
K	382	3	Sorter stands	4
K	383	3	Floor	4
K	384	3	Floor	4
K	385	3	Floor	4
K	386	3	Floor	4
K	387	3	Floor	4
K	388	3	Floor	4
K	389	3	Floor	4
K	390	3	Floor	4
K	391	3	Drain	2
K	392	3	Wooden pallet	5
K	393	3	Wooden pallet	5
K	394	3	Wooden pallet	5
K	395	3	Wall - cooler	1
K	396	3	Floor - cooler	1
K	397	3	Floor - cooler	1

[a] ID number assigned to the sites for labeling and tracking purposes.

[b] Zone 1: Food contact surface. Zone 2: Areas adjacent to food contact surfaces areas. Zone 3 includes surfaces that do not come in contact or are directly associated with (or close from) food. Lastly, Zone 4 refers to the areas outside the processing environment such as lunchrooms, hallways, office areas, etc.

[c] Code number representing the categories of the sites sampled. Number 1 indicates sites in the Cold Storage (CS). Number 2 indicates drains (D). Number 3 and 4 represent wet (WNFCS) and dry nonfood contact surfaces (DNFCS), respectively. Number 5 indicates moving nonfood surfaces (MNFCS) and number five represents sites outside packinghouse (OP).CS = Every surface (every sample taken) from a cold storage room. D = All drains (trench drain, regular drain, cap drains, etc.). WNFCS = Non-food contact surfaces that may get wet and are stationary. DNFCS = Non-food contact surfaces that stay dry, stationary. MNFCS = All equipment that is moved during operation (Forklifts, cars, pallets, bins, squeegees, etc.). OP = Areas where workers take their break, rooms outside of packinghouse.

Supplemental Table A2. Representative isolates in packinghouses sampled

Packinghouse	*Listeria*-positive samples[a]	Total representative isolates[b]	Samples with 1 isolate	Samples with 2 isolates	Samples with 3 isolates
A	24	34	17	4	3
B	21	25	17	4	0
C	19	24	15	3	1
D	11	13	9	2	0
E	10	10	10	0	0
F	7	7	7	0	0
G	4	9	0	3	1
H	4	4	4	0	0
I	1	1	1	0	0
J	1	1	1	0	0
K	0	0	0	0	0
Total	**102**	**128**	**81**	**16**	**5**

[a] Samples included all *Listeria* positive samples (*L*. spp. and *L. monocytogenes*).

[b] Representative isolates were defined as having a unique allelic type (AT) per sample.

Supplemental Table A3. New Allelic types found in the study.

Isolates[a]	Allelic Type[b]	Genus	Species
FSLS10-3407	112	*Listeria*	*monocytogenes*
FSLS10-3413	260	*Listeria*	*monocytogenes*
FSLS10-3421	261	*Listeria*	*marthii*
FSLS10-3459	262	*Listeria*	*monocytogenes*
FSLS10-3465	202	*Listeria*	*monocytogenes*
FSLS10-3495	134	*Listeria*	*welshimeri*
FSLS10-3503	219	*Listeria*	*welshimeri*
FSLS10-3513	263	*Listeria*	*marthii*
FSLS10-3516	264	*Listeria*	*marthii*
FSLS10-3519	265	*Listeria*	*marthii*
FSLS10-3583	266	*Listeria*	*innocua*
FSLS10-3590	267	*Listeria*	*welshimeri*
FSLS10-3603	268	*Listeria*	*innocua*
FSLS10-3615	195	*Listeria*	*monocytogenes*

[a] Isolate code given by Cornell's Food Safety Lab.

[b] New allelic type assigned to the isolate by Cornell's Food Safety Lab for future recognition, storing and analyzing purposes.

Supplemental Table A4 – Total *Listeria monocytogenes* and *L*. spp. isolates from the 24 and 48 hours enrichments.

			***Listeria monocytogenes* isolates[c]**		***Listeria* spp. (excluding *L. monocytogenes*) isolates**	
Packinghouse	**Visit[a]**	**Sample ID[b]**	**24-hour (AT)[d]**	**48-hour (AT)**	**24-hour (AT)**	**48-hour (AT)**
D	4	7	3400 (81)	3401 (81)		
D	4	8	3402 (57)	3403 (79)		
D	3	11	3404 (79), 3405 (79)			
D	4	16		3406 (59)		
D	3	17	3407(112), 3408(112)	3409 (112)		
D	4	17	3410 (59)			
D	1	18		3411 (260), 3412 (260), 3413 (260)		
D	4	18	3414 (79)			

D	4	19	3415 (79)	3416 (59)		
D	2	20		3417 (79), 3418 (79)		
D	2	31				3419 (11), 3420 (11)
C	2	46			3421 (261)	3422 (261)
C	3	46		3423 (112), 3424 (112)		
C	4	46			3425 (31)	3426 (31)
C	3	47				3427 (55), 3428 (55)
C	2	48		3429 (79), 3430 (79), 3431 (79)		
C	2	49		3432 (64), 3433 (64)		
C	2	50		3434 (79)		
C	2	52	3435 (260), 3436 (260)	3437 (79)		
C	2	53	3438 (112)	3439 (112), 3440 (260)		

C	3	53	3441 (64), 3442 (64)	3443 (64), 3444 (64)		
C	4	53	3445 (260)	3446 (260)		
C	3	54		3447 (64)		
C	3	58	3448 (64)	3449 (112), 3450 (57)		
C	2	63		*3451 (112), 3452 (112),* 3453 (112), 3454 (260), 3455 (260)		
C	2	64		3456 (64), 3457 (64)		
C	4	64	3458 (262)	3459 (262)		
C	2	65	3460 (260)	3461 (260)		
C	4	65	3462 (262)	3463 (262)		
C	2	69		3464 (202), 3465 (202)		
G	3	72		3466 (80), 3467 (79)		
G	3	77		3468 (79), 3469 (96)		3470 (134)

G	2	78	3471 (112), 3472 (57)	3473 (57), 3474 (112), 3475 (112), 3476 (57)		
G	2	88	3477 (57), 3478 (57)	3479 (57), 3480 (112)		
B	3	108	3481 (112)			
B	2	110				3482 (12)
B	4	110				3483 (6)
B	2	111			3484 (12), 3485 (12)	
B	2	118			3486 (24)	
B	2	121			3487 (23)	
B	4	123	3488 (57)			
B	4	124	3489 (57)		3490 (6)	3491 (6)
B	2	125			3492 (24)	3493 (6)
B	3	125		3494 (59)	3495 (134)	3496 (134)

B	4	125	3497 (57)			
B	4	126				3498 (109)
B	4	131		3499 (57)		
B	4	133	3500 (57)	3501 (57)		
B	1	134				3502 (6)
B	3	134				3503 (219) , 3504 (53)
B	4	134				3505 (6)
B	2	135			3506 (133)	3507 (133)
B	2	137			3508 (24)	
B	2	138			3509 (134)	3510 (134)
B	4	140				3511 (109)
A	2	142	3512 (79)	3514 (79)	3513 (263)	3515 (263)

A	3	143				3516 (264)
A	1	144		3517 (57), 3518 (57)		
A	4	144			3519 (265)	3520 (265)
A	1	145	3521 (57)			
A	2	145	3522 (64)	3524 (64)	3523 (24)	
A	4	145			3525 (265)	3526 (265)
A	1	146	3527 (64)			
A	2	146	3528 (57)	3529 (64), 3530 (57)	3531 (24)	
A	3	147	3533 (61)	3534 (57), 3535 (61)		3536 (89)
A	4	148	3537 (57)	3538 (57)		
A	3	149	3539 (79), 3540 (79)	3541 (79), 3542 (79)		
A	3	152	3543 (61)	3545 (61), 3546 (61)	3544 (31), 3547 (31)	3548 (31)

A	3	155				3549 (31), 3550 (31)
A	2	164				3551 (31), 3552 (31)
A	4	164	3553 (57), 3554 (57)			
A	1	166		3555 (57)		
A	2	166	3556 (61)	3557 (61), 3558 (112)		3559 (12)
A	2	167				3560 (12)
A	3	168	3561 (57), 3562 (57)	3563 (61)	3564 (12)	
A	4	168			3565 (47)	3566 (47)
A	1	174	3567 (112), 3568 (112)	3569 (112), 3570 (112)		
A	2	174		3571 (96), 3572 (96), 3573 (96)		
A	3	174			3574 (89)	3575 (89)
F	2	179	3576 (61)	3577 (61), 3578 (61)		

F	2	183			3579 (23), 3580 (23)	
F	1	186				3581 (44), 3582 (44)
F	2	206			3583 (266)	
F	2	207				3584 (23)
F	1	209				3585 (22)
F	2	209	3586 (61), 3587 (61)	3588 (61), 3589 (61)		
E	1	247				3590 (267)
E	2	259	3591 (59)			
E	3	260			3592 (23)	3593 (23)
E	1	261			3594 (37)	
E	1	262	3595 (58), 3596 (58)	3597 (58), 3598 (58)		
E	2	265	3599 (59), 3600 (59)	3601 (59), 3602 (59)		

E	4	270				3603 (268), 3604 (268)
E	2	273				3605 (37)
E	3	273				3606 (31), 3607 (31)
E	4	273			3608 (23)	
H	2	292				3609 (33)
H	2	303				3610 (33)
H	1	307			3611 (12)	
H	1	308			3612 (12)	3613 (12), 3614 (12)
I	2	344	3615 (195), 3616 (195)			
K	2	387		3617 (119), 3618 (119)		

[a]Visit in which the sample was found positive for *Listeria.*

[b]ID number assigned to the sites for labeling and tracking purposes.

[c]A four digit number was assigned to each Listeria isolate by the Cornell Food Safety Lab for future recognition, storing and analyzing purposes.

[d]Number in the parenthesis indicates isolates allelic type (AT).

Supplemental Table A5. Allelic Types Present in Representative Isolates.

Packinghouse	Allelic Type	No. of isolates[a]	Species
D	11	2	*L. innocua*
	57	1	*L. monocytogenes*
	59	3	*L. monocytogenes*
	79	7	*L. monocytogenes*
	81	2	*L. monocytogenes*
	112	3	*L. monocytogenes*
	260	3	*L. monocytogenes*
		21	
C	31	2	*L. innocua*
	55	2	*L. welshimeri*
	57	1	*L. monocytogenes*
	64	10	*L. monocytogenes*
	79	5	*L. monocytogenes*
	112	8	*L. monocytogenes*
	202	2	*L. monocytogenes*
	260	9	*L. monocytogenes*
	261	2	*L. marthii*
	262	4	*L. monocytogenes*
		45	
G	57	6	*L. monocytogenes*
	79	2	*L. monocytogenes*
	80	1	*L. monocytogenes*
	96	1	*L. monocytogenes*
	112	4	*L. monocytogenes*
	134	1	*L. welshimeri*
B		**15**	
	6	6	*L. innocua*
	12	3	*L. seeligeri*
	23	1	*L. innocua*
	24	3	*L. seeligeri*
	53	1	*L. innocua*
	57	6	*L. monocytogenes*
	59	1	*L. monocytogenes*
	109	2	*L. innocua*
	112	1	*L. monocytogenes*
	133	2	*L. welshimeri*
	134	4	*L. welshimeri*
	219	1	*L. welshimeri*
		31	

A	12	3	*L. seeligeri*
	24	2	*L. seeligeri*
	31	7	*L. innocua*
	47	2	*L. welshimeri*
	57	13	*L. monocytogenes*
	61	8	*L. monocytogenes*
	64	4	*L. monocytogenes*
	79	6	*L. monocytogenes*
	89	3	*L. welshimeri*
	96	3	*L. monocytogenes*
	112	5	*L. monocytogenes*
	263	2	*L. marthii*
	264	1	*L. marthii*
	265	4	*L. marthii*
		63	
F	22	1	*L. innocua*
	23	3	*L. innocua*
	44	2	*L. innocua*
	61	7	*L. monocytogenes*
	266	1	*L. innocua*
		14	
E	23	3	*L. innocua*
	31	2	*L. innocua*
	37	2	*L. innocua*
	58	4	*L. monocytogenes*
	59	5	*L. monocytogenes*
	267	1	*L. welshimeri*
	268	2	*L. innocua*
		19	
H	12	4	*L. seeligeri*
	33	2	*L. innocua*
		6	
I	195	2	*L. monocytogenes*
		2	
J	119	2	*L. monocytogenes*
		2	

[a] Total number of isolates that were prepared for characterization by sequencing of the *sigB* (n=218).

Supplemental Table A6. Lineages of *Listeria monocytogenes* isolates.

Listeria monocytogenes isolates[a]	
Allelic Type	Lineage
57	II
58	I
59	I
61	I
64	I
79	IIIA
80	IIIA
81	IIIA
96	IIIA
112	TBD[b]
119	II
195	TBD
202	TBD
260	TBD
262	TBD

[a] A total of 128 representative isolates were identified in this study of those 78 were *L. monocytogenes*.

[b] Lineage to be determined (TBD). Currently, allelic type (AT) is not found in the Food Microbe Tracker data base.

Supplemental Table A6. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained in each visit from produce packinghouses (n=11)

	No. of positive samples (% prevalence)		
Visit	*Listeria* [a]	*L. monocytogenes*[b]	*L.* spp.[c]
1	14 (3.5)	7 (1.8)	7 (1.8)
2	45 (11.4)	23 (5.8)	22 (5.6)
3	26 (6.6)	14 (3.5)	12 (3.0)
4	17 (4.3)	16 (4.0)	11 (2.8)
Total	102 (6.4)	60 (3.8)	52 (3.3)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria: L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria,* excluding *L. monocytogenes.*

Percentage prevalence was calculated as follows: No. of positive samples / total samples taken per visit (n=396).

Supplemental Table A7. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained in visit one from each produce packinghouses (n=11).

Packinghouse	Total samples	No. of positive samples (% prevalence) Visit 1		
		Listeria[a]	*L. monocytogenes*[b]	*L.* spp.[c]
A	35	5 (14.3)	5 (14.3)	0 (0.0)
B	35	1 (2.9)	0 (0.0)	1 (2.9)
C	35	0 (0.0)	0 (0.0)	0 (0.0)
D	35	1 (2.9)	1 (2.9)	0 (0.0)
E	35	3 (8.6)	1 (2.9)	2 (5.7)
F	35	2 (5.7)	0 (0.0)	2 (5.7)
G	35	0 (0.0)	0 (0.0)	0 (0.0)
H	34	2 (5.7)	0 (0.0)	2 (5.7)
I	36	0 (0.0)	0 (0.0)	0 (0.0)
J	46	0 (0.0)	0 (0.0)	0 (0.0)
K	35	0 (0.0)	0 (0.0)	0 (0.0)
Total	396	14 (3.5)	7 (1.8)	7 (1.8)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria: L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria,* excluding *L. monocytogenes.*

Percentage prevalence in each packinghouse was calculated as follows: No. of positive samples / total samples collected

Supplemental Table A8. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained in visit two from each produce packinghouses (n=11)

Packinghouse	Total samples	No. of positive samples (% prevalence) Visit 2		
		Listeria[a]	*L. monocytogenes*[b]	*L.* spp.[c]
A	35	11 (31.4)	5 (14.3)	6 (17.1)
B	35	8 (22.9)	0 (0.0)	8 (22.9)
C	35	10 (28.6)	9 (25.7)	1 (2.9)
D	35	2 (5.7)	1 (2.9)	1 (2.9)
E	35	3 (8.6)	2 (5.7)	1 (2.9)
F	35	5 (14.3)	2 (2.7)	3 (8.6)
G	35	2 (5.7)	2 (5.7)	0 (0.0)
H	34	2 (5.9)	0 (0.0)	2 (5.9)
I	36	1 (2.8)	1 (2.8)	0 (0.0)
J	46	1 (2.2)	1 (2.2)	0 (0.0)
K	35	0 (0.0)	0 (0.0)	0 (0.0)
Total	396	45 (11.4)	23 (5.8)	22 (5.6)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria: L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria,* excluding *L. monocytogenes.*

Percentage prevalence in each packinghouse was calculated as follows: No. of positive samples / total samples collected

Supplemental Table A9. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained in visit three from each produce packinghouses (n=11).

Packinghouse	Total samples	No. of positive samples (% prevalence) Visit 3		
		Listeria[a]	*L. monocytogenes*[b]	*L.* spp.[c]
A	35	10 (28.6)	4 (11.4)	6 (17.1)
B	35	4 (11.4)	2 (5.7)	2 (5.7)
C	35	5 (14.3)	4 (11.4)	1 (2.9)
D	35	2 (5.7)	2 (5.7)	0 (0.0)
E	35	2 (5.7)	0 (0.0)	2 (5.7)
F	35	0 (0.0)	0 (0.0)	0 (0.0)
G	35	3 (8.6)	2 (5.7)	1 (2.9)
H	34	0 (0.0)	0 (0.0)	0 (0.0)
I	36	0 (0.0)	0 (0.0)	0 (0.0)
J	46	0 (0.0)	0 (0.0)	0 (0.0)
K	35	0 (0.0)	0 (0.0)	0 (0.0)
Total	396	26 (6.6)	14 (3.5)	12 (30)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria: L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria,* excluding *L. monocytogenes.*

Percentage prevalence in each packinghouse was calculated as follows: No. of positive samples / total samples collected

Supplemental Table A10. Frequency of environmental samples positive for *Listeria*, *L. monocytogenes*, and *Listeria* spp. obtained in visit four from each produce packinghouses (n=11).

Packinghouse	Total samples	No. of positive samples (% prevalence) Visit 4		
		Listeria[a]	*L. monocytogenes*[b]	*L.* spp.[c]
A	35	5 (14.3)	2 (5.7)	3 (8.6)
B	35	10 (28.6)	5 (14.3)	5 (14.3)
C	35	4 (11.4)	3 (8.6)	1 (2.9)
D	35	6 (17.1)	6 (17.1)	0 (0.0)
E	35	2 (5.7)	0 (0.0)	2 (5.7)
F	35	0 (0.0)	0 (0.0)	0 (0.0)
G	35	0 (0.0)	0 (0.0)	0 (0.0)
H	34	0 (0.0)	0 (0.0)	0 (0.0)
I	36	0 (0.0)	0 (0.0)	0 (0.0)
J	46	0 (0.0)	0 (0.0)	0 (0.0)
K	35	0 (0.0)	0 (0.0)	0 (0.0)
Total	396	17 (4.3)	16 (4.0)	11 (2.8)

[a] *Listeria* refers to the genus level and includes all species of *Listeria.*

[b] *L. monocytogenes* referred only to the species of *Listeria: L. monocytogenes.*

[c] *Listeria* spp., referred to all species of *Listeria,* excluding *L. monocytogenes.*

Percentage prevalence in each packinghouse was calculated as follows: No. of positive samples / total samples collected

Supplemental information A11. Phylogenetic tree for *Listeria monocytogenes* isolates

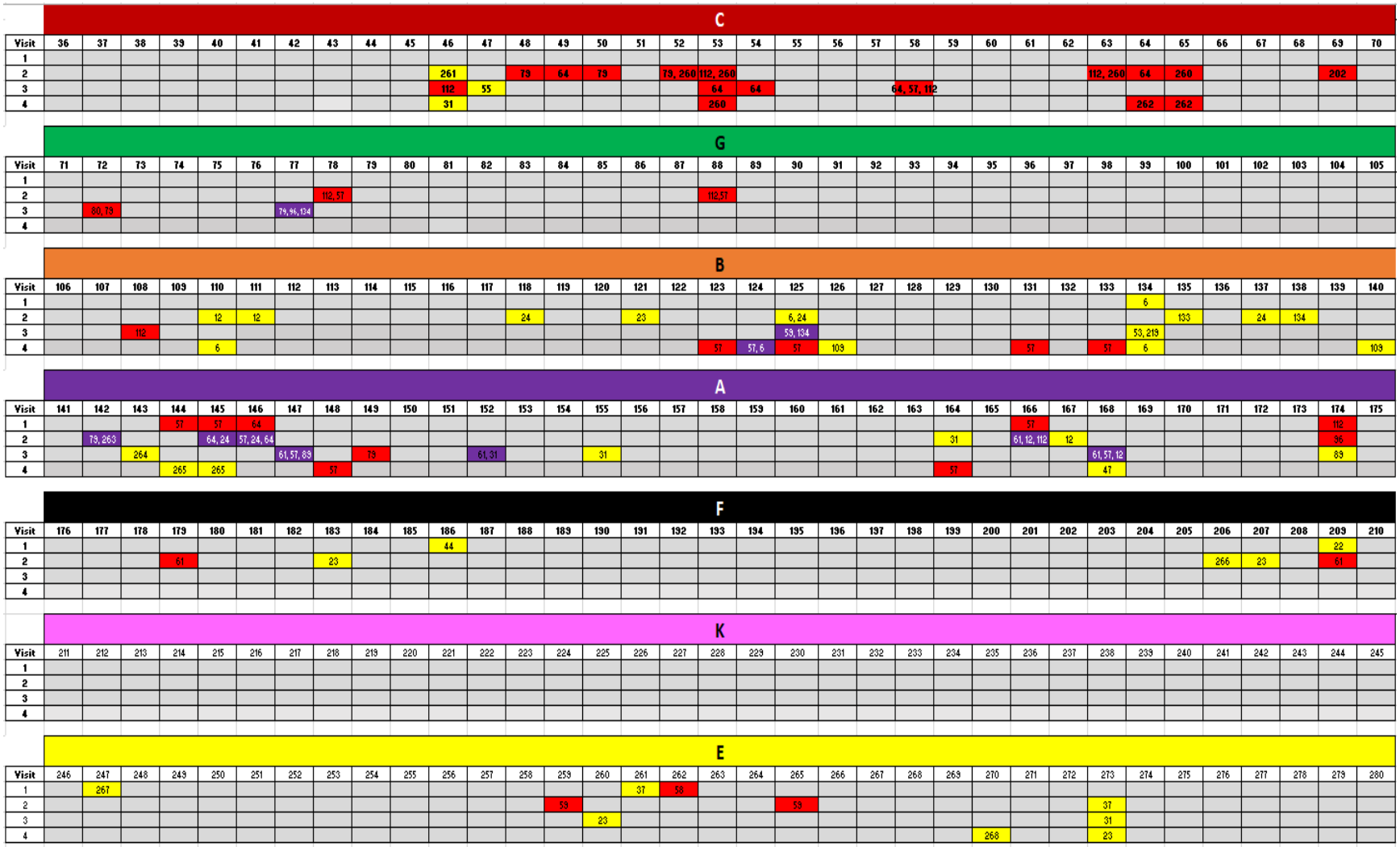
https://drive.google.com/open?id=1s_NG13YeIQUYD0TbwQ6vcLvfW7dIn8SZ

Supplemental information A12. Phylogenetic tree for *Listeria* spp. isolates

https://drive.google.com/file/d/1lSfTpw8cTdlbolE5U9pHHAzd-qCHDxb-/view?usp=sharing

Supplemental information A13. Phylogenetic tree for *Listeria* isolates from packinghouse A.

https://drive.google.com/file/d/1pQNBXR1IhcaJlY_oBJIqWkVXC0NQS388/view?usp=sharing



C																																						
Visit	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
1																																			
2											261		79	64	79		79, 260	112, 260										112, 260	64	260				202	
3											112	55						64	64				64, 57, 112												
4											31							260											262	262					

G																																						
Visit	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
1																																			
2								112, 57										112,57																	
3		80, 73					79,96,134																												
4																																			

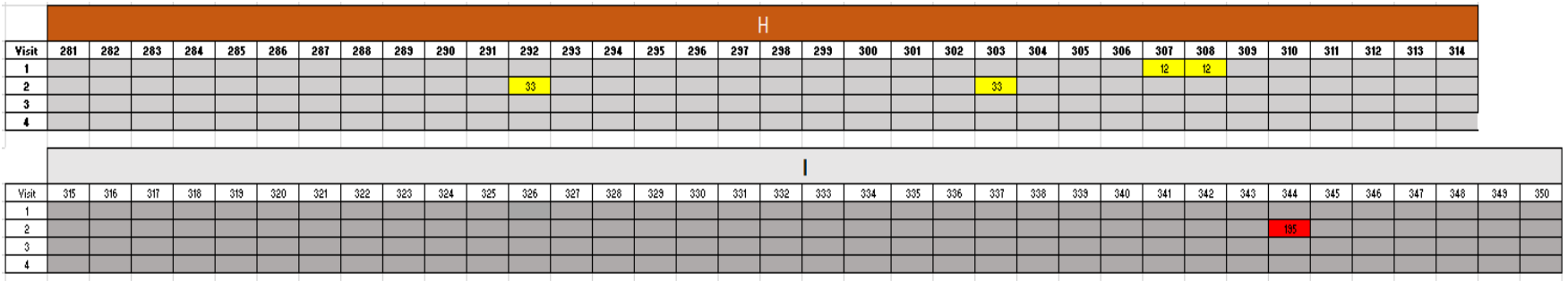
B																																						
Visit	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140
1																													6						
2					12	12							24			23				6, 24										133		24	134		
3			112																	53, 134									53, 213						
4					6													57	57, 6	57	109					57		57	6						109

A																																						
Visit	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175
1				57	57	64																				57								112	
2		79, 263			64, 24	57, 24, 64																		31		61, 12, 112	12							36	
3			264				61, 57, 83		79			61, 31			31													61, 57, 12							83	
4				265	265			57																57				47							

F																																			
Visit	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
1											44																							22	
2				61				23																							266	23		61	
3																																			
4																																			

K																																			
Visit	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245
1																																			
2																																			
3																																			
4																																			

E																																						
Visit	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280
1		267														37	58																			
2														59						59								37								
3															23													31								
4																									268			23							



Sample positive for *L. monocytogenes*

Sample Positive for *L.* ssp. (other than *L. monocytogenes*)

Listeria co-isolation

AT Allelic type

Supplemental Figure A6. Listeria prevalence in the sites of the 11 produce packinghouses facilities during the 4 visits.

Appendix B: Supplemental Tables for Chapter 4

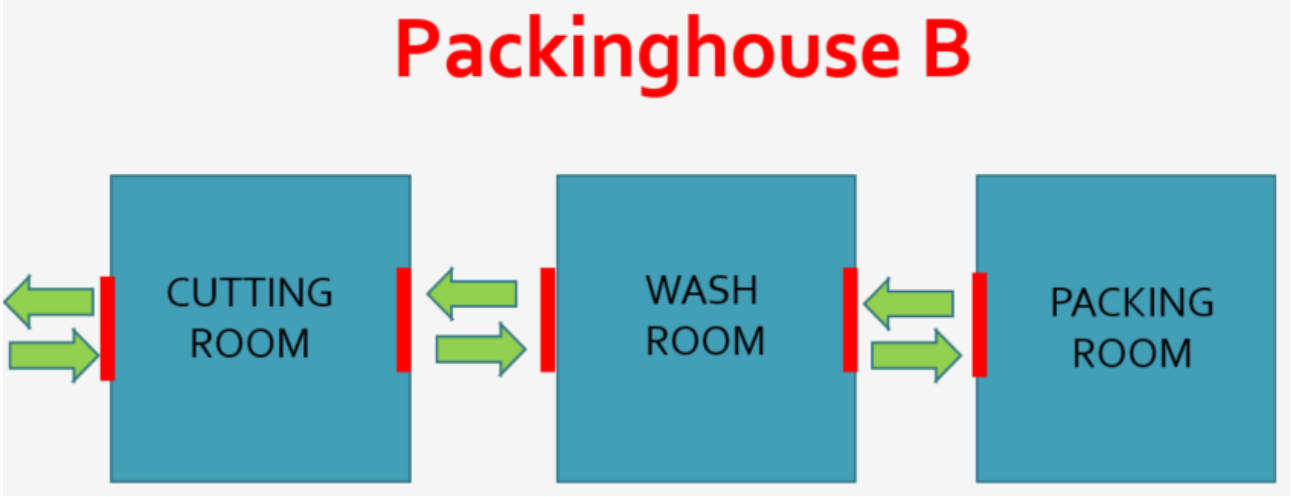


Figure B1: Packinghouse B layout and traffic flow during visits 1-4. Green arrows represent workers', equipment', and product flow. Red bars represent doors in the facility.

Figure B2: Current packinghouse B layout and traffic flow. Green arrows represent workers', equipment', and product flow. Red bars represent doors in the facility.

Figure B3. Pictures of some of the Listeria positives in packinghouse A.