CENTER for PRODUCE SAFETY

CPS 2019 RFP
FINAL PROJECT REPORT

Project Title
Factors affecting persistence of *Listeria monocytogenes* need to be identified for evaluation and prioritization of interventions

Project Period
January 1, 2020 – December 31, 2020 (extended to March 31, 2021)

Principal Investigator
Martin Wiedmann
Cornell University
Department of Food Science
341 Stocking Hall
Ithaca, NY 14853-7201
T: 607-254-2838
E: mw16@cornell.edu

Co-Principal Investigator
Renata Ivanek
Cornell University
Department of Population Medicine and Diagnostic Sciences
Ithaca, NY 14853-6401
T: 607-253-4383
E: ri25@cornell.edu

Objectives

1. *Conduct a systematic review of published and unpublished data and literature to identify modifiable factors that may contribute to resident Listeria in produce packing and fresh-cut facilities and relevant interventions, assess the validity of these findings based on the strength of evidence and prioritize interventions for assessment in Objectives 2 and 3 using expert elicitation.*
2. *Use controlled experiments and observational studies to validate selected interventions identified in Objective 1 in produce packing and fresh-cut facilities with resident Listeria.*
3. *Validate selected interventions that are challenging to validate experimentally (e.g., extensive facility modifications) using our previously developed agent-based model.*

Funding for this project provided by the Center for Produce Safety through:
WSDA SCBGP grant# K2869

FINAL REPORT

Abstract
Listeria, and especially persistent *Listeria*, in produce operations can lead to product contamination, posing public health and business risks. As such, a rapid review was conducted to identify locations of persistent *Listeria*, risk factors for persistent *Listeria*, and interventions to eliminate persistent *Listeria* in food processing environments. The review revealed only one of the 32 included studies assessed persistent *Listeria* in produce operations, identifying a need for future studies conducted in produce operations. In addition, of the 32 studies, only 13 included a definition of persistence; however, the definition of persistence was highly variable from study to study. This finding indicates a need to standardize the requirements for persistent *Listeria*. The five sites mentioned by the greatest number of included studies where persistent *Listeria* was isolated from were floors, drains, conveyor belts, slicers, and tables. In addition, there were only 9 studies that discussed risk factors for persistent *Listeria* and 11 studies that tested interventions for the elimination of persistent *Listeria*. As such, additional research is needed to better understand *Listeria* persistence in food processing facilities, particularly in produce operations. In addition to the review, a longitudinal study was conducted in four apple packinghouses to test interventions to control *Listeria* in a real-world environment; the interventions were identified using newly developed a produce specific root cause analysis (RCA) tool. For instance, an updated forklift cleaning and sanitation protocol was tested in three of the four packinghouses, which appeared to significantly reduce (P=0.04) the log odds of the forklifts testing positive for *Listeria*. In addition, updated cleaning and sanitation protocols of the wet areas and wax areas in the packinghouse were tested. These updates revealed that equipment breakdown appears to be necessary to control *Listeria* populations, especially in cases where *Listeria* is repeatedly isolated from the same sites. Furthermore, agent-based models were utilized to assess interventions for the elimination of persistent *Listeria* that were too time or cost intensive to test under real-world conditions. These models can be used by industry to identify which interventions are worth testing in produce operations to eliminate persistent *Listeria*. Overall, this project (i) provides industry with a produce-specific root cause analysis (RCA) tool for *Listeria* findings, (ii) identified key areas in produce facilities where cleaning and sanitation weaknesses favor *Listeria* presence (e.g., forklifts and produce waxing areas), and (iii) further emphasizes the importance of equipment breakdown for sanitation.

Background
Listeria monocytogenes is a foodborne pathogen known to exist in a wide variety of environments, including natural (39), agricultural (15, 41, 46, 47), urban (39), and food processing environments (48). While *L. monocytogenes* exists in diverse environments, produce contamination that leads to recalls and outbreaks is often traced back to the packing and processing environment (31).

While *L. monocytogenes* may enter produce operations (i.e., packinghouses or processing facilities) on raw materials, via employees acting as fomites (e.g., employee boots), via transportation crates or vehicles, etc., recalls and outbreaks are typically linked back to contamination of the packing and processing environment (31, 43). There are 3 types of *Listeria*: (i) persistent *Listeria*, (ii) persistent transient *Listeria*, and (iii) transient *Listeria*, with both persistent and persistent transient *Listeria* referring to cases of repeat isolation of *Listeria* in a produce operation. *L. monocytogenes* that survives in the packing and processing environment over an extended period is referred to as “persistent *L. monocytogenes*” (16). Persistent *L. monocytogenes* represents elevated public health (e.g., outbreaks) and business

(e.g., recalls) risks. Furthermore, once *L. monocytogenes* becomes "persistent" within a processing environment, it can often be difficult to eliminate because it is present in a "niche" within the equipment or facility itself that can be difficult to clean and sanitize (43). It can also be challenging to identify where the persistent *L. monocytogenes* is living within the processing environment and develop interventions to eliminate the *L. monocytogenes* (43). "Persistent transient *Listeria"* refers to the case of repeated isolation of a diverse population of *Listeria* (i.e., *Listeria* of several different subtypes) from a given site; while this *Listeria* does not necessarily represent *Listeria* living in a produce operation, it does indicate continual introduction of *Listeria* to the given site and a subsequent lack of *Listeria* control (1). While persistent transient *Listeria* is less of a risk compared to persistent *Listeria*, persistent transient *Listeria* can still lead to more sporadic finished product contamination if not addressed. On the other hand, *L. monocytogenes* that enters the production environment but is eliminated through cleaning and sanitation efforts is referred to as "transient *L. monocytogenes*;" transient *L. monocytogenes* represents the lowest risk.

In order to identify and eliminated persistent or persistent transient *Listeria* from produce operations, continued sampling of the facility is necessary, and the root cause of contamination must be identified. This information should then be utilized to identify interventions to control *Listeria* in the produce operation; however, identifying interventions that truly address the root cause of contamination can be difficult. As such, a complete list of previously tested interventions should be complied and tested to assist produce operations in prioritizing which interventions to implement. The objectives of this study were to:

(i) Conduct a systematic review of published and unpublished data and literature to identify modifiable factors that may contribute to resident *Listeria* in produce packing and fresh-cut facilities and relevant interventions, assess the validity of these findings based on the strength of evidence and prioritize interventions for assessment in Objectives 2 and 3 using expert elicitation.

(ii) Use controlled experiments and observational studies to validate selected interventions identified in Objective 1 in produce packing and fresh-cut facilities with resident *Listeria*.

(iii) Validate selected interventions that are challenging to validate experimentally (e.g., extensive facility modifications) using our previously developed agent-based model.

Research Methods and Results

Objective 1 Methods

Literature search. The review protocol is published on the Center for Open Science website (https://osf.io/6k83y/). Studies were gathered to be assessed for inclusion in this rapid review by conducting a search in Food Science and Technology Abstracts (FSTA) in Web of Science. The use of only one database was determined to be sufficient in this case, as all journals cited three or more times in a previous scoping review of *Listeria* in food processing environments (48) are indexed in FSTA. The following search terms were used to identify studies in FSTA: "*Listeria*," "*L. monocytogenes*," or "listeriosis" and "food-processing industry," "food manufacturing facility," "food production plant," "food production facility," "food-processing," "food processing," "processing plant," "processing environment," "processing equipment," "packinghouse," "food factory," "food industry," "fresh-cut facility," "food operation," or "packinghouse." In addition, a grey literature search was performed by searching the previously funded projects by the Center for Produce Safety (6).

Inclusion criteria and identification of studies. De-duplication of repeat studies, abstract screening, and full text review were performed using Covidence (10). For a study to have been

deemed relevant for inclusion in this rapid review, it must meet the following inclusion criteria: (i) take place in a food operation (including produce fresh-cut facilities or packinghouses) with description of environment/plant (including dairy, meat, retail meat, delicatessens, fish, produce packinghouses and fresh-cut produce); (ii) include sampling of specified/ described surfaces and microbiological testing of samples; (iii) report samples evaluated for *Listeria*; (iv) specifically refer to persistence, permanent, residence, recurrence, dispersal, or other relevant terms in our proposed study; (v) be original research or a review of original research; (vi) have matching subtypes for at least 3 sampling events over at least 2 months; (vii) use a substantive subtyping method [i.e., amplified fragment length polymorphism (AFLP), multi locus variable-number tandem repeat analysis (MLVA), Multi virulence locus sequence typing (MVLST), pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), restriction endonuclease analysis (REA), Ribotyping, and whole genome sequencing (WGS)]; and (viii) be in English.

Even though *L. monocytogenes* is the only species of *Listeria* pathogenic to humans, this review was interested in persistence of all species of *Listeria*. *Listeria* spp. is often used as an index for *L. monocytogenes*, as other *Listeria* spp. often inhabits similar environments to *L. monocytogenes* (8). As such, persistence data on *Listeria* spp. can provide useful insights into persistence of *L. monocytogenes*.

To identify relevant studies, an abstract review was performed; 2 people reviewed each abstract. For all studies with disagreements between abstract reviewers, the entire team met and discussed if the study should move on to the next step. After abstract review, full text review was performed; 2 people reviewed each full text. The same protocol was utilized for disagreements between the full text reviewers.

Data extraction. Data extraction was performed on all studies that were deemed relevant after full-text review. One person performed data extraction and data checks. **Table 1.1** shows the information pulled from each study.

Objective 1 Results

Literature search and study screening results. A rapid review was conducted to compile information on where *Listeria* persisted within processing/packing environments, as well as risk factors for persistence and interventions implemented to eliminate persistent *Listeria*. While the main goal of this review was to identify studies that assessed persistence in produce operations (packinghouses and fresh-cut facilities), all food production environments were included to gather a more comprehensive list of risk factors and interventions. In total, 1,656 studies were identified through the literature search; after de-duplication, 1,654 remained (i.e., one pair of duplicate studies was removed). Abstract screening eliminated 1,390 of the 1,654 studies, leaving 264 studies for full text review. Full text review eliminated 232 studies. The top three reasons why studies were excluded were because (i) they did not find matching subtypes at least 3 times over 2 months (N=70 studies), (ii) they did not describe sampling sites (N=63), and (iii) they did not use a substantive subtyping method (N=48 studies) (**Table 1.2**). To provide more transparent information on persistent *Listeria* in food processing environments, future studies should consider including information on locations of persistent *Listeria*; this will allow readers to better utilize the findings in their studies. In addition, future studies should consider using highly discriminatory typing methods (e.g., WGS) to allow for a better understanding of persistent *Listeria* in food processing environments.

There is a need for additional studies that assess persistence in produce operations and that assess Listeria spp. persistence in addition to L. monocytogenes. Of the 32 studies deemed relevant in the current review, (i) 12 were conducted in seafood or smoked seafood processing

facilities (5, 9, 11, 13, 14, 19, 21, 22, 26, 35, 36, 45), (ii) 10 were conducted in meat or poultry slaughter or processing facilities (4, 18, 24, 27, 28, 29, 32, 37, 40, 44), (iii) 4 were conducted in cheese processing facilities (7, 17, 23, 33), (iv) 2 were conducted in sandwich or deli meat processing facilities (2, 3), (v) 1 was conducted in a produce facility (34), and (vi) 3 were conducted in un-specified facilities or multiple product type facilities (12, 25, 30) (**Table 1.3**). Therefore, there are a large number of studies that focus on seafood and meat and poultry operations. On the other hand, only 1 study assessed *Listeria* persistence in produce operations, indicating additional studies are needed in produce operations.

Only 1 (24) of 32 studies assessed *Listeria* spp. persistence, whereas all 32 studies specifically assessed *L. monocytogenes* persistence (Table 1.3). There is an opportunity for additional studies to include *Listeria* spp. subtyping in future persistence studies. As *Listeria* can serve as an index organism for *L. monocytogenes* (8), subtyping *Listeria* spp. in addition to *L. monocytogenes* in future studies can provide additional information on common persistence locations, persistence risk factors, and interventions for elimination of persistent *Listeria*.

The majority of studies assessed L. monocytogenes persistence established using PFGE. To sample the processing facilities, 15 used sponges, 7 cotton swabs, 2 used both cotton swabs and sponges, 1 used clothes, and 7 did not specify their sampling tool (**Table 1.3**). For subtyping, 18 studies utilized PFGE (2, 3, 4, 9, 11, 12, 13, 17, 19, 22, 28, 29, 32, 33, 35, 37, 40, 44), 5 utilized ribotyping (7, 21, 23, 26, 36), 4 utilized RAPD (5, 24, 27, 45), 1 utilized MVLST (34), 1 utilized AFLP (25), 3 utilized multiple subtyping methods (14, 18, 30) (Table 1.3). Of the 3 studies that utilized multiple subtyping methods, 2 utilized WGS (14, 30); however, Madden et al. (30) only utilized WGS for multi-locus sequence typing (Table 1.3).

The definitions of persistence used by past studies are highly variable. Thirteen of the 32 studies provided a definition of persistence within their articles. However, the definitions provided by these studies are variable. The most common definition specified by 3 studies defined persistence as isolation of the same subtype ≥2 times over ≥6 months (11, 17, 30). Two studies defined persistence as isolating the same subtype ≥3 times over ≥3 months (25, 37). Two studies also defined persistence as a subtype being isolated on all sampling events (3, 12). The following definitions were given by 1 study each: ≥2 times with no specified time frame (34), ≥2 times over ≥1 year (18), ≥2 times over a 13-year period (14), ≥5 times over ≥3 months (29), ≥6 times over ≥2 months (13), and 5 to 6 times over ≥1 year (44). Regardless, all 32 relevant studies were required to have at least 1 subtype isolated ≥3 times over ≥2 months to be included in the current study.

The majority of studies (23 out of 32 studies) sampled both food contact surfaces and non-food contact surfaces; 4 studies only sampled food contact surfaces (4, 7, 9, 14), 3 studies only sampled non-food contact surfaces (11, 34, 40), and 2 studies did not specify (25, 26) (**Table 1.3**). The five most common sites where persistent *Listeria* was isolated from were floors (20 studies), drains (14 studies), conveyor belts (13 studies), slicers (9 studies, plus an additional 11 studies mentioned other cutting machines), and tables (8 studies). While these sites are likely to harbor *Listeria*, the number of studies that mentioned persistent *Listeria* was isolated from these top five sites may be biased by the fact that these sites were commonly sampled among all relevant studies. Other locations where persistent *Listeria* was isolated from include, boots/ boot covers (14, 44), brooms and squeegees (2, 27, 34, 45), ceilings (18), condensate line (21), crates (19, 21, 23, 25, 32, 33), cutting boards (3, 32, 44), floor cracks (34), gloves (2, 24, 45), hoses (2), puddles (30, 34), packaging areas/ equipment (22, 25, 32, 33), scales (3, 9), shelves (2, 17), trolleys and carts (3), walls (18), wheels (cart and conveyor belt) (12, 34), and various other pieces of processing equipment and areas specific to different product type (2, 3, 7, 11, 17, 19, 22, 24, 26, 27, 29, 37, 40, 44).

Persistence risk factors. In total, 9 studies mentioned risk factors for persistent *Listeria*; 5 studies mentioned equipment cleanability (2, 7, 24, 29, 35), 5 studies mentioned lack of compartmentalization (including compartmentalization of employees, equipment, lines, etc.) (3, 19, 25, 26, 29), 2 mentioned cleaning routines/protocols (25, 29), 2 mentioned raw materials/ raw material areas (19, 25), 2 mentioned product type (25, 29), 1 mentioned dripping condensate (24), and 1 mentioned high-pressure systems that facilitate spread of particles (24).

In addition, 2 studies stated the temperature of the equipment can also facilitate persistence, as one study saw increased persistence in an N2 chiller due to the cool temperatures (24) and the other study saw decreased persistence in peeling equipment due the higher temperatures caused by the steam used at this step (29). Therefore, the nature of the equipment can also play a role in the likelihood of persistent *Listeria* being present.

Interventions to eliminate persistent Listeria from processing facilities. Eleven studies tested interventions to eliminate persistent *Listeria* from processing facilities (**Table 1.4**). For instance, 4 studies mentioned utilizing deep cleaning activities (2, 26, 29, 40); the effect on persistent *Listeria* was variable from study to study (Table 1.4). This variability may be due to lack of adequate information on the drivers of persistence and elimination of persistent *Listeria* strains; however, it may also be caused by other differences between the processing environments investigated in each of these studies. Three studies also utilized further disassembly of equipment during cleaning and sanitation (7, 19, 35); 2 of the 3 studies saw elimination of the persistent subtype and the 1 of the 3 studies saw a reduced prevalence but an unknown effect on persistence (Table 1.4). Therefore, the results from disassembly of equipment interventions in most cases indicate it may be an effective strategy for eliminating persistent *Listeria* from processing environments.

Objective 2 Methods

Packinghouse characteristics and study design. Four apple packinghouses were enrolled in this longitudinal study to test the effect of various interventions on a reduction in *Listeria* persistence and transient persistence in the packing environment; the packinghouses were designated as packinghouse A, B, C, and D. See **Table 2.1** for details on each of the packinghouses.

To identify persistent and persistent transient *Listeria* populations in the packinghouses, and to test the effectiveness of the interventions and controlling *Listeria*, environmental sampling was performed. Sampling took place from November 11th, 2020 to March 30th, 2021; between 7 and 9 samplings were conducted in each packinghouse. See **Table 2.2** for a timeline of the samplings and intervention implementation.

Root cause analysis and intervention implementation. To identify interventions to implement in each of the packinghouses, root cause analysis (RCA) was performed. RCA was performed according to the protocol described by Belias and Wiedmann (1). Briefly, a 1.5h meeting was held over zoom with members of the Cornell team and representatives from each packinghouse (e.g., plant managers, maintenance managers, sanitation managers, food safety and quality managers). RCA was performed separately for each packinghouse, but common issues between the packinghouses were identified to determine if common interventions may be effective across packinghouses. The RCA steps are provided in **Figure 2.1** and the fishbone diagram used in the RCA is provided in **Figure 2.2**. At the meeting, the results of sampling 1, sampling 2, and sampling 2.5 were reviewed for each packinghouse to identify sites with repeat *Listeria* positives. The major bones (i.e., (i) company practices/ food safety culture, (ii) personnel, (iii) facilities, (iv) cleaning and sanitation, (v) produce introduction, (vi) packinghouse equipment, and (vii) produce processing equipment) were prioritized by the most likely to contribute to persistent or transient persistent *Listeria* (Figure 2.2). Then, 3 or 4 minor bones

(i.e., the bones within each major bone) were identified as the most likely to contribute to persistent or persistent transient *Listeria* (Figure 2.2), and a "five whys" procedure was performed to identify the root cause of each. After the root causes were identified, interventions were developed to address each root cause.

In packinghouse A, the following interventions were implemented: (i) updated cleaning and sanitation protocols for the wet end (i.e., bin dump, flume, brushes, and wax area) using foamers, (ii) redesigning drain grates so they could be removed for cleaning and sanitation, and (iii) replacing a wooden bin with a metal bin for collecting leaves and rotten apples. In packinghouse B, the following interventions were implemented: (i) updated forklift cleaning and sanitation protocol, and (ii) updated floor cleaning and sanitation protocol. In packinghouse C, the following interventions were implemented: (i) updated forklift cleaning and sanitation protocol, (ii) removal of catch pans under brush beds prior to cleaning and sanitation, (iii) use of high-dose PAA (325 ppm) for brush bed sanitation, and (iv) removal of the pressure washer from the cleaning and sanitation protocol in the wax area and replace its use with brushes and more employees. In packinghouse D, the following interventions were implemented: (i) updated forklift cleaning and sanitation protocol, (ii) use of quaternary ammonium compound (quat) powder around forklift stops, and (iii) updated protocol for cleaning and sanitation of the wax area. All interventions were implemented in the last two weeks of January 2021.

Sample collection. In total 1,339 environmental samples were collected across the entire study (398, 234, 394, and 313 samples were collected from packinghouses A, B, C, and D respectively). On each full sampling visit (samplings 1, 2, 3, 4, 5, and 6; **Table 2.2**), between 34 and 64 samples were collected from each packinghouse. All sites were zones 2–4; some common sampling sites included equipment frames, footings, drains, floor wall junctures, floor cracks, storage rooms (controlled atmosphere, final product, etc.), forklifts, forklift stops, leaf bins, etc. Samplings 1 and 6 were performed by members of the Cornell team, whereas the remaining samplings were performed by packinghouse personnel (the same people swabbed all packinghouses at each sampling).

All environmental samples were collected using sterile sponges hydrated with Dey Engley neutralizing buffer (3M, Saint Paul, MN); sample collectors changed their gloves between the collection of each sample. All samples were collected during production, except for the samples collected in "sampling 2.5" which were collected pre-production (Table 2.2). All samples were shipped overnight on ice to the Cornell Food Safety Lab for processing.

Sample processing and Listeria isolation. All samples were processed with 24h of collection, except: (i) packinghouses A, B, and D samples for sampling 2.5 were processed within 48h, (ii) all sampling 4.5 samples were processed within 72h, and (iii) all sampling 6 samples were processed within 96–144h due to shipping delays.

A modified FDA BAM protocol was used for *Listeria* isolation (20). Briefly, 90 mL of buffered *Listeria* enrichment broth (BLEB; BD, Franklin Lakes, NJ) was added to each sponge sample. The samples were then stomached for 1 min at 230 RPM and incubated at 30°C for 48h. After 4h of incubation, 360 µL of *Listeria* selective enrichment supplement (LSES; Oxoid, Basingstoke, UK) was added to each sample. Then, 50µL of each sample enrichment was plated on modified Oxford agar (MOX; BD) and *Listeria monocytogenes* plating media (LMPM; Biosynth International, Itasca, IL) at both 24h and 48h into incubation. All MOX plates were incubated at 30°C for 48h and all LMPM plates were incubated at 35°C for 48h. Up to six characteristic *Listeria* colonies were selected from each positive sample and sub-streaked on brain heart infusion agar; characteristic *Listeria* colonies are pewter and dimpled on MOX and round, blue (*L. monocytogenes*, *L. ivanovii*, or hemolytic *L. innocua*) or white colonies (other *Listeria* species) on LMPM.

Listeria subtyping. Confirmation of all presumptive *Listeria* isolates was performed using PCR of a portion of the *sigB* gene, followed by subsequent sequencing of the PCR product for species identification and allelic type (AT) assignment for a preliminary assessment of the *Listeria* subtypes present in the packinghouses. The protocol for *sigB* PCR and sequencing used in the current study was described by Sullivan and Wiedmann (42).

In addition, the *sigB* AT data was utilized to identify which isolates would be selected for further subtyping by whole genome sequencing (WGS). All *sigB* ATs isolated from a given packinghouse at least eight times and on at least three sampling events were identified; then, one representative isolate of each of these *sigB* ATs from each positive sample was selected for WGS. In total, WGS was performed on 286 isolates (140, 10, 63, and 73 from packinghouses A, B, C, and D, respectively). Results from WGS are not finalized, and as such, will not be discussed further in this report.

APC testing. In order to determine if using high-dose PAA (325 ppm) was more effective for brush bed sanitation as compared to a quat-based sanitizer (Geron IV; Anderson Chemical Company, Litchfield, MN), APC testing of the brush beds was performed before and after cleaning and sanitation. Specifically, a weekly rotation of the two sanitizers was performed (e.g., week 1 the quat-based sanitizer was used every day, week 2 high-dose PAA was used every day, week 3 the quat-based sanitizer was used every day, etc.). On a randomly selected day, 6 brushes were swabbed using swabs hydrated with 10 mL Letheen Broth (3M) prior to cleaning and sanitation. Then, after cleaning and sanitation, the same 6 brushes were swabbed again. This sampling scheme was repeated two times when the quat-based sanitizer was used and one time when the high-dose PAA was used.

All samples were transported on ice to an independent testing lab and processed within 24h of arrival. The samples were diluted, and 1 mL of each dilution was plated on APC Petrifilm (3M). The plates were incubated at 35°C for 48h.

Water sampling. Samples from the air conditioning water tanks were collected and tested for *Listeria*. The tanks that hold the water used in the air conditioning units are open, and in several cases, leakage was observed from the air conditioning units into cold storage rooms. As such, we were interested in determining if the tank water was a potential source of *Listeria* in the packing environments. At each sampling, 1 L of water was collected from each water tank (2, 1, 2, and 1 water tanks were sampled in packinghouses A, B, C, and D, respectively). In total 30 water samples were collected. The timeline of water sampling is provided in Table 2.2. The water samples were shipped on ice overnight and processed with 72h of arrival.

To process the samples, the water was vacuum filtered through 0.45-µm filters. The filters from each sample were then aseptically transferred to separate Whirl-Pak bags. The same enrichment, plating, isolate selection, and subtyping procedures were used as described above for the environmental *Listeria* samples.

Statistical analysis. All data was cleaned, visualized, and analyzed in R version 4.0.0 (38). Mixed effects logistic regression was performed using the lme4 package to determine if there was a difference in the log odds of a sample being *Listeria* positive before and after intervention implementation in each of the packinghouses. Only samples collected during production were included in the analyses; the forklift only sample collections were also excluded from these models. A separate model was fit for each packinghouse. Intervention status (was the sample collected before or after intervention implementation) and date of sampling were tested for inclusion as fixed effects and site was forced in the model as a random effect. The model outcome was the percentage of samples positive for *Listeria*. Univariable logistic regression was

first performed, and any variable with a *p*-value <0.1 were tested for inclusion in the multivariable logistic regression model. To identify the final multivariable logistic regression model for each packinghouse, backwards selection was performed to identify the model with the lowest Akaike information criterion (AIC). For a model to be selected as the final model, its AIC value had to be at least 2 less than the next simplest model.

Mixed effects logistic regression was performed to determine if the log odds of a forklift sample being *Listeria* positive decreased after implementing the new cleaning and sanitation protocol in packinghouses B, C, and D. The same protocol for the regression analysis was used as described above, except site nested within packinghouse was included as a random effect. A Fisher's exact test was also performed to determine if there was an association between a forklift sample being positive for *Listeria* and if the sample was collected in the week after the cleaning and sanitation intervention or if it was collected two weeks after the cleaning and sanitation intervention; only samples collected after intervention implementation were included in this analysis.

Objective 2 Results

Listeria prevalence and population in environmental samples. The overall percent of *Listeria* (*Listeria* spp. including *L. monocytogenes*) positive samples in packinghouse A, B, C, and D was 31% (124/398), 5% (12/234), 16% (62/394), and 24% (74/313), respectively, across all samplings (**Figure 2.3**). *L. monocytogenes*, *L. innocua*, *L. seeligeri*, and *L. welshimeri* were isolated from the packinghouses. All species were isolated from all packinghouses, except *L. welshimeri* was not isolated from packinghouse B. In addition, *L. monocytogenes* was only isolated from 1 sample (N= 234 in total) in packinghouse B.

In packinghouse A, the percent of *Listeria* positive samples before and after intervention implementation was 33% (62/187) and 25% (49/197), respectively. In addition, during the sampling conducted before production but after cleaning and sanitation (pre-interventions) the percent of *Listeria* positive samples was 58% (14/24); the high percent of positive samples is likely attributed to the fact that only sites that were positive in prior samplings were sampled (Figure 2.3). In packinghouse B, the percent of *Listeria* positive samples before and after intervention implementation was 3% (4/116) and 7% (8/111), respectively. In addition, <17% (0/6) of samples were positive for *Listeria* in the pre-production sampling (the sampling was conducted after cleaning and sanitation) (Figure 2.3). In packinghouse C, the percent of *Listeria* positive samples before and after intervention implementation was 27% (50/188) and 5% (10/186), respectively. In addition, 13% of samples were positive for *Listeria* in the pre-production sampling (Figure 2.3). In packinghouse D, the percent of *Listeria* positive samples before and after intervention implementation was 25% (36/146) and 20% (30/149), respectively. In addition, 53% of samples were positive for *Listeria* in pre-production sampling (Figure 2.3).

While there was a decrease in the log odds of a sample testing *Listeria* positive after intervention implementation in packinghouses A, C, and D compared to before intervention implementation, the decrease was only significant in packinghouse C ($P<0.001$) according to mixed effects logistic regression at a significance level of 0.05; however, the decrease was marginally significant in packinghouse A ($P=0.07$) (**Table 2.3**). Interestingly, there was an increase in the log odds of a sample testing *Listeria* positive after intervention implementation in packinghouse B compared to before intervention implementation; however, this increase was not significant ($P=0.31$) (Table 2.3). We suspect this increase was due to a change in sampling sites following sampling 2.5 in packinghouse B, which revealed additional areas of concern in the packinghouse that were not addressed in the root cause analysis. As such, this highlights that RCA must be a dynamic process that allows for changes to be made as more information becomes available (e.g., through continued sampling).

Sites that were repeatedly positive across packinghouses were forklift stops, forklifts, drains, floor wall junctures (especially those without proper curbing to prevent pooling of water at the base of the wall), rot bins and the floor under rot bins, floors cracks and puddles under air conditioning units in cold storage and controlled atmosphere storage rooms, and wax area equipment frames. In addition, in packinghouse A, a portion of the packinghouse had wooden walls that were repeatedly positive (samples were collected from 2 sites on the wooden walls: one was positive on 5 out of 7 sampling events and the other was positive on 3 of 7 sampling events). In packinghouse B, there was an open PVC pipe that drained the catch pans directly under the brush bed area to drain water from the catch pan to the drain; this site was positive with the same *sigB* AT on 3 out of 4 sampling events, indicating persistence. The PVC pipe is difficult to reach, which likely prevented it from being properly cleaned and sanitized and facilitated the development of persistent *Listeria*. In packinghouse C, catch pans under brush beds, a PVC pipe that drained the catch pans, and a cross bar under the brush beds were also repeatedly positive for *Listeria*. In packinghouse D, 8 out of 8 samples collected in the waxing area were positive on at least one sampling event; the same *sigB* AT was also isolated from all positive sites in this area, providing evidence of persistence. These sites included a decal sticker on top of the waxer and a chain that was set into the equipment frame, making it difficult to clean and sanitize; similar sites were also positive in packinghouse A. We hypothesized the buildup of wax in the area trapped the *Listeria* cells and protected them during cleaning and sanitation. Regardless, similar sites or areas should be considered for inclusion in environmental monitoring programs in produce operations.

Forklift interventions. Repeated *Listeria* positives were found on the forklifts in all packinghouses over the course of the study. For instance, in packinghouse A, four of the five forklift sites were positive for *Listeria* at least once, and three of the four positive sites were positive on more than one sampling date. In packinghouse B, three of the five forklift sites were each positive on one sampling occasion. In packinghouse C, seven of the eight forklift sites were positive at least once and four of the seven sites that were positive were positive on more than one sampling event. In packinghouse D, three out of four forklift sites were positive on at least one sampling event and one of the three positive sites was positive more than once.

An updated cleaning and sanitation protocol was evaluated in packinghouses B, C, and D, which included the use of foamers for applying the cleaner and sanitizer. To test the effectiveness of the protocol and to determine the frequency of cleaning and sanitation required to control *Listeria* on the forklifts, forklift cleaning and sanitation was performed monthly (i.e., the first Saturday or Sunday of a given month). Then, sampling of the forklifts was performed on the week after cleaning and sanitation (to determine if the cleaning and sanitation was effective) and two weeks after cleaning and sanitation (to determine if cleaning and sanitation should be performed once a month or twice a month).

Overall, there were significantly lower log odds of a sample being positive for *Listeria* after implementing the new forklift cleaning and sanitation protocol (P=0.04), indicating the new cleaning and sanitation protocol was effective. There was no significant association between a forklift sample being positive for *Listeria* and if that sample was collected in the week following the forklift cleaning and sanitation intervention or if it was collected two weeks following cleaning and sanitation. The lack of a significant association may indicate there was truly no further change in *Listeria* presence on the forklifts in the two weeks following cleaning and sanitation, and that cleaning and sanitizing forklifts once a month is sufficient. However, only 26 samples were collected at the two-week time point, and therefore, additional sampling is required to determine if this is truly the case at a sufficient level of power.

Updated cleaning and sanitation protocols. Through root cause analysis, deficiencies in the cleaning and sanitation protocols were identified in all packinghouses. The updated protocols excluded high-pressure hoses (and replacing their use with additional employees, brushes, and low-pressure hoses), used foamers for applying cleaners and sanitizers, and ensured the order of chemical application was correct.

In packinghouse A, prior to intervention implementation, pressure washers with heated water were used for cleaning and sanitation of the wet end of the packing area (i.e., the bin dump, the flume, brush beds, and wax area). As an intervention, an updated cleaning and sanitation protocol was implemented in the wet end of the packing area, which included foamers for applying the cleaner and quat-based sanitizer and elimination of pressure washers from the protocol. While there was a marginally significant decrease in the log odds of a sample testing positive for *Listeria* after intervention implementation (P=0.07) (described above), there was still a high percent of samples positive for *Listeria* (i.e., 25%, 49/197). In addition, while there were *sigB* ATs repeatedly isolated from the packing environment, they are common *sigB* ATs (i.e., isolated from more than one site and packinghouse), and as such, WGS data is needed to truly understand the effectiveness of the intervention at eliminating persistent *Listeria*. Furthermore, there likely was not a dramatic decrease in the percent of *Listeria* positive samples following implementation of the new cleaning and sanitation protocol because *Listeria* was likely present within multiple niches within the equipment and/or facility that cannot be easily reached, even when using enhanced cleaning and sanitation. Therefore, a complete breakdown of equipment prior to cleaning and sanitation is likely required to better reduce the levels of *Listeria* in this packinghouse.

In packinghouse C, there were two catch pans and a PVC pipe draining the first catch pan that were positive prior to intervention implementation; the first catch pan was positive on 2 of 4 pre-intervention samplings, the PVC that drained the first catch pan was positive on 3 of 4 pre-intervention samplings, and the second catch pan was positive on 1 of 4 pre-intervention samplings. In addition, while there were *sigB* ATs repeatedly isolated from these sites pre-intervention, these *sigB* ATs were also isolated multiple times from other packinghouses; this indicates they may be common and whole genome sequencing is required to identify if they were persisting in this area. As an intervention to reduce *Listeria* in this area, the catch pans were removed from under the brush beds weekly and fully taken apart (there were pieces on each catch pan where two pieces of metal overlapped and could be harboring *Listeria*). After implementation of this intervention, only 1 sample from the PVC pipe draining the first catch pan was positive (N= 3 total post-intervention samples from this site). In addition, zero of the six catch pan samples were positive after intervention implementation. This, in addition to the significant reduction of *Listeria* in the packinghouse, indicates this intervention was likely effective.

In packinghouse C, all 5 samples collected in the waxing area were positive after the first sampling (i.e., from a drain, a floor wall juncture, a puddle by the floor wall juncture, a floor crack, and a wire tray under the wax brushes). After this sampling, a second set of wax brushes was obtained, and the two sets of brushes were switched weekly. When a given set of brushes was not being used, the brushes were soaked in a quat-based sanitizer and allowed to dry completely over the course of the week. After initiating this new protocol, only 1 sample in this area was positive for *Listeria* (i.e., a floor crack under the waxer) on sampling 3 (before implementation of the remaining interventions). Since the *Listeria* obtained from all wax area sites in the first samplings had the same two *sigB* ATs as the *Listeria* isolated from the floor crack under the waxer in the third sampling, we hypothesized the *Listeria* may be persisting in the wall where *Listeria* was isolated from during the first sampling (the wall has no curb, and the floor is sloped towards the wall facilitating water pooling). However, WGS results are needed to confirm this hypothesis, as both *sigB* ATs are common. As such, the pressure washer originally

used to clean the wax area was eliminated (i.e., to prevent potential spread of *Listeria* from the wall/floor to the waxing equipment) and replaced with brushes (one set for the equipment frame and one set for the walls). Additional employees were also allocated for cleaning this area to account for the additional time it would take for cleaning due to elimination of the pressure washer. After implementation of the brushes and adding cleaning and sanitation employees, *Listeria* was not isolated from this area. However, due to the infrequent isolation of *Listeria* from this area, more extensive sampling over a longer time period is necessary to determine the true effectiveness of this intervention.

In packinghouse D, all 8 samples within the wax area were positive on at least one sampling event. In addition, the same *sigB* AT was isolated from all 30 positive samples from the wax area. While this *sigB* AT is a commonly isolated AT, the consistent isolation of the AT from this area provides evidence for persistence. Prior to intervention implementation cleaning and sanitation was performed by: (i) using an alkaline cleaner, (ii) using a wax stripping chemical, then (iii) sanitation. The use of the wax stripping chemical after the alkaline cleaner makes total elimination of the organic matter and apple juices trapped under the wax build up difficult (i.e., because the wax acts as a barrier, so the detergent is unable to reach the organic matter and juices to remove them from the equipment). This can help to facilitate persistence. As such, a preliminary intervention was tested to determine if using the wax stripping chemical prior to the alkaline cleaner would help reduce the *Listeria* in this area. However, continued persistence was observed in this area. The lack of effectiveness was not an unexpected result, as the widespread persistence observed in this area indicates *Listeria* harborage points were present in the equipment, which allows the *Listeria* to evade cleaning and sanitation. As such, to eliminate this *Listeria* population, a complete breakdown and deep cleaning event of this area is likely needed.

Brush bed cleaning and sanitation protocols. In packinghouse C, repeat *Listeria* positives were found in the catch pans below the brush beds, as well as other sites in the area (e.g., a PVC pipe draining the first catch pan, cross bars under the brush beds). As such, it was hypothesized that *Listeria* may be living on the brush beds, and repeatedly contaminating the surrounding area. There are two flumes and two brush beds in the area; the first flume uses PAA as an antimicrobial agent in the wash water, and the second uses chlorine as an antimicrobial agent in the wash water. There is a brush bed after each flume. To determine if an alternative sanitizer would be effective at reducing *Listeria* in the area, high-dose PAA (325 ppm) and a quat-based sanitizer (Geron IV) were used on a weekly rotation, and the effectiveness of the sanitizers was tested using APC testing before and after cleaning and sanitation. As testing of the high-dose PAA was only performed on one day, no statistical analyses were performed. The median reduction in APC for the swabs collected when the quat-based sanitizer was used was 2.4 $\log_{10}$ CFU/swab, whereas the median increase in APC for the swabs collected when the high-dose PAA was used was 0.2 $\log_{10}$ CFU/mL (**Figure 2.4**). However, it should be noted that the lack of an observed reduction with the high-dose PAA may be false, as one of the brushes swabbed was above the limit of detection before and after cleaning and sanitation. While there was an increase in $\log_{10}$ CFU/mL after cleaning and sanitation with high-dose PAA, there was a lower starting level of bacteria on the brushes and there were more variable log reductions (e.g., there was a reduction in counts on some brushes and an increase on others). Therefore, the true effect of each sanitizer is not clear. Regardless, the reductions seen on brushes in harder-to-reach areas appeared to be less than those of fully exposed brushes. As such, more time should be spent on adequately cleaning and sanitizing hard-to-reach brushes rather than testing alternative sanitizers.

With that said, there was a reduction in *Listeria* positive samples in this wet area (i.e., the area that contained the flumes and brush beds) before and after intervention implementation

(16% (16/101) before and 2% (2/94) after; 32 of the 62 total sites where samples collected in this packinghouse were from the wet area). Regardless, additional work would be needed to fully understand the impact of the sanitizer rotations on *Listeria* presence. However, changes to the cleaning and sanitation protocols of the catch pans were included as part of the interventions and may be responsible for the reduction in *Listeria* positives.

Water sampling. Water samples were collected from the open water tanks used in the air conditioning system in each packinghouse. The water from 2 tanks were treated with an antimicrobial, whereas the water from 4 tanks were untreated. In total, 13% (4/30) of water samples were positive for *Listeria*; <11% (0/9) of treated water tank samples and 19% (4/21) of untreated water tank samples were positive for *Listeria*, respectively (**Table 2.4**). Therefore, while this water appears to be sporadically positive, it may serve as a source of *Listeria* in the packing environment (e.g., due to leaks in the air conditioning systems) when positive. Produce operations should consider treating the water in air conditioning systems if the system is prone to leakage, especially in areas where leakage may come in contact with exposed product.

See **Table 2.5** for a summary of findings on persistence of *Listeria* in produce operations.

Objective 3 Methods

Agent-based models (ABMs) of 2 produce packinghouses developed as part of a previous CPS project “Modeling tools for design of science-based *Listeria* environmental monitoring programs and corrective action strategies” (Barnett-Neefs et al., in preparation) have been modified for the purpose of this project. Each model was created using NetLogo 6.2.0 and simulated the introduction and spread of *Listeria* into each facility and across interior agents. Specifically, two apple packinghouses were represented in ABMs, using data collected from observation, expert opinion, and literature. Model parameters that were represented as probability distributions were controlled by a global random seed independent from the rest of the model to ensure a repeatable stream of values was chosen from the distribution between simulations of modeled scenarios. Each iteration within a scenario was also controlled with a local random seed to further ensure repeatability during simulations. Agents represented key pieces of equipment or surfaces within the facilities, which included observed attributes such as surface area, cleanability, water level, hygienic zone, and were modelled alongside connectivity to other agents and local presence of environmental water on the floor or ceiling and traffic activity (referred to as a “patch”). Contamination introduction was possible via three independent methods: (i) contamination on incoming product, represented by contaminating a designated “receiving” agent whereby product would enter the facility; (ii) zone 4 contamination of agents and patches within specific distances of designated doors, representing staff entry; and (iii) random contamination of any agent/patch within the model, representing less easily predicted phenomena such as ceiling drips. Each simulated facility was given a weekly sanitation schedule based on the corresponding facility’s real sanitation schedule, which dictated which agents were cleaned and how frequently. The models operated in hourly progressions of time units. Some of the agents in the model for each facility were characterized as inherently uncleanable, i.e., at risk of niche forming if they become contaminated; these sites are thereafter referred as “niche sites”. This means that if such an agent becomes contaminated with *Listeria*, the facility’s routine sanitation would not be able to clean it and the agent would remain contaminated, thus forming a contamination niche. Agents characterized as “niche sites” represented equipment designed in a way that makes them difficult to thoroughly clean as part of the routine sanitation events. In reality, these sites would be cleaned following dismantlement of the entire equipment to access normally inaccessible parts (i.e., deep cleaning).

For persistence modelling, both agent-based models were run for a virtual period of 8 weeks with simulated environmental monitoring for the presence of *Listeria* performed once a week at midday. Additionally, each agent's contamination status (both *Listeria* presence/ absence and *Listeria* concentration if contaminated) was tracked on an hourly basis to establish continuous contamination histories (i.e.: the "true" agent history as opposed to inference based on simulated environmental monitoring). In addition to the baseline conditions, two different versions of introduction conditions were modelled: (i) all three *Listeria* introduction routes represented but all "niche sites" simulated as cleanable, simulating a situation where such equipment in the facility is redesigned to prevent niche formation, (ii) the removal of all *Listeria* introduction routes within the model, but a randomly selected "niche site" contaminated at the start of the simulation. "Persistent" contamination was defined as contamination (according to "true" contamination history) that remained following two major sanitation events within the model. The actual time taken for a contaminated site to be deemed "persistently" contaminated varied between models, with Model A performing weekly sanitation (i.e.: at least two weeks were needed before contamination could be declared persistent), and Model B performing a combination of daily and weekly sanitation for different agents (i.e.: only two days were required for some, and two weeks for others). Agents that showed reoccurring contamination were termed "transient" when identified using "true" contamination history. "Apparent persistence" was used when the persistence phenomena was observed using simulated environmental sampling. With the establishment of functional persistence models and methods to measure critical relevant occurrences (i.e., being able to identified between "true persistence", "apparent persistence" and "transient" contamination), these ABMs also served as platforms to test the performance of selected corrective action strategies.

Objective 3 Results

Preliminary analysis of the model indicated (i) the strong likelihood of reoccurring contamination of agents that would have been classified as "apparent persistence" according to weekly simulated environmental monitoring, but comparison against hourly monitoring showed this be misidentification of persistent transient contamination (**Figure 3.1**). This implies that it is possible misidentification of an agent's contamination behavior (i.e., "persistent transient" mistaken for "apparent persistence") could lead to the incorrect handling of controlling agent contamination, as a persistent transient contamination may be more effectively prevented by preventing cross-contamination rather than modifying agent cleanability. However, this does not eliminate the occurrence of "true" persistent contamination (**Figure 3.2**). (ii) In a scenario where all incoming contamination was prevented, a preexisting *Listeria* harborage point with connections to the rest of the model could rapidly contaminate the rest of the system and establish *Listeria* within other accessible niches (**Figure 3.3A** and **3.3B**). However, as *Listeria* transmission within a facility is dependent on cross-contamination occurrences, *Listeria* harborage points that have either no or very few connections to other agents (i.e., compartmentalization) would result in relatively reduced facility-wide contamination (**Figure 3.C**). These initial findings suggest that models can be used to assess different interventions and help focus implemented interventions on (i) sites that show "true persistence" and (ii) sites that are most likely to lead to more widespread contamination (due to their connectivity).

Outcomes and Accomplishments

A rapid review was conducted, as laid out by objective 1 of this project, which identified 1,656 studies to be assessed for inclusion. These 1,656 studies were reduced to 32 studies that met all inclusion criteria. A summary of locations persistent *Listeria* was isolated from, risk factors identified by previous studies that lead to persistent *Listeria*, and interventions to eliminate persistent *Listeria* were compiled. In addition, deficiencies in the current knowledge of persistent *Listeria* in produce operations, and food processing environments, were identified.

A longitudinal study was conducted, as laid out by objective 2 of this project, to test identified interventions in a real-world setting. Environmental sampling and intervention implementation was performed in four apple packinghouses. While testing was not performed in any fresh-cut produce operations, the use of multiple apple packinghouses allowed for testing of similar interventions across operations; the replication of interventions allowed us to determine which were effective across space. Updated forklift cleaning and sanitation protocols were tested in three of the packinghouses, updated cleaning and sanitation protocols of the wet area of the packinghouse were tested in two of the packinghouses, and updated cleaning and sanitation protocols of the was areas were tested in two packinghouses, among other interventions. A total of 1,339 environmental samples and 30 water samples were tested for *Listeria*. From those samples, 1,419 isolates were collected and subjected to *sigB* PCR and sequencing, and 286 isolates were subjected to WGS to get a comprehensive understanding of persistent and persistent transient *Listeria* populations in produce operations. The testing of water from air conditioning systems also identified another source of *Listeria* in produce operations. APC testing was also performed to determine if different sanitizer chemistries were more effective at reducing microbial populations on brush beds.

Two previously constructed agent-based models of packinghouses (Barnett-Neefs et al., in preparation) were modified to verify the effect of interventions on persistent *Listeria*, as laid out by objective 3 of this project. In this context “validation” means that there was no statistically significant difference between model predictions about *Listeria* presence in different locations in the simulated facilities and independent historical data from environmental monitoring in those same facilities. Based on the successful confirmation of model validity, the model can be used for prediction and analysis of *Listeria* dynamics in the facilities. We extended the prediction time horizon in those models from two weeks in the original model to eight weeks to be able to evaluate *Listeria* persistence in the facilities. The project allows hourly evaluation of *Listeria* dynamics at each location in a facility, synchronous evaluation of multiple locations in the facility (also at 1 hour time intervals), and evaluation of all agents over multiple model iterations. This 3-dimensional evaluation (agents x hours x iteration) provided a unique new view of possible *Listeria* dynamics in a facility that would never be possible based on data from environmental monitoring only. However, we are continually reminded of the computational requirements that agent-based models carry. To manage that we had to simplify a cross-contamination function in the model where the number of *Listeria* exceeds the computational capacity of the software used to stop the models from crashing. Additionally, we have not yet been able to evaluate selected interventions using those models. The agent-based models described here are now used in a new collaboration as part of the USDA/NIFA funded “Artificial Intelligence Institute for Next-Generation Food Systems” with University of California – Davis. As part of this new collaboration our agent-based modeling framework serves as a basis for a new project on statistical inference and learning for food safety assessment and prediction under privacy and resource constraints.

Summary of Findings and Recommendations

See **Table 2.5** for a summary of the summary and observations of the learnings of the current study.

Common sites of persistent *Listeria* from the rapid review (objective 1) and sites of repeat isolation from the longitudinal study (objective 2) include floor drains, floor wall junctures (including floors and walls in separate samples), forklifts, forklift stops, tables, slicers, wax area equipment frames, catch pans, PVC pipes, among other sites. Industry should consider these sites in their own routine and for-cause investigation sampling plans when a lack of *Listeria* control is identified. Academia should also include similar sites in future studies of persistence in produce operations and should consider studies that develop additional intervention strategies to eliminate persistent *Listeria* from these sites.

Monthly cleaning and sanitation of forklifts, which utilizes foamers for application of cleaning and sanitation chemicals, appears to be effective at controlling *Listeria* on forklifts. However, testing in each individual operation is recommended to confirm a monthly frequency is truly sufficient due to differences from operation to operation.

Cleaning and sanitation protocols that involve breaking down equipment to expose potential niches appear to be effective for eliminating persistent *Listeria* or repeat isolation of *Listeria*, based on the rapid review and the longitudinal study.

Alternative sanitizer chemistries may be more effective than one another; however, identifying locations on a piece of equipment that are difficult to reach may be more important for sufficient cleaning and sanitation. Additional research is needed to confirm this hypothesis.

Agent-based models provide an alternative strategy for testing resource intensive interventions for eliminating persistent *Listeria* from produce operations. However, modifications made to the models to allow for tracking persistence require large amounts of computing power.

Additional studies are needed that assess *Listeria* persistent in produce operations, and universal definitions of persistence should be developed to facilitate comparison of findings across studies. These areas represent topics that should be considered in future studies.

APPENDICES

Publications and Presentations

Belias, A., Sullivan, G., Wiedmann, M., and Ivanek, R. 2021. Factors that contribute to persistent *Listeria* in food processing facilities and relevant interventions: a rapid review. *In preparation*.

Budget Summary

The following is a summary of the funds expended as of April 30, 2021:

Category	**Budget**	**Funds Expended to Date**
Salary & wages	44,135.00	43,113.97
Compensation, graduate, & sabbatical	13,672.00	13,664.00
Employee benefits	(1,411.00)	3,998.60
Travel – domestic	2,514.00	2,513.80
Materials & supplies	34,120.00	33,989.83
Services	0	18,435.04
Other direct expenses	30,839.00	4,000.31
Limitation of funds	0	0
Indirect expenses	3,325.00	3,220.22
Total	**127,194.00**	**122,935.77**

Tables and Figures

Table 1.1. Categories used to extract data from the papers deemed relevant for inclusion.

Category	Description
Study type	Review or original research (observational or experimental)
Facility type	Facility type (e.g., packing house, fresh cut produce, dairy, etc.); include number of facilities/ each type of facility
Country	Country
Date	Range of dates when study was conducted
Sampling scheme	Length of study, collection frequency, when during processing, sample number
Sampling zone	Zones samples collected from (1-4, or FCS vs. NFCS)
Controls	If applicable (yes/ no, if yes, what type of controls)
Randomization	If applicable (yes/ no, if yes, what type of randomization)
Swabbing method	Sponges, cotton swabs
Detection method	Microbiological methods for enrichment and isolation
Species	Subtyping only for *L. monocytogenes*, or all *Listeria* spp.
Prevalence	Prevalence of *Listeria* spp. and/ or *L. monocytogenes* (plant level)
Subtyping method	Subtyping method
Authors – persistence?	Did the authors define persistence?
Authors – definition	If yes, definition of persistence (copy and pasted from paper)
Observed persistence	Number of sampling events a given subtype was isolated during over what period of time
Persistence locations	Locations of persistent *Listeria* (sampling sites positive for persistent strains)
Persistence risk factors	Risk factors for persistent *Listeria* (if applicable)
Interventions	Interventions tested to eliminate persistent *Listeria* (if applicable) – include conclusions for each of the interventions

Table 1.2. Description of study exclusion reasons from the full text[a] review stage.

Reason	Number of studies
No matching subtypes found at least 3 times over 2 months	70
No description of sampling sites	63
Did not use a substantive[b] subtyping method	48
No full text article could be located	19
Not original research or a review of original research	12
Lab studies	10
Only discussed food product testing or modeling	7
Not in English	2
Conducted in the wrong environment	1

[a]In total, 264 studies were assessed at the full text review stage for eligibility. Of those, 232 were excluded and 32 were deemed relevant for inclusion.

[b]Subtyping methods classified as "substantive" are amplified fragment length polymorphism (AFLP), multi locus variable-number tandem repeat analysis (MLVA), multi virulence locus sequence typing (MVLST), pulse field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), restriction endonuclease analysis (REA), Ribotyping, and whole genome sequencing (WGS)

Table 1.3. Characteristics of the studies deemed relevant for inclusion.

Study	Facility type (No. of facilities)	Country[a]	Study length[a]	Collection frequency[a]	Timing[a,b,c]	No. of samples[a]	Sample sites[a,d]	Swabbing method[a]	Species[e]	Subtyping method[f]
Blatter et al., 2010	Sandwich (1)	Switzerland	12 months	Twice/ week	During production and after C&S	2,028	Both	Swabs	LM	PFGE
Bolocan et al., 2015	RTE meat (1)	Romania	10 months	every 2-3 months	During production and before C&S	166	Both	Sponges	LM	PFGE
Camargo et al., 2015	Slaughterhouse (1)	Brazil	NS	13 total samplings	Before and during production	178	FCS	Sponges	LM	PFGE
Cao et al., 2006	Frozen shrimp (1)	NS	6 months	Every 2 months	NS	NS	Both	Sponges	LM	RAPD
Cesare et al., 2007	Cheese (1)	Italy	2 months	6 samplings	NS	52	FCS	Swabs	LM	Ribotyping
Ciccio et al., 2012	Cold-smoked salmon (1)	Italy	6 years	10 samplings	NS	95	FCS	Sponges	LM	PFGE
Cruz & Fletcher, 2011	Mussels (3)	New Zealand	2 years	NS	NS	37,241	NFCS	NS	LM	PFGE
Dalmasso & Jordan, 2013	Unspecified (1)	Ireland	10 months	Every 1-2 months	During production and before C&S	205	Both	Sponges	LM	PFGE
Dauphin et al., 2001	Smoked salmon (3)	NS	4-7 months	NS	During production and after C&S	32	Both	NS	LM	PFGE
Fagerlund et al., 2016	Salmon (1)	Norway	14 years	NS	Some after C&S	NS	FCS	Cloths	LM	MLVA and WGS
Fox et al., 2011	Cheese (16)	Ireland	2 years	NS	During production	NS	Both	Sponges	LM	PFGE
Giovannacci et al., 1999	Pork slaughter and cutting (5)	France	1 year	Every 2 weeks to every 2 months	During production and before and after C&S	NS	Both	NS	LM	PFGE and RAPD
Gudmundsdottir et al., 2006	Cooked shrimp (2)	Iceland	3.5 years	NS	During production and after C&S	466	Both	Swabs	LM	PFGE
Hoffman et al., 2003	Smoked fish (2)	NS	6 months	Approx. weekly	Beginning of production	512	Both	Sponges	LM	Ribotyping

Study	Facility type (No. of facilities)	Country[a]	Study length[a]	Collection frequency[a]	Timing[a,b,c]	No. of samples[a]	Sample sites[a,d]	Swabbing method[a]	Species[e]	Subtyping method[f]
Johansson et al., 1999	Fish (1)	Finland	14 months	NS	NS	163	Both	Swabs	LM	PFGE
Kabuki et al., 2004	Cheese (3)	US	6 months	4 samplings	NS	246	Both	Sponges	LM	Ribotyping
Keeratipibul & Techaruwichit, 2012	Cooked chicken (1)	Thailand	16 weeks	3 days a week, 6 times/ day	During production	7,643	Both	Swabs	L. spp.	RAPD
Keto-Timonen et al., 2007	RTE foods (1)	NS	8 years	Approx. weekly	During production and after C&S	4,554	NS	NS	LM	AFLP
Klaeboe et al., 2006	Raw seafood (1) & smoked seafood (1)	Norway	8 months	Twice daily approx. every 4 weeks	NS	NS	NS	NS	LM	Ribotyping
Lawrence & Gilmour, 1995	Poultry (1)	Ireland	6 months	Monthly	NS	NS	Both	NS	LM	RAPD
Lunden et al., 2002	Meat (3)	NS	NS	NS	NS	NS	Both	NS	LM	PFGE
Lunden et al., 2003	Meat (4)	NS	Several years	NS	NS	NS	Both	Sponges and swabs	LM	PFGE
Madden et al., 2018	Multiple products (24)	Ireland	18 months	Every 2 months	During production	1,203	Both	Sponges	LM	PFGE and WGS[g]
Melero et al., 2019a	Poultry (1)	Spain	1 year	10 samplings	During production and before C&S	250	Both	Sponges	LM	PFGE
Melero et al., 2019b	Cheese (1)	Spain	1 year	Every 1.5 months	During production and before C&S	413	Both	Sponges	LM	PFGE
Murugesan et al., 2015	Fresh-cut Mushroom (1)	NS	1 year	3 sampling events	During production	255	NFCS	Sponges and swabs	LM	MVLST
Nakamura et al., 2006	Cold-smoked fish (1)	Japan	2 years	Every season	NS	191	Both	Swabs	LM	PFGE
Norton et al., 2001	Cold-smoked fish (3)	US	6 months	5 samplings	NS	206	Both	Swabs	LM	Ribotyping
Ortiz et al., 2010	Pork (1)	Spain	3 years	Monthly	NS	801	Both	Sponges	LM	PFGE

Study	Facility type (No. of facilities)	Country[a]	Study length[a]	Collection frequency[a]	Timing[a,b,c]	No. of samples[a]	Sample sites[a,d]	Swabbing method[a]	Species[e]	Subtyping method[f]
Stessl et al., 2020	Meat (1)	Austria	5 years	6 samplings	NS	256	NFCS	Sponges	LM	PFGE
Veghova et al., 2017	Meat (1)	Slovakia	4 years	15 samplings	After production	196	Both	Sponges	LM	PFGE
Vongkamjan et al., 2017	Seafood (1)	Thailand	1.5 years	17 samplings	NS	372	Both	Sponges	LM	RAPD

[a]NS = not specified
[b]Timing refers to the time during production when samples were collected.
[c]C&S = cleaning and sanitation
[d]Either food contact surfaces (FCS), non-food contact surfaces (NFCS), or both.
[e]LM= *L. monocytogenes*, L. spp.= *Listeria* spp. (including *L. monocytogenes*)
[f]Acceptable subtyping methods are amplified fragment length polymorphism (AFLP), multi locus variable-number tandem repeat analysis (MLVA), multi virulence locus sequence typing (MVLST), pulse field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), restriction endonuclease analysis (REA), Ribotyping, and whole genome sequencing (WGS).
[g]WGS was only used for multi-locus sequence typing.

Table 1.4. Interventions tested to eliminate persistent *Listeria* from the processing environment in relevant studies

Study	Persistence locations	Intervention description	Intervention conclusions
Blatter et al., 2010	Slicers, conveyor belts, spattles, gloves, bread feeding machine, water hoses, air hoses, squeegee, floor, glove dispenser, drain, shelf, floor	Specialized deep cleaning and sanitation of more difficult to clean equipment (exact procedures were not provided)	There was a reduction in *Listeria* prevalence after implementation in the difficult to clean equipment (slicers and a bread slicing machine)
Cesare et al., 2007	Ripening boxes, cloths, salting equipment	Steaming of cheese ripening boxes and cloths Spraying equipment with a 0.3% sodium hypochlorite solution Disassembly of equipment before cleaning and sanitation	Effectiveness was not specified Not effective at eliminating persistent *Listeria* (67% of equipment samples were positive after implementation No *Listeria* positive samples after implementation
Delmasso & Jordan, 2013	Drains, floors, wheels of trolleys or other mobile equipment, boots in the changing room, floor of a storeroom, and in the washroom	Improved cleaning and sanitation protocols, which included a PAA sanitation step (no additional details were given)	Two out of 3 persistent PFGE type were eliminated and there was a reduction in the *L. monocytogenes* prevalence. The 3rd PFGE type was still found, but its prevalence was reduced
Dauphin et al., 2001	Floors, other locations were not specified.	An improved cleaning and sanitation program was implemented on the floors, which included hot steam, hot air, and hot water	*L. monocytogenes* was not found for 5 months following implementation
Gudmundsdottir et al., 2006	Floor, cooking equipment, pumping system to cooker, tubs, forklifts, conveyor belts, peeling machines, belt to peeling machine	Disassembly of the peeling machine before cleaning and sanitation	The persistent PFGE type was no longer found in the high-risk area of the plant following implementation
Klaeboe et al., 2006	Salting area, skinning machine (before and after smoking), portioning machine, filleting machine, slicing machine	A deep cleaning and sanitation event was conducted. This event included cleaning, disinfecting, and drying for 3 weeks during a production stop. The following interventions were also implemented: strict enforcement of the zones in the production area, removal of waste from the floor, and reduction of external traffic entering the production area	There was a reduction in 1 out of 2 of the persistent ribotypes after implementation. There was an increased in the 2nd persistent ribotype after implementation
Lunden et al., 2002	Exterior surface of the dicer, conveyor belt after dicer, cutting blades, control panel, tool kit. The persistent PFGE type was not found anywhere before the dicing	Deep cleaning and sanitation events were performed, which included daily and thorough dismantling of the dicer, alkali-acid-alkali washes	The 1 persistent PFGE type was no longer found in the plant after implementation

Study	Persistence locations	Intervention description	Intervention conclusions
	machine/ dicing area or in the raw product	every second week, and heating of small parts	
Murugesan et al., 2015	The persistent MVLST type was isolated from a floor crack and a floor crack at a trench drain on all 3 sampling events. It was also isolated from the following on at least one of the sampling events: floor cracks, equipment frame, floor under conveyor, pool of water at end of trench drain, steps, floor mat, crevice under hand washing station, squeegee, hand truck wheel, wheel of conveyor	Updates to cleaning and sanitation activities were made, including use of quaternary ammonium compound powder on floors, filling in of floor cracks, elimination of pressure washers during cleaning and sanitation, among other changes	There was a significant reduction in *Listeria* spp. prevalence in the plant, but the persistent MVLST type was still isolated after implementation
Nakamura et al., 2006	Slicing machine, floor in brining room, floor (water) in raw fish processing area, floor in drying room, floor in thawing room, floor (water) in front of brining room, floor in smoking room, washing conveyor belt after brining,	Disassembly of the slicing machine during cleaning and sanitation	There was a decrease in the percent of *L. monocytogenes* positive samples, however, there was an unknown effect on persistence
Ortiz et al., 2010	Cutting room equipment, slaughter room, loin marinating machine, grinding machine	Compartmentalization of the cutting room to limit transfer of *Listeria* to this area and updated cleaning and sanitation programs	There was no clear effect of the changes
Stessl et al., 2020	Drains (storage rooms, raw material processing areas, heat treatment areas, corridors) and overshoes	A basic cleaning and sanitation event and an intensified (deep) cleaning and sanitation event were performed Returning to normal production after a construction event (wall breaks throughs were closed, return to normal work and cleaning and sanitation)	There was a decrease in *Listeria* prevalence after implementation of both cleaning and sanitation events. Persistent PFGE types were still isolated There was an increase in *Listeria* prevalence after returning to normal production. Persistent PFGE type were still isolated

Table 2.1. Characteristics of the apple packinghouses (PH) included in the longitudinal study.

PH	Size - Ft^2 (m^2)	No. of Lines	Packing season	Packing schedule	Cleaning and sanitation frequency[a]	Cleaning and sanitation personnel
A	85,000 (7,896)	2	September to April	5 days/ wk	Daily	Line workers
B	Not provided	1	September to February	5 days/ wk	Daily	Line workers
C	36,000 (3,344)	1	September to March	5 days/ wk	Daily	Separate crew
D	40,400 (3,753)	1	September to March	5 days/ wk	Zone 1 daily, complete cleaning and sanitation weekly	Line workers

[a]Frequency before intervention implementation.

Table 2.2. Timeline of samplings and intervention implementation in the longitudinal study in the 4 apple packinghouses (PH).

Date(s)	Event	PH	Samples collected	Timing
11/11/20 & 11/13/20	Sampling 1	All	All environmental; water (C only)	During production
12/2/20 & 12/4/20	Sampling 2	All	All environmental; water	During production
12/14/20 & 12/15/20	Sampling 2.5	All	Environmental samples positive in samplings 1 & 2	Pre-production
1/12/21 & 1/14/21	Sampling 3	All	All environmental; water	During production
1/18/21 to 1/29/21	Intervention implementation	All	NA	NA
2/2/21 & 2/4/21	Sampling 4	All	All environmental; water	During production
2/15/21	Sampling 4.5	B, C, D	Forklifts only	During production
3/2/21 & 3/4/21	Sampling 5	All	All environmental; water	During production (A, C, D); after season (B)
3/15/21	Sampling 5.5	C & D	Forklifts only	During production
3/39/21 & 3/30/21	Sampling 6	All	All environmental; water (A, C, D)	During production (A); after season (B, C, D)

Table 2.3. Results of mixed effects logistic regression explaining the relationship between intervention status (i.e., if a sample was collected before or after intervention implementation) and the log odds of a sample being positive for *Listeria* in each of the packinghouses. Sampling site was included in each model as a random effect[a].

PH	Variable	Log-odds (95% confidence interval)	P-value
A	Intercept	-0.944 (-1.49, -0.45)	<0.001
	Intervention status (after)[b]	-0.48 (-1.01, 0.04)	0.07
B	Intercept	-4.59 (-9.15, -2.98)	<0.001
	Intervention status (after)[b]	0.69 (0.63, -2.14)	0.31
C	Intercept	-1.37 (-1.98, -0.87)	<0.001
	Intervention status (after)[b]	-2.10 (-2.98, -1.33)	<0.001
D	Intercept	-1.53 (-2.32, -0.88)	<0.001
	Intervention status (after)[b]	-0.40 (-1.07, 0.26)	0.24

[a]For the packinghouse A model, the residual variance and standard deviation for the site are 1.832 and 1.354, respectively. For the packinghouse B model, the residual variance and standard deviation for the site are 3.689 and 1.921, respectively. For the packinghouse C model, the residual variance and standard deviation for the site are 1.446 and 1.202, respectively. For the packinghouse D model, the residual variance and standard deviation for the site are 2.593 and 1.61, respectively.
[b]Intervention status refers to if the sample was collected before or after intervention implementation. “Before” is the baseline in all models.

Table 2.4. *Listeria sigB* allelic types (ATs) isolated from the water samples in each apple packinghouse (PH) on each of the samplings.

PH	ID	Treated[a]	S1[b]	S2[b]	S3[b]	S4[b]	S5[b]	S6[b]
A	#1	Yes	NS	-	-	-	-	-
	#2	No	NS	12	-	-	-	-
B	#1	Yes	NS	-	-	-	-	NS
C	#1	No	61	-	-	-	NS	-
	#2	No	62	-	6	-	-	-
D	#1	No	NS	-	-	-	-	-

[a]"Yes" indicates the water is treated with an antimicrobial and "No" indicates the water is not treated with an antimicrobial
[b]"NS" indicates a sample was not collected at the given sampling. "-" indicates a sample was negative for *Listeria* at the given sampling. S1, S2, S3, S4, S5, and S6, indicate samplings 1 through 6, respectively.

Table 2.5. General observations and recommendations on persistent *Listeria* for industry based on the observed results from the current study.

Area	Observation or recommendation
Wax area	*Listeria* persistence is common in the wax area of packinghouses and relevant produce processing facilities, as *Listeria* can be trapped under wax and evade cleaning and sanitation processes and chemicals. Breakdown of equipment is likely necessary to eliminate *Listeria* persistence in these areas.
High-pressure hoses	High pressure hoses can cause *Listeria* spread due to increased splash. Elimination of high-pressure hoses during the cleaning and sanitation process can be replaced by an increase in employees and brushes designated to specific pieces of equipment to reduce *Listeria* spread.
Equipment breakdown	Persistent *Listeria* is likely to be present in niches within the equipment that cannot be reached during regular cleaning and sanitation activities. If persistent *Listeria* is present, equipment breakdowns prior to cleaning and sanitation are likely necessary for elimination.
Air conditioning water	Open tanks that store water used in the air conditioning system can be contaminated with *Listeria*. Antimicrobial treatment of the water may be necessary, especially in air conditioning systems prone to leakage, to prevent this route of *Listeria* transfer in the operation.
Floor wall junctures	Presence and persistence of *Listeria* at floor wall junctures is possible, especially when there are no wall curbs or there is not proper slopping of floors away from walls to prevent pooling of moisture that supports *Listeria* growth.
Forklifts	Monthly cleaning and sanitation of forklifts, which utilizes foamers for application of cleaning and sanitation chemicals, appears to be effective at controlling *Listeria* on forklifts. However, testing in each individual operation is recommended to confirm a monthly frequency is truly sufficient due to differences from operation to operation.
Brush beds	While there may be marginal differences in the microbial reductions caused by different sanitizer chemistries on brush beds, identification of brushes that are in difficult to reach areas is necessary to ensure they are being properly cleaned and sanitized.

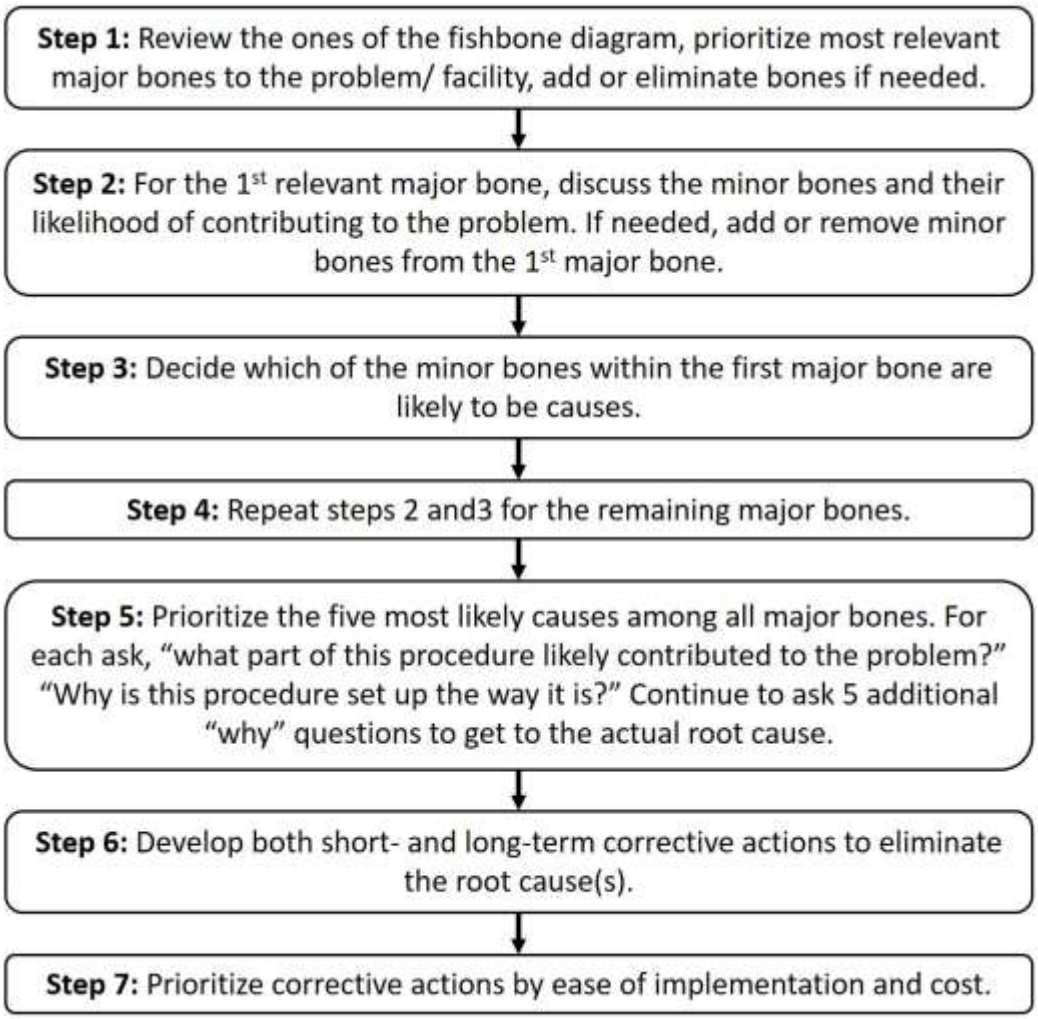


Figure 2.1. Steps for conducting a root cause analysis. Major bones refer to the seven broad categories in the fishbone diagram in Figure 2.2. Minor bones refer to the sub-categories within each of the seven major bones.

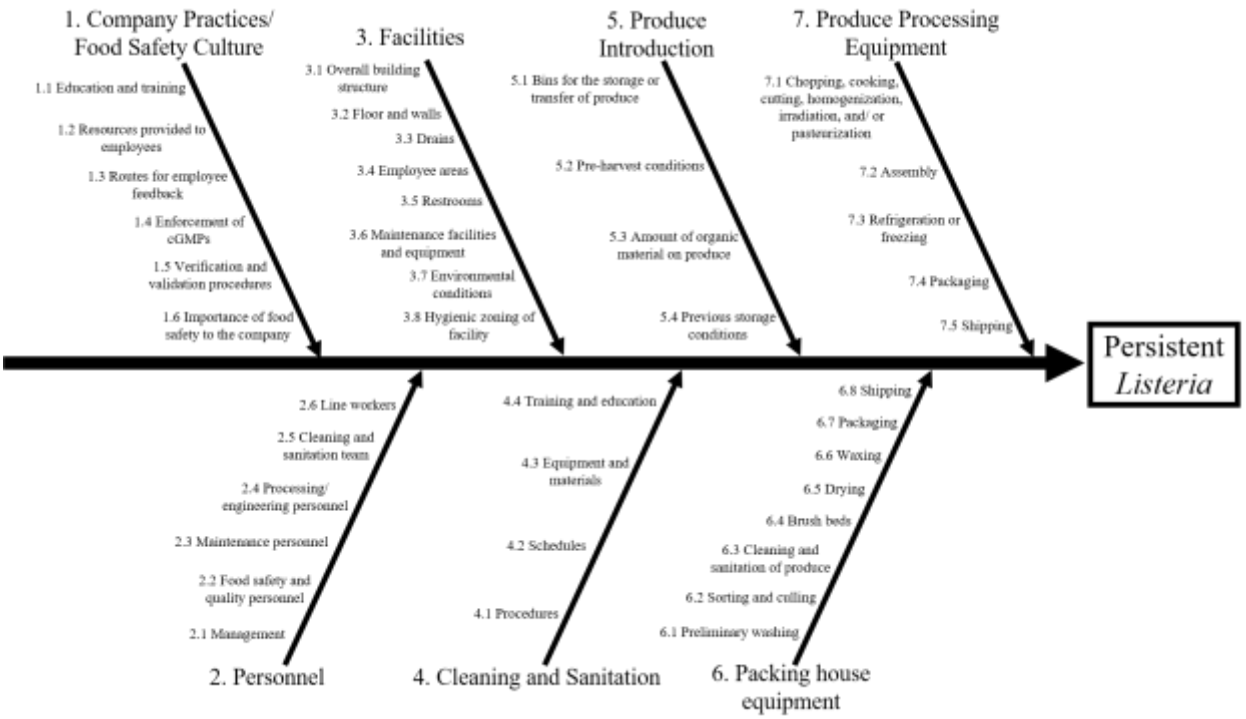


Figure 2.2. Fishbone diagram used in the brainstorming of identifying the root cause of persistent *Listeria* in produce operations.

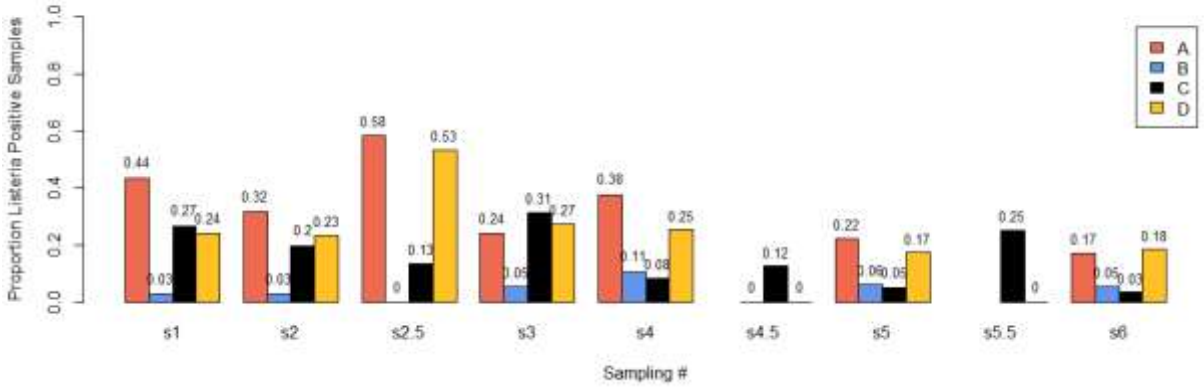


Figure 2.3. The percent of positive *Listeria* (*Listeria* spp. including *L. monocytogenes*) samples from each packinghouse in each of the samplings. Samplings 1, 2, 3, 4, 5, and 6 were conducted during production; sampling 2.5 was conducted pre-production but after cleaning and sanitation; and samplings 4.5 and 5.5 were only of forklifts.

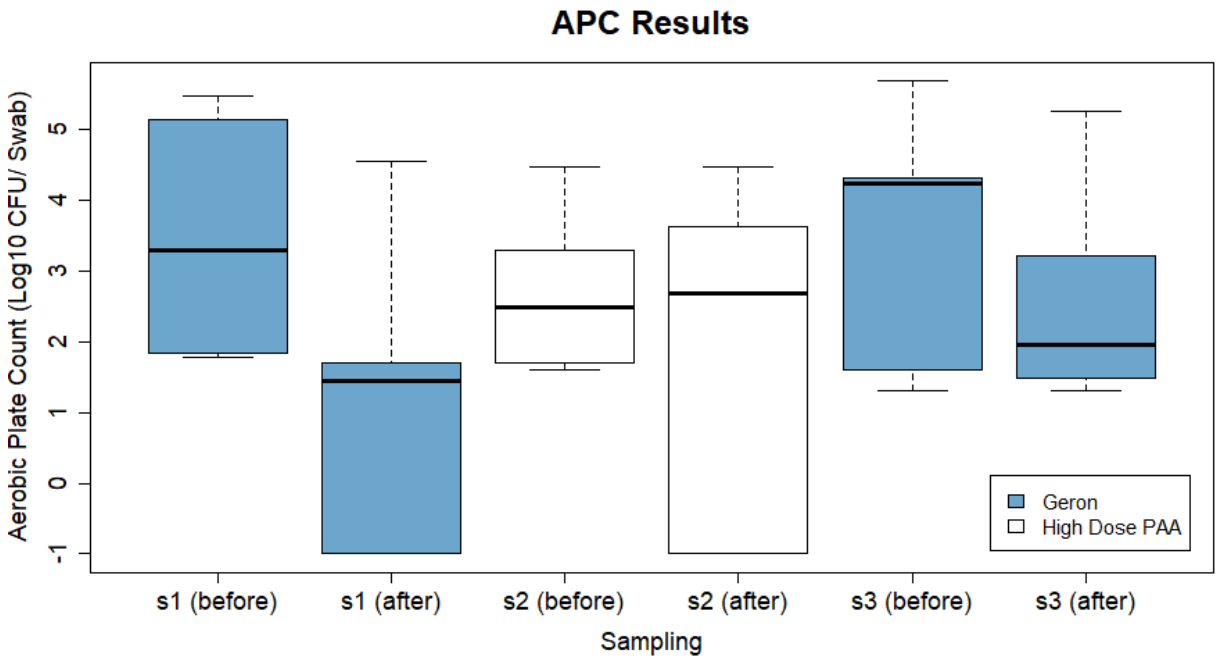
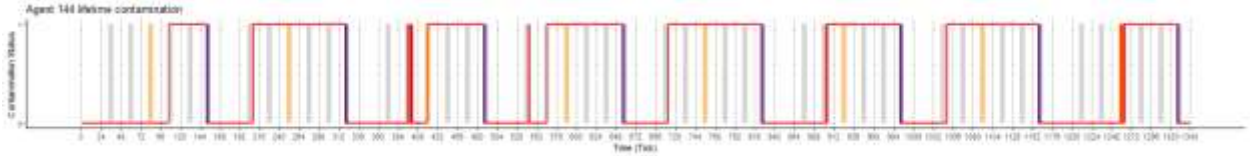
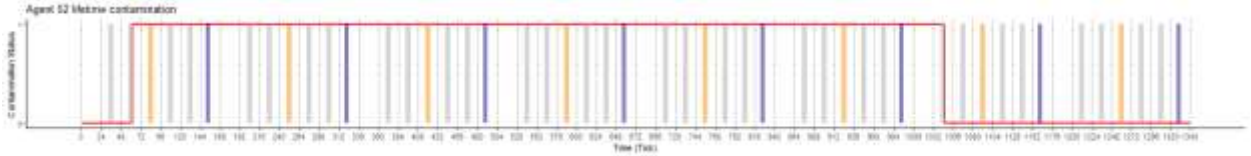


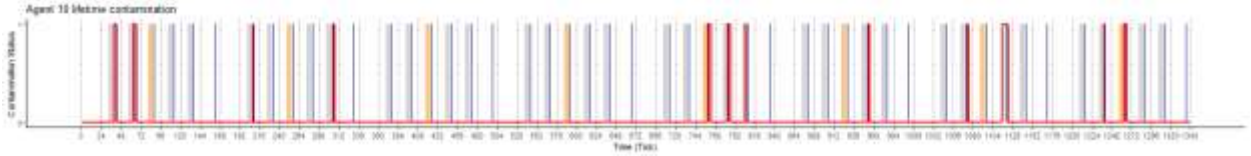
Figure 2.4. Aerobic plate count results from the brush beds before and after cleaning and sanitizing with high-dose peracetic acid (PAA, 325 ppm) and Geron IV (quaternary ammonium compound-based sanitizer).



1B: Agent 52 (belt in drying area) from Model A undergoing weekly sanitation that exhibits persistent contamination. There is a large tunnel that surrounds the belt, but the belt (and surrounding tunnel) is in the main packaging area/



1C: Agent 10 (belt in dryer room area) from Model B undergoing daily sanitation that exhibits transient contamination 11 times, but no persistent contamination. The dryer room are is isolated from the rest of the packing line.



1D: Agent 170 (box sticker printer in tray packing area) from Model B undergoing weekly sanitation that exhibits transient contamination 8 times, but no persistent contamination.

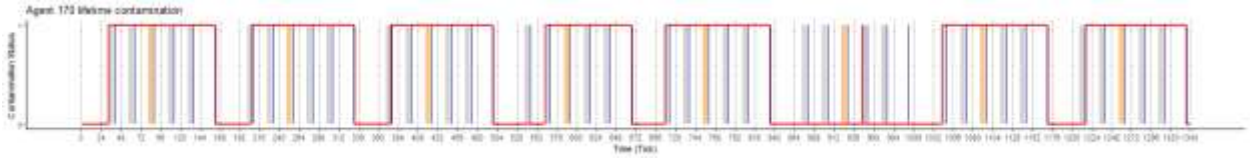


Figure 3.1. Hourly contamination status of agents in a scenario where all niche sites are simulated as cleanable surfaces and the facility is undergoing weekly or daily sanitation compared against contamination status inferred from simulated environmental monitoring. The contamination status (0: negative, 1: positive) of agents were observed hourly over the course of 8 weeks (work shifts highlighted in grey and sanitation events in blue) and compared against contamination status at the times (Wednesdays at midday) when simulated environmental monitoring was performed (orange)

1A: Agent 144 (belt in tray-packing area) from Model A undergoing weekly sanitation that exhibits transient contamination on 10 separate occasions, but no persistent contamination.

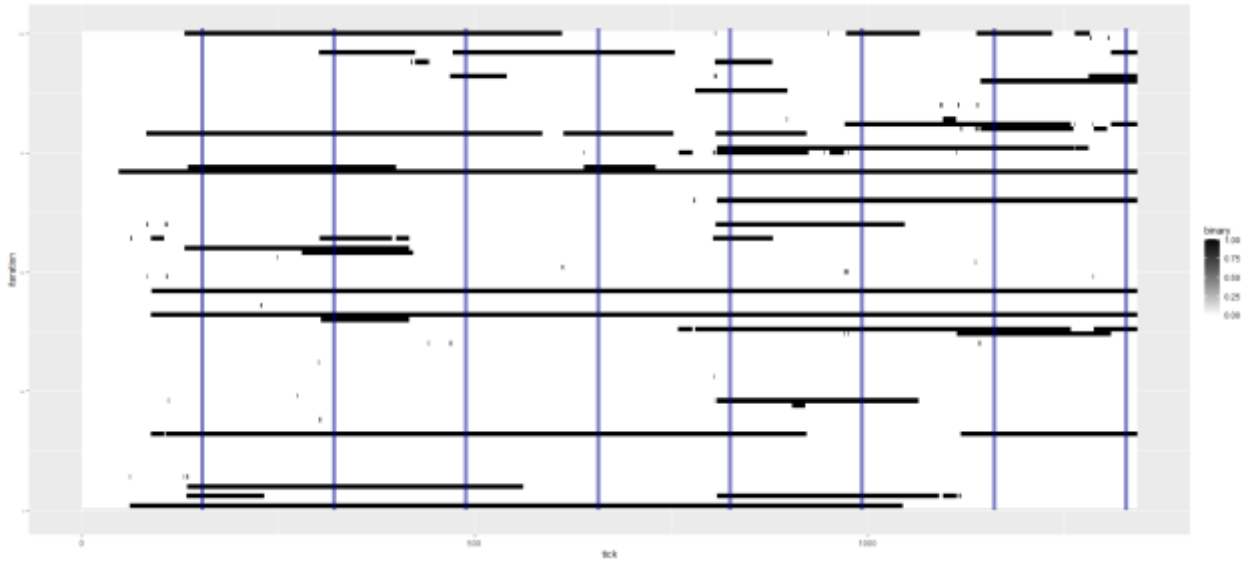


Figure 3.2. Heatmap of contamination status for Model A's agent 52 across all 100 iterations in a scenario where all niche sites are simulated as cleanable surfaces. Agent 52 (belt room in drying area) exhibited "true" persistence occurring in 13 of 100 iterations. Weekly sanitation events highlighted in blue.

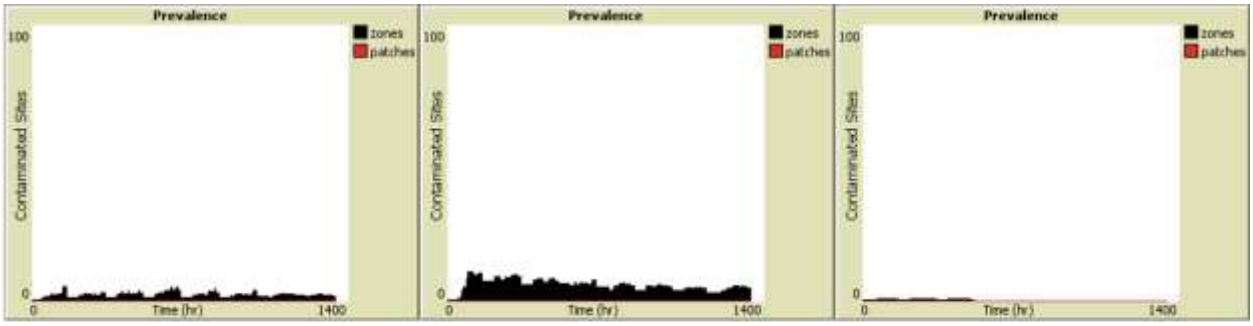


Figure 3.3.A. Initial contamination of agent 97 (produce scanner prior to final sorting): maximum agent prevalence of 5% across the entire facility caused by contamination of this niche site.

Figure 3.3.B Initial contamination of agent 49 (drain in cleaning area of packinghouse): maximum agent prevalence of 10% across the entire facility caused by contamination of this niche site.

Figure 3.3.C Initial contamination of agent 21 (isolated mop holder station): maximum agent prevalence of 0.4% across the entire facility caused by contamination of this niche site.

Figure 3.3. NetLogo's interface tracking model-wide prevalence of contaminated agents over eight simulated weeks in a scenario where all modes of *Listeria* introduction into the facility are prevented (i.e., no introduction via raw materials, Zone 4 and random events) and a single random niche agent is simulated as the source of *Listeria* contamination. A randomly selected niche agent was contaminated at the start of each scenario to track prevalence of agent contamination over time.

References cited

1. Belias, A. & Wiedmann, M. (2021). Hazards, risks, and challenges of *Listeria* in the food supply. *Food Safety Management in Practice*, In press.
2. Blatter, S., Giezendanner, N., Stephan, R., & Zweifel, C. (2010). Phenotypic and molecular typing of *Listeria monocytogenes* isolated from the processing environment and products of a sandwich-producing plant. *Food Control*, 21(11), 1519–1523.
3. Bolocan, A. S., Oniciuc, E. A., Alvarez-Ordonez, A., Wagner, M., Rychli, K., Jordan, K., & Nicolau, A. I. (2015). Putative cross-contamination routes of *Listeria monocytogenes* in a meat processing facility in Romania. *Journal of Food Protection*, 78(9), 1664–1674. https://doi.org/10.4315/0362-028X.JFP-14-539
4. Camargo, A. C., Dias, M. R., Cossi, M. V. C., Lanna, F. G. P. A., Cavicchioli, V. Q., Vallim, D. C., Pinto, P. S. de A., Hofer, E., & Nero, L. A. (2015). Serotypes and pulsotypes diversity of *Listeria monocytogenes* in a beef-processing environment. *Foodborne Pathogens and Disease*, 12(4), 323–326. https://doi.org/10.1089/fpd.2014.1875
5. Cao, J., Clarke, M., Witkowsky, R., Lu, H., Sayedahaman, A., Levin, R. E., & McLandsborough, L. A. (2006). Concentrations and tracking of *Listeria monocytogenes* strains in a seafood-processing environment using a most-probable-number enrichment procedure and randomly amplified polymorphic DNA analysis. *Journal of Food Protection*, 69(3), 489–494. https://doi.org/10.4315/0362-028X-69.3.489
6. Center for Produce Safety (2021). Funded research projects. Retrieved from https://www.centerforproducesafety.org/funded-research-projects.php. Accessed March 9, 2021.
7. Cesare, A. de, Manfreda, G., Macri, M., & Cantoni, C. (2007). Application of automated ribotyping to support the evaluation of *Listeria monocytogenes* sources in a Taleggio cheese producing plant. *Journal of Food Protection*, 70(5), 1116–1121.
8. Chapin, T. K., Nightingale, K. K., Worobo, R. W., Wiedmann, M., & Strawn, L. K. (2014). Geographical and meteorological factors associated with isolation of *Listeria* species in New York State produce production and natural environments. *Journal of Food Protection*, 77(11), 1919–1928. https://doi.org/10.4315/0362-028X.JFP-14-132
9. Ciccio, P. di, Meloni, D., Festino, A. R., Conter, M., Zanardi, E., Ghidini, S., Vergara, A., Mazzette, R., & Ianieri, A. (2012). Longitudinal study on the sources of *Listeria monocytogenes* contamination in cold-smoked salmon and its processing environment in Italy. *International Journal of Food Microbiology*, 158(1), 79–84. https://doi.org/10.1016/j.ijfoodmicro.2012.06.016
10. Covidence (2021). Retrieved from https://www.covidence.org/. Accessed January 1, 2021.
11. Cruz, C. D., & Fletcher, G. C. (2011). Prevalence and biofilm-forming ability of *Listeria monocytogenes* in New Zealand mussel (*Perna canaliculus*) processing plants. *Food Microbiology*, 28(7), 1387–1393.
12. Dalmasso, M., & Jordan, K. (2013). Process environment sampling can help to reduce the occurrence of *Listeria monocytogenes* in food processing facilities. *Irish Journal of Agricultural and Food Research*, 52(1), 93–100.
13. Dauphin, G., Ragimbeau, C., & Malle, P. (2001). Use of PFGE typing for tracing contamination with *Listeria monocytogenes* in three cold-smoked salmon processing plants.

International Journal of Food Microbiology, 64(1/2), 51–61. https://doi.org/10.1016/S0168-1605(00)00442-6

14. Fagerlund, A., Langsrud, S., Schirmer, B. C. T., Moretro, T., & Heir, E. (2016). Genome analysis of *Listeria monocytogenes* sequence type 8 strains persisting in salmon and poultry processing environments and comparison with related strains. *PLoS ONE*, 11(3). https://doi.org/10.1371/journal.pone.0151117
15. Falardeau, J., Johnson, R. P., Pagotto, F., & Wang, S. (2017). Occurrence, characterization, and potential predictors of verotoxigenic *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* in surface water used for produce irrigation in the Lower Mainland of British Columbia, Canada. *PLOS ONE*, 12(9), e0185437. https://doi.org/10.1371/journal.pone.0185437
16. Ferreira, V., Wiedmann, M., Teixeira, P., & Stasiewicz, M. J. (2014). *Listeria monocytogenes* persistence in food-associated environments: epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, 77(1), 150–170. https://doi.org/10.4315/0362-028X.JFP-13-150
17. Fox, E., Hunt, K., O'Brien, M., & Jordan, K. (2011). *Listeria monocytogenes* in Irish Farmhouse cheese processing environments. *International Journal of Food Microbiology*, 145(Suppl. 1), S39–S45. https://doi.org/10.1016/j.ijfoodmicro.2010.10.012
18. Giovannacci, I., Ragimbeau, C., Queguiner, S., Salvat, G., Vendeuvre, J. L., Carlier, V., & Ermel, G. (1999). *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. *International Journal of Food Microbiology*, 53(2/3), 127–140. https://doi.org/10.1016/S0168-1605(99)00141-5
19. Gudmundsdottir, S., Gudbjornsdottir, B., Einarsson, H., Kristinsson, K. G., & Kristjansson, M. (2006). Contamination of cooked peeled shrimp (*Pandalus borealis*) by *Listeria monocytogenes* during processing at two processing plants. *Journal of Food Protection*, 69(6), 1304–1311. https://doi.org/10.4315/0362-028X-69.6.1304
20. Hitchins, A. D., Jinneman, K., & Chen, Y. (31 October 2017). Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods, Chapter 10. In Bacteriological analytical manual (BAM). U.S. Food and Drug Administration, Washington, DC.
21. Hoffman, A. D., Gall, K. L., Norton, D. M., & Wiedmann, M. (2003). *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. *Journal of Food Protection*, 66(1), 52–60. https://doi.org/10.4315/0362-028X-66.1.52
22. Johansson, T., Rantala, L., Palmu, L., & Honkanen-Buzalski, T. (1999). Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish products and in a production plant. *International Journal of Food Microbiology*, 47(1/2), 111–119. https://doi.org/10.1016/S0168-1605(99)00019-7
23. Kabuki, D. Y., Kuaye, A. Y., Wiedmann, M., & Boor, K. J. (2004). Molecular subtyping and tracking of *Listeria monocytogenes* in Latin-style fresh-cheese processing plants. *Journal of Dairy Science*, 87(9), 2803–2812. https://doi.org/10.3168/jds.S0022-0302(04)73408-6
24. Keeratipibul, S., & Techaruwichit, P. (2012). Tracking sources of *Listeria* contamination in a cooked chicken meat factory by PCR-RAPD-based DNA fingerprinting. *Food Control*, 27(1), 64–72.

25. Keto-Timonen, R., Tolvanen, R., Lunden, J., & Korkeala, H. (2007). An 8-year surveillance of the diversity and persistence of *Listeria monocytogenes* in a chilled food processing plant analyzed by amplified fragment length polymorphism. *Journal of Food Protection*, 70(8), 1866–1873. https://doi.org/10.4315/0362-028X-70.8.1866

26. Klaeboe, H., Rosef, O., Fortes, E., & Wiedmann, M. (2006). Ribotype diversity of *Listeria monocytogenes* isolates from 2 salmon processing plants in Norway. *International Journal of Environmental Health Research*, 16(5), 375–383. https://doi.org/10.1080/09603120600869406

27. Lawrence, L. M., & Gilmour, A. (1995). Characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. *Applied and Environmental Microbiology*, 61(6), 2139–2144. https://doi.org/10.1128/AEM.61.6.2139-2144.1995

28. Lunden, J. M., Autio, T. J., & Korkeala, H. J. (2002). Transfer of persistent *Listeria monocytogenes* contamination between food-processing plants associated with a dicing machine. *Journal of Food Protection*, 65(7), 1129–1133. https://doi.org/10.4315/0362-028X-65.7.1129

29. Lunden, J. M., Autio, T. J., Sjoberg, A. M., & Korkeala, H. J. (2003). Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *Journal of Food Protection*, 66(11), 2062–2069. https://doi.org/10.4315/0362-028X-66.11.2062

30. Madden, R. H., Hutchison, M., Jordan, K., Pennone, V., Gundogdu, O., & Corcionivoschi, N. (2018). Prevalence and persistence of *Listeria monocytogenes* in premises and products of small food business operators in Northern Ireland. *Food Control*, 87, 70–78. https://doi.org/10.1016/j.foodcont.2017.12.020

31. Malley, T. J. V., Butts, J., & Wiedmann, M. (2015). Seek and Destroy Process: *Listeria monocytogenes* process controls in the ready-to-eat meat and poultry industry. *Journal of Food Protection*, 78(2), 436–445. https://doi.org/10.4315/0362-028X.JFP-13-507

32. Melero, B., Manso, B., Stessl, B., Hernandez, M., Wagner, M., Rovira, J., & Rodriguez-lazaro, D. (2019a). Distribution and persistence of *Listeria monocytogenes* in a heavily contaminated poultry processing facility. *Journal of Food Protection*, 82(9), 1524–1531. https://doi.org/10.4315/0362-028X.JFP-19-087

33. Melero, B., Stessl, B., Manso, B., Wagner, M., Esteban-Carbonero, O. J., Hernandez, M., Rovira, J., & Rodriguez-Lazaro, D. (2019b). *Listeria monocytogenes* colonization in a newly established dairy processing facility. *International Journal of Food Microbiology*, 289, 64–71. https://doi.org/10.1016/j.ijfoodmicro.2018.09.003

34. Murugesan, L., Kucerova, Z., Knabel, S. J., & LaBorde, L. F. (2015). Predominance and distribution of a persistent *Listeria monocytogenes* clone in a commercial fresh mushroom processing environment. *Journal of Food Protection*, 78(11), 1988–1998. https://doi.org/10.4315/0362-028X.JFP-15-195

35. Nakamura, H., Tokuda, Y., Sono, A., Koyama, T., Ogasawara, J., Hase, A., Haruki, K., & Nishikawa, Y. (2006). Molecular typing to trace Listeria monocytogenes isolated from cold-smoked fish to a contamination source in a processing plant. *Journal of Food Protection*, 69(4), 835–841. https://doi.org/10.4315/0362-028X-69.4.835

36. Norton, D. M., McCamey, M. A., Gall, K. L., Scarlett, J. M., Boor, K. J., & Wiedmann, M. (2001). Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish

processing industry. *Applied and Environmental Microbiology*, 67(1), 198–205. https://doi.org/10.1128/AEM.67.1.198-205.2001

37. Ortiz, S., Lopez, V., Villatoro, D., Lopez, P., Davila, J. C., & Martinez-Suarez, J. V. (2010). A 3-year surveillance of the genetic diversity and persistence of *Listeria monocytogenes* in an Iberian pig slaughterhouse and processing plant. *Foodborne Pathogens and Disease*, 7(10), 1177–1184. https://doi.org/10.1089/fpd.2010.0535
38. R Core Team. (2020). R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
39. Sauders, B. D., Overdevest, J., Fortes, E., Windham, K., Schukken, Y., Lembo, A., & Wiedmann, M. (2012). Diversity of *Listeria* species in urban and natural environments. *Applied and Environmental Microbiology*, 78(12), 4420–4433. https://doi.org/10.1128/AEM.00282-12
40. Stessl, B., Szakmary-Brandle, K., Vorberg, U., Schoder, D., & Wagner, M. (2020). Temporal analysis of the *Listeria monocytogenes* population structure in floor drains during reconstruction and expansion of a meat processing plant. *International Journal of Food Microbiology*, 314. https://doi.org/10.1016/j.ijfoodmicro.2019.108360
41. Strawn, L. K., Gröhn, Y. T., Warchocki, S., Worobo, R. W., Bihn, E. A., & Wiedmann, M. (2013). Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Applied and Environmental Microbiology*, 79(24), 7618–7627. https://doi.org/10.1128/AEM.02831-13
42. Sullivan, G. & Wiedmann, M. (2020). Detection and prevalence of *Listeria* in U. S. produce packinghouses and fresh-cut facilities. *Journal of Food Protection*, 83(10):1656-1666.
43. Tompkin, R. B. (2002). Control of *Listeria monocytogenes* in the food-processing environment. Journal of Food *Protection*, 65(4), 709–725. https://doi.org/10.4315/0362-028X-65.4.709
44. Veghova, A., Minarovicova, J., Korenova, J., Drahovska, H., & Kaclikova, E. (2017). Prevalence and tracing of persistent *Listeria monocytogenes* strains in meat processing facility production chain. *Journal of Food Safety*, 37(2). https://doi.org/10.1111/jfs.12315
45. Vongkamjan, K., Benjakul, S., Vu, H. T. K., & Vuddhakul, V. (2017). Longitudinal monitoring of *Listeria monocytogenes* and *Listeria* phages in seafood processing environments in Thailand. *Food Microbiology*, 66, 11–19. https://doi.org/10.1016/j.fm.2017.03.014
46. Weller, D., Wiedmann, M., & Strawn, L. K. (2015). Irrigation is significantly associated with an increased prevalence of *Listeria monocytogenes* in produce production environments in New York State. *Journal of Food Protection*, 78(6), 1132–1141. https://doi.org/10.4315/0362-028X.JFP-14-584
47. Wilkes, G., Edge, T., Gannon, V., Jokinen, C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E., & Lapen, D. R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research*, 43(8), 2209–2223. https://doi.org/10.1016/j.watres.2009.01.033
48. Zoellner, C., Ceres, K., Ghezzi-Kopel, K., Wiedmann, M., & Ivanek, R. (2018). Design elements of *Listeria* environmental monitoring programs in food processing facilities: a scoping review of research and guidance materials. *Comprehensive Reviews in Food Science and Food Safety*, 17, 1156-1171.