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Root Cause Analysis Can be Used to Identify and Reduce a Highly Diverse *Listeria* Population in an Apple Packinghouse: A Case Study

ABSTRACT

Root cause analysis (RCA) was utilized to identify *Listeria* elimination strategies in an apple packinghouse. While most of the *Listeria* was not persistent according to *sigB* allelic typing (i.e.,16 allelic types were isolated from 22 positive samples), the same packinghouse sites were continually positive. The root cause was identified as a limited understanding of how to eliminate *Listeria*. Based on these findings, we provided instructions on proper *Listeria* elimination strategies and supported implementation, including (i) increasing cleaning and sanitation from once to twice a week, (ii) use of quaternary ammonium compound (quat) powder around forklift stops and floor cracks, and (iii) removal of a dead-end pipe. Five samplings were conducted to test intervention effectiveness. While increased cleaning and sanitation frequency did not significantly reduce the log-odds of a site testing *Listeria* positive [11% (4/35) before and 13% (18/137) after, $P = 0.787$ by logistic regression], the site-specific interventions appeared to have controlled the *Listeria* at the respective sites. Specifically, after utilizing quat powder, *Listeria* was not isolated from the forklift stops or floor crack (0/5 samples positive after versus 6/10 samples positive before intervention). Therefore, this study provides a roadmap for performing RCA and implementing interventions for controlling *Listeria* in packinghouses.

INTRODUCTION

Listeria contamination of fruits and vegetables is an issue that can be traced back to contamination from pre-harvest or post-harvest sources (e.g., packinghouses, processing plants). There have been 32 recalls caused by *L. monocytogenes* contamination of fruit and vegetable products in the U.S. in 2019 and 2020 *(38)*. For example, there was a recall of apples in 2019 caused by *L. monocytogenes* contamination, which involved 2 bulk bins and 2,297 cases of apples *(37)*. In addition, there have been several *L. monocytogenes* illness outbreaks linked to produce *(8, 9, 10, 11, 12, 13)*. A *L. monocytogenes* illness outbreak linked to cantaloupes in 2011 *(8)* and a *L. monocytogenes* illness outbreak linked to caramel apples in 2014 *(10)* were of particular relevance; both outbreaks were linked to contamination in the packing environment *(10, 24)*.

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Packinghouses (i.e., a facility that washes, sorts, culls, grades, and/ or packages produce but does not perform any processing steps) *(15, 17, 27, 33, 35)* and food processing facilities *(5, 16, 19, 22, 25, 32)*, in general have been shown to often harbor *Listeria*. *Listeria* can be brought into the packing environment on produce or produce bins (via the preharvest environment), with employees serving as fomites, via forklifts or other transportation vehicles, among other routes *(16, 17, 28, 30, 31, 39, 40)*. Once *Listeria* has been transferred into a packinghouse, it can contaminate different pieces of equipment and the packinghouse structure itself (e.g., floors, walls, or drains). A lack of *Listeria* control in a packinghouse can be identified through environmental sampling when the same sites within a packinghouse are repeatedly positive. Through further subtyping (e.g., using pulsed field gel electrophoresis or whole genome sequencing) of the *Listeria* present in "repeat positive" sites, two scenarios can be identified: (i) "persistent *Listeria*" and (ii) "persistent transient *Listeria*." "Persistent *Listeria*" refers to the *Listeria* not removed from the packing environment (e.g., through cleaning and sanitation, facilitated by proper sanitary design of equipment), allowing it to survive and replicate over time and facilitating its spread in the packing environment as well as possible contamination of finished product *(16)*. On the other hand, "persistent transient *Listeria*" describes the scenario when the same sites within the packing environment are continuously positive but not necessarily with the same subtype of *Listeria* (e.g., due to continuous re-introduction of *Listeria* from the same sources) *(4)*. Direct traceback of product contamination to persistent transient *Listeria* populations is more difficult compared to persistent *Listeria* populations because of the larger number of subtypes present. However, similar to persistent *Listeria*, persistent transient *Listeria* can still lead to product contamination and subsequent recalls and illnesses. There is a third scenario referred to as "transient *Listeria*," which refers to the *Listeria* that enters the processing environment but is eliminated through routine cleaning and sanitation activities.

To avoid recalls and outbreaks it is necessary to identify sites of persistent *Listeria* and persistent transient *Listeria* within the packing environment and to subsequently implement corrective actions that eliminate contamination. However, only performing superficial "corrective" actions (e.g., re-cleaning and sanitation) may not truly eliminate contamination, leading to recurrence of contamination linked to the same root cause (e.g., an introduction vector that was not addressed). Therefore, identifying the root cause of the contamination or persistent *Listeria* in the packing environment is essential *(36)*. As such, this study aimed to develop, implement, and test a protocol for performing root cause analysis (RCA) to identify interventions to eliminate *Listeria*. While the study reported here used an apple packinghouse as a model system, the RCA protocol and procedures will be broadly applicable to different produce and food processing operations.

MATERIALS AND METHODS

Packinghouse characteristics and study design

An apple packinghouse in the northeastern U.S. was investigated in this case study to evaluate a root cause analysis (RCA) procedure for reducing or eliminating *Listeria* in produce operations. The packinghouse is approx. 25,000 ft^2 (approx. 2,300 m^2), has a single packing line, and runs from September to May each year. The packinghouse is regulated under the U.S. FDA rule for preventive controls for human food *(1)*, as >50% of apples they pack come from growers not under their management; the packinghouse is not located on a primary production farm *(2)*. The packinghouse performed daily dry cleaning (i.e., removal of leaves and debris from the equipment and floor) and performed full "wet" cleaning and sanitation weekly. The same employees who worked on the line during production also performed cleaning and sanitation activities.

Initial data on *Listeria* detection in the same packinghouse investigated in the case study reported here was reported by Sullivan and Wiedmann *(33)*, who designated this facility as "packinghouse A." This previously reported data was utilized in the current study (i) as proof of *Listeria* presence in the packinghouse, (ii) for the identification of sampling sites with a higher likelihood of being positive for *Listeria*, and (iii) as evidence in the RCA. In addition to this previously collected data, sample collection was also performed as part of the current study to test the effectiveness of the interventions.

In the current study, root cause analysis was performed to help identify interventions for reducing or eliminating persistent *Listeria* in the packing environment. To test the effectiveness of selected interventions, sampling of the packinghouse environment was performed before and after intervention implementation (see *Fig. 1* for a timeline of sampling and intervention implementation).

Root cause analysis and intervention implementation

RCA was performed using a fishbone diagram and the "5 why's procedure," utilizing the procedure recently described by Belias and Wiedmann *(4)*. RCA was performed in a 1.5 h meeting with the packinghouse manager, the quality assurance manager, the maintenance manager, and two members from the Cornell team (i.e., the RCA team) on October 25th, 2019. Briefly, a pre-constructed fishbone diagram was utilized as a brainstorming tool in the RCA *(Fig. 2)*; this fishbone diagram was adapted from Belias and Wiedmann *(4)* and modified to be specific to produce operations (i.e., packinghouse and processing facilities). There are 7 major bones in the fishbone diagram that represent high-level categories that can lead to *Listeria* problems: (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. The apples were not further processed, so the major bone "vii: produce processing equipment" was deemed

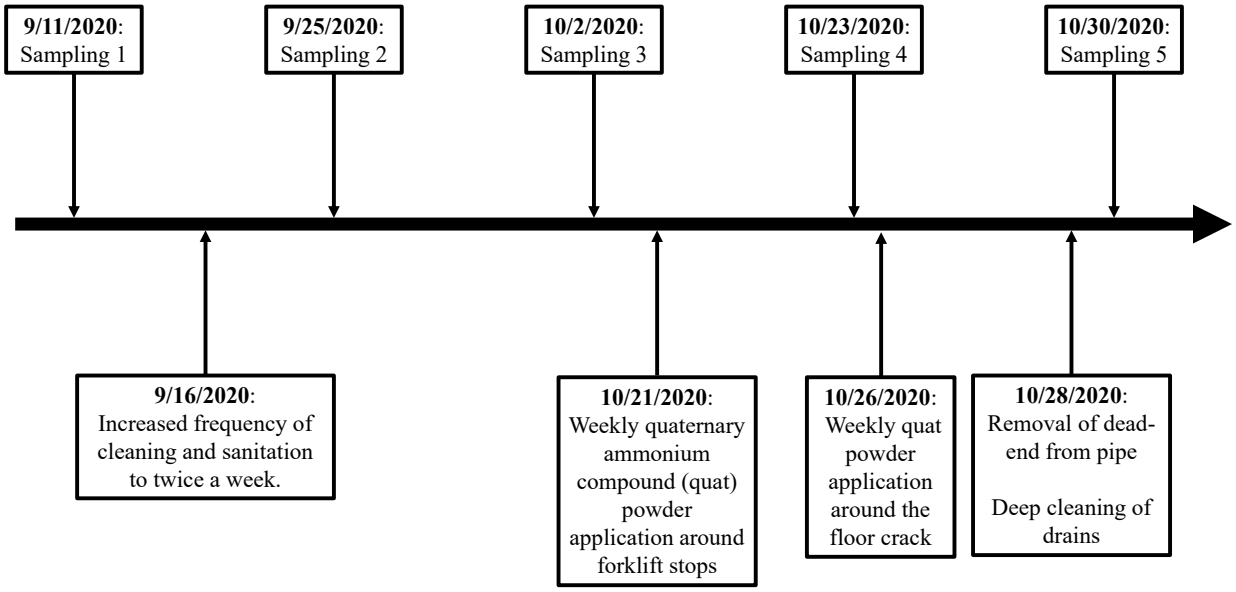

FIGURE 1. Sampling events and intervention implementation timeline for eliminating *Listeria* in the apple packinghouse. The root cause analysis for intervention identification was performed on October 25th, 2019.

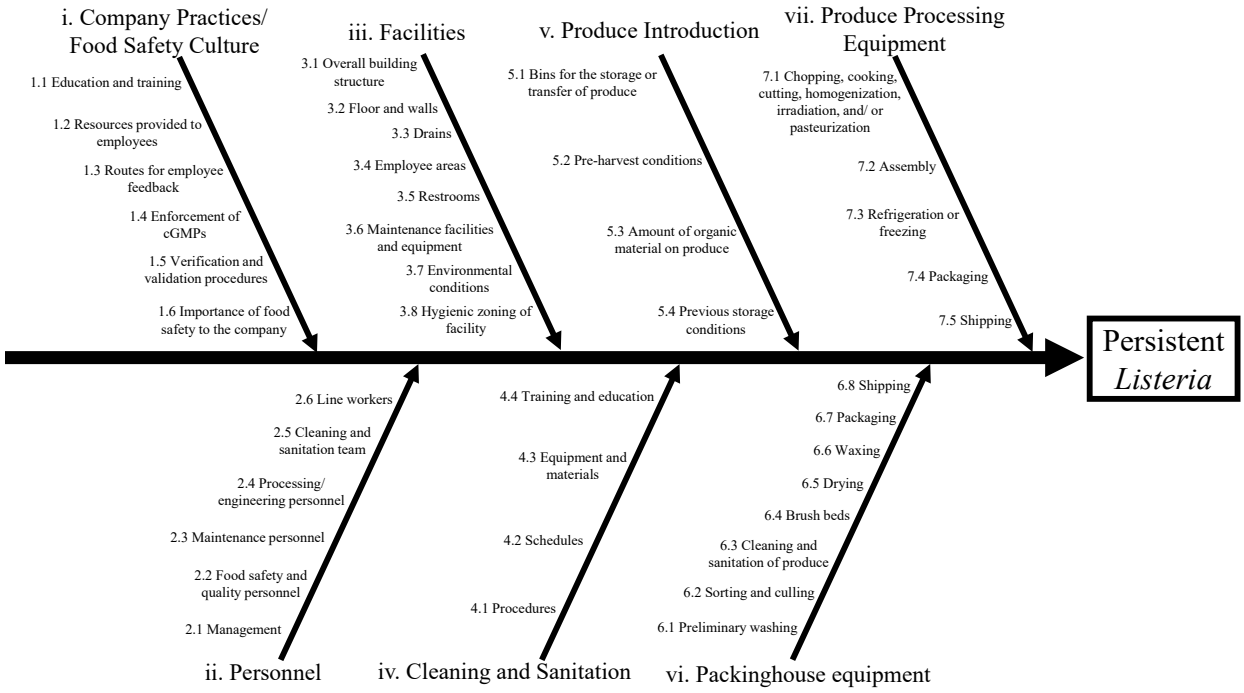

FIGURE 2. Fishbone diagram utilized in root cause analysis to identify interventions to eliminate *Listeria* in the packinghouse environment. This fishbone diagram was adapted from: Belias, A. and M. Wiedmann. 2021. Hazards, risks, and challenges of *Listeria* in the food supply. Food Safety Management in Practice. In press.

irrelevant and was therefore removed. Within each of these major bones, there are minor bones specifying more specific qualities of a produce operation. As with the major bones, these were modified, removed, or added based on relevance; for instance, forklifts and forklift stops were added as minor bones under the "facilities" major bone.

Based on the RCA, the following interventions were tested: (i) increasing cleaning and sanitation from once a week to twice a week, (ii) weekly (applied on Monday of each week) use of quat powder around forklift stops and floor cracks, (iii) a site-specific niche (a dead-end pipe) removal, and (iv) implementing a deep cleaning protocol in the drains.

Sample collection

To test the effectiveness of intervention implementation, we performed sampling of the packinghouse environment *(Fig. 1)* in addition to previous pre-intervention sampling reported by Sullivan and Wiedmann *(33)*. Five samplings were performed from September 1st, 2020 to October 30th, 2020, including one sampling before and 4 samplings after intervention implementation. All sampling events were performed on Fridays to test if the second cleaning and sanitation event in a given week (performed on Wednesdays) was associated with a lower log odds of a sample testing positive for *Listeria*; in pre-intervention sampling a higher percent of *Listeria* positive samples were seen in end-of-week samples as compared to early-in-week samples. On each visit, 35 samples were collected from the "wet-area" of the packing house (i.e., the area with the bin dump, flume, brush beds and waxing equipment); only 32 samples were collected on the final sampling event (October 30th) because 3 sampling sites were no longer present due to removal of the dead-end pipe. All samples were collected from zones 2 and 3 (i.e., no food contact surface samples were collected). The sampling sites included: (i) 11 PVC pipe samples, (ii) 9 drain samples, (iii) 2 forklift stop samples, (iv) 1 floor crack sample, and (v) 12 equipment frame samples *(Table S1)*. Samples were collected using sponges hydrated with Dey-Engley broth (3M, Saint Paul, MN). Sampling was performed at least 2 h into production. All samples were transported back to the lab on ice, then stored at 4°C until processing.

***Listeria* enrichment and isolation**

All samples were processed within 24h of collection using a modified version of the FDA BAM method *(18)*. Briefly, 90 mL of buffered *Listeria* enrichment broth (BLEB, BD, Franklin Lakes, NJ) was added to each sponge sample, followed by stomaching at 230 RPM for 1 minute and incubation at 30°C for a total of 48h. After the initial 4h of incubation, 360 µL of *Listeria* selective enrichment supplement (LSES, Oxoid, Basingstoke, UK) was added. At 24h and 48h into incubation, 50 µL of the enriched samples are streaked for isolation onto modified Oxford agar (MOX, BD) and *Listeria monocytogenes* plating medium (LMPM, Biosynth International, Itasca, IL). The MOX plates were incubated at 30°C for 48h and the LMPM plates were incubated at 35°C for 48h. After incubation, characteristic *Listeria* colonies were sub-streaked from the MOX and LMPM plates onto brain heart infusion agar plates (BHI, BD); characteristic *Listeria* colonies on MOX are dimpled and pewter and characteristic *L. monocytogenes* colonies on LMPM are round and blue. Up to 16 characteristic colonies per sample were sub-streaked onto BHI, such that up to 4 colonies were selected from 24h MOX plates, 24h LMPM plates, 48h MOX plates, and 48h LMPM plates. The BHI plates were incubated at 37°C for 24h. *sigB* sequencing and allelic typing

PCR and subsequent sequencing of a 660 bp fragment of *sigB* was performed on all characteristic *Listeria* colonies sub-streaked to BHI for species identification; allelic type (AT) assignment based on the *sigB* sequence was performed for a preliminary assessment of the *Listeria* subtypes present in the packinghouse *(23)*. PCR and sequencing were performed according to the protocol described by Sullivan and Wiedmann *(33)*. All isolates confirmed as *Listeria* were stored as 15% glycerol stocks at -80°C.

Statistical analysis

All data visualization, cleaning, and analyses were performed in R version 4.0.0 *(26)*. Logistic regression was performed to determine if there were significant differences in the percent of samples positive for *Listeria* before and after increasing cleaning and sanitation frequency to twice a week. Date of sampling and if the sample was collected before or after increased cleaning or sanitation were tested for inclusion in the models as potential explanatory factors. The model outcome was the percentage of samples positive for *Listeria*. To identify which of the explanatory factors were associated with the outcome, univariable logistic regression was first performed. Any variable with $P < 0.1$ was then included in a multivariable regression model. To identify which explanatory factors should be included in the final multivariable logistic regression model, backwards selection was performed to identify the model with the lowest Bayesian Information Criterion (BIC) value. For a model to be selected as the final model, its BIC value had to be at least 2 less than the next simplest model.

In addition, the relationship between the increased cleaning and sanitation frequency and the percent of *Listeria* positive samples was also assessed using the sampling data collected over multiple seasons before intervention implementation to determine if the interventions were effective compared to historical data. To do so, the dataset in the current study was combined with the *Listeria* presence/absence data reported by Sullivan and Wiedmann *(33)* for the same packinghouse. However, the sites from Sullivan and Wiedmann *(33)* included in the analyses in the current study

were reduced to only include sites collected from the "wet-end" of the packinghouse, as this was the only area sampled in the current study. In addition, only samplings on Thursdays or Fridays were included in the analyses performed here to determine the difference in samplings conducted after the second weekly cleaning and sanitation event (performed on Wednesdays). Logistic regression was used to determine the relationship between the log odds of a *Listeria* positive sample and if the sample was collected before or after increasing the frequency of cleaning and sanitation. The same procedure for logistic regression described above was used. No statistical analyses were performed to test the quat powder, pipe removal, and drain cleaning interventions because there were too few samples.

RESULTS

Root cause analysis

At the RCA meeting an RCA team was assembled, which included the packinghouse manager, the quality assurance manager, the maintenance manager, and 2 members of the Cornell team. The RCA team reviewed the historical results to identify instances of repeat *Listeria* isolation (i.e., both persistent and persistent transient) in the packinghouse and opportunities for corrective actions. The historical results showed, that while there were persistent *Listeria* strains present in the packinghouse in the first years of sampling (2017 and 2018; based on whole genome sequencing data), the persistent strains were no longer present in the following year (2019) *(34)*. However, there was a pattern of persistent transient *Listeria*, as indicated by the repeat isolation of *Listeria* of non-matching subtypes (according to WGS) from the same sites (e.g., drains, dead-end pipe and catch pan area, forklift stops) within the packinghouse. In addition, the historical results showed (i) the majority of the positives in the packinghouse were from the wet-end (i.e., the area with the dump tank, flume, brush beds, and waxing equipment), (ii) there tended to be a greater percentage of positive samples in samplings conducted at the end of the week compared to the beginning of the week (which is closer to the weekly cleaning and sanitation, which was originally performed on Saturday or Sunday), (iii) the deep square drains in the packinghouse were commonly positive, however after a deep cleaning event during the first year of sampling, a decrease in drain positives was observed, (iv) forklift stops at the dump tank loading area were commonly positive, and (v) sites by the catch pan below the brush bed, which is drained by a dead-end pipe, were commonly positive.

Next, brainstorming was performed by reviewing the fishbone diagram *(Fig. 2)*. Major bones (i.e., bones "i" to "vi"; see *Fig. 2*) were reviewed and prioritized in order of importance; the following 3 major bones were prioritized in this case: (iii) facilities, (iv) cleaning and sanitation, and (vi) packinghouse equipment. Within each of these major bones, the relevant minor bones were discussed to determine their likelihood of contributing to the repeat isolation of *Listeria*. After review and discussion of historical results with the RCA team, the minor bones that were potential causes requiring further explorations were determined. Once this was completed for all relevant minor bones, the 4 minor bones most likely to be the root cause of the repeat *Listeria* positive sites were identified. The 4 minor bones selected as being the most likely contributors to repeat isolation of *Listeria* in this case were (i) cleaning and sanitation protocols and schedules, (ii) the catch pan area (under the "packinghouse equipment" major bone), (iii) forklift stops (under the "facilities" major bone), and (iv) drains (under the "facilities" major bone). For each of these 4 minor bones, the "5 why's procedure" was performed by asking: "what part of this procedure likely contributed to the persistent *Listeria*?" and "why is this procedure set up the way it is?" Then, we continually asked 5 additional "why" questions to get to the actual root cause. For instance, if the identified problem was a persistent *Listeria* population at the end of the catch pan and dead-end pipe, the "5 Why" questions may be: (i) Why is the persistent *Listeria* population found in this area? Because the *Listeria* is living in the dead-end; (ii) Why is the *Listeria* living in the dead-end? Because moisture, apple juices/ organic matter, and *Listeria* cells get trapped in the dead-end; (iii) Why do these things get trapped in the dead-end? Because there is no easy way for these things to be removed from the dead-end (iv) Why is there no easy way for these things to be removed from the dead-end? Because it is difficult to get cleaning and sanitation chemicals, as well as brushes for mechanical cleaning, to reach the dead end; and (v) Why is the pipe and dead-end designed the way it is and why is a dead-end pipe in use in the facility? From there, long- and short-term corrective actions to eliminate the root cause(s) were identified. These corrective actions were then prioritized based on the cost and ease of implementation. A description of the root causes, long and short-term corrective actions that were identified, and the interventions tested in the packinghouse can be found in *Table 1*. See *Fig. 1* for the intervention implementation schedule.

Listeria population

Overall, 13% (22/172) of samples were positive for *Listeria* spp. (including *L. monocytogenes*) across the 5 sampling events performed as part of the study reported here. The 22 positive samples came from 9 sampling sites; these sites include (i) 3 sites from a single dead-end PVC pipe that drained the catch pan under the brush beds, (ii) 2 forklift stops, (iii) 1 floor crack, (iv) and 3 sites within drains (2 sites within a single square drain and 1 site from a trench drain leading into the square drain) *(Table 2)*.

The *Listeria* species isolated included *L. monocytogenes*, *L. innocua*, and *L. seeligeri*; *L. monocytogenes* was isolated from 11 samples, *L. innocua* was isolated from 5 samples, and *L. seeligeri* was isolated from 15 samples (with a total of 22

TABLE 1. List of root causes and corrective actions identified, and interventions tested to control repeatedly isolated *Listeria* in the packinghouse

Identified Problem	Minor Bone	Root Cause	Corrective Actions[a]		Interventions[b]
			Short-term	Long-term	
Increase in *Listeria* positives over the course of a week (between cleaning and sanitation events)	Cleaning and sanitation protocols and schedules	Not knowing how frequently cleaning and sanitation must be performed to control *Listeria*	(1) Increasing the frequency of cleaning and sanitation of the wet end to more than once a week (2) Improved cleaning and sanitation protocols (e.g., use of foamers to apply chemicals)	Hiring additional employees for a cleaning and sanitation (as opposed to having the line workers stay after their shifts)	Increasing cleaning and sanitation frequency of the wet end to twice a week (once on the weekend and once on Wednesday)
A persistent transient *Listeria* population around the forklift stops	Forklift stops	The forklift stops trap moisture and nutrients underneath to support *Listeria* growth and are difficult to clean and sanitize	(1) Use of quat[c] powder around forklift stops (2) Seasonal removal and deep cleaning of the forklift stops	Raise forklift stops to facilitate easier cleaning and sanitation	Weekly quat[c] powder application around the forklift stops
A persistent transient *Listeria* population in a floor crack	Floor crack	Floor cracks created when drains were constructed were never filled in	Use of quat[c] powder around floor crack	Filling in crack to eliminate the harborage point	Weekly quat[c] powder application in the floor crack
A persistent *Listeria* population present in the catch pan and a dead-end pipe that drains the catch-pan	Catch pan area under brush beds	The catch pain area has several plastic-metal and metal-metal junctures, as well as a dead-end pipe that are difficult to clean and sanitize	Removal of the dead-end from the pipe	Redesign the catch pan area to eliminate juncture points that can harbor *Listeria*	Removal of the dead-end from the pipe
Persistent and persistent transient *Listeria* populations in a square drain and a connected trench drain under the bin dump/ flume	Drains	The drains were not designed to be easily cleaned and sanitized	(1) Use of a cleaner sanitizer in a deep cleaning event (2) Use of quatc powder around the drain or quatc ring in the drain	Installation of a diaphragm pump in the square drains to facilitate draining and prevent the buildup of organic matter	Use of a cleaner sanitizer to perform deep cleaning of the drains

[a]Corrective actions refer to actions that were identified as potential solutions to address each root cause. These corrective actions were not all implemented in the current study due to time and budget constraints but provide examples of additional steps that can be taken to control *Listeria* associated with the identified root causes.

[b]Interventions refer to the actions taken in the current study to control *Listeria* associated with the identified root causes.

[c]quat = quaternary ammonium compound.

TABLE 2. *Listeria sigB* allelic types (ATs) isolated on each sampling date from the positive samples in the packinghouse

Site ID[a]	Site Description	*sigB* ATs Historically Isolated from Each Site[b]	*sigB* ATs Isolated on Each Sampling Date[c,d]				
			Sampling 1: 9/11/20	Sampling 2: 9/25/20	Sampling 3: 10/2/20	Sampling 4: 10/23/20	Sampling 5: 10/30/20
305	Pipe outlet	12, 61, 67	-	-	9	-	-
306	Dead-end portion of PVC pipe	Not sampled	-	-	9	-	-
309	Pipe inlet	12, 61, 67	-	-	9	9	9
318	Forklift stop, full bins	-	-	-	12	-	-
319	Forklift stop, empty bins	3, 57	23, 57, 61, 67	-	20, 24	-	-
320	Floor crack by a trench drain[e]	Not sampled	23, 61	163	-	31	-
328	Square drain, PVC pipe in drain[f]	2, 3, 12, 58, 60, 61, 103	22, 57, 67	-	4, 12, 58	-	58
329	Square drain, outer edge[f]	2, 3, 12, 58, 60, 61, 103	22, 57, 67	57, 61	7, 20, 57	-	20, 24, 58
335	Trench drain	1, 9, 57, 58, 61	-	7, 57, 60	3	20, 24	3, 20, 24, 57, 58

[a]Full descriptions of all sampling sites can be found in *Table S1*.

[b]Historical results were obtained from the following study: Sullivan, G. and M. Wiedmann. 2020. Detection and prevalence of *Listeria* in U.S. produce packinghouses and fresh-cut facilities. *J. Food Prot.* 83(10):1656–1666.

[c]*Listeria sigB* ATs 57, 58, 60, 61, 67, and 103 are *L. monocytogenes; Listeria sigB* ATs, 22, 23, and 31 are *L. innocua*, and *Listeria sigB* ATs 1, 2, 3, 4, 7, 9, 12, 20, 24, and 163 are *L. seeligeri.*

[d]"-" indicates the sample was negative on the given sampling date.

[e]Refers to a different trench drain than that described as site 335.

[f]Historical isolates represent both sites in the square drain: PVC pipe in the drain and the outer edge. Therefore, the same ATs are listed in the historical column for both sites.

positive samples). Of the 22 positive samples, 9 had 2 species present (4 samples were positive for *L. monocytogenes* and *L. innocua*, while 5 were positive for *L. monocytogenes* and *L. seeligeri*).

sigB allelic typing was performed on a total of 199 isolates obtained from the 22 positive samples; these isolates yielded 16 *sigB* allelic types (ATs) *(Table 2)*. Between 1 and 5 *sigB* ATs were isolated from each sample (mean=2 ATs per sample and standard deviation=1 AT per sample). In many cases, different *sigB* ATs were isolated from a given site on different sampling dates *(Table 2)*. For instance, 6 *sigB* ATs were isolated from a forklift stop (Sample ID 319); each AT was only isolated on one sampling date *(Table 2)*. In addition, among the 12 *sigB* ATs isolated from 2 sites within a single square drain and 1 site within the trench drain leading into the square drain, 6 ATs were only isolated on one sampling date *(Table 2)*, suggesting a persistent transient population. In the catch pan area, *sigB* AT 9 (*L. seeligeri*) was isolated on 3 sampling dates from the inlet to the dead-end pipe, on 1 sampling date from the dead-end of the pipe, and on 1 sampling date from the outlet of the dead-end pipe *(Table 2)*, suggesting persistence.

Intervention effectiveness

The effectiveness of each intervention was tested by performing sampling before and after intervention implementation *(Fig. 1)*. For the increased cleaning and sanitation frequency intervention (from once a week to twice a week), one sampling was conducted prior to and four samplings were conducted after implementation; 11% (4/35) of samples were *Listeria* positive before and 13% (18/137) of samples were *Listeria* positive after implementation. There was no significant difference in the log odds of a sample testing positive for *Listeria* before compared to after implementation ($P = 0.787$ based on logistic regression; *Table S2*). In addition, date of sampling ($P = 0.866$) was not

significant *(Table S2)*. Since only 1 sampling was conducted prior to implementation, the percent of positive samples after implementation was also compared to the percent of positive samples from historical samplings in the same wet-area in this packing house *(33)*; only samplings conducted later in the week (after Wednesday when the 2nd weekly cleaning and sanitation was performed) were included in the analyses. Based on these historical results, 31% (25/81) of samples were *Listeria* positive prior to compared to 13% (18/137) of samples positive after intervention implementation. Based on univariable logistic regression, both (i) date of sampling ($P < 0.001$) and (ii) if the samples were collected before or after intervention implementation ($P = 0.037$) were significant. However, only date of sampling was retained in the final model *(Table S3)*.

To test the effectiveness of an intervention that involved weekly application of quat powder around the forklift stops, 3 samplings were performed before implementation and 2 samplings were performed after implementation. One of the 2 forklift stops was positive on sampling 1 (9/11/20) and sampling 3 (10/2/20); no *sigB* AT was isolated from the forklift stop on more than one sampling date, indicating the presence of a persistent transient *Listeria* population *(Table 2)*. Following the implementation of weekly quat powder application (samplings 4 and 5), *Listeria* was not isolated from this forklift stop *(Table 2)*. The other forklift stop was positive on sampling 3 (10/2/20) and was also not positive after application of quat powder *(Table 2)*. To test the effectiveness of weekly quat powder application to a floor crack repeatedly positive for *Listeria* in the current study, 4 samplings were performed before and 1 sampling was performed after implementation. *Listeria* was isolated from the crack on sampling 1 (9/11/20), sampling 2 (9/25/20), and sampling 4 (10/23/20); no *Listeria* with the same *sigB* AT was isolated from the crack on more than one sampling date, indicating the presence of a persistent transient *Listeria* population *(Table 2)*. In the sampling after implementation of the quat powder intervention (sampling 5), *Listeria* was not isolated from the floor crack *(Table 2)*. Therefore, the weekly quat powder application around the forklift stops and floor crack appear to be effective at controlling *Listeria*, as *Listeria* was no longer isolated from these sites following implementation of this intervention.

As the initial study in this facility *(33)* found repeat positives in the outlet from the same dead-end pipe sampled in the current study (5/8 samples of the dead-end pipe were *Listeria* positive from October 2017 to April 2019 in the previous study), we also tested whether the removal of the dead-end portion of the pipe would lead to elimination of presumptively persistent *Listeria* in this site; 4 samplings were performed before and 1 sampling was performed after. *Listeria* was isolated from the dead-end and the pipe outlet on sampling 3 (10/2/20) but was not isolated in the sampling after removal of the dead-end (i.e., sampling 5). However, *Listeria* was isolated from the pipe inlet both before removal of the dead-end (samplings 3 and 4; 10/2/20 and 10/23/20, respectively), as well as after (i.e., sampling 5; 10/30/20) *(Table 2)*. Interestingly, only *sigB* AT 9 was isolated from the dead-end pipe (the pipe inlet, outlet, and the dead-end) in the current study, suggesting a persistent *Listeria* *(Table 2)*. Since *sigB* AT 9 was isolated from the pipe inlet even after removal of the dead-end portion, *sigB* AT 9 was likely persistent and not remediated through intervention implementation; *sigB* AT 9 was not isolated from any other sites during the current sampling but was isolated from the trench drain leading into the square drain in 2018 *(33)*.

To test the effectiveness of a one-time deep cleaning event using a chlorinated cleaner in the square drain and a trench drain leading into the square drain with persistent transient *Listeria* (6 out of 12 *sigB* ATs isolated from the 3 drain sites were isolated only on 1 sampling date), 4 samplings were conducted prior to intervention implementation and 1 sampling (sampling 5) was conducted after. *Listeria* was isolated from at least 1 of the 2 sites within the square drain on sampling 1 (9/11/20), sampling 2 (9/25/20), sampling 3 (10/2/20), and sampling 5 (10/30/20). *Listeria* was also isolated from the trench drain on sampling 2 (9/25/20), sampling 3 (10/2/20), and sampling 4 (10/23/20), as well as on sampling 5 (10/20/20) *(Table 2)*. Therefore, *Listeria* was still isolated from all drain samples following utilization of a chlorinated cleaner in the drains. While all 6 *sigB* ATs isolated from the 3 drain sites on the sampling after using the chlorinated cleaner had also been isolated from at least one of the drain sites prior to intervention implementation, the large number of *sigB* ATs isolated from these sites (3 ATs from the 2 square drain sites and 5 ATs from the trench drain) is still indicative of a persistent transient population *(Table 2)*. The exceptions would be the re-isolation of *sigB* AT 57 (isolated on 4 out of 5 samplings) and *sigB* AT 20 (isolated on 3 out of 5 samplings) which may indicate persistence *(Table 2)*. However, further subtyping (e.g., using whole genome sequencing) would be needed to confirm these hypotheses.

DISCUSSION

Persistent or persistent transient *Listeria* contamination of packinghouse environments represent a considerable challenge for industry. Historical data *(33, 34)*, as well as additional sampling data collected as part of this study, indicated both persistent and persistent transient *Listeria* populations were present in the investigated packinghouse. Specifically, *Listeria* was commonly isolated from forklift stops, a dead-end pipe, and drains. In addition, based on historical results, the percentage of positive samples was higher at the end of the week compared to the beginning of the week (i.e., after the weekly cleaning and sanitation was performed). As such, this study used the packinghouse as a model to (i) implement a protocol for performing root cause analysis (RCA) to identify interventions to eliminate

Listeria, and (ii) to implement interventions and perform sampling to determine the effectiveness of the interventions. The following interventions were identified through RCA, implemented, and tested: (i) increasing cleaning and sanitation from once a week to twice a week, (ii) use of quat powder around forklift stops and floor cracks, (iii) a site-specific niche (a dead-end pipe) removal, and (iv) implementation of a deep cleaning protocol in the drains. The site-specific interventions (e.g., use of quat powder) appeared to be more successful at eliminating *Listeria* from the apple packinghouse compared to increasing the frequency of cleaning and sanitation. This apple packinghouse case study provides an example of how RCA could be performed to eliminate or reduce persistent or persistent transient *Listeria* populations from produce operations.

A persistent and persistent transient Listeria population was present in the apple packinghouse investigated in the current study

Overall, our findings show the facility used in the study reported here had a number of sites with evidence for persistent transient *Listeria*, as well as at least 1 site with evidence of persistence. While most of the *Listeria* in the packinghouse in the current study represents persistent transient *Listeria*, the same *sigB* ATs were isolated ≥3 times from the square drain and the dead-end pipe, indicating potentially persistent *Listeria*. The dead-end pipe represented 3 sampling sites (the pipe inlet, the pipe outlet, and the dead-end portion of the pipe). In the dead-end pipe, *sigB* AT 9 was isolated on 3 sampling events from the pipe inlet and on 1 sampling event from the dead end and the pipe outlet. While a typing method with better discriminatory power (e.g., pulsed field gel electrophoresis or whole genome sequencing) would be needed to confirm these isolates as truly the same, the repeat isolation of this AT within one single pipe (and the lack of isolation of any other *sigB* ATs from this site) is likely to indicate persistence. This pipe is a likely site for persistence, as the dead-end portion can accumulate apple debris and other organic matter that can support the growth of *Listeria*. Consequently, cleaning and sanitation can be a challenge because it is difficult to reach the dead-end portion. In addition, the inlet to the pipe is taped into a catch pan; the adhesive from the tape and the portion of the PVC pipe that overlaps with the metal catch pan (on the inside of the PVC pipe) can also act as harborage sites. While only one study was identified which listed plastic tubing as a harborage point for persistent *Listeria (20)*, several studies have suggested equipment that is difficult to clean is a risk factor for persistent *Listeria (6, 14, 21)*. Our findings provides further support of the importance of sanitary design in controlling persistent *Listeria* populations in processing environments and suggest that complete root cause analyses should include a consideration as to why equipment with poor sanitary design is present in a given facility.

In addition, the site that included a square drain and a connected inflow trench drain, showed evidence for persistent and persistent transient *Listeria* populations. In the square drain and the connected trench drain, *sigB* AT 57 was isolated during 4 sampling events. While this may indicate persistence of this subtype, *sigB* AT 57 is a common *sigB* AT *(15, 28, 30, 33, 40)* and has been shown to be highly diverse *(3)*. As such, it is possible this does not truly represent a persistent *Listeria*. In addition, *sigB* AT 20 was isolated from at least 1 of the 3 drain sites during 3 sampling events, which may indicate persistence; while not as common as *sigB* AT 57, *sigB* AT 20 was also isolated from a forklift stop on 1 sampling in the current study and was isolated in 2 previous studies *(3, 28)*. Drains have been listed as harborage points for persistent *Listeria* in several previous studies *(7, 19, 20, 29)*. However, additional subtyping (e.g., whole genome sequencing) is still needed in the current study to confirm persistence. In addition to the persistent population, the isolation of a highly diverse population of *Listeria* from the 3 drain sites (i.e., 12 *sigB* ATs were isolated from the 3 sites across the 5 samplings) indicates there is also a persistent transient *Listeria* population present in the drain. The drain is located under the dump tank and flume, which deposits large amounts of organic matter and debris into the drain. Organic matter and soils originating from outdoor environments (e.g., similar to those environments apples are grown in) are a known source of *Listeria (28, 30, 31, 39, 40)* and are therefore a likely contributor of the diverse *Listeria* population present in this drain. Furthermore, the drain is deep and has poor drainage, which creates a large number of harborage points that are difficult to clean and that have sufficient moisture and nutrients to support *Listeria* growth; this can lead to both the persistent and persistent transient *Listeria* populations.

A persistent transient *Listeria* population was isolated from one of the forklift stops sampled in the current study, as 6 different *sigB* ATs were isolated from the forklift stop and no AT was isolated more than once. The presence of a persistent transient *Listeria* population at the forklift stops can be expected, as the forklifts often go outside to pick up bins of apples to be run on the packing line. In this packinghouse there are no control measures (e.g., door foamers) for the forklift wheels that would prevent *Listeria* transfer into the packinghouse, and the outdoor environments in the northeast have been shown to harbor diverse *Listeria* populations *(3, 28)*. As such, this could facilitate the transfer of the observed persistent transient *Listeria* population in the current study. Sullivan and Wiedmann *(33)* also identified forklift stops as harborage sites for *Listeria*; 1 out of 2 of the forklift stops positive for *Listeria* in this prior study was the same forklift stop as discussed in the current study.

A persistent transient *Listeria* population was also isolated from the floor crack samples in the current study, as *Listeria* of 4 *sigB* ATs were isolated from the floor crack and no AT was isolated more than once. This floor crack is directly

adjacent to a trench drain and was created when the drain was installed in the packinghouse. This is consistent with the findings of Murugesan et al. *(25)*, who repeatedly isolated *Listeria* from a floor crack next to a trench drain. Therefore, while all floor cracks are likely harborage points, a floor crack's proximity to the drain may increase the likelihood of *Listeria* being present in the floor crack itself (i.e., due to potential splash from the drain into the floor crack). However, more extensive sampling is required to determine if floor cracks adjacent to other high-risk areas (e.g., drains) are at a higher likelihood of becoming contaminated.

As such, PVC pipes (especially those with dead ends), drains, forklift stops, and floor cracks area a few examples of sites that should be included in environmental monitoring programs in produce operations. The contamination patterns (i.e., the diverse *Listeria* population and repeat *Listeria* isolation from the same sites) in the packinghouse investigated in the current study represent "persistent *Listeria*" and "persistent transient *Listeria*" populations. Persistent *Listeria* poses public health and business risks (e.g., recalls) because as the *Listeria* survives in the packing environment over time it can grow; as the *Listeria* grows it is more likely to be transferred to other areas in the packing environment (e.g., by employees or mobile pieces of equipment) and eventually contaminate product. Since persistent *Listeria* represent a single strain of *Listeria*, finished product contamination can be traced back to the packing environment through environmental and product testing and subsequent subtyping of isolates (e.g., via whole genome sequencing). While it is more difficult to link final product contamination to the environment when a persistent transient *Listeria* population is present (compared to persistent *Listeria*), it can represent an instance of continuous introduction of *Listeria* from the same sources. As such, a persistent transient population may indicate more stringent supplier verification programs are required, and in serious cases the identification of alternative suppliers may be needed. Control measures may also be necessary to prevent transfer of *Listeria* from employees, forklifts, distribution trucks, or storage crates, among other routes, into the packing environment (e.g., captive boot programs, compartmentalization of forklifts).

It is important to note a highly diverse persistent transient *Listeria* population may hide a persistent population present in the packing environment (i.e., if there are a large number of *Listeria* subtypes present at any given site, the chance of identifying the persistent subtype is less likely as compared to if only the persistent subtype was present). Regardless, identifying a persistent *Listeria* that is covered up by a persistent transient *Listeria* population is still possible through large sampling efforts, such as "swab-a-thons" that subtype multiple isolates from each positive site. However, as we characterized up to 16 isolates from each positive sample, it is unlikely that true persistence was "covered up" by persistent transient strains in this case.

Root cause analysis can be utilized to identify interventions to eliminate or reduce *Listeria* populations in the apple packing environment; however, multiple iterations of testing and intervention implementation may be required to reduce Listeria populations

RCA was utilized to identify likely root causes of frequent repeat *Listeria* detection in the apple packinghouse in the current study; a previous study as well as this study reported these issues represent a combination of (i) persistent *Listeria* (e.g., in a catch pan with an outflow pipe with a dead-end) as well as (ii) persistent transient *Listeria* (e.g., at the forklift stops). RCA provided a formal process for identification of possible root causes associated with (i) overall high frequency of *Listeria* detection and (ii) different areas where repeat isolation of *Listeria* was an issue. The identified root causes were then used to identity interventions deemed likely to reduce overall frequent *Listeria* isolation as well as frequent site specific isolation of *Listeria*. Overall, the facility implemented one plant-wide intervention and four site specific interventions, which are discussed in detail below. The RCA and subsequent interventions were successful for the forklift stops and floor crack, but further iterations of the RCA are required to control the *Listeria* populations in the drain and dead-end pipe, and to account for the increase in *Listeria* positives in the second half of the week between cleaning and sanitation events. In addition, a multipronged approach that targets multiple interventions at the same time is likely needed to effectively reduce or eliminate persistent and persistent transient *Listeria* populations. In addition, further root cause analyses that explore the reasons for why certain practices were not implemented (e.g., selection of equipment with sanitary design) will be needed for long-term successful *Listeria* control.

The plant-wide intervention tested in the current study was to increase the cleaning and sanitation frequency from once to twice a week; this was implemented to address the increase in percentage of *Listeria* positive samples observed when sampling was conducted at the end of the week. No significant change in the log odds of isolating *Listeria* after intervention implementation was observed in the 2020 sampling results. Increasing the frequency of cleaning and sanitation can also increase the amount of moisture present in the packinghouse. The increase in moisture can allow for an increase in the growth of *Listeria* and may explain the lack of a significant reduction in *Listeria* following implementation of this intervention. In addition, while the adequacy of the cleaning and sanitation protocol in the packinghouse was assessed, no changes in the protocol were made besides increasing the frequency. It is possible improvements in how cleaning and sanitation are performed (e.g., use of a foamer to apply cleaners and sanitizers) may be needed to further reduce the percent of *Listeria* positive sites in the wet area of the packinghouse. However, as only one sampling was conducted prior to increased cleaning and sanitation in the 2020 sampling events, the lack of a

significant relationship may also be a function of chance (due to low sample size) or a fluctuation in the percent of positive samples just prior to intervention implementation for some unrecorded reason (e.g., lower than usual prevalence of *Listeria* on the incoming apples). When taking into account historical results, while both increased cleaning and sanitation frequency and date of sampling were significant for the log odds of a sample testing positive for *Listeria* according to univariable regression, only date of sampling was retained in the multivariable model. This is due to the fact that the date of sampling and increasing cleaning and sanitation frequency are related with one another (i.e., increased cleaning and sanitation was only performed on dates in 2020), and the date of sampling was better able to explain differences in the log odds of a sample testing positive for *Listeria*. There was a decrease in *Listeria* positive samples as time went on. This is consistent with the findings of Sullivan and Wiedmann *(33)*, where there was a lower percent of *Listeria* positive samples in the second half of the packing season compared to the first half of the packing season in all three packinghouses investigated, when comparing sites that were repeatedly positive. Therefore, as sampling of the packing environment continued, the percent of positive samples decreased. This may indicate the packers utilized information on which sites were at a higher likelihood of testing positive to implement corrective actions (e.g., more time spent during cleaning and sanitation) as the studies progressed. These findings also illustrate the difficulty of assessing the effectiveness of interventions without extremely large sampling data sets or without adequate pre-intervention sampling to determine the true percent of *Listeria* positive sites in a facility prior to implementation and if there was a meaningful change in the percent of *Listeria* positive samples after implementation.

Two of the four site-specific interventions did appear to be successful at controlling *Listeria*. For example, *Listeria* was no longer isolated from 2 forklift stops and 1 floor crack after weekly quat powder application at these sites. Murugesan et al. *(25)* also tested the effectiveness of using quat powder to reduce *L. monocytogenes* in a mushroom processing environment. While there was a reduction in the number of sites positive for *L. monocytogenes* in the facility, some floor sites were still positive; the authors hypothesized this was due to harborage of *Listeria* in the porous concrete *(25)*. This likely indicates quat powder is effective against persistent transient *Listeria*, but not against persistent *Listeria* because elimination of persistent *Listeria* requires true elimination of the niche. Therefore, while quat powder is not expected to eliminate *Listeria*, it can be used as a short-term solution for sites that are difficult to modify (e.g., floor cracks and forklift stops) to reduce their ability to harbor and spread *Listeria*. Quat powder is expected to prevent *Listeria* spread (i.e., the quat powder serves as a barrier that inactivates *Listeria* cells freed from the original site upon vibrations or contact with mobile equipment or employees) from the site to other sites within the packing or processing environments. However, while this intervention appeared to be successful at controlling persistent transient *Listeria* in two locations (e.g., forklift stops and the floor crack) in the current study, it must be stated that sampling was performed over a relatively short time frame (approx. 2 months). As such, continued sampling over a longer period is required to confirm the long-term effectiveness of the intervention. It is also important to note that this study does not provide evidence that widespread use of quat powders on the floors of a facility will eliminate repeatedly isolated *Listeria* populations, rather long-term interventions (e.g., improved equipment and facility design) would likely be more effective in this case.

A chlorinated cleaner for "deep-cleaning" of floor drains identified as a site with both a persistent and a persistent transient *Listeria* population was also tested. Assessment of this intervention is highly relevant as floor drains are common sites of persistent or persistent transient *Listeria* in packing or processing environments *(15, 17, 33)*. Cleaner sanitizers (such as the chlorinated cleaner used in the current study) are typically used in areas with a high likelihood of being contaminated, as they prevent the spread of *Listeria* to other sites during the cleaning process. The drains (both the square drain and the trench drain leading into it) are located right below the dump tank, and as such, they are commonly covered in a large amount of organic matter from splash out of the dump tank putting them at high risk for *Listeria* contamination. The implemented intervention did not appear to be effective, however, only 1 sampling was performed after intervention implementation. Therefore, continued use of the cleaner sanitizer may be necessary to see an effect, especially because the large amount of debris in the drain may take several cleaning rounds before it is removed. In addition, because of the high-risk nature of this site (i.e., the large amount of debris present and the poor sanitary design of the drain), it is possible complete elimination of *Listeria* may not be possible without re-designing the drain to eliminate niches and improve cleanability. Rather, measures to control spread of *Listeria* out of the drain should be utilized in the short term. Some control measures could include the use of a quat powder around the drain, a quat ring in the drain, and monitoring the drain for flooding that would disperse the *Listeria* to other areas of the packinghouse. Use of high-pressure hoses should also be avoided, especially in drains, as the high-pressure water facilitates splash and subsequent *Listeria* transfer from the drain to equipment. Installing a pump in the drain to improve its ability to drain could also improve cleanability.

We also identified a dead-end pipe that appeared to have been contaminated with a persistent *Listeria*. While *Listeria* was no longer isolated from the pipe outlet after the removal of the dead-end portion of the pipe, the same subtype was still isolated from the pipe inlet. As such, it appears that while

the dead-end portion does have potential to harbor *Listeria*, it was not the (only) harborage point in this case. Rather, the persistent *Listeria* is likely harbored in the pipe inlet (or upstream). More specifically, the pipe inlet is taped inside a hole in the catch pan (the tape is in the inside of the pipe inlet), which possibly allowed for the attachment of *Listeria* and made it difficult to eliminate the *Listeria* through cleaning and sanitation. In order to eliminate the *Listeria*, complete removal of the pipe prior to cleaning and sanitation or replacing the pipe for a new one at regular frequencies would be necessary. This specific situation illustrates how RCA, in many cases, must be an iterative process where the initial root cause or associated intervention identified may not be the correct one. In this case, it could be argued the root cause was not correctly identified (if the root cause was "a dead-end pipe") or the root cause was correctly identified (insufficient procedures to assure sanitary facility and equipment design), but the intervention was not correctly selected or implemented. As such, the root cause analysis should be reviewed with the newly acquired data (i.e., sampling data from the pipe and surrounding area following removal of the dead-end) to determine what further interventions should be applied. For instance, an appropriate intervention for the root cause of "insufficient procedures to assure sanitary facility and equipment design" would be to (i) correct all sanitary design deficiencies (including the aforementioned tape that holds the inflow pipe in place) and (ii) implement proactive procedures to assure sanitary equipment and facility design going forward. However, it should be noted that removal of all sanitary design deficiencies may take time, and as such, changes to the most critical pieces of equipment (i.e., most likely to lead to product contamination if *Listeria* was to develop harborage at the site) and the least expensive or time-consuming changes should be prioritized first.

CONCLUSIONS

The aim of this study was to utilize a RCA procedure to identify, implement, and test interventions to eliminate *Listeria* from an apple packinghouse with both persistent and persistent transient *Listeria* populations. RCA was found to be a useful strategy for identifying the initial cause of *Listeria* contamination in the packinghouse, in that 2 out of 4 site-specific interventions were effective at reducing *Listeria* populations from the respective sites. Specifically, the use of quat powder was effective at preventing *Listeria* isolation from (i) forklift stops and (ii) a floor crack. However, the use of a cleaner sanitizer in drains and the removal of a dead-end pipe did not eliminate persistent or persistent transient *Listeria* populations. These instances indicate, that while they were not initially successful, use of an iterative process to test several possible options to identify the true root cause or identify and implement effective interventions is often necessary to truly control *Listeria* in the packing environment. Regardless, the RCA protocol tested in this study can be used by other packinghouses, produce processing facilities, or, with modifications, other food processing facilities to identify, implement, and test interventions for reducing or eliminating *Listeria* repeat isolation from within the operations.

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SUPPLEMENTARY INFORMATION

TABLE S1. Description of sampling sites within the apple packinghouse.

Site ID	Site description
301	Inside of large PVC by rot bin next to flume.
302	Inside of small PVC pipe under end of flume before rollers. S of site #301
303	Inside of small PVC by chemical barrels. N of site #'s 301 and 302
304	Inside of large PVC by start of flume near bin submerge
305	Inside of PVC in NW corner at end of brush bed
306[a]	Inside dead-end (removed to swab) of PVC in NW corner. Same pipe as site #305
307[a]	Same as 306
308[a]	Same as 306 and 307
309	Top of PVC pipe in NW corner (part that connects with the catch pan)
310	Catch pan at N end of the brush bed by NW corner. Crack/ metal juncture
311	Plastic/ metal joints supporting south end of brush bed by west platform stairs
312	Metal cross beams with wires wrapped around them under the brush bed. Two by stairs at the SE part of the platform
313	Large screws under brush-bed (4 total). The four on the E side of brush bed
314	Plastic/ metal juncture that supports brush bed. Under 2nd set of fans. Accessed from the NW corner
315	Gap between box for brush bed motors and catch pan in NW corner under the brush bed.
316	Frame of brush bed on NW side. By second set of fans
317	Large screws under brushes (directly). 4 in total under second set of fans
318	Forklift stop – full bins, by bin loader
319	Forklift stop – empty bin return
320	Floor crack on NW side of bin return
321	Trench drain by NW corner PVC pipe. 3rd grade from corner
322	Square drain by dryer – PVC pipe in center
323	Square drain by dryer – outer edge
324	PVC pipe (inside) by juice reject belt
325	Square drain between packing area and brush beds – PVC outlet
326	Square drain between packing area and brush beds – outer edge
327	Square drain by pelletizer – outside/ top
328	Square drain by flume/ bin dump – PVC pipe
329	Square drain by flume/ bin dump – outer edge
330	Flume connection flange – south-most by half wall
331	Metal frame lip/ gap at rollers after flume. W side, on platform
332	Overflow door by flume (inner section) by leaf pile
333	Condensation on PVC pipe under flume, at U-bend
334	Four large screws under the brush bed. Last four before fan box, near stairs. E side
335	Small trench leading to square drain by flume

[a]Samples were not collected from these sites during sampling 5 (10/30/21), as the dead-end was removed from the pipe.

TABLE S2. Univariable[a] logistic regression model results explaining the relationship between *Listeria* isolation in the packinghouse and date of sampling and intervention status (i.e., if the sample was collected before or after increasing the frequency of cleaning and sanitation to twice a week[b]) in the current study.

Model	Variable	Log-odds (95% CI[d])	*P*-value
Date of sampling	Intercept	38.1 (-427.1, 509.1)	0.872
	Date of sampling	-0.002 (-0.03, 0.02)	0.866
Intervention status	Intercept	-2.0 (-3.3, -1.1)	<0.001
	After intervention implementation[c]	0.2 (-0.9, 1.5)	0.787

[a]Only univariable regression models were fit, as no variables were significant at a level of $P<0.1$ by univariable regression.
[b]Prior to increasing the cleaning and sanitation frequency, cleaning and sanitation was performed once a week.
[c]The baseline of the intervention status variable is before intervention implementation (i.e., when cleaning and sanitation was performed once a week).
After intervention implementation, cleaning and sanitation was performed twice a week.
[d]95% CI= 95% confidence interval.

TABLE S3. Final logistic regression model results explaining the relationship between *Listeria* positive isolation in the packinghouse and date of sampling when combining historical data[a] from the packinghouse with the data collected as part of the current study.

Variable	Log-odds (95% CI[b])	*P*-value
Intercept	22.0 (8.3, 35.5)	0.001
Date of sampling	-0.001 (-0.02, -0.001)	<0.001

[a]The historical data was reported in Sullivan and Wiedmann (2020).
[b]95% CI= 95% confidence interval