PREVALENCE OF *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES* IN FROZEN FOOD MANUFACTURING ENVIRONMENTS

by

BRITTANY FRAN MAGDOVITZ

(Under the Direction of Mark A. Harrison)

ABSTRACT

Listeria monocytogenes is a ubiquitous organism which presents challenges in controlling and monitoring the pathogen in the frozen food industry. This research was focused on understanding the prevalence of *Listeria* spp. and *L. monocytogenes* in the frozen food processing environment and on raw vegetables used in these facilities. The first activity was a survey of the frozen food industry to understand the design of processing facilities and their environmental monitoring practices. *Listeria* spp. sampling was most commonly performed weekly on non-food contact surfaces, with floors, drains, and walls as areas with the highest frequency of positive sampling sites. Subsequently, data was collected using a triple-blinded method that provided a safe harbor for collection of sensitive information from industry participants. Environmental monitoring observations from 27 facilities provided 42,799 results for *Listeria.* Zones 3 and 4 had a higher probability of having *Listeria* positive sampling sites compared to zone 2 for routine environmental monitoring samples. The most prevalent *Listeria* positive sites within a facility were drains (4.0%), pumps (3.9%), troughs (3.6%), chutes (2.5%), and containers (2.3%). The last activity determined the prevalence of *Listeria* spp. and *L. monocytogenes* on raw vegetables arriving at frozen food facilities. A total of 290 samples were collected, with 96 and 17 samples

positive for *Listeria* spp. (33.1%) and *L. monocytogenes* (5.9%), respectively. Enumeration data for the 96 *Listeria* spp. samples indicated 82 samples had greater than 100 MPN *Listeria* spp./g and 14 samples less than 100 MPN *Listeria* spp./g. The *Listeria* prevalence on raw produce and in processing environments provides industry information that can be used for more accurate quantitative risk assessments for controlling *Listeria* in frozen food facilities.

INDEX WORDS: *Listeria monocytogenes*, *Listeria* spp., frozen food, environmental monitoring, and food manufacturing facilities

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DEDICATION

I would like to dedicate this dissertation to my amazing family. To the strongest woman I know, my mother, I want to thank you for your constant love and support that has helped guide me to this point. You continue to motivate me every day to reach for the stars. To my father, although you are not here to see my accomplishments, I hope to represent the title as the next Dr. Magdovitz proudly. Your love, dedication, and passion for education inspired me to further my knowledge in the field of science. To my brothers and sisters-in-law, I am grateful for all your encouragement and guidance throughout this process. And lastly, to my dog, Snowball, and my adorable niece, you both put a smile on my face every day. To all of you, thank you for everything that you do for me.

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CHAPTER 1

INTRODUCTION

Information about *Listeria monocytogenes* within the food industry is critical as this ubiquitous pathogen is found to occur in diverse environments *(3, 4, 10)*. *Listeria* is a psychrotroph that can reside in refrigerated food processing facilities and can be a problem for the frozen food industry *(8, 9)*. Two recent listeriosis outbreaks in the United States, one associated with ice cream and the other with frozen vegetables increased the concern for *L. monocytogenes* in frozen foods *(5, 11)*. These outbreaks can have detrimental impacts on the frozen food industry, as the market value is expected to exceed $306 billion (U.S. dollars) in 2020 *(1)*.

Prevalence studies for *L. monocytogenes* on produce found 0.29% to 25.4% positive samples depending on the commodity, product type, location, and analysis methods *(2, 6, 7, 12)*. In the U.S., guidance has been developed for the industry to help reduce *L. monocytogenes* contamination on products and in processing environments. These guidelines help focus on improved environmental monitoring programs to actively seek and remove *Listeria* within food processing facilities *(13, 14)*.

This dissertation seeks to evaluate the prevalence of *Listeria* within the frozen food industry's processing environment. An overview of the industry's environmental monitoring practices was assessed to understand issues the industry is facing (Chapter 3). A blinding method to collect sensitive food safety data to provide a safe harbor between researchers and industry participants was developed (Chapter 4). This blinding method was used in the collection of

information and samples from the frozen food industry to allow for the evaluation of environmental monitoring observations from various frozen food processing facilities (Chapter 5), and for detection of *Listeria* prevalence on raw produce arriving at frozen food facilities (Chapter 6).

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CHAPTER 2

LITERATURE REVIEW

Listeria monocytogenes

Listeria monocytogenes was first a concern as an animal pathogen as early as 1926; the first reported human listeriosis case was in 1929 *(43, 45, 54)*. In the early 1980s, coleslaw was the first food product implicated with a listeriosis outbreak associated as a foodborne illness *(53)*. This outbreak had a total of 41 cases and 18 deaths, most of the cases (83%) were prenatal cases *(54)*. After food was found to be a vehicle for *L. monocytogenes* contamination, the food industry became aware of *L. monocytogenes* as a cause of foodborne illness in humans.

L. monocytogenes is a gram-positive, rod-shaped bacterium *(25, 28)*. *Listeria* can grow at temperatures from 0-45°C, but optimal temperature for growth is 30-37°C *(22, 31)*. Cells can be killed with temperatures greater than 50°C *(39)*. Average generation time for multiple strains of *L. monocytogenes* at 4, 10, and 37°C were found to be 43, 6.6, 1.1 hours, respectively with a lag time of 151, 48, 7.3 hours *(8, 22)*. Survival during freezing depends on the rate of freezing and the food substrate *(22)*. Optimal *Listeria* growth occurs between pH 5-9 and at a water activity ≥0.97 *(22, 39)*. *L. monocytogenes* has been found to be relatively resistant to certain stressors on bacteria including acidic environments, freezing, salt concentration above 10%, and drying *(31, 64)*.

Listeria can be found in a wide range of environments, including soil, sewage, fecal material, vegetation, water, and raw food commodities *(32)*. Summer weather has been linked to higher occurrences of listeriosis than colder winter weather *(18, 23)*. Contamination in the food

processing environment can become frequent due to the ubiquitous presence of *Listeria* throughout the environment *(27, 32, 44, 65)*. This can be a challenge for food processors. Food products that are commonly associated with contamination of *L. monocytogenes* are ready-to-eat foods, which include deli meats, soft cheeses, raw milk, and vegetables *(32)*. These products are processed so the consumer can eat them without performing additional kill steps. Some RTE foods that receive a kill step (e.g., cooking, possibly blanching) can be contaminated with *L. monocytogenes* from the processing environment after the kill step has occurred, whereas RTE foods lacking a kill step may be contaminated with the pathogen from the environment at any point before consumption. If *L. monocytogenes* contaminated products are held under conditions that allow growth of *Listeria*, then the problem can increase *(39)*.

The main concern with *L. monocytogenes* is for high-risk individuals including people who are old, young, immunocompromised, and pregnant *(22, 31)*. In a healthy individual, listeriosis can be a mild illness, but the pathogen can cause gastroenteritis, meningitis, or brain infections when it crosses the epithelial barrier of the intestinal tract, the blood brain barrier, or the feto-placental barrier *(32)*. The onset time for symptoms of listeriosis ranges from 9 to 48 hours for gastrointestinal illness while more invasive illnesses can take up to 70 days *(39)*. Listeriosis is commonly caused by consumption of contaminated food products, with an estimated 1,600 illnesses per year in the United States *(15)*. The mortality rate is approximately 20% *(15)*. Listeriosis is the third highest leading cause of death for foodborne illness, killing 260 people per year *(15)*. Hospitalization rates for people who contract the illness are high for people within the susceptible population (i.e., old, young immunocompromised, and pregnant). *L. monocytogenes* continues to become more of a critical threat to public health and to food processors *(15)*. The severity and high fatality rate for *L. monocytogenes* establishes a need for

preventive measures; however, the characteristics of the microorganism make it difficult to expect all food to be free of *Listeria* contamination *(22)*.

Prevalence of *Listeria monocytogenes* with Produce

Lack of information on prevalence and concentration of *L. monocytogenes* on produce in the U.S. is due to different aspects of regulatory authority and the zero tolerance regulatory action limit for the presence of the pathogen in ready-to-eat foods. Although there is a lack of information on *L. monocytogenes* prevalence in the U.S. for RTE products, some studies have focused on baseline prevalence from various commodities. In 1988, a study was published showing no isolation of *L. monocytogenes* from samples of fresh produce (lettuce, potato peels, corn husks, broccoli stems, cabbage, carrot peels, cauliflower stems, mushroom stems, spinach, and beet peels) and frozen green beans, pea pods, green peas, and spinach in the U.S. *(47)*. Improvements in detection methods, sampling procedures, and scientific knowledge of *Listeria* has led to more detection of the pathogen on various food commodities. In 2010-2013, a study focused on ready-to-eat products in the U.S. provided an overview of multiple food groups (meat, dairy, produce, seafood, and combination foods) with an overall *L. monocytogenes* prevalence of 0.37%. For the produce categories, raw cut vegetables had 18 *L. monocytogenes* positive samples of 1,689 (1.07%) with a concentration of <0.036 MPN/g to 330 CFU/g *(36)*.

Prevalence information in the U.S. may be lacking for certain food commodities. However, research on *L. monocytogenes* prevalence has been performed in other areas of the world. A study in Spain focused on fresh-cut fruit and vegetables and whole vegetables for indicators and pathogens. For *L. monocytogenes*, non-detectable limits occurred with all of their commodities (i.e., carrots, spinach, corn salad, sprouts, and a variety of leafy greens), except in fresh-cut lettuce and mixed salads which had a prevalence of 3.4% and 0.8%, respectively *(1)*.

Another Spanish study tested fresh fruit, raw whole vegetables, and ready-to-eat vegetables. *Listeria* was not detected from fruit, but the prevalence with vegetables was 2.7% for *Listeria* spp. and 0.9% for *L. monocytogenes (7).* A third study in Spain, focused specifically on vegetables that were fresh, modified atmosphere packaging (MAP), or frozen. Of the 191 samples, 8 (4.2%) *L. monocytogenes* positive samples were isolated with over 100 cfu/g in all cases. Frozen samples had the highest prevalence at 8.3%, compared to fresh (1.4%) and MAP vegetables (4.3%) *(41)*.

An Italian study during 2005-2007, compared different preventive strategies (i.e., strict GMPs and HACCP, chlorine wash step, and physical microbial reduction) on RTE and whole vegetables. From 699 samples, only 2 (0.29%) of the RTE samples were positive for *L. monocytogenes* while no whole vegetables were positive *(21)*. The positive samples were found by using the BAX PCR method, but not confirmed through traditional cultural methods. The study outlined that the first producers who implemented strict HACCP and GMP plans provided products with higher microbiological quality than the other microbial reduction processes *(21)*.

A study in Santiago, Chile found 88 out of 347 (25.4%) samples of frozen vegetable salads were positive for *L. monocytogenes (20).* Raw or cooked ready-to-eat vegetable salads from the supermarket revealed 22 out of 216 (10.2%) *L. monocytogenes* positive samples with no positives from the 154 samples of minimally processed salads *(20)*. Additionally, this study enumerated a randomly chosen set of 20 positive samples by plate count and reported 90% had less than 10 cfu/g *(20)*. The MPN technique was performed for another 34 samples *(20)*. For the MPN results, 12 had ≥ 1,100 MPN/g, 5 were in the range of 93-240, 8 were in-between 3-23, and 9 were less than 3.0 MPN *L. monocytogenes*/g *(20)*. The MPN technique is designed for detecting low numbers, and this study suggests MPN methodology is more suitable than plate

count for enumeration due to the lower detection level. Twelve of the contaminated frozen samples in this study were cooked, and no *L. monocytogenes* was recovered after cooking step was applied *(20)*.

A study conducted in Botswana tested 1,324 food samples from supermarkets and street vendors and found 57 (4.3%) *L. monocytogenes* positive samples *(42)*. From all the supermarket samples, the highest prevalence for *L. monocytogenes* was found in frozen cabbage at 10% *(42)*. A Korean study tested 244 samples of fresh cut produce, RTE, and ready-to-cook foods for *L. monocytogenes* and found only one sample to be positive (0.4%) in the ready-to-eat category *(19)*. Meat, dairy products, fresh vegetables, seafood, and RTE foods were collected from Thai supermarkets. Of the 380 samples, 64 (16.8%) were positive for *Listeria* spp. and 18 (4.7%) were positive for *L. monocytogenes*. Of the 90 fresh vegetable samples, *Listeria* spp. and *L. monocytogenes* were detected in 21 (23.3%) and 3 (3.3%) of the samples, respectively *(57)*.

These studies show *Listeria* and *L. monocytogenes* prevalence on produce varies from 0.29% to 25.4%. There are variables between the studies that can affect the prevalence including location in the world, processing methods applied, sample types, and analysis methods, but these studies show a need for additional information on the prevalence and number of *Listeria* and *L. monocytogenes* on products to be used in quantitative risk assessments *(7, 20, 21, 57)*.

Listeriosis Outbreaks

Improper food handling observed in studies show that consumers frequently implement unsafe food handling practices *(51)*. Information about consumer food safety practices is inconsistent and more educational efforts are needed to help inform consumers how to reduce their risk of foodborne illness *(50)*. The average cost for all foodborne illness exceeds $1,110 per case and the national cost for the U.S. is over $55 billion *(52)*. The average annual cost of illness

associated with *L. monocytogenes* in 2009 was $2.6 billion *(30)*. The largest *L. monocytogenes* outbreak based upon hospitalization and mortality occurred in 2017 in South Africa with the deli meat, polony *(46)*. The cost valuation for the 204 listeriosis fatalities exceeded $260 million (U.S. dollars) *(46)*. Hospitalization cost for the 1,034 cases exceeded $10 million and cost per case varied by age of patient *(46)*. The largest hospitalization cost was associated with babies at $15,840 per case *(46)*. The extensive focus on food safety practices helps to reduce morbidity, mortality, and the large cost of foodborne illness outbreaks.

Major listeriosis outbreaks vary by commodity, number of cases, severity, and location *(32)*. Outbreaks associated with *L. monocytogenes* linked to produce in the U.S. began in 1979 in Maryland with an outbreak linked to an unknown food source narrowed down to either raw vegetables or pasteurized milk, causing 20 cases and 3 deaths *(29)*. Additionally, in Texas in the early 1990s, there was an outbreak of *L. monocytogenes* linked to frozen vegetables as frozen broccoli and cauliflower were the potential source of infection *(55)*. The largest outbreak of listeriosis linked to fruit in the U.S. was in 2011-2012 with 147 cases and 33 deaths. The source of the outbreak was cantaloupes from Jensen Farms. The outbreak was linked to 28 different states and caused 143 people to be hospitalized *(13)*. Another, fruit linked *L. monocytogenes* outbreak was associated with caramel apples in 2014. This outbreak caused 35 cases with 34 hospitalization and 7 deaths across 12 states *(5)*. In 2015, an outbreak linked listeriosis to frozen products as ice cream from Blue Bell caused 10 cases in 4 states, which led to 3 deaths *(49)*. The following year, a listeriosis outbreak was linked directly to frozen vegetables, which created more concern for *L. monocytogenes* in frozen products *(14)*. CRF Frozen Foods in Pasco, Washington products were linked to 9 cases of listeriosis causing 3 deaths in 4 states *(14)*.

Outside the U.S., there have been multiple cases of vegetable linked listeriosis outbreaks *(32)*. In Australia in 1978-1979, an outbreak caused 12 neonatal listeriosis cases with no associated deaths *(34)*. Products were later recalled, but there were 9 deaths linked to that outbreak *(24)*. Sheep and cattle were linked to a listeriosis case in South Africa in 1999 after the animals were feed poor quality unmarketable potatoes *(56)*. In Brazil, there have been several foodborne illnesses involving produce. Although no outbreaks for *L. monocytogenes* have been found in the country, studies in Brazil have shown a prevalence of 3.03% in raw and ready-to-eat produce *(12)*.

Frozen Food Industry

The frozen food industry has grown as consumers' interests have evolved. According to the Allied Market Research, by 2020, the frozen food market is expected to reach $306 billion (U.S. dollars) *(4)*. In 2018, the food sales market for frozen food grew over two percent in both dollars and units *(2)*. The market drivers to the growth within this market are changing lifestyles and food habits of consumers, increases in convenience for food consumption, and growing number of working professionals *(4)*.

Traditionally, frozen vegetables were used in food preparation where consumers fully cooked or heat treated (microwave) the products. Due to this additional step by consumers, frozen vegetables have been considered to be not-ready-to-eat (nRTE). However, some of these frozen vegetables can be consumed subsequent to thawing and without an additional lethality step, such as the use of peas in a salad or spinach in a smoothie. With the frozen food industry's growth there are areas of miscommunication by marketing and misunderstanding from consumers about the intended use of products. Hence, there is a need to ensure the

microbiological safety of these products and to prevent contamination from pathogens, such as *L. monocytogenes*, regardless of whether frozen foods are considered RTE or nRTE *(37).*

Listeria can survive at cold temperatures including freezing temperatures, which may not significantly reduce cell numbers. The food matrix and freezing rate of the product affects the ability of *Listeria* to survive during freezing *(39)*. Foodborne illnesses from *L. monocytogenes* is a concern in the frozen food industry because of its ability to survive freezing operations with minimal loss in viability during storage. Across a two-year period (June 2017 – June 2019) there were 39 human food recalls for frozen food products found on FDA's website, which lists recalls associated with FDA regulated products. Out of those, 12 recalls were related to *L. monocytogenes (63).* Recalls related to frozen products for *L. monocytogenes* can be related to multiple factors, including new scientific information, evolving consumer practices, and updated government guidance focusing on actively searching for *L. monocytogenes* within food processing facilities *(62).*

Environmental Monitoring

Elimination of *L. monocytogenes* from a processing facility is almost impossible, but facilities can reduce the prevalence of the pathogen in their food and the processing environment *(9)*. Food processing facilities have used cleaning and sanitation methods to minimize the risk of pathogens in the environment. The issue with this practice is it can minimize the risk of the pathogen in the facility but does not prevent pathogens from contaminating the food. Sanitation procedures alone cannot prevent outbreaks from occurring. A primary source of *L. monocytogenes* on ready-to-eat foods is through recontamination of the pathogen during or after processing steps *(59).* Strict cleaning and sanitation procedures are highly recommended for the

reduction of contamination, but more intense measures need to be taken to reduce and eliminate the problem.

Environmental monitoring is a procedure implemented in a food processing facility to understand the cleaning and sanitation program, as well as develop procedures to reduce pathogen contamination within the processing facility's environment. An environmental monitoring program helps document areas within the environment which maybe be a source of potential contamination of food product processed in the facilities. A processing facility should have a detailed environmental control program that incorporates effective cleaning and sanitation. Environmental monitoring plans are established by companies based upon characteristics of the products manufactured, pathogens of concern, and the type of processing environment. Government guidance suggests using preventive measures that include an environmental monitoring plan in the processing area especially for ready-to-eat foods *(35)*. This preventive measure is recommended in the Preventive Controls for Human Food, Produce Safety Rule, and other guidance documents implemented by the FDA. Additionally, third-party certifications such as Global Food Safety Initiative (GFSI), require a preventive control measure for contamination and environmental monitoring plans. More focus within environmental monitoring plans are placed on ready-to-eat areas of the processing environment. Ready-to-eat areas or post-lethality areas (which can be RTE areas) are a focus of environmental sampling as these areas have a higher risk of potential recontamination on the product based upon a risk based approach to sampling. Pathogen recontamination in a post-lethality area is a recognized condition of listeriosis outbreaks *(35)*.

For products under the jurisdiction of the FDA, the most recent draft guidance in 2017 for *L. monocytogenes* provides detailed descriptions for the industry to help establish an

environmental monitoring plan *(62)*. It is suggested that processing areas be divided into zones 1 to 4 with zone 1 defined as a food contact surface, directly in contact with the food product, i.e., equipment, conveyor belts, and tables. Zones 2, 3, and 4 are nonfood contact surfaces progressively farther away from food contact surfaces with zone 2 in very close proximity to food products and food contact surfaces and zone 4 in remote areas outside of the processing area *(62)*. For a well-designed environmental monitoring plan, samples should be collected and documented in all areas of the facility to help identify sources of concern. The plans should be documented through written procedures which focus on risk-based approaches from scientifically valid sources and are catered to each individual facility *(62)*. The procedures should specify sampling sites, timing of sampling, frequency of the sample collection, etc. Meticulous consideration should be taken to determine the locations of the sample sites as *Listeria* can be found to adhere to many surfaces found in processing environments including stainless steel, aluminum, and polycarbonate *(10)*. Additionally, procedures should identify organisms of concern and if the focus is on indicator organisms or pathogen testing, as well as the analytical methods used for testing. Testing can be conducted in-house or by an outside commercial laboratory. Sampling should be monitored and properly documented to understand areas of concern within the facility. Facilities should modify environmental monitoring procedures based upon the analysis of sampling data and focus the plan on a risk-based sampling approach *(62)*.

United States Government Regulations for *L. monocytogenes*

U.S. policy related to *L. monocytogenes* as an "adulterant" in ready-to-eat food products is among the most rigid in the world. Further, the zero-tolerance policy for this pathogen considers a RTE product containing detectable *L. monocytogenes* in a 25 g sample to be subject to a recall and/or a seizure *(31)*. Regulating agencies elsewhere in the world vary on regulations

associated with *L. monocytogenes.* Some countries such as Canada and Germany, separate food into risk-based categories and provide separate regulations based upon the products ability to support the growth of *L. monocytogenes (31).* This allows for more high risk foods to have zero-tolerance for *L. monocytogenes* and less risky food to have an acceptable limit. The zero-tolerance approach for *L. monocytogenes* in RTE foods adopted by U.S. food industry regulators encourages higher performance standards in manufacturing facilities but may have a detrimental effect on environmental monitoring practices because facilities are prone to conduct fewer tests to reduce the possibility of collecting positive results for *L. monocytogenes (37, 66).*

The U.S. regulating agencies established regulations for *L. monocytogenes* in response to the public health concerns after the 1980s outbreaks linked *Listeria* to food products *(54).* The food code in 1993 provided recommendations for sanitation, employee practices, cooking times and temperatures to prevent the spread of *L. monocytogenes (54).* In 1987, U.S. Department of Agriculture (USDA) established the zero-tolerance policy for *L. monocytogenes* in ready-to-eat meat and poultry products and initiated testing for the pathogen in these products *(40).* Around the same time period, the U.S. FDA established the presence of *L. monocytogenes* as an adulterant in other foods and as such, a violation of the Federal Food, Drug and Cosmetic Act *(6, 54).* Studies showed a decrease in listeriosis cases for the years following the enactment of government regulations in the late 1980s, which helped to support the idea that improvement in the industry from regulatory pressure improved public safety *(58).*

Additional regulations have been implemented by both the USDA and FDA to further guide the industry in prevention methods for *L. monocytogenes* including requiring detailed Hazard Analysis Critical Control Points (HACCP) plans, Sanitation Standard Operating Procedures (SSOPs), and more recently, companies should have a Preventive Controls Qualified

Individual on staff to ensure compliance with the Preventive Controls for Human Foods *(33)*. FDA updated their 2008 guidance for controlling *L. monocytogenes* in RTE foods in 2017 to a new draft guidance document to establish more effective environmental monitoring procedures, allowing the first environmental *Listeria* spp. positive test to be an indicator that there is a problem without triggering an automatic recall *(61, 62)*. The "seek and destroy" method is the current approach by the FDA for environmental monitoring wherein facilities are encouraged to find the problem and couple that with intensified cleaning and sanitizing activities to reduce contamination potential in their facility *(38)*. This is considered to be a major shift in FDA's approach to regulating this pathogen.

FDA attempted to move towards a regulatory limit for *L. monocytogenes* for certain food categories similar to other countries' regulations, such as Canada with a three category system for ready-to-eat foods based upon the health risks of the products associated with *L. monocytogenes (26).* A focus was placed on determining the dose-response that would cause listeriosis indicating if below a 100 cfu/g would not be harmful to a healthy population *(6, 16)*. Risk assessments and models were conducted to collect more scientific information, and the guidance document in 2008 further clarified the definition of RTE products *(61)*. Limited understanding and lack of scientific evidence for a quantitative level that is not of concern for susceptible populations has led the regulation to stay with a zero-tolerance policy for ready-to-eat products *(6, 54)*.

Additionally, after the 2015 outbreak associated with ice cream, *L. monocytogenes* was detected at low levels ranging from 0.15 - 7.1 MPN/g from the contaminated products *(17)*. This helps to further the idea that more scientific evidence is needed to establish a dose-response of *L. monocytogenes* for susceptible and healthy populations *(48)*. The FDA and USDA continue to

follow the zero-tolerance approach for *L. monocytogenes* in ready-to-eat products with a focus on preventive controls including environmental monitoring to reduce the pathogen contamination throughout the farm to fork process *(6)*.

Corrective Actions for Positive *Listeria* Results in Processing Environments

The FDA guidance for the food industry released in 2017 helps to clarify practices that should be implemented to reduce the pathogen throughout the food processing environment *(61, 62)*. Former industry practices did not include vigorously looking for *L. monocytogenes* because if a positive was found there would be large repercussions. This was due to the zero-tolerance policy that if *L. monocytogenes* is found in an RTE product or on a food-contact surface then the company must perform a recall of the entire production load that tested positive *(61)*. Since there was no set guidance on how often to test for *Listeria*, limited testing provided fewer opportunities for a recall.

A new shift in the industry is to focus on creating sampling plans that emphasize finding areas that harbor *Listeria* spp. and *L. monocytogenes* called the "seek and destroy" method *(38)*. Production facilities are able to continue production after finding a positive for *Listeria* spp. in the environment on food contact or non-food contact surfaces (not for *L. monocytogenes* on food-product surfaces, the zero-tolerance rule still applies, and products must be recalled if positive), but facilities should implement proper corrective action procedures to mitigate the problem. *(62)*. After a *Listeria* spp. positive is found, there are detailed procedures outlined in the draft guidance for the next steps companies should take. FDA recommends a company should include in their written procedures for the environmental monitoring plan, what steps should be taken for corrective actions, and who is responsible for taking those steps *(62)*. Corrective action steps include intensively cleaning and sanitizing the area of the positive test

and retesting the area for a number of consecutive days to confirm that the contamination is removed. If consecutive tests remain positive, then a problem area of concern is identified, and a root cause analysis should be performed. Depending on the location of the reoccurring positive samples, steps should be taken to stop production and hold product until a root cause analysis determines the source of the problem and removes it. The action should be taken quickly if the reoccurring positive samples are on food contact surfaces *(62)*.

Suggestions from industry organizations focus on preventive methods of reducing the pathogen's presence in the food processing environment by implementing extensive sanitation standard operating procedures, proper employee training, and an effective environmental monitoring plan. With these in place, some areas of concern will be discovered and need to be remedied. Proper corrective actions for a positive pathogen result, first includes intensive cleaning and sanitization of the area of the positive result. Also, vector sampling which is sampling areas around the positive site should be performed to see if the pathogen is transient or a consistent issue in various areas of the processing facility. A crucial aspect for corrective action protocols is to determine the root cause of the sample with an established food safety team from several disciplines of the company represented in the group *(3)*.

Environmental monitoring within the industry is evolving based upon new scientific information and varies across all facilities. The USDA suggest sampling between 3 to 5 food contact surface samples per line during sampling, in comparison to Health Canada's suggestion for sampling approximately 10 food contact surfaces, but the sampling frequency depends on the complexity of the processing line *(60, 66)*. FDA on the other hand, provides recommendations to determine if *Listeria* control measures are effective and adequate based upon size of processing plant, product flow, product, processing methods, and previous data collection *(66)*. *L.*

monocytogenes is not just limited to processing environments and can be found in all types of food environments. A study found 25 out of 30 retail environments that sell produce tested positive for *L. monocytogenes* with an overall prevalence of 4.4% *(11)*. *Listeria* is a ubiquitous organism that can be found in all environments *(9)*. Preventive control measures from the farm, processing environment, retail, and consumers are needed to ensure the pathogen is diminished and does not cause future foodborne illness in healthy and susceptible populations.

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CHAPTER 3

EVALUATING ENVIRONMENTAL MONITORING PROTOCOLS FOR *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES* IN FROZEN FOOD MANUFACTURING FACILITIES

Magdovitz, B. F., Gummalla, S., Thippareddi, H., & Harrison, M. A. (2020). Evaluating environmental monitoring protocols for *Listeria* spp. and *Listeria monocytogenes* in frozen food manufacturing facilities. *Journal of Food Protection*, 83:172-187.

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ABSTRACT

Food processors face serious challenges due to *Listeria monocytogenes* contamination. Environmental monitoring is used to control *L. monocytogenes* from the processing environment. Although frozen foods do not support the growth of *L. monocytogenes*, the moist and cold conditions in frozen food production environments are favorable for growth of *L. monocytogenes*. The purpose of the study was to determine the current state of awareness and practices applied across a variety of frozen food facilities related to environmental monitoring for *Listeria.* A survey tool was created to elicit information on existing environmental monitoring programs within the frozen food industry. The topics included cleaning and sanitizing applications and frequency, microbiological testing, and environmental areas of concern. The survey was reviewed by academic and industry experts with knowledge of microbiology and frozen food processing and was field tested by industry personnel with extensive knowledge of environmental monitoring. The survey was distributed and analyzed electronically via Qualtrics among 150 frozen food contacts. Data were gathered anonymously with a response rate of 31% (n=46). The survey indicated that facilities are more likely to test for *Listeria* spp. in environmental monitoring zones 2 to 4 (nonfood contact areas) on a weekly basis. The major areas of concern in facilities for finding *Listeria*-positive results are floors, walls, and drains. At the time of the survey, few facilities incorporated active raw material and finished product testing for *Listeria*; instead, programs emphasized the need to identify presence of *Listeria* in the processing environment and mitigate potential for product contamination. Recognition of environmental monitoring as a key component of a comprehensive food safety plan was evident, along with an industry focus to further improve and develop verification programs to reduce prevalence of *L. monocytogenes* in frozen food processing environments.

HIGHLIGHTS

- Environmental monitoring practices vary throughout the frozen food industry.
- Areas of concern of processing facilities for *Listeria* are floors, drains, and walls.
- *Listeria* spp. sampling most commonly performed weekly on nonfood contact surfaces.

The goal for an effective food safety plan is to prevent harborage of pathogens in the production environment and reduce the potential for cross-contamination and consequently adulteration of the food being processed. Manufacturing facilities implement environmental monitoring programs as a means of verification to support their food safety plans. Effective environmental monitoring programs can provide evidence that an operation's food safety plan is contributing to the company's ability to produce a safe product. Factors that affect environmental monitoring plans include the design of the program to seek and destroy for pathogens of concern and the effectiveness of corrective actions that ensue any positive findings *(20)*.

Cleaning and sanitation practices are followed to remove food residues and to reduce or eliminate microorganisms from food contact surfaces and the food processing environment. Presence and growth of *Listeria* in a food processing facility can be an indication of unsatisfactory cleaning and sanitation procedures *(5)*. If a niche area harboring pathogens such as *Listeria* is found, effective corrective actions to remove the contamination should be performed *(20)*. Although effective cleaning and sanitation programs help to produce a safe product, other factors may increase the risk of potential product contamination such as development of *Listeria monocytogenes* growth niches and harborages. These may include, but are not limited to, poorly designed equipment and facility infrastructure, lack of personnel hygiene, and absence of validated processes *(13)*. Because of the complexity of factors that impact the prevalence and growth of *Listeria* in frozen food manufacturing facilities, the application of well-designed environmental monitoring programs is paramount.

Traditionally, frozen vegetables are used in food preparation where the products are heat treated, fully cooked, or both and hence considered to be not ready-to-eat. However, some of these frozen vegetables can be consumed subsequent to thawing and without an additional

lethality step, such as use of peas in a salad. Hence, there is a need to ensure the microbiological safety of these products and prevent contamination with *L. monocytogenes*. In Portugal, Mena et al. *(17)* reported *L. monocytogenes* contamination of frozen vegetables to be 14.8 to 22.6%. As determined by the National Food Processors Association study, *L. monocytogenes* prevalence in multiple products including cheeses, salad, seafood, and lunch meat was 1.82% of the total samples collected *(8)*. High-risk populations for listeriosis include pregnant women, children, persons with immunocompromised conditions (e.g., cancer, HIV infection, dialysis, organ transplantation), and the elderly *(3, 7, 9, 18)*. Although freezing the product can prevent growth of *L. monocytogenes*, storage at improper temperature can allow products to thaw and permit the growth of *Listeria (12)*. Controlling for hazards through good manufacturing practices helps to prevent bacterial contamination from harborage sources *(6)*. Increased focus on effective personnel training and proper design of equipment and facilities are necessary to reduce *Listeria* prevalence in food manufacturing facilities *(1)*. The objective of the study was to determine the level of awareness and practices used in the frozen food industry related to environmental monitoring for *Listeria* spp. and *L. monocytogenes*. The survey aimed to identify areas of concern in processing environments through the interpretation of industry-generated data.

MATERIALS AND METHODS

An electronic survey was created to provide an overview of the protocols for environmental monitoring that the frozen food industry implements in their manufacturing facilities. The survey was distributed through a listserv of food safety professionals working in frozen food manufacturing facilities. The participation in the research was voluntary, and no compensation was provided to encourage responses. Questions were written to establish an

understanding of the operation, processes, and a general design of each facility while maintaining anonymity of participants.

Qualtrics (Provo, UT) software was used in the development and formatting of the questions in the electronic survey. The design of the survey was adapted to participants' responses. The software customized the survey outline based upon the answers provided to prior questions. This allowed the survey to provide follow-up questions for more in-depth responses. For example, if a participant indicated they implemented a protocol included in a specific question, the survey would follow up with a question asking about the frequency of the practice. If a participant indicated that they were not executing the protocol, the survey would continue with the next question.

The survey was designed with different question formats to ensure the questions provided comprehensive information. Figure 3.1 is an example of the survey that participants completed. Several questions were multiple choice where one or more answers could be chosen for each response. Other questions were designed as a matrix table, from which the participant could choose from multiple answer choices. The last type of question was an open-ended question for which participants provided a typed entry to the question. These were used sparsely and mostly as supplement questions to reduce the time and effort required to participate in the survey.

The survey was reviewed at different stages by academics and industry personnel with knowledge of food microbiology and commercial frozen food processing before implementation. The University of Georgia's Human Subjects Office reviewed and approved the standards and safety associated with the survey. In addition, legal counsel representing the frozen food industry reviewed the survey to ensure anonymity protection to all participants. Before the survey was

distributed, a pilot test was performed using a small group of industry experts (n=5) with extensive knowledge of environmental monitoring in commercial food processing facilities. Feedback provided from the pilot test was used to improve the survey.

The questions focused on various aspects that are important to designing an effective environmental monitoring program. The survey was divided into sections to depict the generic layout of the processing facility, sanitation protocols, and details on the environmental monitoring program being implemented in the facility. The section on the layout of the facility includes volume and size of the facility, design of the floor and drains, and conditions of the processing areas. Sanitation and cleaning questions are based upon good manufacturing practices and current industry practices. The environmental monitoring section focuses on testing protocols for product and environmental surfaces.

Distribution of the final Qualtrics survey link was done via email to members of the American Frozen Food Institute with a statement indicating the purpose of the research. All responses were collected and compiled through the Qualtrics Web site, allowing participation in the survey to be anonymous to the researchers. The analysis of the data was performed with Qualtrics and Excel (Microsoft, Redmond, WA) to analyze the percentages of responses versus the total respondents for each question.

RESULTS

Of the 150 frozen food contacts that received the survey through a listserv, 46 contacts participated by completing a survey (31% response rate). In total there were 80 responses for categories of frozen foods, including vegetables (n=39) and fruits (n=17) as the leading foods produced (Figure 3.2). Other facilities produced frozen meat, poultry, entrées, dessert, pizza, potato products, and appetizers. About half of the respondents manufacture at least two of the

categories of food surveyed within one facility. The most common combinations of categories within a facility were vegetables with fruit, potatoes and appetizers, and entrée combined with meat and poultry. The processing facilities that participated were inspected by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture, Food Safety Inspection Service, or both.

The definition of ready-to-eat (RTE) and not ready-to-eat (nRTE) was still in flux at the time of the study, but each participant defined their products based upon the intended use of their product. Survey participants defined their products based upon the manufacturer's determination of the product, by nRTE packages including cooking instructions on the package for consumers to follow, whereas RTE products are prepared to ensure the quality and taste of the final product is optimal for the consumer. Sixty-one percent of responders defined their products as nRTE and 28% as RTE. Eleven percent of responses produce both RTE and nRTE and stated the cleaning and sanitation procedures are the same for all their products.

Categories for volume of facilities in the survey was defined as small ($1 to $10 million in production per year), medium ($10 to 100 million per year) and large (> $100 million per year). These categories were established by literature and advice from industry professionals. Fifty-four percent (n=25) were categorized as medium-sized facilities, 37% percent (n=17) as large, and 9% percent (n=4) as small.

Another measure of the facility is the square footage of the entire facility and the area of the processing room(s). Categories for the entire facility size included small (< 2,400 m^2), medium (2,400 to 10,000 m^2) and large (> 10,000 m^2). No facilities were categorized as small in this survey, whereas 53% were medium-sized facilities (n=25) and 47% (n=22) were classified as large. Square footage of only the processing areas were defined as small (< 1,100 m^2),

medium (1,100 to 4,600 m^2) and large (> 4,600 m^2). Similar to the previous question, 60% (n=28) of companies were classified as medium, 38% (n=18) as large, and 2% (n=1) as small.

Information was collected from the responders on the facility design and age. Facilities in this survey were older, with no facilities surveyed that were less than 10 years old. Thirty-nine responses included 19 facilities over 30 years old, 15 facilities 20 to 30 years old, and 5 facilities 10 to 20 years old. Only seven of the facilities performed a renovation less than a year ago, whereas 10 facilities performed a renovation over 15 years ago. Eight percent (n=3) of the facilities have never performed a renovation to their facility. Of the renovations performed within the past 10 years, nine were renovated 1 to 5 years ago and eight were renovated 5 to 10 years ago.

All the facilities in this survey produced frozen food products. Facility layouts varied based upon company. Twenty-seven of 39 processing areas did not have a refrigerated processing area, whereas the other 12 processing areas were refrigerated. A majority of the facilities had either coated concrete floors (n=24) or epoxy-coated floors (n=20). The other flooring types were tile (n=2) and noncoated concrete floors (n=4). Of the facilities surveyed, 30 had trench drains, whereas 13 had cup drains. Sixty-four percent (n=23) had three or fewer drains per 100 m^2 in the processing area. In addition, 36% (n=13) had four or more drains per 100 m^2 of the processing area.

Implementation of good manufacturing practices is essential in all food manufacturing facilities and the employees should be trained periodically *(18).* Key components to an environmental monitoring plan are the cleaning and sanitization steps. Cleaning and sanitation occur most commonly during pre- and postshift. The results from the survey revealed that ~50% of the respondents performed cleaning and sanitation preshift, whereas the other half of the

respondents cleaned and sanitized postshift (Figure 3.3). Some respondents only clean during pre- or postshift, whereas some facilities indicated they clean preshift, midshift, and postshift. Some use additional cleaning times including midshift, multiple times during the shift, and weekly.

Eighty-seven percent (n=33) of responders indicated that they have performed validation of their cleaning procedures. Validation steps included visual inspection by quality assurance technicians followed by ATP, aerobic plate count (APC), allergen swabs, or a combination after cleaning but before production starts. Additional cleaning was performed until ATP or APC swabs were within the appropriate range designated by the facility in case the standards were not met subsequent to initial cleaning and sanitation. Other validation measures performed by the facility management included academic reviews, professional reviews from a third-party laboratory, and in-plant historical reviews of current and past-process controls. These validation measures ensure that the cleaning trends follow an established pattern to confirm the facility conforms to their standards.

Environmental monitoring plans were based on the zone concept and defined by FDA *(23)*. Zone 1 was classified as food contact surfaces, whereas zones 2, 3, and 4 are nonfood contact surfaces. Sanitizer and cleaning compounds used differed between facilities. Detergent and water with soap were described as the most common cleaning compounds. Detergents were used in zones 1 (n=28) and 2 (n=28) more than in zone 3 (n=25) and 4 (n=19); however, water with soap was consistent throughout all zones (Figure 3.4). Solvent-based cleaners were consistently used throughout all the zones (n=14 per zone).

Sanitizers used most by facilities were quaternary ammonium compounds followed by peroxyacetic acid (Figure 3.5). Other sanitizers less commonly used in the facilities included

hypochlorite, chlorine dioxide, and iodophors. The responses showed that sanitizers were applied in zones 1 (n=73) and 2 (n=65) at a higher proportion than in zones 3 (n=60) and 4 (n=41). To determine how sanitizers were applied the survey delved into each sanitizer's application method. The application methods for sanitizers include spraying the sanitizer onto the equipment, using liquid and water as a clean-in-place process or soaking the equipment, and foaming the surface. Quaternary ammonium compounds were most commonly applied by spray method (n=21), followed by liquid and water (n=16) and foam (n=12) applications. Peroxyacetic acid was applied by the spray (n=15) and liquid and water (n=14) methods. Five responders applied chlorine samples as liquid and water and one as gas. Hypochlorite was applied by foam (n=10), spray (n=7), and liquid and water (n=6).

Environmental monitoring practices focus on testing for indicator organisms and pathogens to ensure that facilities are reducing risk to their consumers. Indicators used in zone 1 included APC (n=24) and ATP (n=29), followed by coliforms (n=18) (Figure 3.6). In zones 2 (n=31), 3 (n=32), and 4 (n=30) *Listeria* spp. were most commonly monitored. The facilities that test for *L. monocytogenes* indicated they also test for *Listeria* spp. The responses for the "other microorganisms" category stated the processors tested for *Salmonella* and/or yeast and molds.

Frequency of testing indicators and pathogens within establishments was based on scientific literature and individual company policy. APC and ATP were tested preshift in all zones, with a focus on zone 1 (Figures 3.7 and 3.8). Coliforms were tested weekly in all zones and preshift in zone 1 (Figure 3.9). Approximately two-thirds of the respondents that tested for ATP or APC also indicated they test for coliforms. *Listeria* spp. were most commonly tested weekly in zones 2 to 4 (n=58). Almost every respondent combined indicator testing for APC or ATP in zone 1 with testing for *Listeria* spp. in zone 2 to 4. Additional frequencies for *Listeria*

spp. in zones 2 to 4 for were midshift (n=32), preshift (n=21) and monthly (n=28) (Figure 3.10). *L. monocytogenes* was also indicated as being tested weekly in zones 2 to 4 (n=18) (Figure 3.11). Within the other organisms category, the most commonly tested organism was *Salmonella,* and it was tested weekly in zones 2 to 4 (Figure 3.12). The facilities that tested for other microorganisms were commonly facilities that tested for *Listeria* spp. as well.

Supplemental to environmental sampling, some facilities performed final product testing for *Listeria* and *L. monocytogenes* as part of their program. More than 70% of respondents indicated they do not test for *Listeria* spp. or *L. monocytogenes* in raw materials (n=26) or products during processing (n=25). However, 47% (n=17) of respondents indicated they do not test finished product for *Listeria*. For finished products, 8% (n=3) tested for *Listeria* spp., 27% (n=10) tested for *L. monocytogenes,* and 17% (n=6) tested for both. All respondents who stated they test for *Listeria* species in final product indicated that when a positive result was found further testing was performed to determine whether the positive sample was *L. monocytogenes.*

For both *Listeria* spp. and *L. monocytogenes,* the areas of concern in the manufacturing environment for a positive test result were drains, floors, and walls. For *Listeria* spp., survey respondents indicated the most common areas to focus on for environmental monitoring were drains (n=27), floors (n=25), and walls (n=4) (Figure 3.13). The most common areas for *L. monocytogenes* during environmental monitoring were also drains (n=13), floors (n=9), and walls (n=9) (Figure 3.14).

DISCUSSION

The survey provides an overview of current frozen food industry practices related to environmental monitoring. The data revealed there is an industry focus on current environmental monitoring programs to improve and develop extensive practices to reduce prevalence of *L.*

monocytogenes in frozen food processing environments. There were variations in responses related to specific practices. This could be due to differences in facilities, types of products processed, company policies, or a combination. It could also indicate some uncertainty within the food industry as to the best environmental monitoring practices.

Product type. A factor that can affect the design of an environmental monitoring plan can be the type of food being processed. RTE foods require a more extensive plan than nRTE foods because there are no additional postprocess preventive control steps required with RTE foods for consumers to reduce contamination levels. A challenge study in Europe found it difficult to establish a distinct difference between RTE foods that do support the growth of *Listeria* versus products do not support the growth of *Listeria (2)*. RTE foods such as deli meats have higher prevalence of *L. monocytogenes* (5%) in finished products *(14)*. Consumers' use of the product may vary from the manufacturers' intended use for the product, as some consumers may eat an nRTE product without further cooking assuming the product is to be consumed as an RTE product. Manufacturers should continue to add detailed information and try to educate the consumers through improved communication methods to ensure that the food products are consumed in the way intended by the manufacturer.

Size, age, and design of facility. The facilities in the survey defined their production capacity predominately as medium and large by volume in dollars of production per year and size based upon area of production facility in square meters. Larger facilities may have more experience in designing a food safety program; this experience can provide guidance for smaller facilities that are beginning to design their protocols. Most of the facilities surveyed were more than 30 years old. These facilities are most likely operating under conditions that were designed without new technological improvements that could provide better means for reducing hazards in

the food being processed in them. The design of the facility may be based upon the technology and industry practices when the facility was built *(19)*. Older facilities may increase their production capacity more than what they were originally designed for owing to higher demand for products. The higher demand for the products leads to increased production and an increased need for better and faster sanitation and cleaning procedures *(16)*.

Drains and floors. Floors in the survey mostly contained coated concrete or epoxy-coated floors. The epoxy coating is a good balance of cost and durability because it helps to adjust to thermal expansion exposure of extreme temperature *(6)*. A majority of the surveyed facilities (n=30) were designed with trench drains compared to cup drains. Trench drains have a high capacity for flow, but the extensive open grating requires a higher need for cleaning because microorganisms can spread across the drain *(6)*. Facility design should include adequate number of drains to provide proper removal of water. The participants indicated more facilities (64%) have fewer than three drains per 100 m^2. Cleaning of the drains should not be performed when food is exposed to the environment. A clean-in-place system can be used to clean drains similar to other equipment *(6)*.

Cleaning and sanitation. Cleaning and sanitation should be implemented in a facility to provide clean manufacturing operations to produce safe and wholesome products *(21)*. Basic procedures for cleaning and sanitation include application of a cleaning compound to remove residue, followed by a sanitizer to reduce the microbial load *(16)*. The cleaning compound is applied first because its efficiency in removing the soil and food residue on food contact surfaces can affect the effectiveness of sanitizer applied subsequently. Factors that affect the cleaning and sanitation performance are time, temperature, concentration, surface type, material, and workers performing cleanup *(16)*. Deep cleaning that includes additional time and labor compared with a

traditional facility cleaning can help reduce *L. monocytogenes* prevalence in facilities with a high prevalence of *L. monocytogenes,* by up to 26% *(10)*. Most of the facilities indicated they performed a validation of their cleaning procedures. Monitoring and verification of cleaning and sanitation protocols varied based on individual facility's procedures *(11)*. The descriptions of the cleaning validation protocols provided a wide range of answers including indicator and pathogen testing, third-party consulting, and academic or in-plant reviews. The differences in the validation methods indicate discrepancy in industry practices and encourage more data to determine the preferred method of validating protocols.

Indicator organism. ATP swab results are used as a good indicator for facility standards and provide motivation for the cleaning crew as an incentive to achieve higher benchmarks *(10)*. Data indicated that indicator organisms including ATP, APC, and coliforms are monitored in zone 1, the food contact surface. Indicator organisms are used by the industry to monitor the cleaning and sanitation procedures performed in the facility. Testing for APC and ATP is commonly used indicator of cleanliness of the surface but does not detect presence of pathogens. Although indicator testing does not detect pathogens, the testing can help to determine areas of concern for the presence of pathogens if APC or ATP identifies sections of improper cleaning and sanitation practices. These testing methods are used preshift to help verify the effectiveness of the cleanup before a new production shift is about to start. The ATP test results (relative light units) can correlate with sanitation effectiveness and provide real-time measurement of microorganisms on a surface *(16)*.

Frequency of testing for pathogens. Data also identified several facilities were testing for the presence of *Listeria* spp. or *L. monocytogenes* post sanitation or before production (preshift). With a preshift testing model, a positive result would indicate there is contamination

of the surface before production. Most facilities use indicator organisms or ATP testing preshift as a verification of the effectiveness of the cleaning and sanitation program. There are two issues with conducting preshift *Listeria* spp. or *L. monocytogenes* testing. (i) It may indicate there is uncertainty concerning the effectiveness of the cleaning and sanitation program. This should prompt the food safety team to review these programs and identify potential limiting factors, such as revalidation of sanitation effectiveness, retraining of sanitation personnel, or identification of appropriate chemicals and processes. (ii) It is recommended these facilities review their sampling strategy on the timing of sample collection. The midshift testing protocol helps to determine pathogen contamination during the production shift and not before production. A robust environmental monitoring plan focused on eliminating the pathogen of concern should test for the pathogen at the highest frequency of finding a positive. The guidance documents from the FDA suggest testing for pathogens during shift approximately 3 to 4 hours into production *(23)*. Collecting environmental monitoring samples 3 to 4 hours into production allows *L. monocytogenes* (if present) to emerge from harborage sites to contaminate food contact surfaces, products, and the environment *(23)*.

***Listeria* and *L. monocytogenes* testing.** *Listeria* spp. and *L. monocytogenes* were frequently tested in zones 2 to 4, the nonfood contact surfaces, on a weekly basis. Facilities monitor for food contact surfaces and nonfood contact surfaces, but the frequency of monitoring and collection times for this activity are determined on an individual basis *(25)*. Few facilities test for *Listeria* in raw materials or products during production, with greater emphasis on monitoring placed on preventing product contamination in the processing environment. The areas of concern in facilities for finding *Listeria*-positive results are floors, drains, and walls.

The zero-tolerance approach for *L. monocytogenes* in RTE foods adopted by the U. S. food industry regulators encourages higher performance standards in manufacturing facilities but may have a detrimental effect on environmental monitoring practices because facilities are prone to conduct fewer tests to reduce the possibility of collecting positive results for *Listeria* and *L. monocytogenes (24)*. The FDA released a new draft guidance document in 2017 updating their 2008 document to establish more effective environmental monitoring procedures, allowing the first environmental *Listeria*-positive test to be an indicator that there is a problem without triggering an automatic recall *(22, 23)*. This approach by the FDA is promoting the "seek and destroy" method in environmental monitoring wherein facilities are encouraged to find the problem and couple that with intensified cleaning and sanitizing activities to reduce the contamination potential in their facility *(15)*.

Industry and data trends. Industry continues to advocate for improvements in environmental monitoring programs. The pathogen environmental monitoring programs are meaningful assessments of the effectiveness of a facility's food safety plans *(4)*. The responses in the survey vary due to differences in environmental monitoring plans. This is because each food safety plan is individualized per facility. Collecting data across the industry of current environmental monitoring practices can help improve food safety plans in all organizations. The collection of data from current implemented effective food safety and environmental monitoring plans provides information to new facilities designing their individualized protocols.

Listeria-related recalls within the frozen food industry have created greater concern for mitigating *Listeria* contamination problems within the food processing environment. The survey collected information about facilities including age of the facility, time of most recent renovations, products produced, production size, and production volume. Because most of the

facilities classified as medium and large in volume and size, the information from their responses can help smaller processors that are trying to develop their environmental monitoring plan. Although these data are only from frozen food facilities, they can provide insight for *Listeria* prevention practices in other segments of the food industry.

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Figure 3.1: The survey distributed to frozen food processors focused on environmental monitoring practices

The Frozen Food Foundation has funded the research project *Prevalence and Concentration of Listeria monocytogenes and an Indicator Organism (Listeria spp.) in Frozen Food Manufacturing Environments and Products and Development of Sampling Plans for Environmental and Product Sampling*. The potential presence of *Listeria monocytogenes* in food processing facilities is an important area of focus for the food industry.

Survey responses will be used in the development of quantitative risk assessments for evaluating (1) the potential risk of product contamination due to *Listeria* and (2) the fate of the pathogen during processing, distribution, and final consumer preparation. Specifically, the questions in this survey will help establish the occurrence of *Listeria* and *L. monocytogenes* in the processing facility environment, including on food contact and nonfood contact surfaces, raw materials, and finished products. The University of Georgia's Institutional Review Board (IRB) has determined that the proposed activity is not research involving human subjects as defined by DHHS and FDA regulations.

If your company has more than one facility, please choose a representative facility and answer this survey based on that operation. Do not provide any information that would identify your company or facility.

Your assistance is greatly appreciated. This survey takes about 15 minutes to complete. If you have questions or comments, please contact us.[a]

[a] Questions with circles represent questions where participants are limited to only 1 response. Questions with squares represent questions where participants can choose multiple responses. Questions with neither are open ended responses.

Q1 What food products are processed in the facility? (Choose all that apply)

- ❑ Vegetable
- ❑ Fruit
- ❑ Meat
- ❑ Poultry
- ❑ Entrée
- ❑ Dessert
- ❑ Pizza
- ❑ Potato
- ❑ Appetizer
- ❑ Other

Q2 Are the food products produced in the facility ready-to-eat (RTE) or not ready-to-eat (NRTE) foods? (Recognizing that FDA's definitions for these categories are in flux, please base your response on your own intended use for the food).

- ❍ Ready-to-eat (After responding GO TO Q4)
- ❍ Not ready-to-eat (After responding GO TO Q4)
- ❍ Both (If this is your response, GO TO Q2a)

Q2a How do your cleaning and sanitizing procedures differ between the RTE and NRTE products?

Q3 Answer the remainder of the questions in the survey based upon the RTE products produced in the facility.

Q4 Approximately what size is the facility based upon volume of production?

- ❍ Very Small (less than $1 million in production per year)
- ❍ Small ($1 - $10 million in production per year)
- ❍ Medium ($10 - $100 million in production per year)
- ❍ Large (more than $100 million in production per year)

Q5 Approximately what size is the facility based upon area of square footage?

- ❍ Small (less than 25,000 square feet)
- ❍ Medium (25,000 - 100,000 square feet)
- ❍ Large (more than 100,000 square feet)

Q6 Approximately how many square feet is the processing area?

- ❍ Small (less than 12,000 square feet)
- ❍ Medium (12,000 - 50,000 square feet)
- ❍ Large (more than 50,000 square feet)

Q7 Provide a general description of the processing flow in the facility.

Q8 Identify the preventive controls in place in the facility.

Q9 Provide a general description of the storage of raw materials and ingredients in the facility.

Q10 Approximately how old is the facility?

- ❍ Less than 5 years old
- ❍ 5-10 years old
- ❍ 10-20 years old
- ❍ 20-30 years old
- ❍ Older than 30 years

Q11 Approximately how long ago was the last major renovation of the facility?

- ❍ Less than 1 year
- ❍ 1-5 years
- ❍ 5-10 years
- ❍ 11-15 years
- ❍ More than 15 years
- ❍ No previous renovations

Q12 Is the processing area refrigerated?

- ❍ Yes
- ❍ No

Q13 What type of floor surface is in the processing area?

- ❑ Coated concrete floors
- ❑ Tile
- ❑ Epoxy coated floor
- ❑ Other

Q14 What type of drains are in the processing area?

- ❑ Cup drains
- ❑ Trench drains
- ❑ Other

Q15 How many floor drains per 1,000 square feet are in the processing area?

- ❍ 1
- ❍ 2
- ❍ 3
- ❍ 4
- ❍ 5
- ❍ Greater than 5

Q16 How often does cleaning and sanitizing occur in the facility? (Choose all that apply)

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly
Cleaning	❑	❑	❑	❑	❑
Sanitizing	❑	❑	❑	❑	❑

Q17 Have you performed a validation of your cleaning procedures?

- ❍ Yes
- ❍ No

Q17a If Q17 is “Yes”: What have you done to validate your cleaning procedures?

Q18 For the following questions please refer to following definitions from the FDA's *Listeria* draft guidance:

- Zone 1: Food contact Surfaces (e.g., utensils, table surfaces, slicers, pipe interiors, tank interiors, filler bowls, packaging and conveyors, hoppers)
- Zone 2: Nonfood contact surfaces that are in close proximity to food and food-contact surfaces (e.g., equipment housing or framework, and some walls, floors or drains in the immediate vicinity of FCSs carts)
- Zone 3: More remote nonfood contact surfaces that are in or near the processing area and could lead to contamination of Zone 1 and 2 (e.g., Forklifts, hand trucks and carts that move within the plant and some walls, floors or drains not in the immediate vicinity of FCSs)
- Zone 4: Nonfood contact surfaces, remote areas outside of the processing area, from which environmental pathogens can be introduced into the processing environment (e.g., locker rooms, cafeterias, and hallways outside the production area or outside areas where raw materials or finished foods are stored or transported).

Q19 What cleaning compounds are used in each zone? (Choose all that apply)

	Water and Soap	Detergent	Solvent Cleaners	Other
Zone 1	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑

Q20 What sanitizers are used in each zone? (Choose all that apply)

	Chlorine Dioxide	Hypochlorites	Iodophors	Quaternary Ammonium Compounds (Quats)	Peroxyacetic Acid	Other
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q20a If chlorine dioxide used: How is the chlorine dioxide applied?

- ❑ Spray
- ❑ Liquid and water
- ❑ Gas
- ❑ Foam
- ❑ Other

Q20b If hypochlorites used: How are the hypochlorites applied?

- ❑ Spray
- ❑ Liquid and water
- ❑ Gas
- ❑ Foam
- ❑ Other

Q20c If iodophors used: How are the iodophors applied?

- ❑ Spray
- ❑ Liquid and water
- ❑ Gas
- ❑ Foam
- ❑ Other

Q20d If quaternary ammonium compounds (Quats) used: How are the quaternary ammonium compounds (Quats) applied?

- ❑ Spray
- ❑ Liquid and water
- ❑ Gas
- ❑ Foam
- ❑ Other

Q20e If peroxyacetic acid used: How is the peroxyacetic acid applied?

- ❑ Spray
- ❑ Liquid and water
- ❑ Gas
- ❑ Foam
- ❑ Other

Q20f If other sanitizers used: What other sanitizers are used in the facility and how are they applied?

Q21 Does your facility conduct samples for the following? (Choose all that apply)

	Aerobic Plate Counts	Coliforms	Fecal Coliforms	Generic *E. coli*	*Listeria* species	*Listeria monocytogenes*	ATP	Other microorganisms
Zone 1	❑	❑	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑	❑	❑

Q21a If your facility tests samples for aerobic plate counts, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21b If your facility tests samples for coliforms, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21c If your facility tests samples for fecal coliforms, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21d If your facility tests samples for *E. coli*, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21e If your facility tests samples for *Listeria* species, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21f If your facility tests samples for *Listeria monocytogenes*, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21g If your facility tests samples for ATP, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q21h If your facility tests samples for other microorganisms, what other microorganisms are tested for in the facility?

Q21i If your facility tests samples for other microorganisms, what is the frequency of conducting tests?

	Pre-shift	Mid-shift	Multiple times during a shift	Post-shift	Weekly	Monthly
Zone 1	❑	❑	❑	❑	❑	❑
Zone 2	❑	❑	❑	❑	❑	❑
Zone 3	❑	❑	❑	❑	❑	❑
Zone 4	❑	❑	❑	❑	❑	❑

Q22 Is the final product tested for *Listeria* species or *Listeria monocytogenes*?

- ❍ *Listeria* spp.
- ❍ *Listeria monocytogenes*
- ❍ Both
- ❍ Neither

Q22a For Q22, if a positive test for *Listeria* species is found in the final product, does further testing occur to determine if the sample is *Listeria monocytogenes*?

- ❍ Yes
- ❍ No

Q23 Are the raw materials or ingredients tested for *Listeria* species or *Listeria monocytogenes*?

- ❍ *Listeria* spp.
- ❍ *Listeria monocytogenes*
- ❍ Both
- ❍ Neither

Q23a For Q23, if a positive test for *Listeria* spp. is found in the raw materials or ingredients, does further testing occur to determine if the sample is *Listeria monocytogenes*?

- ❍ Yes
- ❍ No

Q24 Is the product tested during processing for *Listeria* species or *Listeria monocytogenes*?

- ❍ *Listeria* spp.
- ❍ *Listeria monocytogenes*
- ❍ Both
- ❍ Neither

Q24a For Q24, if a positive test for *Listeria* species is found during processing, does further testing occur to determine if the sample is *Listeria monocytogenes*?

- ❍ Yes
- ❍ No

Q25 In which areas of the facility are positive tests for *Listeria* spp. most common? (Choose all that apply)

- ❑ Raw Materials
- ❑ Final Products
- ❑ Food contact Utensils
- ❑ Conveyor belts
- ❑ Drains
- ❑ Sinks
- ❑ Floors
- ❑ Walls
- ❑ Doors
- ❑ Other

Q26 In which areas of the facility are positive tests for *Listeria monocytogenes* most common? (Choose all that apply)

- ❑ Raw Materials
- ❑ Final Products
- ❑ Food contact Utensils
- ❑ Conveyor belts
- ❑ Drains
- ❑ Sinks
- ❑ Floors
- ❑ Walls
- ❑ Doors
- ❑ Other

Q27 If you have any additional comments or questions, please feel free to write them here. Do not provide any identifying information.

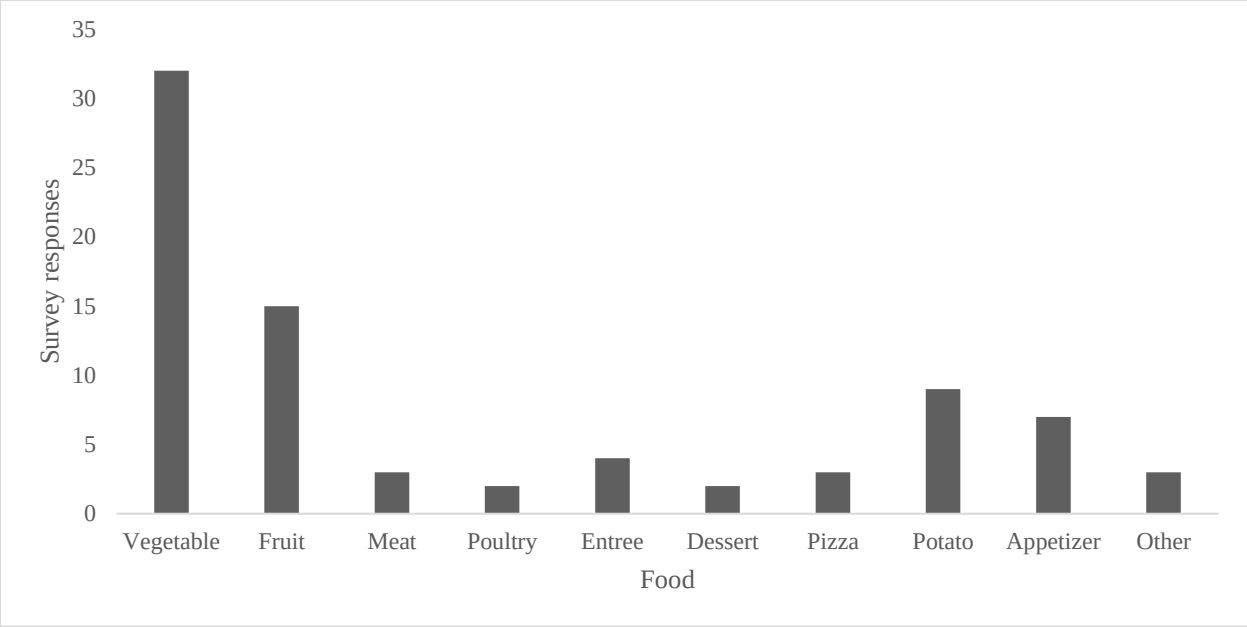

Figure 3.2. Type of food products processed in surveyed frozen food facilities (n=46; respondents could choose more than 1 item)

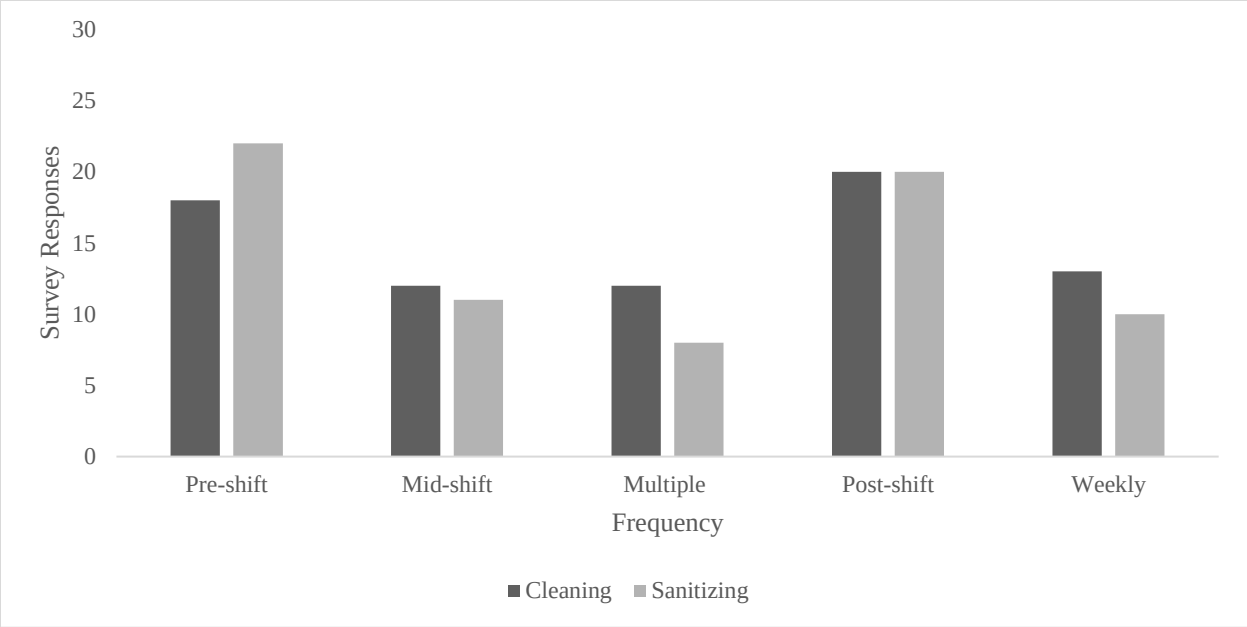

Figure 3.3. Frequency of cleaning and sanitation practices in the surveyed frozen food facilities (n=36; respondents could choose more than 1 item)

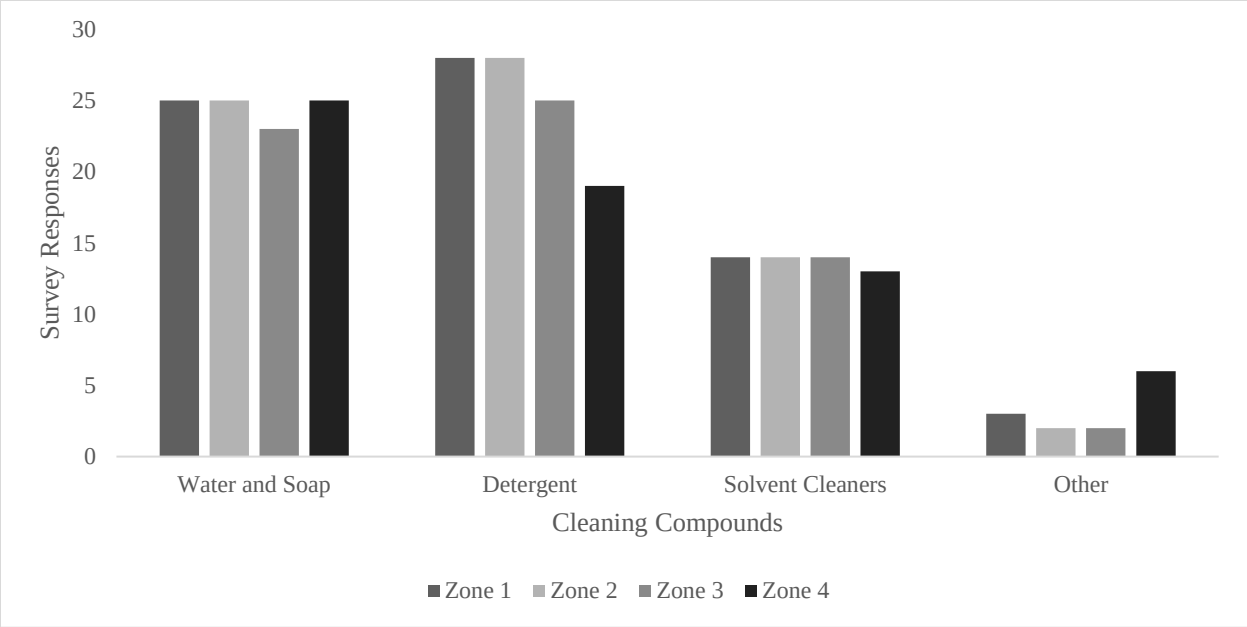

Figure 3.4. Types of cleaning compounds used in surveyed frozen food facilities categorized by hygienic zones (n=36; respondents could choose more than 1 item)

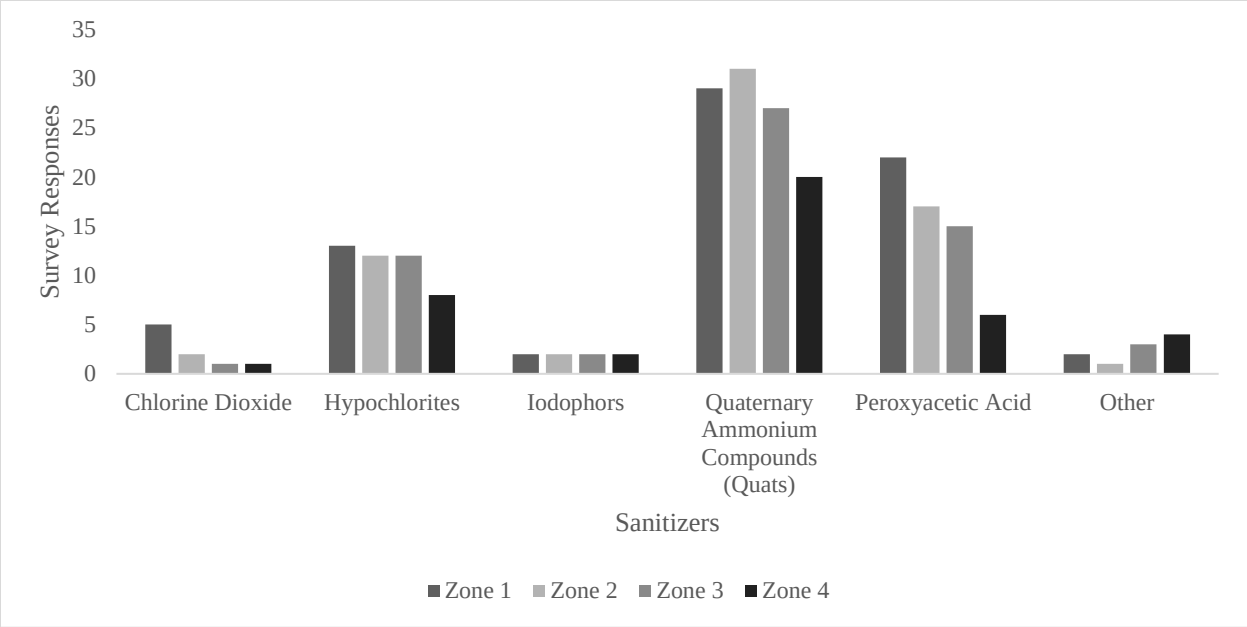

Figure 3.5. Different sanitizers used in surveyed frozen food facilities classified by hygienic zones (n=36; respondents could choose more than 1 item)

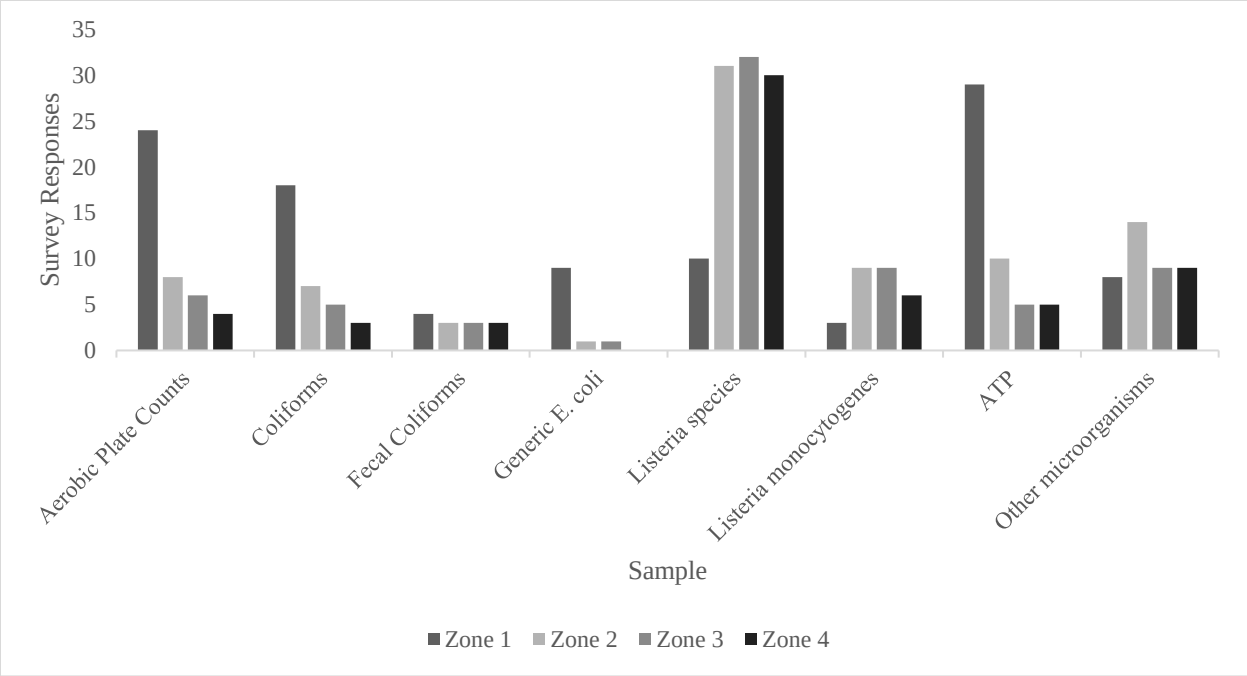

Figure 3.6. Environmental monitoring for indicator microorganisms and pathogen surveyed frozen food facilities grouped by hygienic zones (n=36; respondents could choose more than 1 item)

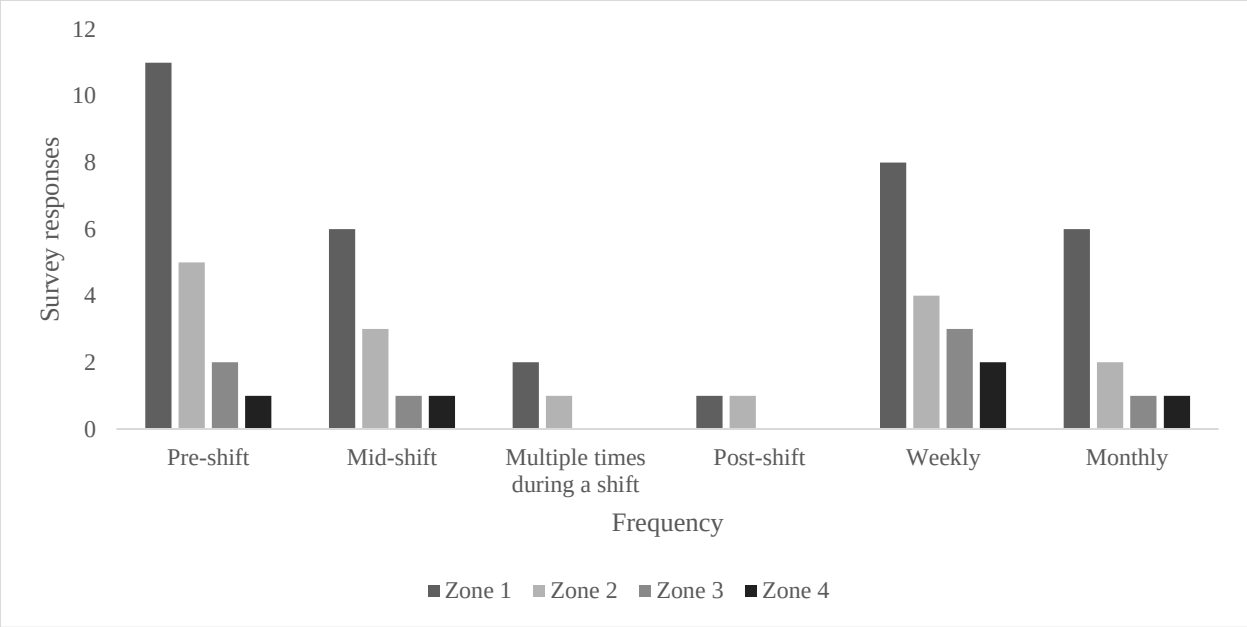

Figure 3.7. The frequency of aerobic plate counts (APC) samples tested by hygienic zones in surveyed frozen food facilities (n=23; respondents could choose more than 1 item)

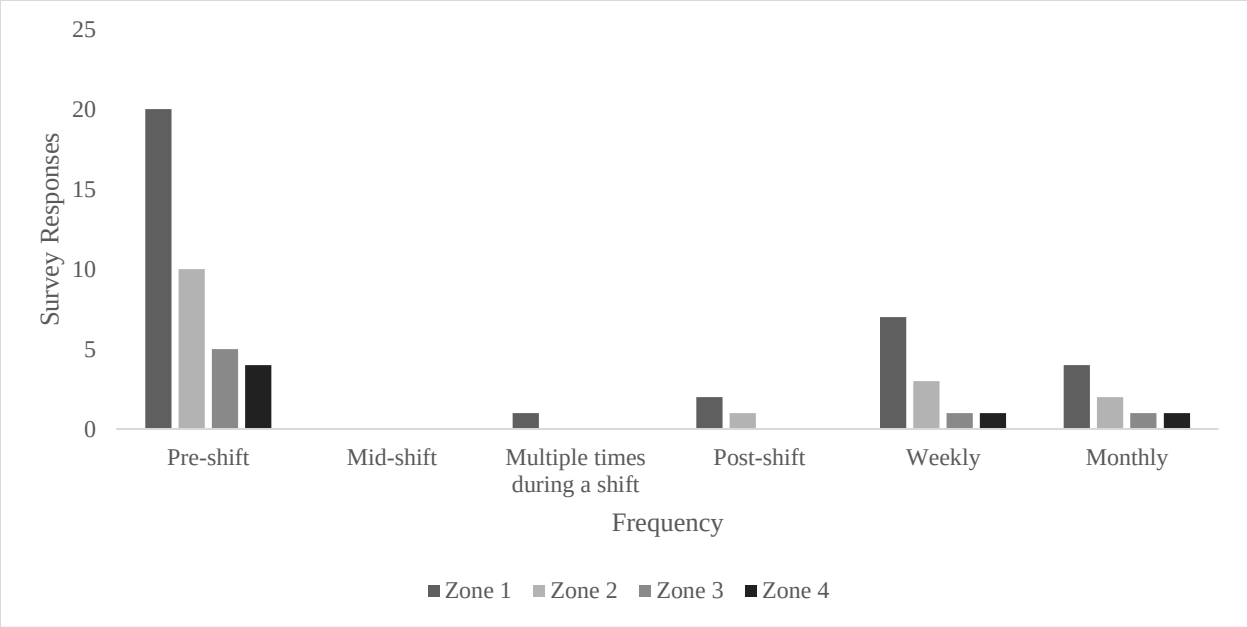

Figure 3.8. The frequency of adenosine triphosphate (ATP) samples tested by hygienic zones in surveyed frozen food facilities (n=28; respondents could choose more than 1 item)

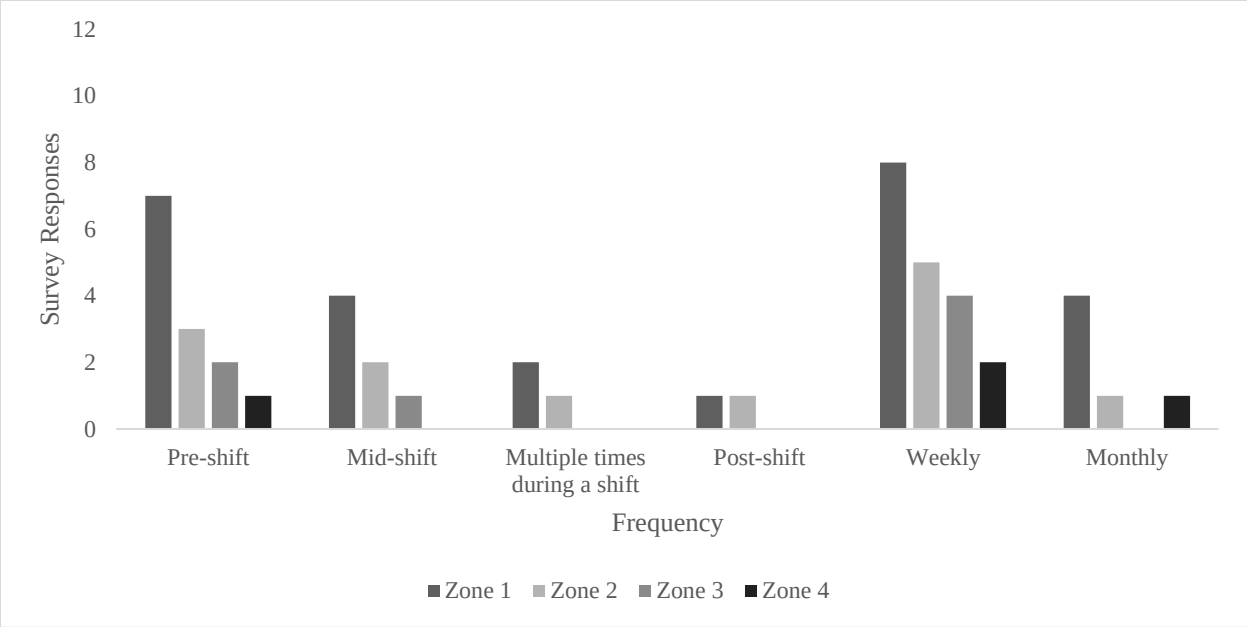

Figure 3.9. Coliform sample collection frequency based upon hygienic zones in surveyed frozen food facilities (n=17; respondents could choose more than 1 item)

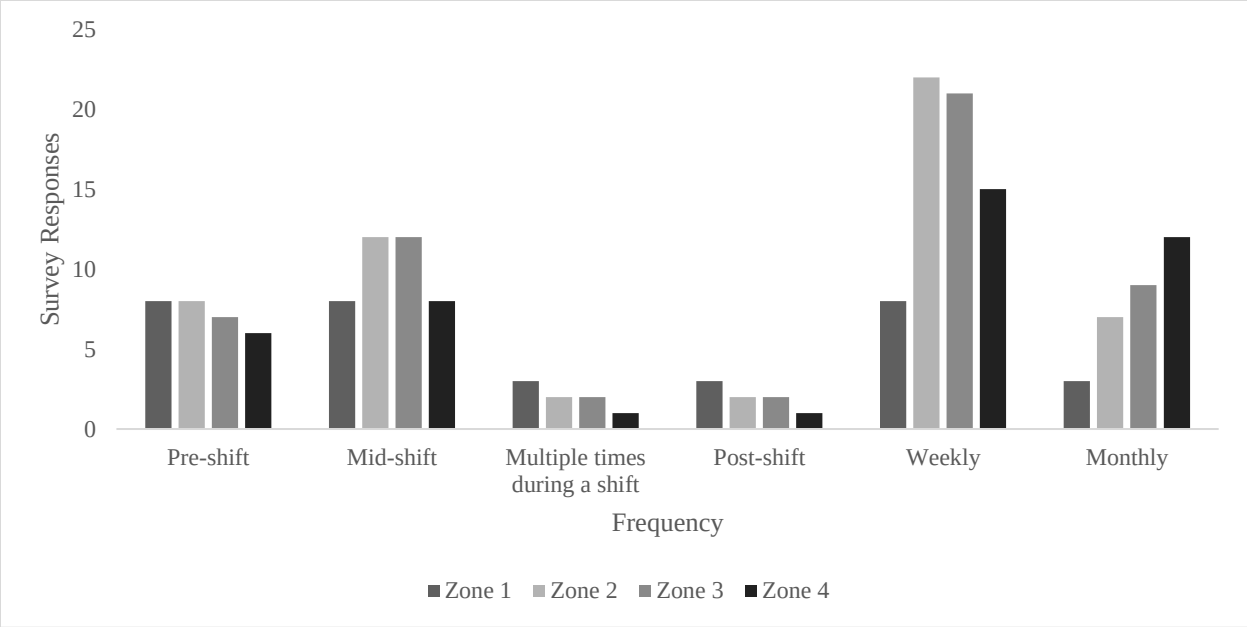

Figure 3.10. Frequency of *Listeria* spp. sampling by hygienic zones in surveyed frozen food facilities (n=33; respondents could choose more than 1 item)

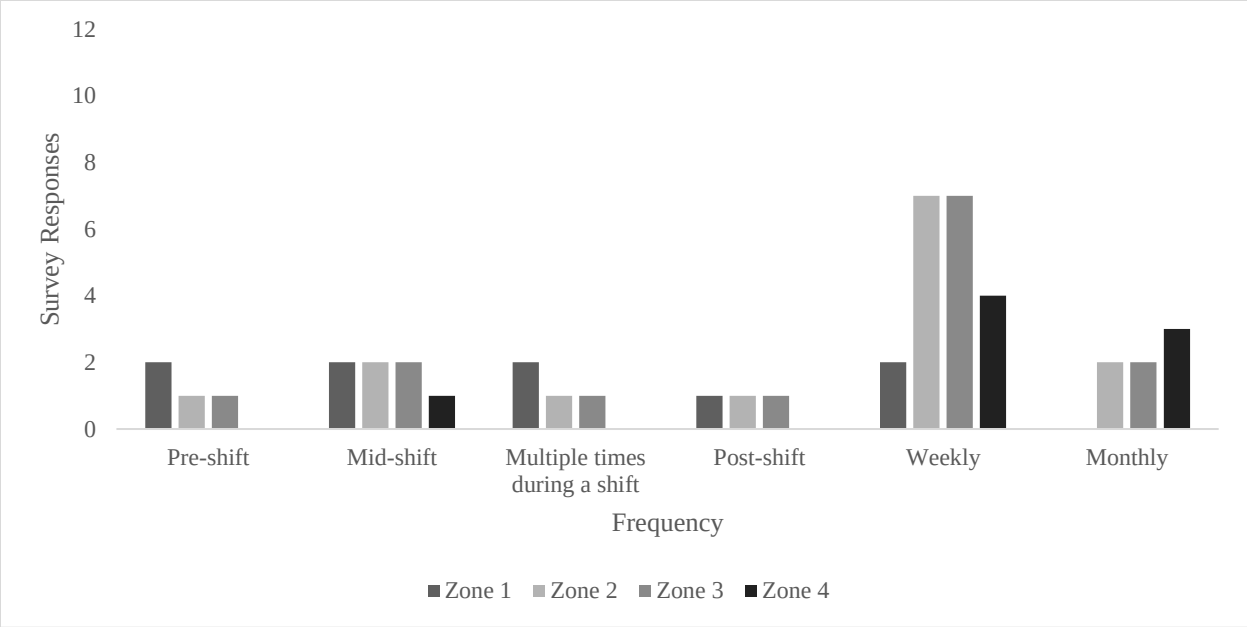

Figure 3.11. Frequency of *Listeria monocytogenes* sample collection in surveyed frozen food facilities categorized by hygienic zones (n=9; respondents could choose more than 1 item)

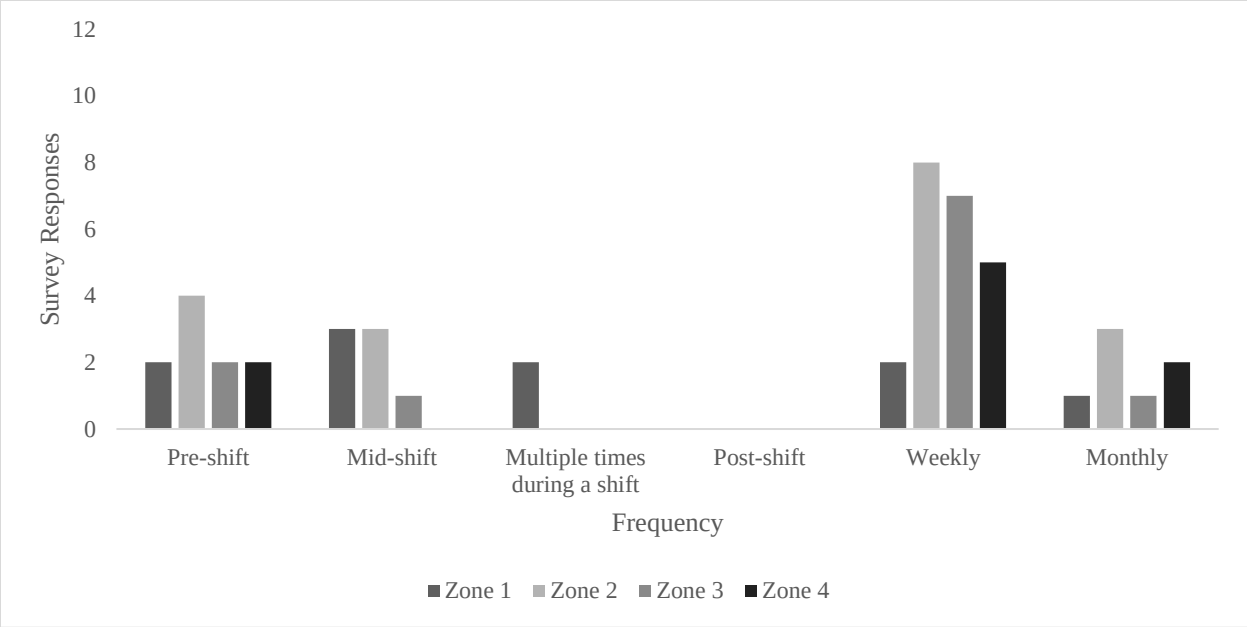

Figure 3.12. Sample collection frequency of "other microorganisms" in surveyed frozen food facilities by hygienic zones (n=11; respondents could choose more than 1 item)

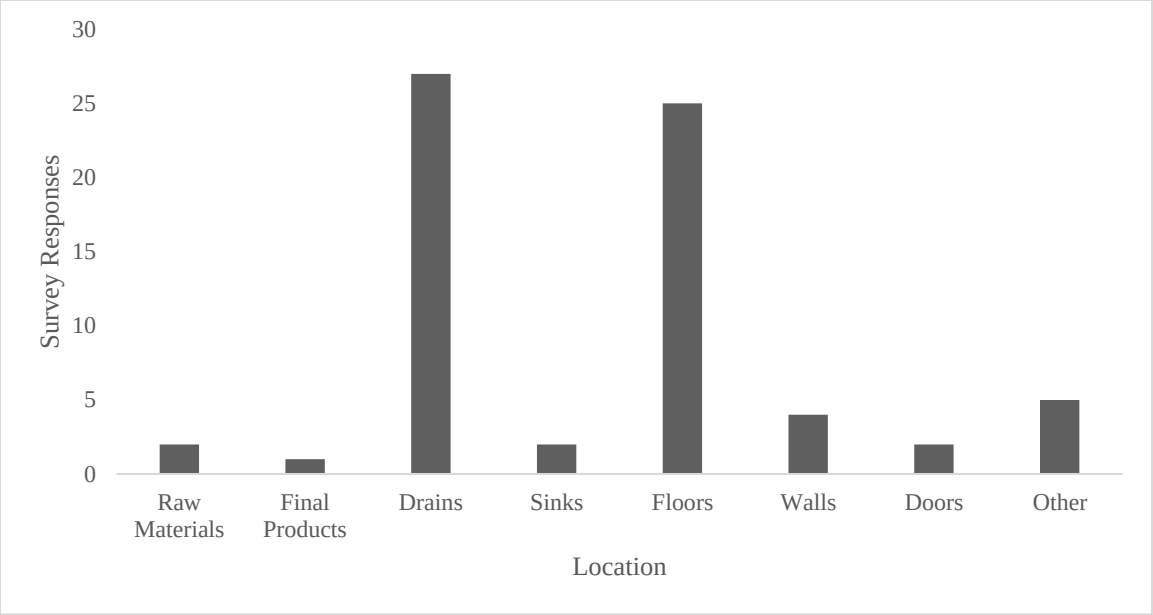

Figure 3.13. Areas of concern within the surveyed frozen food facilities for *Listeria* spp. presence (n=34; respondents could choose more than 1 item; only sites with at least 1 response are shown)

Figure 3.14. Areas of concern within the surveyed frozen food facilities for *Listeria monocytogenes* presence (n=24; respondents could choose more than 1 item; only sites with at least 1 response are shown)

CHAPTER 4

BLINDING PROTOCOLS FOR ACQUISITION OF POTENTIALLY SENSITIVE FOOD SAFETY INFORMATION

Magdovitz, B. F., Gummalla, S., Thippareddi, H., and Harrison, M. A. Blinding protocols for acquisition of potentially sensitive food safety information.

To be submitted to the *Journal of Food Protection.*

ABSTRACT

Difficulties in addressing research problems can revolve around the data collection process from private entities. Potential issues can arise when collecting food samples or food safety data from industry or third-party sources because of concerns about the distribution or exposure of potentially sensitive information. Industry is cautious of its involvement in research projects because effects on production levels, capital investment, regulatory inquiries, unwarranted publicity, or other legal issues can arise depending on the nature of the information gathered, and the possible inadvertent information release into the public domain. Well-designed clinical trials with animals or humans use blinding methods to reduce bias in the analysis. This project applied a similar strategy to sensitive data acquisition in the effort to gather meaningful food safety related data while assuring the information provided was not at risk. To obtain materials and records directly from participating frozen food companies that would provide insight into current industry practices without potential downsides for participating companies, blinding methods for collecting electronic data and material samples were created. Analysis of food safety concerns using industry data and the distribution of findings can be of assistance industry-wide in conducting risk assessments and developing improved research-based food safety plans. The method described was designed to collect data using blinding protocols to reduce bias and prevent traceback of the information to the original source. The benefit of blinding protocols promotes industry participation and creates data collection with anonymity of the original source that can improve reliability of the research and the applicability of the conclusion to the industry. These blinding protocols are suitable for use in future food safety research projects involving data within and between different segments of the food industry and could be used to encourage collection of valuable industry samples and data.

HIGHLIGHTS

- A method was created to collect food safety data without traceback to the original source.
- The blinding protocols describe collection for material and electronic data samples.
- Blinding protocols encourage industry participation and maintain anonymity.

To facilitate unbiased collection of material samples such as food, microbiological samples, or food safety data such as prevalence and concentration of chemical, physical, or microbiological hazards from food companies and processing facilities, it is important to have trust in the protocols developed between the industry participants and the independent researchers conducting the study. Concerns over the collection of potentially sensitive data from individual food manufacturers, companies, and entities have precluded industry participation in key research initiatives aimed at generating baseline data representative of a broader community. In the case of food companies, there may be legal concerns related to regulatory compliance, unwarranted publicity, or other legal issues that could limit their participation in sample or data collection studies. Involvement in research projects is generally muted if participation could implicate the company in a problem that would not otherwise arise had they not contributed to the study. A key tenet for a company participating in research should be that it derives more tangible benefit from their involvement than potential risks and costs associated with sharing of their samples and data. This dynamic creates a challenge for researchers when recruiting participants for these studies and to collect valuable samples and data for further analyses. For example, to develop appropriate food safety plans aimed at mitigating the risks associated with the presence of *Listeria monocytogenes*, it is desirable to have robust data pertaining to the occurrence and distribution of this pathogen in foods and food processing environments. However, there is tension with providing this data, as companies in the food industry may face potential regulatory action, other legal action, or unwarranted publicity if this pathogen is found in certain products or areas within the processing environment. Yet companies often have enormous amounts of historical and ongoing data that could be useful in evaluating pathogen prevalence and risk assessments and aid in development of food safety strategies. By blinding

the source of the data, and thus eliminating potential regulatory or unwarranted publicity implications, companies are more likely and willing to participate in industry-wide efforts to develop important scientific knowledge aimed at improving the safety of foods.

Blinding protocols for research in clinical studies involving humans or other animals can be used to reduce bias in analysis *(1)*. Blinding in a clinical study is defined as the concealment of a group or medicine to one or more individuals involved or conducting the study *(7)*. Clinical trials use randomization and blinding to minimize bias. These methods protect the identity of participants and the integrity of the study. A study in the Journal of the American Medical Association demonstrated the variability in the definitions of blinding terminology used by physicians and in textbook descriptions *(5)*. There remains variability in the definition of blinding which can lead to confusion and misunderstanding of the term used in clinical studies *(11)*. A double blind method in a clinical trial blinds both the researcher and participant to the information involved in the trial *(3)*. Studies have shown that improper concealment throughout the study can lead to biases in the analyses *(10)*.

Food manufacturers face a unique challenge with involvement in research projects because outcomes can affect their production activity and revenue. In the United States, current regulatory policy for foodborne pathogens like *Listeria monocytogenes* considers a zero-tolerance approach in ready-to-eat (RTE) foods, i.e., allows for no detectable presence of the pathogen in a prescribed amount of food or on food contact surfaces through the use of approved analytical methods *(12-15)*. Thus, industry participants are hesitant to participate in projects that involve foodborne pathogens, especially those subject to the zero-tolerance approach by regulatory agencies. Yet, these same companies may be very interested in developing best practices to limit and reduce problems related to this pathogen in the food supply, as there is a

huge opportunity to learn from the ample data companies can provide. Industry reluctance to participate in a project that actively seeks to understand the prevalence and levels of *Listeria* motivated the design of these blinding protocols. The objective was to design a method that encourages participation from industry in food safety-related projects. As part of this project, blinding methods were developed to collect electronic and material samples anonymously to build a strong aggregate data set from multiple facilities.

MATERIALS AND METHODS

Methods were developed to provide a safe harbor data collection, which established a guided depository where individuals can submit information without identifying participants. This method for anonymous data collection was created to obtain a substantial data set from multiple companies for a study involving the presence of *L. monocytogenes* on raw food products and on nonfood contact surfaces in frozen food processing facilities. This aggregate data set was analyzed to help improve protocols addressing *Listeria* occurrence currently employed in food processing environments.

Potential food safety professional participants were identified via subscription to a frozen food industry listserv. A packet of information was distributed to potential participants that included the goals and intentions of the research project. Members from various disciplines involved in research (including industry, researchers, and legal counsel) helped to create the packet to provide clear and concise instructions. The packet included a flowchart describing the blinding protocols and process to prevent traceback of the data submitted, as well as a description of how anonymity of participants would be safeguarded. The packet outlined the company's role in the study, all of which allowed for a full understanding of the blinding steps involved in the data and sample collection process before agreeing to partake in the research.

Electronic data collection. The method provided multi-level blinding steps for collection of electronic data from anonymous participating companies (Figure 4.1). The data were submitted for evaluation of food safety protocols for improvement of industry practices. To recruit participants, an initial email with a general description of the research project and the requirements of a participating company's involvement was sent out to a listserv of food safety professionals through legal counsel. The companies were provided a packet of information describing the researchers' goals and the blinding steps that would be taken generally throughout the project and specifically with each data set. The participants who elected to partake in the research and provide data responded directly and only to legal counsel. First, legal counsel compiled a list of interested participating companies and assigned each participant with a unique identifier to eliminate names and other identifying information. Assignment of the unique identifier was the first blinding step to detach the company's identifying information. All documentation and lists of unique identifier codes were kept on paper by legal counsel and destroyed at the end of the data collection period to eliminate any form of traceback to the original contributor of the data.

Second, an Excel "Data Collection Form" was sent to the participants. The Data Collection Form was designed by researchers, legal counsel, and industry representatives to ask the participants detailed information about the locations where the environmental monitoring samples were collected and the methods to collect and analyze samples. The Data Collection Form was distributed to each potential participant individually from legal counsel to avoid identifying which companies were participating. Once complete, the form was sent back to legal counsel via a thumb drive. No data was transmitted electronically to avoid associating data with e-mail addresses. The thumb drives were not identified via name or company, but rather by

unique company identifiers. As noted, the paper list of unique identifiers was destroyed by legal counsel, so there was no way to trace which data came from which companies. Legal counsel erased all metadata associated with the forms (Excel files) to remove any data, such as names, characters, logos, or descriptions that could identify the participants. Importantly, there was no connection between the research participants and the university research team as the flow of all information and dialogue from industry participants was directed to and funneled through legal counsel.

After the legal counsel removed all identifying attributes via metadata, the Data Collection Form was emailed from legal counsel to the project investigator identified as Researcher 1 with the unique company identifier as the first code. Once Researcher 1 received the Data Collection Form, Researcher 1 changed the unique identifier to a random six-digit code (obtained using a random number generator). The coding changes were recorded on paper and then the paper copy was destroyed after the data collection was completed. After the random six-digit code was assigned, Researcher 1 sent the Data Collection Form to another project investigator, Researcher 2, via e-mail. Researcher 2 then performed the analysis on the data. Third codes were then applied to the individual data sets before any presentation or publication of the data. The third code was a random five-digit number using a new list of randomly generated numbers. As with the first two codes, the coding was kept on paper and destroyed before publication of the data.

This coding system and triple-blinding protocol provided robust assurance of anonymity to the participating companies, data, and the researchers. The multiple coding system provides substantial difficulty for someone to trace the data back to the original source. At this point, as all paper references to coding changes have been destroyed, it would be almost impossible to

trace the information back to participants. Our study used a legal counsel team as the third party to avoid communication between the participating companies and the research team to maintain anonymity. For future studies, the legal counsel could be replaced with a separate third-party source that is not involved in the project if desired.

Material data collection. As with the electronic data, a method was established to allow for the collection of physical samples by a participating company that were shipped anonymously to a researcher for analysis (Figure 4.2). This method focused on collecting material samples from perishable food products; however, the method can be modified for any ingredient or product samples that would be shipped to a research team where blinding protocols are desired. Three separate samples representing a lot were collected by employees of the participating companies and placed in a non-labeled bag. The collected samples were labeled with an initial code to be able to group the samples together. The generic codes labeled on the bags were "A, B, C" or "1, 2, 3". These steps were repeated for additional lots of the same product or other types of product. Once ready to ship, all bags were placed in an unlabeled, insulated polystyrene foam container inside an unlabeled, outer shipping carton and sealed. These labeling schemes reduced any form of identification based upon more detailed coding or writing on the samples. The boxed products were then shipped to the researchers.

Before the samples were collected and shipped, a predetermined shipping and return address was established for every package sent and received. The shipping address was the location of the lab conducting the sample analysis. No tracking information was provided to the recipient. The predetermined return address was a third-party trade organization that was involved with the design of the project, to avoid inclusion of the sample provider's address. This allowed participants of the study to avoid inclusion of identifying information on the packages.

After receipt of the samples by the lab, all evidence of the original label and shipping barcodes were eliminated. After Researcher 1 removed all codes from the package and the product, samples were aseptically removed and placed into a new container and simultaneously assigned a new second code. The coding, established before the study was conducted, varied based upon the products collected. The second code maintained the order of each commodity's arrival to the lab by designating each new sample collection as a new lot. For example, V1L1S1 would refer to V1 as Vegetable 1 (each commodity was designated their own letter), L1 would signify lot 1, and S1 was a sample within the lot. Researcher 2 received the samples with the second blinding code. Researcher 2 then analyzed the sample, compiled the data, and applied a third blinding code before any presentation and publication of the data. The third blinding code was established with a five digit random number generator per each commodity, keeping the letter of the commodity at the beginning of the code (i.e., V57324, where V represents the letter designated to the commodity in code 2). Separating the samples by commodity allows the presentation of data across all products and per each commodity. Similar to the electronic data, this method ensured the resulting data was triple blinded by the time the researcher published the information. In the same way as the electronic data, all the coding and documentation was kept handwritten on paper and destroyed at the end of the data collection process to eliminate traceback to the contributor of the data.

RESULTS AND DISCUSSION

This blinding protocol was developed to facilitate a research project involving the collection of aggregate environmental monitoring data from numerous frozen food processors to allow industry-wide participation and analysis of environmental monitoring programs practiced in the industry. By evaluating current industry practices, the study identified a need to institute

an industry-wide standard for competency as it relates to assessing *Listeria* prevalence across different food commodities and food manufacturing facilities. An anonymous electronic survey conducted specifically within this segment of the food industry demonstrated interest in improving the current status of environmental monitoring *(8)*. However, when approached about providing individual environmental monitoring data, the initial participation was very low. Companies were hesitant to contribute in situations where data could be traced backed to their operations, and potentially implicated in regulatory enforcement or other legal actions. This hesitancy to participate due to potential traceability provided the need to design protocols to ensure blinding.

The systematic blinding protocols developed for this environmental monitoring data collection promoted higher levels of participation in the research compared to the initial response before blinding protocols were developed. A larger data set of current practices was obtained for analysis, which in turn will help to develop guidance applicable to the industry to alleviate food safety-related issues in manufacturing. One major concern with providing environmental monitoring information for a zero-tolerance pathogen was the possibility an operation might delete data representing positive pathogen findings. However, the anonymous collection allowed the industry to provide truthful information without having to cherry-pick or self-select data to redact problems occurring within facilities. This further reduced bias in the dataset as the industry participants did not have incentive to remove data that could be misrepresented to the public or confound the broader analysis. The blinding protocol helped to prevent any extrapolation or misrepresentation of information attributed to a company, as it provided confidence that there can be no traceback to the original source.

Developing successful blinding methods used in research requires detailed descriptions of the process and detailed instructions for participants to follow. For example, limitation of sizing or other characteristics in methodology reports may inadvertently eliminate details that can cause a participant or reader to conclude the methods were deficient. Thus, a uniform method of blinding to ensure methodologies are consistent across all research projects is necessary *(4, 6, 9)*. Blinding methods, when conducted properly, provide anonymity to the participants, encourage participants to contribute to the study, and reduce the concern of legal or regulatory implications. Further, this also reduces any researcher obligation to report potential harmful results found in the analysis, as there is no way to know the origin of the data. Blinding methods eliminate traceability making it difficult to obtain additional knowledge of the original source after the material or information is sent. Thus, it is critical to address the concerns of interest during the planning stages, so the proper questions are asked of the participants.

The methods described in this paper are focused on electronic data collected from routine and investigative environmental monitoring activities conducted to address a company's food safety protocols and the collection of physical samples of perishable food. However, the methods can be modified for collection of any form of electronic or physical data intended to be blinded for research purposes. Additionally, when collecting data, it is critical to make certain that multiple participants are in each category and subcategory of the study. If only one participant of a specific product type provides samples, traceback to the original source is simple and blinding techniques are irrelevant. This project required at least three participants in a category or subcategory.

Before studies are conducted, all participants and researchers should understand the blinding process and its impact on the research. The mutual understanding and trust between the

participating companies and the researchers will be the foundation for protecting the anonymity of the participants and avoiding legal exposure due to traceback. The information packet provided to the participants at the outset of the study explained the role of each entity in the project and the proper protocols to be followed to ensure the blinding methods were properly implemented. In applying blinding methodologies to study development, parties must carefully consider the different strategies of applying multi-level coding to avoid duplication or other situations that could inadvertently compromise the blinding *(2)*.

To ensure that studies appropriately incorporate consistent methodologies, there are some variations that should be considered before implementing blinding methods. First, the procedures should clearly specify all roles of those responsible for and involved in the blinding methods. This includes outlining which personnel will be responsible for each role (i.e., define personnel as Researcher 1 and Researcher 2). No one person should perform more than one role to adequately apply anonymous coding and eliminate the potential for traceback. Additionally, the procedures should outline substitute personnel for these positions if someone is unavailable. If this is not clearly specified before the study begins, there is the potential for overlap in roles which can compromise the anonymity of the blinding system. Second, all personnel involved in the study should be trained to prevent improper coding, labeling, and handling of the samples. One way to train personnel includes conducting a mock trial before samples are collected to educate all contributors of their specific role. If changes are necessary, these changes should be made and then revised information packets should be distributed to all members involved in the research study. For this study, several mock trials with the blinding methods were conducted for both the electronic and material data collection with specified functions established to ensure a

smooth functioning system when the material samples and data were collected. Carefully designed flowcharts distributed to the participants were a useful aid to outline procedures.

Data collection may be derived from historical data which is information companies have previously compiled from routine facility activities. When collecting historical data from a participant, variations in the descriptors used by individuals at the processing facilities who inputted the data can present challenges. In the current study, specific challenges arose due to the diversity in the data collection and the layout of the collection format. For the requested dataset, researchers established categories with a finite number of responses so participants could arrange the data in a simplified manner (i.e., the Data Collection Form provided categories such as pre-, post- or during-lethality to choose from in a column for sampling location within the facility). This organized the responses into specific categories, which further provided easier analysis of the results. Alternatively, responses to categories that were open ended, e.g., equipment descriptions of specific environmental monitoring sample sites within the facilities, varied considerably. In conducting studies, care should be taken to ensure the categories are clarified before the collection form is completed to limit response variability in the participants' answers. As shared in the methods section, a packet was provided with instructions outlined for the Data Collection Form to drive towards more consistency in the responses from participants. Additionally, participants should be encouraged to complete the data requests in their entirety. Incomplete responses can be challenging as the blinding protocol innately restricts the researcher's ability to request the participating company for any clarifications or additional information.

Before use of blinding protocols in a research project, a diverse list of possible risks associated with possible blinding failures should be established and clearly communicated to

participants *(2)*. The research team should consider all potential unplanned blinding errors. This list should then be used to develop more complete protocols to reduce complications in the blinding of the study. The proactive approach in anticipating errors in a blinding protocol helps to prevent errors in the methods from occurring throughout the research collection process *(2)*.

Overall, this method allows for anonymous data collection from participants while eliminating traceback to the original source. While there is still trust between academic investigators and industry participants in studies involving access to facilities to gather study samples, blinding the source of sampling information should increase transparency of key information surrounding industry practices in an approach that exposes companies to limited risk of regulatory consequences or unwarranted publicity. Ultimately, this may increase participation across the industry leading to a larger group of data contributors, greater number of data sets, more robust data, and a higher confidence in the analysis as a representative set of the industry.

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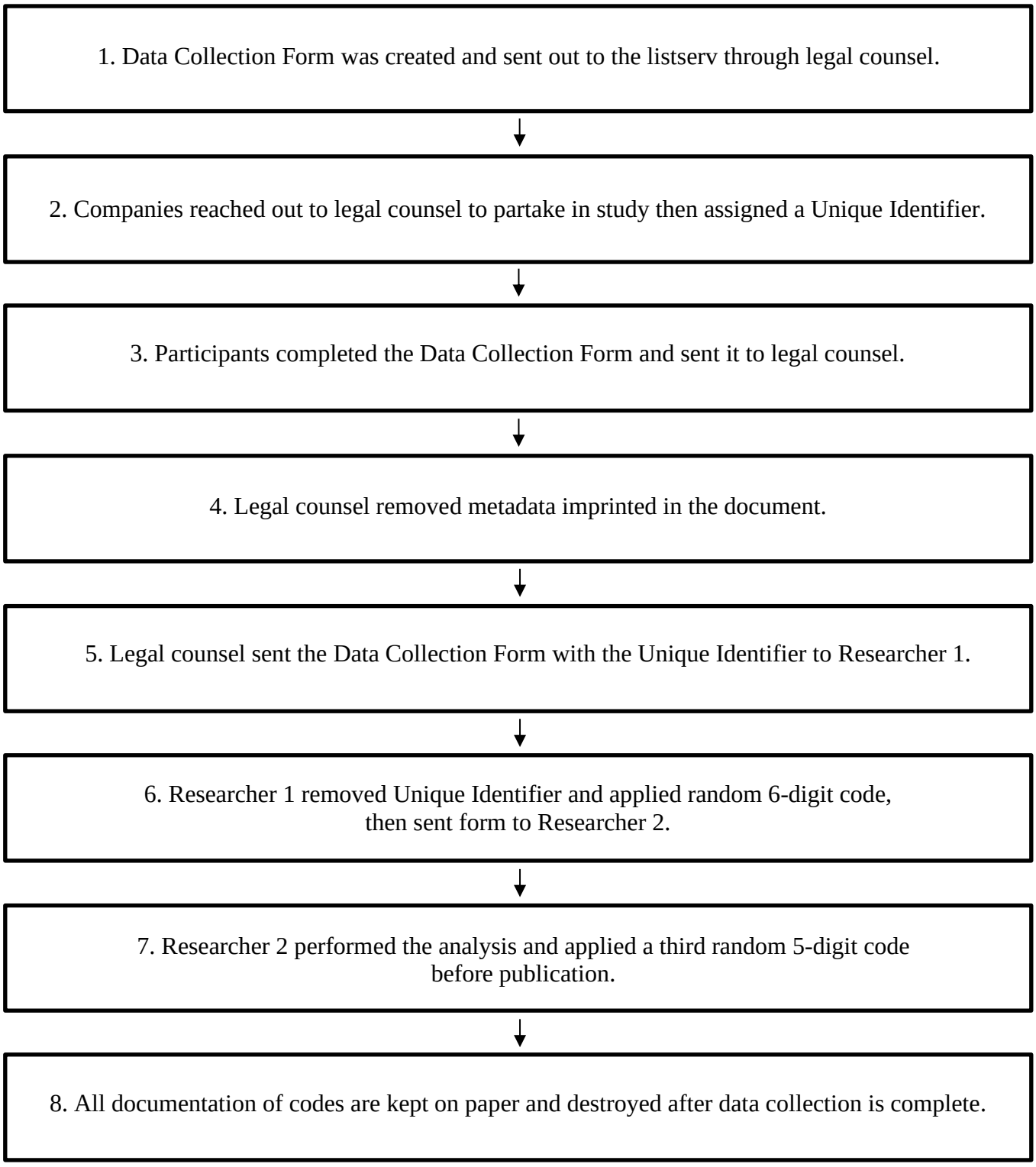

Figure 4.1: Flowchart of blinding protocols for electronic data collection

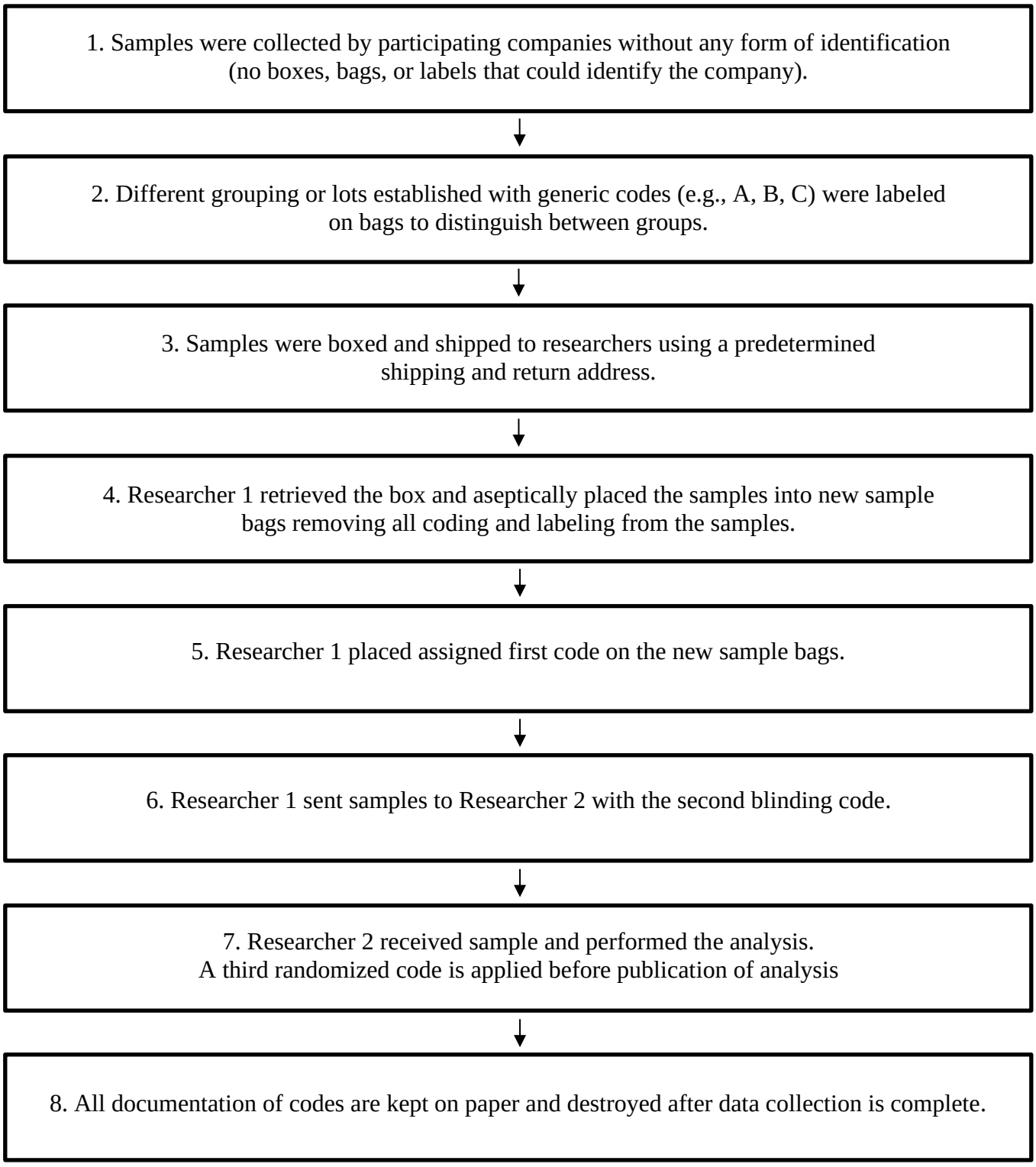

Figure 4.2: Flowchart of blinding protocols for material sample data collection

CHAPTER 5

ANALYZING AGGREGATE ENVIRONMENTAL MONITORING DATA FOR *LISTERIA* SPP. IN FROZEN FOOD MANUFACTURING ENVIRONMENTS

Magdovitz, B. F., Gummalla, S., Thippareddi, H., Garren, D. M., Berrang, M. E., and Harrison, M. A. Analyzing aggregate environmental monitoring data for *Listeria* spp. in frozen food manufacturing environments.

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Abstract

Food processors face serious challenges due to the ubiquity and prevalence of *Listeria monocytogenes* in production facilities. Environmental monitoring for *Listeria* within the industry is important and detection of *Listeria* spp. is often used as an indicator for the potential presence of *L. monocytogenes* in the food processing environment. Historical environmental monitoring data from the frozen food manufacturing industry was compiled and analyzed to evaluate the adequacy of current practices in mitigating risks of *L. monocytogenes* in the processing environment and to determine if there are trends that could be used to further refine industry practices. A method to collect anonymous data for analysis to build a strong aggregate data set from multiple facilities was used. Information included general descriptions of each facility and specific information about individual environmental monitoring test results from zones 2-4, nonfood contact surfaces. The general information collected from facilities included the size of the facilities and how environmental monitoring samples were collected and analyzed. For each individual sample, information collected included the area or equipment sampled and the result of the sample. Descriptors were provided to allow for grouping of similar results. Historical data collected spanned six months to a year of environmental monitoring samples. Twenty-seven facilities provided 42,799 environmental monitoring observations. Zones 3 and 4 had a higher probability of *Listeria* positive results compared to zone two for routine environmental monitoring samples ($p<0.05$). Pre-lethality and post-lethality production areas had a higher probability of *Listeria* positive results compared to lethality areas for all environmental samples ($p<0.05$). Cold storage locations, i.e., coolers and freezers had a significantly higher probability for a *Listeria* positive result than noncold storage areas ($p<0.01$). Prevalence data from processing operations can provide the industry guidance on focusing sanitation within

processing operations to reduce risk related to *L. monocytogenes.* The data helps to determine areas on which to focus when testing for prevalence of *Listeria* spp. within the food processing environment.

Highlights

- Twenty-seven facilities provided 42,799 environmental monitoring observations.
- Zones 3 and 4 had a higher probability of a *Listeria* positive compared to zone two for routine environmental monitoring samples ($p<0.05$).
- Cold storage locations had a higher probability of a *Listeria* positive than noncold storage areas ($p<0.01$).

1.0 Introduction

The presence of *Listeria* within food manufacturing facilities continues to challenge food processors to determine the best way to remove the pathogen from the processing environment. *Listeria monocytogenes* is a ubiquitous bacterium that can be found throughout food processing environments (Bell & Kyriakides, 2005; Jordan, Dara Leong, & Ordóñez, 2015; Tompkin, 2002). *L. monocytogenes* is a pathogen of concern for public health especially with ready-to-eat products (Goldfine & Shen, 2007; Gombas, Chen, Clavero, & Scott, 2003; Kovačević, McIntyre, Henderson, & Kosatsky, 2012; Leong, Alvarez-Ordóñez, & Jordan, 2014; Warriner & Namvar, 2009). Reduction of *Listeria* prevalence in food processing facilities is one focus of an effective food safety plan (Muhterem-Uyar, et al., 2015). Proper education of food processing workers is vital to ensure the understanding of the dangers of *L. monocytogenes* and their role in preventing cross-contamination by this pathogen (Jordan, et al., 2015).

Documentation of persistence of *L. monocytogenes* in food-processing operations has occurred, but there continues to be a focus on determining the entry of *Listeria* within a facility (Jordan, et al., 2015). Possible contamination from outside sources, such as raw materials and employees, can be a source for the introduction of *Listeria* into a facility (Carpentier & Cerf, 2011; Strydom, Vorster, Gouws, & Witthuhn, 2016). Proper food safety plans should include developed protocols to help eliminate pathogen contamination from outside sources, which include employees changing to personal protective equipment and using sanitizing foot baths (Todd, et al., 2010). Once *Listeria* has entered a facility, harborage sites may remain in a niche location, which can be hard to detect without extensive sampling (Tompkin, 2002). A pathogen free environment in a processing facility is almost impossible to maintain, but proper preventive control measures can reduce pathogen contamination. Control measures should be implemented

throughout all stages of the farm-to-fork process for *L. monocytogenes*, including in the production facility (Luber, et al., 2011). The approach used in food processing facilities to reduce the likelihood of *Listeria* in the environment includes employee education and training, sound sanitation protocols, microbiological testing of raw and finished products, and a proper environmental monitoring plan (Jordan, et al., 2015; Ryser & Marth, 2007; Tompkin, 2002).

Environmental testing in facilities helps to monitor the processing environment to reduce pathogen contamination to food products. This preventive control method requires frequent sampling of food contact and nonfood contact surfaces to track the occurrence and frequency of pathogens within the facility. Well-designed environmental monitoring plans are based upon scientific literature and continue to evolve as data from sampling is evaluated (Zoellner, Ceres, Ghezzi-Kopel, Wiedmann, & Ivanek, 2018).

An electronic survey was conducted as an overview of frozen food manufacturing environmental monitoring practices (Magdovitz, Gummalla, Thippareddi, & Harrison, 2019). Results indicated a focus on environmental monitoring for all who participated, but there was variability within practices that were performed throughout the industry. Variability within food manufacturing environmental monitoring practices provides evidence of the need for a more consistent industry focus on determining best methods to reduce *Listeria* contamination throughout the processing environment (Magdovitz, et al., 2019). The objective of this study was to determine the awareness and practices currently used within the frozen food industry in environmental monitoring sampling plans targeting *Listeria.* Collection of anonymous, historical environmental monitoring data points from multiple processors and facilities provided an industry-generated dataset that was analyzed to determine common practices and trends and to determine areas of concern within a facility.

2.0 Materials and Methods

2.1 Blinding Protocols for Data Collection

Environmental monitoring samples were collected with a triple-blinding protocol to promote participation from participants and maintain anonymity of the data (Chapter 4). Double blinded steps were used to gather data through a legal counsel team to ensure the removal of source identification. A third code was applied before publication to further insure blinded data.

2.2 Design of Data Collection Form

Industry and academic professionals with extensive knowledge of environmental monitoring designed a Data Collection Form to collect historical environmental monitoring data from frozen food processing facilities (Figure 5.1). The form established categories for locations within a facility to allow easy descriptors for companies to use to help categorize locations of sample sites (Table 5.1). Facilities were provided a definition for each category to define when and where the samples were taken. Hygienic zones were defined with the FDA draft guidance for *Listeria* (U.S. Food and Drug Administration, 2008, 2017). Zone 1 is food contact surfaces while zones 2, 3, and 4 are nonfood contact surfaces progressively farther away from food contact surfaces. The current data collection focused on nonfood contact surfaces and only collected environmental monitoring data from zones 2 to 4. Samples were also characterized as routine sampling or corrective actions. Routine samples were collected during regular environmental monitoring testing while corrective actions samples were performed after a positive routine sample was found and a corrective action was implemented, i.e., cleaning and sanitizing the area that yielded the positive sample.

Additionally, generic information about the responding food company was requested to establish the approximate size of the facility, type of commodity processed, processing protocols

implemented within the facility, and their environmental sampling and testing protocols. For the sampling protocol, companies were asked about their laboratory analysis methodology, and whether the lab analysis was done in-house or not. Only facilities using approved AOAC laboratory methods for *Listeria* were used in this study to establish a baseline for consistency across multiple facilities.

2.3 Statistical analysis

Statistical analyses were performed using SAS software (Cary, NC). Preliminary analyses using simple univariate and bivariate analysis were performed in order to finalize the data set of interest. This aided in the removal of missing or incomplete data from the dataset. For each individual environmental monitoring sample, data was analyzed with a generalized linear mixed effects model. Outcomes were normally distributed based upon a binomial distribution, whether or not the test was positive for *Listeria* spp. The random effect accounted for repeated measures within the same facility which represented the differences in the facilities. Mixed effects in the model accounted for the categorical fixed effect and the random effect for variability within facilities. In SAS, the method used was a Laplace optimization with a sandwich estimator for the variance.

Additionally, characteristics of each facility that participated in the data collection provided generic information about their facility. Facility characteristic data was analyzed with several types of models to consider the relationships of each variable to proportions of the tests that were positive for *Listeria.* The model chosen for facility-level variables was a mixed-effect analysis for each independent variable. This is similar to the environmental sampling collection model but considers one-facility level at a time. This allows the model to properly account for repeated measures within facilities and used the full set of outcomes for each individual test and

naturally weighted facilities properly according to the number of observations within the data set. Statistical significance was based upon a p-value less than 0.05 for all data analyses.

3.0 Results

The results varied across all 27 processing facilities, but consistencies were found in *Listeria* positive environmental monitoring sampling sites. A total of 42,799 environmental monitoring results for *Listeria spp.* were analyzed. Positive environmental monitoring results ranged from 0.2% to 12.6% per facility for a period of six months to a year of routine environmental sampling. Facilities were evenly distributed across three categories (less than 2.5%, between 2.5–5%, and greater than 5%) for overall percentage of *Listeria* positive results for routine sampling, with nine facilities in each category.

After a *Listeria* positive routine sample is detected, facilities perform a corrective action procedure, i.e., cleaning and sanitizing the location of the positive result followed by additional testing to see if the problem is corrected. Corrective action sampling, although done less frequently than routine sampling, provided significantly higher percent positive *Listeria* results. This was most likely due to detecting *Listeria* in subsequent samplings until the contamination problem was rectified. When corrective action sampling was included in the analysis, the overall average percentage increased from 3.3% in routine sampling to 4.55% for all sampling. To account for the significant increase in percent positive results when corrective actions sampling was added, all categories were analyzed using two different data sets, by routine only sampling and a combination of routine and corrective action sampling.

When comparing routine sampling results from different zones within facilities, the percentage of *Listeria* positive sampling sites in zone 2 (0.4%) was significantly lower ($\chi^2(2)=8.60$ and $p=0.014$) compared to those from zones 3 (1.1%) and 4 (1.3%). A similar trend

was found when corrective action sampling results were included, with zone 2 (0.7%) having fewer *Listeria* positive results compared to zones 3 (1.5%) and 4 (1.4%).

There was no significant difference ($p>0.5$) for *Listeria* positive results within categories associated with the time of sample collection. Production time determined at what point during processing the samples were collected i.e., pre-, during, or post-production. Production shift focused on whether samples were collected during the first, second, or third shift. These two categories were removed from further analyses due to their lack of statistical significance.

Production area was a category included to determine where in the facility the samples were collected. The classifications for this category were pre-lethality, lethality, post-lethality, and other. Lethality treatments were defined as blanching for vegetable products or a heating step for products that were cooked during processing. Category "other" was used for locations outside of the first three categories listed and for facilities in which the commodity processed did not have a lethality step. The frequency of detecting *Listeria* in different production areas was significant ($p<0.0001$ and $\chi^2(3)=21.16$). Areas for the lethality step (typically blanching or cooking) and those categorized as other have a significantly lower probability of yielding a positive test than pre-lethality and post-lethality areas, at the 0.05 level of significance. The percentage of a positive *Listeria* spp. result for routine sampling within each category were pre-lethality at 1.6%, lethality at 0.7%, post-lethality 1.1%, and other at 0.4%. When corrective action sampling was included, the only two categories to increase were pre-lethality from 1.6% to 2.8% for *Listeria* positive samples and post-lethality from 1.1% to 1.9%.

Using descriptors provided on the data collection form, participants identified the exact location of environmental monitoring sample collected. Locations described by facilities were categorized into 40 regions throughout the production facility. The highest frequency of samples

were collected from floors, equipment, frames, drains, and conveyor belts. These five categories accounted for over 50% of the total samples collected. However, for routine sampling, the top five locations for probability of a *Listeria* positive results were drains (4.0%), pumps (3.9%), troughs (3.6%), chutes (2.5%), and containers (2.3%) (Table 5.2). The probability for a *Listeria* positive sample when corrective actions and routine samples were both included in the data, the top five categories change to squeegee (4.4%), trough (4.2%), drain (3.3%), tools (2.9%), and freezer (2.6%) (Table 5.3).

Samples were also categorized into cold storage locations, defined as sampling sites located within or near the freezer, coolers, chillers, or freezing tunnels. Of the total samples, about 10% were classified into the cold-storage location and there was a significant difference when samples were from routine collection and corrective action ($p=0.0047$ and $\chi^2(1)=8.01$). During routine sampling, the probability of detecting *Listeria* spp. in cold storage areas compared to non-cold storage areas was 1.2% and 0.6%, respectively (1.4% and 0.9% when routine and corrective action samples were combined).

Collection method for each sample was based upon the device used to collect the sample, i.e., sponge or swab. There was no significant difference between collection methods in the recovery of *Listeria* from sampling sites ($p=0.13$ and $\chi^2(1)=2.24$). However, sponges were used more frequently than swabs, 66% compared to 34%, respectively. The probability of collecting *Listeria* positive samples using sponges was 1.5% while for swabs it was 0.5% for routine samples. When corrective action samples were included, the probability of a positive increased 1.8% for sponges and 0.7% for swabs, which is reasonable since areas receiving corrective action are likely to yield subsequent *Listeria* positive results until the contamination source is eliminated.

In addition to the locations for collection of environmental monitoring samples, participants were asked to determine if processing protocols, types of commodities, or method of analysis influences environmental monitoring results. For the dataset, 58% of samples were classified as vegetable producers, 19% of facilities produced entrees of various types, and 23% produced appetizers. There was no significant difference in the collection frequency of *Listeria* positive samples between commodities ($\chi 2(2)=1.53$, $p=0.46$). Facilities were separated by size based on square footage of the facility. Categories were defined by academic and industry professionals: small <12,000 sq. ft. (<1,115 m^2), medium 12,000 – 50,000 sq. ft. (1,115 – 4,645 m^2), and large greater than 50,000 sq. ft. (>4645 m^2). There was a significant ($\chi 2(1)=8.86$, $p=0.003$) difference in the probability of finding a *Listeria* positive site during routine sampling between large facilities (4.4%) and small and medium facilities (1.1%). When corrective action samples were considered with the routine information, frequencies increased to 6.0% for large facilities and 1.6% for small and medium facilities.

The types of processing performed within facilities were categorized to include blanching, individual quick frozen (IQF), or repacking. There was no significant difference in the frequency of finding *Listeria* positive sites between facilities that perform repacking and blanching versus facilities that did not. Facilities that performed IQF processing had a significantly ($p<0.05$) higher probability of producing positive *Listeria* results (3.3%) compared to facilities that did not carry out IQF processing (1.1%). Including corrective action samples, the probability increased to 4.4% for IQF facilities and 1.8% for non-IQF facilities.

To ensure samples were performed with approved methods, facilities were asked about their environmental monitoring sampling methods and who was responsible for the analysis. Samples were analyzed by in-house or 3rd party laboratories. There was a significant

($\chi 2(1)=6.68$, $p=0.0098$) difference between the frequency of detecting *Listeria* spp. from routine sampling by in-house labs (4.6%) compared to 3rd party labs (1.5%). When corrective action samples were considered with routine data for positive samples, in-house labs reported 6.1% *Listeria* positives while 3rd party labs reported 2.1% positives The difference in prevalence due to type of test method used, which included analysis methods such as PCR assays, enzyme linked immunofluorescent assays (ELFA), culture medium, and visual immunoassays, was not statistically significant ($\chi 2(3)=3.56$, $p=0.3134$).

4.0 Discussion

Anonymous and secure collection of data through implementation of blinding protocols likely increased participation by facilities. Twenty-seven facilities provided environmental monitoring data to give an accurate and appropriate analysis of actual industry protocols. Over 40,000 environmental monitoring results provided authentic industry information to understand issues and recognize the needs for improvement to reduce *L. monocytogenes* in processing facilities. This data reveals there is an industry focus on current environmental monitoring programs to improve and develop extensive practices to reduce prevalence of *L. monocytogenes* in food processing environments. The data collected provides an overview of industry practices related to environmental monitoring. There is variability as to when and where environmental monitoring samples are collected within each facility. This indicates an opportunity to assist the frozen food industry in determining the best practices for environmental monitoring focused on reduction of *L. monocytogenes* contamination. Prevalence data from processing operations can provide the industry guidance on focused sanitation locations within the processing operation to reduce the risk of *L. monocytogenes.* These data help to determine focus areas within the environment for detecting prevalence of *Listeria* spp.

4.1 Locations of samples

Samples collected focused on nonfood contact surfaces, zones 2-4. Food contact sampling for zone 1 testing was not included in the collection to allow focus specifically on environmental sampling. To have a sound environmental monitoring program, facilities need to have confidence they are addressing problem areas. There are recommendations that *Listeria* control protocols for zones 2-4 should be addressed effectively before full-fledged sampling of zone 1 (United Fresh Produce Association, 2020). This study focused on zones 2-4 to see how the frozen food industry is addressing these areas. Additionally, while the anonymous collection of information provides a safe harbor for the information, it was felt that removal of zone 1 testing promoted participation by industry participants as some companies preferred not to share sensitive data associated with a zero-tolerance pathogen and some companies do not test for zone 1 in their environmental monitoring plan.

The data for sample collection for zone 4 was only 4% of the total results collected, compared to zones 2 and 3 which were 48% and 47%, respectively. Lower zone 4 sampling frequency might be related to the distance of zone 4 from food contact surfaces and reduced potential cross-contamination in comparison to the other zones. However, zone 4 had a significantly higher probability of positives than zone 2 for routine samples. This data shows that more focus may need to be placed on zone 4 as a potential source of contamination than the industry currently implements.

When considering production time and production shift, information was lacking for several facilities. The lack of information prevented a thorough analysis to determine a difference in these categories, which overall were not significant. The blinding protocols implemented allowed anonymous collection of data but limited researchers from inquiring as to

reasons for missing information. For the production area category, 67% of samples were taken post-lethality. Therefore, regardless of whether products included in this study are currently considered as not ready-to-eat or ready-to-eat, there is more interest in evaluating the post-lethality processing environment than pre-lethality. More focus is set on environmental monitoring programs for the environment surrounding ready-to-eat products as there is no additional kill step to reduce pathogen contamination before a consumer eats the product (Zoellner, et. al., 2018). Only one-third of samples were collected during pre-lethality and lethality steps, showing less focus on environmental monitoring in these areas of the processing environment.

4.2 Areas of concern

Documentation from industry and government agencies establishes a baseline for environmental monitoring focused on floors, walls, drains, and harborage locations (Reinhard, et al., 2018; U.S. Food and Drug Administration, 2017). Within the current data set, these areas are found to have the highest frequency of sampling across most facilities. Although, these areas have the highest frequency of sampling, data shows that these areas may not be the areas of highest concern for a positive *Listeria* result. The current dataset suggests some niche areas with high likelihood of finding positives, which are less frequently sampled within facilities. One example was the squeegee which had the lowest frequency of sampling but the highest probability of a positive for both routine and corrective actions sampling. Squeegees are used for cleaning of wet conditions, which is an environment of concern for harborage of *Listeria*. Additionally, the employee category which included personal protective equipment, i.e., aprons, boots, gloves, etc. ranked in the bottom half of frequency testing but in the top ten for probability of yielding a positive result. This dataset establishes that some areas of concern that are already a

high frequency of focus, i.e., drains and troughs need to continue to be a main focus as the probability of a positive finding remains high, but there are some new areas in which sampling frequency may need to increase to establish a well-designed environmental plan focused on the "seek and destroy" methods for finding positive results (Malley, Butts, & Wiedmann, 2015).

4.3 Facility characteristics

The majority of facilities that participated with this research were classified as large facilities (65%). Size was a factor that was significant; larger facilities had a higher likelihood of positive samples. Larger facilities may have a well-designed "seek and destroy" plan that focuses on areas of higher concern. Additionally, larger facilities were shown to take more samples at greater frequency. The information from large facilities can help small facilities design and develop their environmental monitoring plans.

Facilities that identified using IQF processing had a significantly higher probability of a positive than those facilities that do not perform IQF processing. The facilities who indicated they do not use IQF were mostly repack only facilities, which may indicate all their products are ready-to-eat and have a lower probability of positive result as no raw material is entering their facilities. However, the other two processing methods, blanching and repacking were not significantly different between the facilities that indicated using these processing methods versus those that do not.

4.4 Analysis of samples

Facilities were almost evenly split between third party testing and in-house testing. Most of the third party labs used PCR assays or ELFA methods for analysis, whereas in-house sampling used PCR assays, ELFA, culture medium, and visual immunoassays. All facilities used an approved AOAC method for environmental monitoring testing and there was no significant

difference between these methods. In-house testing may occur more often due to lower cost which could lead to higher positive results. Expected lower costs of conducting in-house analysis might result in a greater number of collected samples being analyzed. This could translate into collecting samples in areas which might otherwise be overlooked because of cost concerns. It is possible that facilities conduct numerous assays in-house and then send selected samples to 3rd party labs for confirmation. There could also be differences in possible variability of interpreting in-house test results. For example, food residues collected on swabs or sponges that are intensely pigmented (e.g., blueberries) could interfere with color interpretation with some rapid assays. When considering whether to use in-house or 3rd party labs to test samples, one should consider the cost per sample, labor cost, proximity of the lab to facility, capitol cost involved with running an in-house lab, and how the testing will be implemented. Once these factors are considered, a company can decide the best methods for their facility to implement.

4.5 Industry practices

Recalls in the frozen food industry for *Listeria* have led to interest in alleviating the problem within the food processing environment. New guidance put forth by the FDA encourages food manufacturing companies to develop a "seek and destroy" environmental monitoring program (U.S. Food and Drug Administration, 2017). This research analyzed established environmental monitoring plans from a variety of frozen food processing facilities to determine prevalence of *Listeria* within the processing environment. Information from established environmental monitoring plans can help to improve and develop environmental monitoring protocols for other processors. Although these data are only from frozen food facilities, they may provide guidance for *Listeria* prevention practices in other segments of the food industry.

Environmental monitoring plans should be focused on randomized locations throughout the facility to help a risk-based plan for future sampling. The results from the current study can be used for that purpose. The top five most tested spots: floors, equipment, frames, drains, and conveyor belts are commonly focused on within the industry as areas of concern. However, the highest frequency of testing does not match to the locations which had the highest probability of a positive result. These data help to focus on areas within facilities which may not be as frequently tested but can harbor pathogens and become a concern. By providing a means to collect aggregate data from the food industry through the blinding protocols used, industry participants were willing to share their findings. Acquiring and analyzing data collected under typical food processing conditions, can help to establish better protocols to use across all facets of the food industry.

5.0 Conclusion

Environmental monitoring for *Listeria* within the food industry is important and detection of *Listeria* spp. is often used as an indicator for the presence of *L. monocytogenes*. Historical environmental monitoring data from the frozen food manufacturing industry was compiled and analyzed to evaluate the adequacy of current practices for detecting *L. monocytogenes* in the processing environment and to determine if there are trends that could be used to further refine industry practices. The historical data collected with blinding protocols spanned six months to a year of industry collected environmental monitoring data. Twenty-seven facilities provided 42,799 environmental monitoring observations. Zone 3 and 4 had a higher probability of *Listeria* positive results compared to zone two when sampled as part of the routine environmental monitoring program ($p<0.05$). Pre-lethality and post-lethality production areas had a higher probability of *Listeria* positive results compared to lethality areas for all environmental samples

($p<0.05$). These data help to determine areas to focus on when sampling for the prevalence of *Listeria* spp. within the food processing environment.

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***Listeria monocytogenes* QRA EXISTING DATA COLLECTION FORM**

DO NOT PROVIDE ANY INFORMATION IDENTIFYING YOUR COMPANY NAME OR LOCATION

Unique Facility Identifier
Type of Facility
Types of Processing
Number of Processing lines in this facility
Production Area Size (Sq. Ft.)
Average Volume (lbs.) of Production per Day (range)
Average Temperature in the Production Area (˚F) (range)
Average Relative Humidity in the Production Area (%) (range)
Sample Collection Device
Details of Sample Collection Method(s)
Approximate Area of Sponge/Swab (l x w)(inches)
Average # of Samples Composited
Method of Dilution or Diluent Type
Method of Microbiological Analysis
Location of Microbiological Testing/Analysis
Average # of Samples Taken Per Week or Month in the Facility
Product Type(s) Manufactured - Frozen Vegetables
Product Type(s) Manufactured - Frozen Fruits
Product Type(s) Manufactured - Other Frozen Foods

Date (DD/MMY Y) or Day of the Week Sample was Collected	Hygienic Zone Non-Food Contact Surfaces (Zones 2, 3, 4)	Production Shift F: First Shift S: Second Shift T: Third Shift	Production Time Pre: Pre-Op or Before Production/ following sanitation D: During production Post: After production N/A:	Type of Equipment (Examples: Conveyor Belts; Freezer, Blancher, Holding Kettles, Rinse and Holding Tanks, Tables, Utensils, Forklifts, Cars, Trolleys, Blenders, Slicers, Peelers, Scrapers, Weigh Scales, Freezing Tunnels, etc.)	Indicate Which Area Sample was Taken From if a Non-Production Area/ Location	Other Sampling Site Information (Examples: Proximity to Drains or Other equipment or Equipment parts- Framework or legs, Overhead Structures, Pipes, Wash Stations, Floor Mats, Trash Receptacles, Etc.	Routine or Post-Corrective Action Sampling R: Routine CA: Post-Corrective Action	Sample Collection Method Sw: Swab Sp: Sponge	Was the Microbiological Test on a Single or Composite Sample	Microbiological Test Results (*Listeria* spp.) Pr: Presumptive P: Positive N: Negative	Other Notes

Figure 5.1: Data Collection Form sent to participants to collect information about environmental monitoring samples

Table 5.1: Terms used by participants to describe equipment and other sites sampled for *Listeria* spp. in frozen food facilities

Equipment Sampling Site Descriptor	Other Sampling Sites
Batter and Breader	Air / Ceiling
Blancher	Chute
Blender, Mixer, and Shaker	Curtain
Cabinet	Door
Container	Drain
Control Panel	Elevator, ladder, stairs, and steps
Conveyor Belt	Employee (PPE)
Dispenser	Entrance and Exit
Equipment	Floor
Eyewash and Handwash Stations	Frame
Freezer	Grate
Hopper	Handrail
Metal Detector	Hose
Scale	Ledge, platform, walkway
Sink	Pipe
Table	Pump
Tank	Squeegee
Tote	Tools
Wastebin	Transportation
	Trough
	Wall

Table 5.2: Probability of collecting *Listeria* spp. positive samples during routine environmental monitoring within frozen food processing facilities

Sampling locations within frozen food processing facilities	Probability of positive *Listeria* spp. sample
Air/ceiling	1.22%
Batter/breader	1.98%
Blancher	0.75%
Blender/mixer/shaker	2.23%
Cabinet	0.00%
Chute	2.52%
Container	2.34%
Control panel	0.63%
Conveyor belt	1.58%
Curtain	0.65%
Dispenser	0.27%
Door	1.05%
Drain	3.98%
Elevator/ladder/stairs/steps	2.29%
Employee (PPE)	2.20%
Entrance/exit	0.90%
Equipment	1.87%
Eyewash/handwash station	1.23%
Floor	2.26%
Frame	1.77%
Freezer	2.12%
Grate	2.15%
Handrail	0.79%
Hopper	1.66%
Hose	1.64%
Ledge/platform/walkway	2.04%
Metal detector	1.76%
Pipe	1.18%
Pump	3.86%
Scale	1.71%
Sink	0.00%
Squeegee	1.30%
Table	0.78%
Tank	1.10%
Tools	1.82%
Tote	1.18%
Transportation	1.86%
Trough	3.60%
Wall	0.74%
Wastebin	1.43%

Table 5.3: Probability of collecting *Listeria* spp. positive samples during routine and corrective action environmental monitoring within frozen food facilities

Sampling locations within frozen food processing facilities	Probability of positive *Listeria* spp. sample
Air/ceiling	1.08%
Batter/breader	2.19%
Blancher	0.53%
Blender/mixer/shaker	2.05%
Cabinet	0.02%
Chute	1.86%
Container	2.08%
Control panel	1.04%
Conveyor belt	1.53%
Curtain	0.55%
Dispenser	0.21%
Door	0.74%
Drain	3.27%
Elevator/ladder/stairs/steps	2.17%
Employee (PPE)	2.32%
Entrance/exit	1.67%
Equipment	1.57%
Eyewash/handwash station	0.99%
Floor	2.30%
Frame	1.83%
Freezer	2.60%
Grate	1.29%
Handrail	0.91%
Hopper	1.52%
Hose	1.19%
Ledge/platform/walkway	1.98%
Metal detector	1.27%
Pipe	1.21%
Pump	2.49%
Scale	0.91%
Sink	0.02%
Squeegee	4.44%
Table	0.54%
Tank	0.75%
Tools	2.93%
Tote	1.44%
Transportation	1.34%
Trough	4.27%
Wall	0.77%
Wastebin	1.12%

CHAPTER 6

PREVALENCE OF *LISTERIA* SPECIES AND *LISTERIA MONOCYTOGENES* ON RAW PRODUCE ARRIVING AT FROZEN FOOD MANUFACTURING FACILITIES

Magdovitz, B. F., Gummalla, S., Garren, D. M. Thippareddi, H., Berrang, M. E., and Harrison, M. A. Prevalence of *Listeria* species and *Listeria monocytogenes* on raw produce arriving at frozen food manufacturing facilities.

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ABSTRACT

Ubiquity of *Listeria monocytogenes* in the environment impacts the broader food industry and presents concerns for frozen food facilities. This study determined the prevalence and population of *Listeria* species and *L. monocytogenes* on raw produce arriving at frozen food facilities. Raw produce was collected using multi-level blinding protocols to ensure anonymity of participants and avoid traceback. Five raw vegetables were selected: corn, carrots, green beans, peas, and spinach. Raw products were collected after arrival at the facilities but before any cleaning or other pre-processing steps. The FDA BAM method for detection of *Listeria* spp. and *L. monocytogenes* was followed, with PCR screening followed by selective plating methods. *Listeria* populations were enumerated from positive samples using MPN methodology. A total of 290 samples were collected, with 96 and 17 samples positive for *Listeria* spp. (33.1%) and *L. monocytogenes* (5.9%), respectively. Enumeration data for the 96 *Listeria* spp. samples indicated 82 samples had greater than 100 MPN *Listeria* spp./g and 14 samples less than 100 MPN *Listeria* spp./g. The prevalence of *Listeria* spp. varied by commodity: spinach (66.7%), peas (50%), corn (32.2%), green beans (22.2%), and carrots (13%). *L. monocytogenes* prevalence was determined in corn (13.6%), peas (6.3%), and green beans (4.2%) arriving at processing facilities. U.S. regulators consider *L. monocytogenes* an adulterant and apply a zero tolerance regulatory action limit for the presence of this pathogen in ready-to-eat foods. Prevalence and pathogen concentration data from raw commodities found in this study can provide the industry information to conduct more accurate quantitative risk assessments and provide a baseline to model and target appropriate pathogen reduction steps during processing.

HIGHLIGHTS

- Prevalence of *Listeria* spp. and *L. monocytogenes* in raw produce was determined
- 96 *Listeria* spp. and 17 *L. monocytogenes* samples were found from 290 produce samples
- 82 produce samples had >100 MPN/g and 14 samples had <100 MPN/g for *Listeria* spp.

There has been heightened focus on pathogen prevalence in fresh produce due to the increase in outbreaks *(17)*. Produce related outbreaks accounted for 0.7% of reported foodborne outbreaks in the 1970s, 6% in the 1990s, 9% from 2002-2005, 16% in 2010-2013, and increased to around 35% in 2019 *(1, 4, 23)*. USDA reported the domestic supply of vegetables intended for freezing in the U.S. in 2018 was over 21 billion pounds, with an additional 6 billion pounds of imported product *(24)*. Every year since the start of this data collection (1970), the production, import, and supply of vegetables for freezing has increased. Trends show that fresh produce consumption in the U.S. is increasing *(13, 24, 28)*.

There are reports of contamination of fresh produce linked to *Listeria monocytogenes (30)*. Worldwide outbreaks from *L. monocytogenes* infections have been reported with fresh produce *(18, 30)*. In Texas, 10 cases of listeriosis were associated with diced celery and five people died *(10)*. One of the largest outbreaks for *Listeria* in produce was associated with cantaloupe in 2011, with 147 cases and 33 deaths *(9)*. An estimated 1,600 people get listeriosis and 260 die each year in the U.S. *(22)*.

The U.S. FDA has provided guidance for reduction of *Listeria* in the food industry including steps to mitigate the pathogen in processing environments *(25, 26)*. *Listeria* prevalence studies on ready-to-eat products in the U.S. from 2010-2013 provided an overview of multiple food groups (meat, dairy, produce, seafood, and combination foods) with an overall prevalence of 0.42%. For the produce categories, 19 of 1,689 (1.12%) raw cut vegetable samples were *Listeria* positive, with enumeration values from <0.036 MPN *Listeria*/g to 330 CFU *Listeria*/g *(16)*. However, a study conducted in the Republic of Ireland found a 9.4% positive *L. monocytogenes* prevalence rate for fresh-cut vegetables *(12)*.

In Santiago Chile, the prevalence of *L. monocytogenes* was 25.4% for frozen vegetable salads and 10.2% for raw or cooked ready-to-eat vegetables *(6).* Additionally, that study enumerated a randomly chosen set of 20 positive samples by plate count and 90% had less than 10 CFU/g. The most probable number (MPN) technique was performed for 34 samples; 12 had greater than 1,100 MPN *Listeria*/g, 5 had between 93-240 MPN/g, 8 had between 3-23 MPN/g, and 9 contained less than 3.0 MPN/g *(6).* The MPN technique is designed for enumerating low numbers, this study shows MPN methodology is more suitable than plate counting for enumeration of *Listeria* in vegetables *(6).*

With the increase in produce associated outbreaks of *L. monocytogenes*, the current research project focused on determining prevalence and concentration of *Listeria* on raw produce arriving at frozen food processing facilities. Such data will aid in the assessment of quantitative risks posed by this pathogen. By knowing more about *Listeria* contamination prevalence and levels on raw incoming product, one can also better access and model the effectiveness of any antimicrobial treatments applied during processing.

MATERIALS AND METHODS

Raw produce samples were collected from various frozen food processing facilities to determine the prevalence and concentration levels of *Listeria* spp. and *L. monocytogenes* on commodities arriving at the facilities. Samples were collected with blinding protocols to encourage industry participation. All samples were collected at the raw product arrival point at facilities prior to cleaning or any further processing.

Blinding protocols. Blinding protocols were established to encourage participation, and to relieve any concern participants might have about the identification of the source of the samples (Chapter 4). A packet was distributed to potential participants through a listserv of food

safety professionals working in frozen food processing. The packet included information about the produce collection process, blinding protocol requirements, and specifications of the produce. Food safety professionals in academia, legal affairs, and the frozen food industry designed and reviewed the packet before distribution to potential participants. Blinding protocols established a triple-blinding method used to collect samples with no traceback to the original source. Double blinding codes were used during collection of samples and a third code was applied before publication and distribution of data.

Produce collection. To ensure produce samples were collected properly using blinding protocols, flowcharts in the packet described materials participants needed in order to collect samples and sample collection protocols (Figure 6.1). Materials needed for collection were sterile gloves, sterile sample collection bags, freezer or gel packs, an insulated foam cooler and outer shipping carton, and the predesignated shipping and return addresses. Samples were collected when raw produce arrived at the facility after unloading from transport vehicles but before any cleaning or further processing. The sample collector collected three separate samples from three different lots. Lots were defined by the facility. A generic coding, i.e., A, B, C, differentiated samples associated with the three different lots. Samples were then collected, placed into sterile sample bags, put into insulated cooler boxes, and shipped to the lab. The predetermined return address used for all shipments was the address of the trade organization associated with the project. This helped to ensure blinding and prevented identification of the source of each sample. The shipping address was to the lab that analyzed the samples. Upon arrival at the lab, Researcher 1 aseptically removed samples from the bags and placed them into new bags with a second code. The second code contained information as to the type of product, lot identifier, and sample number. For example, V2L2S2 would refer to V2 as Vegetable 2 (each

commodity was designated their own letter), L2 would signify lot 2, and S2 was a sample within the lot. Samples were then delivered to Researcher 2 to run the analyses on the samples.

Detection of *Listeria* spp. and *Listeria monocytogenes.* Samples were analyzed using the FDA's *Bacteriological Analytical Manual* procedure for *Listeria* detection *(8, 11, 19).* First, 25 g of product was placed into 225 mL of buffered *Listeria* enrichment broth (Difco, BD Sparks, MD). Most sample types were homogenized in a Stomacher 400 Circulator at 260 rpm for 60 s and incubated at 30°C for 24 to 48 h. For larger commodities (i.e., raw corn on the cob and carrots) a full rinse of the vegetable was conducted with a 1 to 1 dilution weight to volume with buffered *Listeria* enrichment broth. For all samples, after 4 h of incubation, three filter sterilized selective agents were added to achieve final concentration of 10 mg/L acriflavin, 40 mg/L cycloheximide, and 50 mg/L sodium nalidixic acid in the buffered *Listeria* enrichment broth with pyruvate pre-enrichments. Supplements were mixed with the samples and incubated at 30°C for the remainder of the 24-48 h period. After the enrichment period, samples were analyzed with the BAX® Automated System (Hygiena, Camarillo, CA) for detection of *Listeria* spp. For positive samples, a portion of the enriched culture was streaked onto modified Oxford medium (Difco, BD) and CHROMagar *Listeria* (CHROMagar, Paris, France) to obtain isolates for additional identification. The plates were incubated at 35°C for 24-48 h. Presumptive positive isolates were selected from the plates and were streaked for purity onto trypticase soy agar with 0.6% yeast extract (Difco, BD) which were incubated at 30°C for 24 h. The Micro-ID *Listeria* Identification System (Remel, Lenexa, KS) was used to identify the *Listeria* species.

Enumeration method. Positive *Listeria* spp. samples identified with BAX® Automated System (Hygiena) were enumerated following a modified version of the FDA's *Bacteriological Analytical Manual (3, 5).* A 25 g sample from the reserve product of the positive sample was

placed into 225 mL buffered *Listeria* enrichment broth (Difco, BD) and homogenized in a Stomacher 400 Circulator at 260 rpm for 60 s. The MPN dilution scheme used 3 tubes of Fraser broth (Difco, BD) per 4 dilutions. The MPN arrangement delivered equivalent to 1, 0.1, 0.01, and 0.001g sample per aliquot at each respective dilution. Tubes were incubated at 30°C for 24-48 h. Results were analyzed and identified as positive if a dark color change was detected in a test tube. Results were compared to the tables in the BAM Appendix 2 for detection of MPN/g results *(11)*.

RESULTS

Listeria spp. was detected in all five commodities analyzed. There was a total of 290 raw vegetables tested. The produce chosen were raw vegetables collected at frozen food production facilities that were to be further processed into frozen carrots, corn, green beans, spinach, and peas. All vegetables were collected at the receiving docks of the food processing facilities prior to cleaning, trimming, or any other further processing. Across all the commodities, there were 96 *Listeria* spp. (33.1%) samples detected by PCR testing and 17 confirmed *L. monocytogenes* (5.9%) from selective plating and biochemical tests (Table 6.1). Enumeration data for the 96 *Listeria* spp. samples indicated 82 samples had greater than 100 MPN/g while 14 samples less than 100 MPN/g (Figure 6.2). Of the 14 samples with less than 100 MPN/g, 3 of those samples contained less than 10 MPN/g. While *L. monocytogenes* were not enumerated, it is noteworthy that of the 17 samples that contained *L. monocytogenes*, 14 of those were samples that contained >100 MPN/g *Listeria* species while 3 were from samples that contained <100 MPN/g *Listeria* species.

This project was conducted over two different harvest seasons, 2018 and 2019. Samples for the 2018 harvest season provided higher prevalence levels with 191 total samples collected.

Of those, 79 *Listeria* spp. (41.4%) and 16 *L. monocytogenes* (8.4%) samples were detected. From the 99 samples collected in 2019, 17 were positive for *Listeria* spp. (17.2%) and 1 was positive for *L. monocytogenes* (1.0%).

Collection per each commodity varied. Of the 96 green pea samples, 48 *Listeria* spp. (50%) and 6 *L. monocytogenes* (6.3%) positive samples were found. The *Listeria* spp. positive pea samples included 40 samples with greater than 100 MPN/g, 8 were between 10-100 MPN/g, and no samples with less than 10 MPN/g. Of the 6 confirmed *L. monocytogenes* samples, 4 were from samples that had greater than 100 MPN *Listeria* spp./g while 2 were from samples that had between 10-100 MPN *Listeria* spp./g.

For 72 green bean samples, 16 were positive for *Listeria* spp. (22.2%) and 3 for *L. monocytogenes* (4.2%). The *Listeria* spp. enumeration values for green beans revealed 12 samples had more than 100 MPN/g, 2 samples had between 10-100 MPN/g, and 2 samples had less than 10 MPN/g. The 3 samples confirmed to be contaminated with *L. monocytogenes* all contained more than 100 MPN *Listeria* spp./g. A total of 59 corn samples were collected with 19 positive for *Listeria* spp. (32.2%) and 8 positive for *L. monocytogenes* (13.6%). Seventeen *Listeria* spp. positive samples were greater than 100 MPN/g, 1 sample was between 10-100 MPN/g, and 1 sample was less than 10 MPN/g. Of the 8 *L. monocytogenes* positive samples, 7 samples had greater than 100 MPN *Listeria* spp./g with 1 sample between 10-100 MPN *Listeria* spp./g.

The PCR provided indeterminate results with the carrot samples due to interfering substances, which may have been excess dirt on the samples. No *L. monocytogenes* was detected from 54 carrot samples. There were 7 presumptive positive samples for *Listeria* spp. (13%) from selective plating, but they were not confirmed by BAX PCR. For the 7 presumptive positive

samples, they contained more than 100 MPN *Listeria* spp./g. Only one shipment of spinach containing 9 samples was analyzed. This could be due to multiple leafy green outbreaks occurring during the two year collection period of this project. Although the sample size was small, 6 of the 9 samples were positive for *Listeria* spp. (66.7%) and no *L. monocytogenes* was detected. All six samples had greater than 100 MPN *Listeria* spp./g values detected.

DISCUSSION

The 5.9% prevalence for *L. monocytogenes* noted in the current study is similar to other published produce studies with prevalence rates between less than 5% to over 20% *(7, 20, 21, 30)*. The variability in prevalence for produce is due to various factors including product, soil type, farming and processing practices, and methods of collection and detection of *L. monocytogenes (30).*

In the current study, we examined samples across two harvest seasons, 2018 and 2019. The 2018 samples had *Listeria* spp. and *L. monocytogenes* prevalence rates of 41.4% and 8.4%, respectively. In comparison, the 2019 harvest season prevalence rate was 17.2% for *Listeria* spp. and 1.0% for *L. monocytogenes.* With the blinding protocols implemented in the study, there could be no direct questions to the suppliers about the variability between seasons for their produce or whether the same participants sent samples during both harvest seasons. Abiotic and biotic factors in the soil have been shown to make a difference in *Listeria* prevalence *(14, 30).* The variability between prevalence of *Listeria* for different harvest season years could be due to climate conditions, growing locations, and diversity between farms.

In the current study, enumeration of *Listeria* on contaminated produce revealed that of the *Listeria* positive samples, 85% contained more than 100 MPN *Listeria* spp./g. This outcome shows that bacteria coming into the facility on produce should be a large concern for processors.

Worldwide, regulations have set different limits for *L. monocytogenes* for various ready-to-eat products *(15)*. U.S. regulations follow a zero-tolerance rule for ready-to-eat products (no detection of *Listeria* in a 25 g sample). Canada and the EU have set limits, that allow for lower than 100 cfu/g in products that do not support the growth of *L. monocytogenes* and are not associated with consumption from high risk individuals. Although the products in this study were not ready-to-eat and were destined for further processing, the prevalence of *Listeria* entering facilities can be used to target and model steps to reduce the pathogen on the final product. Numbers of *Listeria* on raw produce in this study can complement risk assessment efforts aimed at understanding modes of contamination in processing environments and on finished food products. The population categories were determined based upon regulatory policy in other countries with a limit of 100 cfu/g. The current study compared greater than or less 100 MPN/g and divided further for a very low detection level of less than 10 MPN/g. Enumeration of *Listeria* spp. was done to gain information on the contamination level of raw produce. This was done to provide processors with an idea of the numbers of *Listeria* entering frozen food processing facilities.

Produce collected in the study was not considered ready-to-eat and goes through further cleaning, trimming, and processing before being marketed as frozen vegetables. It is the consumer's responsibility to cook the product before consumption. FDA recommends consumers wash produce if vegetables or fruits have not already been washed before consuming the product *(27)*. Additionally, proper cooking of frozen food products can reduce *L. monocytogenes* to undetectable levels *(6)*. Consumers should follow good handling, washing, and cooking practices with produce to reduce chances of contracting a food-borne illness.

In processing facilities, traditional cleaning and sanitation steps help to reduce the natural contamination load of pathogens on produce *(2).* Deep cleaning of facilities may reduce *L. monocytogenes* prevalence and potentially eliminate the pathogen from facilities. However, effective cleaning and sanitation efforts can be limited due to re-contamination of the processing environment over time *(29).* Additionally, other methods such as environmental monitoring, good manufacturing practices, and sanitation standard operating procedures are implemented in processing facilities as efforts to reduce pathogen contamination in the facilities.

The findings of this study show that produce entering a facility for processing can be frequently contaminated with *Listeria* and must be considered a likely source for the introduction or re-introduction of *Listeria* into a processing facility. Understanding the modes of contamination of *Listeria* is important to help food processors reduce pathogen contamination in the processing environment on food and food contact surfaces. Since contamination located on produce may not be completely washed off, preventative steps need to be monitored and implemented to ensure a safe product *(17).* Cleaning, washing, and further processing steps should incorporate proper preventive controls to help reduce the chance of contamination of the final product. Risk assessments and models using this data will help the industry better recognize the best protocols to use within their processing facilities.

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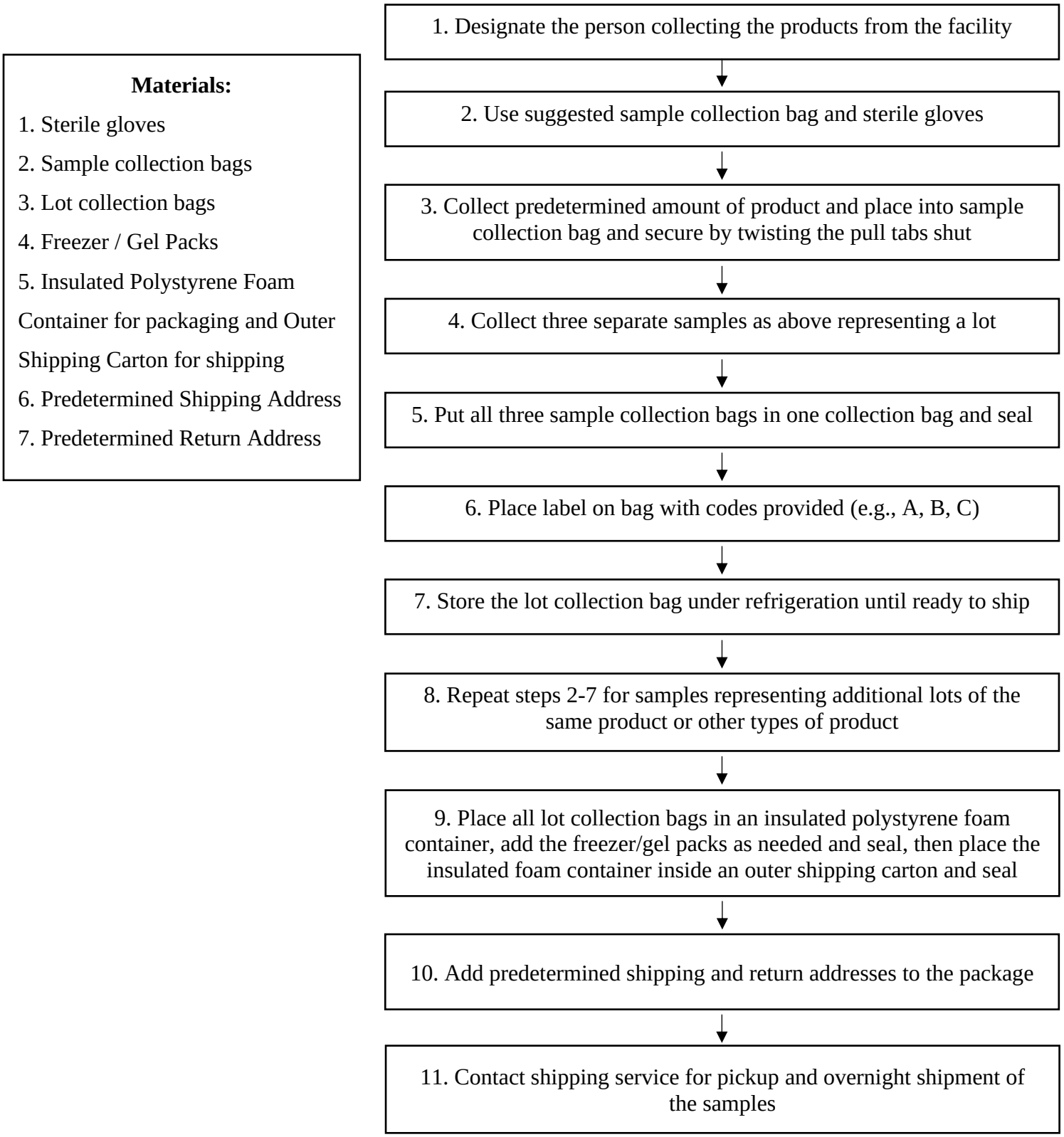

Figure 6.1: Collection protocol for produce arriving at frozen food processing facilities

Table 6.1: Prevalence of *Listeria* spp. and *Listeria monocytogenes* per raw vegetable arriving at frozen food facilities

		Listeria spp. Positives		***L. monocytogenes Positives***	
Vegetables	**Total**	**Number of Positive Samples**	**Percentage of Total Samples**	**Number of Positive Samples**	**Percentage of Total Samples**
Carrots	54	7	13.0%	0	0%
Corn	59	19	32.2%	8	13.6%
Green beans	72	16	22.2%	3	4.2%
Spinach	9	6	66.7%	0	0%
Peas	96	48	50.0%	6	6.3%
Total	290	96	33.1%	17	5.9%

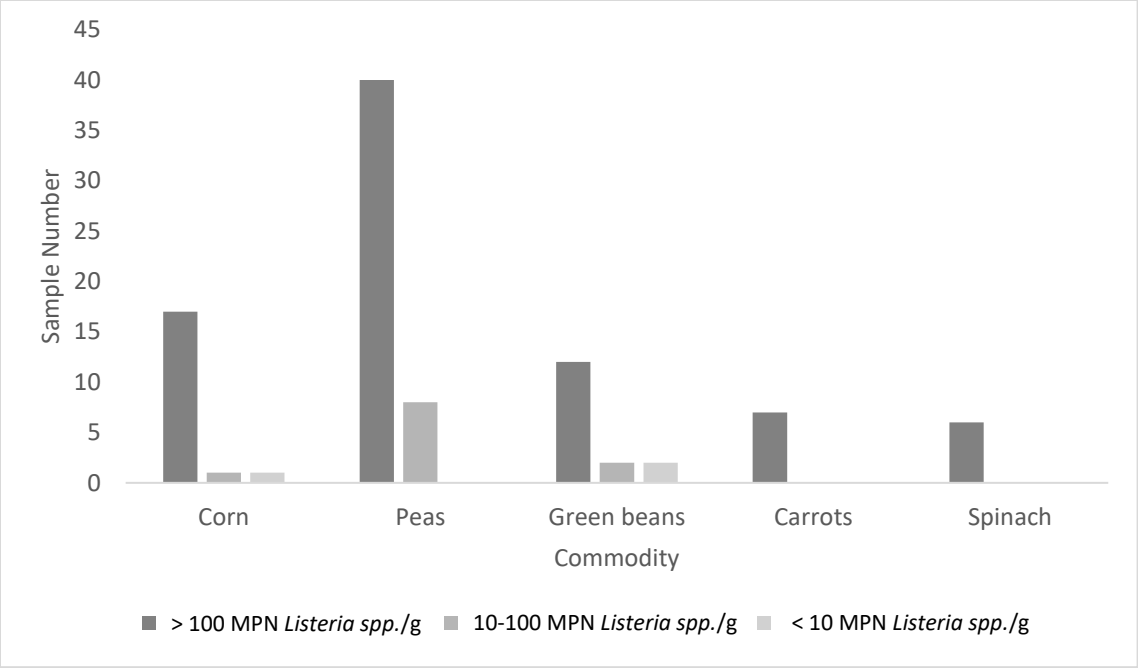

Figure 6.2: Positive samples of *Listeria* species enumeration results per vegetable arriving at frozen food processing facility

CHAPTER 7

CONCLUSIONS

The purpose of this research was to determine the prevalence and concentration of *Listeria* species and *Listeria monocytogenes* in frozen food processing facilities and on raw products entering the facilities. An electronic survey was conducted within the frozen food industry to establish facilities' current practices for preventive controls. This overview determined key differences within the industry that established improved protocols for *L. monocytogenes* reduction within the frozen food manufacturing environments.

To encourage participation within the food industry, all data for this research was conducted through blinding protocols to provide anonymity to the participants. Legal counsel provided guidance on proper steps to conduct a study ensuring privacy to participants. Blinding protocols provided a safe harbor for sensitive food safety data, eliminating the ability to describe the original source of data collected throughout this research project.

Historical environmental monitoring results for *Listeria* data over a six month to a year timespan was collected from various frozen food facilities. Industry focus on pathogen reduction varied per facility's environmental monitoring plan; however, similarities in areas of concern for positive *Listeria* samples were found among all facilities. This research provided a baseline of information from 27 facilities to outline procedures the industry can use to reduce issues in processing facilities. The information can be used to evaluate the potential risk of *Listeria* contamination in food manufacturing environments and to improve quantitative risk assessments.

Population growth levels and occurrence of *L. monocytogenes* on raw products was established for incoming vegetables in frozen food manufacturing facilities. The information can be used as a baseline to model and target appropriate pathogen reduction steps during processing and to improve quantitative risk assessments.

Overall, the research project focused on a broad scope of environmental information to evaluate issues with *Listeria* within the frozen food industry. This information can be used to improve protocols and procedures to reduce *Listeria* contamination within frozen food facilities in the environment and on food products. While the focus was on frozen food processors, the information can also be used to improve protocols within other segments of the food industry.