REDUCING *LISTERIA* CONTAMINATION IN PRODUCE PACKINGHOUSES THROUGH AGENT-BASED MODELING

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ABSTRACT

The complex environment of fresh produce packinghouses can facilitate the spread of pathogens such as *Listeria monocytogenes*, which can enter a facility and establish itself within harborage sites. From here, food being shipped from the packinghouse is at risk of being contaminated with *L. monocytogenes*, endangering public health. Thus, reducing pathogen presence and cross-contamination within these facilities are crucial. The studies presented here demonstrate the development of Agent-Based Models of *Listeria* contamination dynamics in packinghouses and the analysis of contamination behavior. These models were used to compare the effectiveness of different corrective actions on a short-term scale, as well as observe persistent contamination and the effectiveness of corrective actions on reducing it. Overall, results indicate that both data-informed corrective actions and reduction of *Listeria* in incoming raw produce are effective in the short-term, but only the former produces long-term improvement in controlling *Listeria* in the packinghouse environment.

BIOGRAPHICAL SKETCH

Cecil Wilfried Barnett-Neefs was born in Brussels, Belgium in 1994 to Jonathan and Channah Barnett-Neefs. He earned a Bachelor of Science degree in Pharmacology in 2015 from the University of Manchester and continued his studies there to earn a Master of Science degree in Bioinformatics & Systems Biology in 2016. Cecil discovered an interest in applying programming and technology-based solutions to biological problems, seeking to bridge the gap between the two fields. He proceeded to join the Whittaker Lab at Cornell in August 2017 to help design feline coronavirus analysis software and a database system for sample organization. In January 2019, Cecil began his graduate studies at Cornell under the advisement of Dr. Renata Ivanek. In addition to his research, Cecil has been involved with the Cornell Institute for Digital Agriculture and its outreach to student populations. After completing his degree, Cecil intends on continuing to work in applying computational solutions like modeling and IoT to biological fields.

Dedicated to my Opa – It’s not ancient Greek but I suspect you would be proud nonetheless

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Truth be told, if I had all the time in the world and all the pages to write on, I would probably end up writing an acknowledgements section as long as the actual dissertation. However, in the interests of time and sanity I will aim to be somewhat more concise. To all my family, friends, teachers, colleagues, and everyone else who helped me get this far: I am deeply thankful and would not be here without you.

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CHAPTER 1

INTRODUCTION

Listeria monocytogenes is a pathogen of serious concern within the field of food safety, having been implicated in a number of food-borne outbreaks (1–4) and capable of causing a potentially fatal infection (listeriosis) (5,6). Historically, the specific foods associated in these outbreaks in the United States are usually ready-to-eat (RTE) foods that do not undergo a kill-step (either during processing or by the consumer) prior to consumption, as well as cold-stored meat and dairy products (e.g., cantaloupes, deli meats and soft cheeses) (1,7–10). Diagnosis of listeriosis is complicated by the variable severity of its symptoms, as the disease can present with relatively common symptoms such as fever, aching and stiff muscles, nausea, vomiting and diarrhea, or more severe symptoms including abortion, septicemia, meningitis and death in cases of invasive listeriosis (11,12). Despite occurring at a relatively low incidence rate of 0.1 to 10 cases per million annually (depending on the specific country in question) (11), its case-fatality rate of 20-30% is however, of considerable concern (12). Thus, protecting the consumer from exposure is crucial, but also must be executed in an efficient manner to ensure widespread adoption.

Within the fresh produce supply chain in the United States, packinghouses are a key step that involves the receiving and subsequent packing of produce, during which produce undergoes cleaning and culling, before being sorted according to requirements, packed, and shipped from the facility. A single packinghouse can receive, and pack produce from one or more supplier, which may subsequently be distributed to multiple destinations. Therefore, these facilities serve as strategically important locations in *L. monocytogenes* control, as their positions within the produce supply chain can amplify the potential scope of a food-borne outbreak. Within a packinghouse, *L. monocytogenes* is capable of infiltrating the facility via incoming contaminated food (originating from the natural environment (13)) as well as other methods, and potentially cross-contaminating equipment surfaces, employees or produce. Furthermore, *L. monocytogenes*' capacity to grow in more extreme environments (5), such as cold temperatures, can make it more difficult to remove once present inside. Additionally, a key component of this issue is the abundance of potential locations within a facility where this pathogen may survive and grow unimpeded (e.g., facility equipment or drains). Once established within these sites, the pathogen can cross-contaminate the rest of a facility from an internal source. A site that can harbor *L. monocytogenes* over the course of a facility's sanitation events (i.e., current cleaning and sanitation measures do not reduce the microbial population of that site) is termed a "niche" (14). A niche can be located within a number of possible sites within a facility, such as hollow rollers and brush beds (15). Contamination within a niche that remains over an extended period of time may be termed as "persistent" (16), and can remain present in the niche for a considerable duration without explicit intervention. This is in contrast to *L. monocytogenes* that is successfully removed during sanitation, which is termed "transient" contamination.

Given the potential threat of *L. monocytogenes* contamination, packinghouse facilities establish environmental monitoring programs (EMPs, or environmental monitoring (EM)) wherein selected surfaces of the facility are swabbed to determine the presence of *Listeria* spp. (serving as an indicator organism for *L. monocytogenes* presence) (7,8). Results of a facility's EMP can be used to detect niches, as well as the performance of sanitation measures currently in use. An EMP may also inform a facility on changes to make in terms of sanitation measures, or new corrective actions to employ (changes to processes to eliminate unwanted situations like pathogen contamination, such as: changes to sanitation schedule, implementing captive footwear programs etc.). However, given the sheer size and complexity of a packinghouse, it is extremely difficult to design an EMP that will cover every single surface, and decisions must be made on which surfaces to specifically monitor in the interests of time, manpower and money. As a result, it is possible for gaps to appear in an EMP's coverage. A related issue that compounds this matter is the financial cost of experimenting with existing practices, as well as the risk of inadvertently reducing the effectiveness of ongoing measures. Thus, the ability to test potential corrective actions prior to their actual deployment would be a key asset to facilities in these situations. The outcome of a corrective action can drastically vary depending where and how within a facility it may be introduced, as well as what is specifically needed: a corrective action may be needed as a short-term solution to reduce the probability of contamination throughout the facility, or to eliminate contamination from identified contaminated surfaces, some of which may be persistently contaminated niches. Therefore, with a potential gap in EMP coverage it may be difficult to determine how well existing measures or new corrective action are performing. This highlights the need for systems that can expand the understanding of what may be occurring within these unobserved areas of a packinghouse to help ensure the integrity of food safety measures within these facilities.

Several potential decision support tools may be employed in these scenarios to provide guidance; however, an agent-based model (ABM) is one of the more sophisticated options. A facility is represented within an ABM by its interactive surfaces (forming the “agents” of the model), which are capable of interacting with their surroundings (i.e., the environment) and each other, depending on each agent’s individual configuration and overall rules (17). This can allow for the rapid autonomous interaction of agents on a fine spatiotemporal scale, and the observation of a number of agent attributes over time (18,19). Once a “digital twin” of a facility is established in its current conditions (i.e., baseline), the model can be used to investigate bacterial dynamics, including *Listeria* spp., from an *in silico* perspective. Furthermore, modifications can be made to agent behavior and global model parameters to simulate the implementation of various potential corrective actions. An ABM of a packinghouse facility can use facility-specific historical data to model the facility as a whole, allowing not only for the predicting of agent activities outside of a local EMP, but also for the comparison of performance between the expected baseline conditions and potential corrective actions. As these models exist purely *in silico* they can be run relatively quickly to produce results, limited only by computational hardware. This allows for decision-making with the benefits of additional information to compensate for gaps in the EMP, on a spatiotemporal scale fine enough that either a single agent or the entirety of the model could be observed for changes.

This thesis aims to address the need for effective decision support tools designed in providing rapid and comprehensive information in reducing the risk of *L. monocytogenes* contamination within produce packinghouses. The objectives of this thesis are thus to: (i) Construct, validate and analyze ABMs of *Listeria* spp. dynamics in two packinghouses using facility-specific historical data. (ii) Test facility-wide and site-specific corrective actions to quantify their short-term effectiveness on model-wide *Listeria* spp. contamination prevalence and concentration. (iii) Develop a means of tracking and analyzing *Listeria* spp. persistence throughout both ABMs, then evaluate potential corrective action in controlling *Listeria* spp. persistence over long-term durations.

The subsequent chapters of this thesis aim to provide a detailed demonstration of the assembly and validation for two packinghouse models, as well as the investigative capabilities each can provide in a number of potential outcomes, both for their standard baseline setup and in assessing potential corrective actions. Chapter 2 shows the core assembly and short-term predictions for both packinghouse models and includes discussion of challenges faced when creating these ABMs. Chapter 3 shows how these two models, and their accompanying analyses, were modified to investigate *Listeria* spp. contamination persistence patterns, as well as the effectiveness of potential corrective actions both on long-term persistence and in combating persistent *Listeria* spp. contamination. Finally, Chapter 4 will provide conclusions on key finding discussed in this thesis, as well as providing thoughts on challenges and potential research directions.

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CHAPTER 2

USING AGENT-BASED MODELING TO COMPARE CORRECTIVE ACTIONS FOR *LISTERIA* CONTAMINATION IN PRODUCE PACKINGHOUSES

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Key words: agent-based model, *Listeria*, corrective actions, produce, packinghouse, decision support tool

ABSTRACT

The complex environment of a produce packinghouse can facilitate the spread of pathogens such as *Listeria monocytogenes* in potentially unexpected ways. This can lead to finished product contamination and potential foodborne disease cases. There is a need for simulation-based decision support tools that can rapidly test different corrective action scenarios and are able to account for a facility's interior cross-contamination dynamics. Thus, we developed agent-based models of *Listeria* contamination dynamics for two produce packinghouse facilities; agents in the models represented equipment surfaces and employees, and models were parameterized using observations, values from published literature and expert opinion. Once validated with historical data from *Listeria* environmental sampling, each model's baseline conditions were investigated and used to determine the effectiveness of corrective actions in reducing prevalence of agents contaminated with *Listeria* and concentration of *Listeria* on contaminated agents. The evaluated corrective actions included reducing incoming *Listeria*, modification of cleaning and sanitation strategies, and reducing transmission pathways, as well as combinations thereof. Analysis of *Listeria* contamination predictions revealed differences between the facilities despite their inherent similarities in function and design, highlighting that one-size-fits-all approaches may not always be the most effective means for selection and implementation of corrective actions in fresh produce packinghouses. Corrective actions targeting *Listeria* introduced in the facility with raw materials, implementing risk-based cleaning and sanitation, and modifying equipment connectivity were shown to be most effective in reducing *Listeria* contamination prevalence. Overall, our results

suggest that a well-designed cleaning and sanitation schedule, coupled with good manufacturing practices can be effective in controlling contamination, even if incoming *Listeria* spp. on raw materials cannot be reduced. The presence of water within specific areas was also shown to influence corrective action performance. Our findings support that agent-based models can serve as effective decision support tools in identifying *Listeria*-specific vulnerabilities within individual packinghouse facilities and hence may help reduce risks of food contamination and potential human exposure.

INTRODUCTION

Listeria monocytogenes is an environmentally widespread, Gram-positive bacterium known for its ability to grow at refrigeration temperatures (5) and persist in food industry equipment due to the presence of harborage sites and conditions favoring replication of the bacteria (19). Symptoms of *L. monocytogenes* infection can either manifest in the form of relatively lesser signs that include nausea, vomiting, fever and diarrhea, and more severe ones including abortion, meningitis, encephalitis, septicemia and death (5). Though only possessing an incidence rate between 0.1 to 10 cases per 1 million people per year depending on the specific country (11), listeriosis has a case-fatality rate between 20-30% (12), making it a priority in food safety. Within a produce packinghouse facility, introduction of *L. monocytogenes* on incoming raw produce is only one contamination route that needs to be addressed, as re-contamination is possible further along production lines due to the presence of harborage sites within facilities (16). Alternative introduction routes into a facility can include entries via regular staff or equipment movement, or unexpected occurrences (i.e., random events), such as roof leakage due to extreme weather or during specialized equipment repairs. Challenges associated with control of *L. monocytogenes* are further compounded in fresh and ready-to-eat (RTE) foods that do not undergo a kill-step, such as fresh and fresh-cut produce. The interplay between product, equipment surfaces, water and employees can quickly change what may seem like a straightforward product line into far more complicated web of interactions, allowing pathogens like *L. monocytogenes* to spread beyond its initial introduction site to elsewhere within a facility (20). To combat the risk of contamination, facilities can employ environmental monitoring programs (EMPs) to locate pathogen sources, determine pathogen spread throughout the facility, and verify control strategies are effective. EMPs involve the routine collection of sponge and swab samples of strategically selected surfaces within a facility and testing them for *Listeria* spp. as an indicator for conditions that will facilitate *L. monocytogenes* contamination. EMP results play an important role in identifying and implementing control strategies such as cleaning and sanitation programs and hygienic zoning (21), which helps restrict pathogen movement.

A data scarcity due to limited testing as part of a facility's EMP or low prevalence of *Listeria* spp. positive samples detected as part of the EMP can be supplemented by *in silico* tools for more quantitative analysis. Furthermore, a digital decision support tool can assist when determining which corrective actions to pursue within a facility. Due to the structurally complex nature of these facilities, an agent-based model (ABM) is well-suited to this task thanks to its inherent specialization in modeling the interactions of heterogeneous and autonomous "agents" representing the components of the system under study. Simulating a facility *in silico* can be used for a number of objectives, such as better understanding of pathogen movement, interpreting results of EMPs, and evaluating interventions or capital improvements in the facility. One such tool is "Environmental monitoring with an Agent-Based Model of *Listeria*" (EnABLe) (18), which has already been shown to allow for analysis of *Listeria* spp. transmission in the slicing and packaging room of a smoked seafood facility. EnABLe's flexible systems not only allow for the establishment of a model replica (sometimes referred to as a "digital twin") of a real food production environment, but the rapid manipulation of any number of model parameters or agent-specific values as well. This inherent modularity can be an incredibly powerful asset in the development and evaluation of different corrective actions. Moreover, the establishment of a model aids in identifying targeted interventions that can be specifically applied to higher risk areas of a facility to mitigate contamination. Thus, the objective of this study was twofold: (i) to construct and validate ABMs for two produce packinghouses using historical sampling data and (ii) use these validated ABMs to test sets of facility-wide and site-specific corrective actions to quantify their effectiveness in reducing *Listeria* spp. contamination in wet and dry areas of the packinghouses.

METHODS

Data from two produce packinghouse facilities were used to create an ABM for each facility (models “Facility A” and “Facility B”). ABMs were constructed in NetLogo 6.2.0 (22) following the general structure of the EnABLe model developed by Zoellner et al. (8). The models were run with one-hour time steps for a period of two virtual weeks, with the first week allowing *Listeria* to potentially become introduced and spread in a facility and simulated environmental monitoring (EM) being performed in the second week. For corrective action scenarios, each corrective action was started from the beginning of the simulation and ran for the entire two weeks. For both facilities, an ABM was constructed of the main room where packing operations were performed. While the two packinghouse facilities have variations in layout and size, both facilities can be broken down into similar production steps as briefly summarized: produce is brought into the facility via crates carried by forklifts and loaded into a flume system. Once loaded, raw produce is then transferred to a cleaning area for culling and waxing. Produce is then sorted according to size and appearance and is directed accordingly to either a reject area or the appropriate hand-packing area (trays and bags in Facility A; only trays in Facility B). Production as well as cleaning and sanitation shifts were modeled based on information provided by the facilities, with Facility A performing weekly cleaning and sanitation separately on two separate days, and Facility B performing daily cleaning and sanitation on each workday as well as extended cleaning and sanitation on Saturday. Both facilities operated on a single shift during workdays (Monday-Friday) with a half hour break in the middle of the shift.

Model Construction & Specifications

Each model had two types of agents: equipment and employees. Agents' attributes included the following fixed characteristics:

(i) Position (defined by x and y coordinates to represent position in 2D plane)

(ii) Distance from floor (i.e., "height" in cm, used to calculate interaction order for agents sharing the same position)

(iii) Zone category as per proximity to food products (Zone 1: Food-Contact Surfaces (FCS); Zone 2: Non-Food-Contact Surfaces (NFCS) in close proximity to food and FCS; Zone 3: NFCS not in close proximity to food or FCS (23))

(iv) Cleanability (i.e., whether *Listeria* is removed from the agent during a routine cleaning step or not)

(v) Cleaning frequency

(vi) Surface area (cm^2)

Additionally, each agent had a number of time-varying attributes to track:

(i) *Listeria* quantity (both in terms of the absolute number of CFU and concentration per surface area (in CFU/cm^2) on an agent)

(ii) Frequency of contamination from specific sources over the course of the simulation: (a) raw incoming food material, (b) random introduction occurrences that could affect anywhere in the facility, or (c) "Zone 4" (7) introduction (i.e., introduction from areas outside the packing room), which had a more localized effect near actively used doorways

(iii) Agent water level (consisting of three levels: 1: no water; 2: damp to the touch; 3: visible water on agent)

(iv) Niche formations over the course of the simulation (defined as *Listeria* spreading onto an "uncleanable" agent, which is an agent that due to its design cannot be effectively cleaned during routine cleaning and thus remains contaminated once *Listeri*a spreads on it) or temporary niche formations (defined a contamination of an otherwise "cleanable" agent that remained contaminated after routine cleaning) and how frequently these occur

(v) Sampling over the course of the simulation (if the agent has been sampled by the simulated EMP)

Table 2.1 provides a summary of the modeled agents and their characteristics. Agents were grouped based on their location in the production area (Loading, Cleaning, Sorting, Reject, Bag Packing, Tray Packing, and an Other group that included a collection of agents not fitting elsewhere, such as quality control workstations and computer workstations) and by presence of water within the facility area: “wet” (Loading and Cleaning) and “dry” (Sorting, Bag/Tray Packing, Reject and Other). Following the establishment of the agent list, the contact structure among agents was created by assigning (i) directed and (ii) undirected links. The presence of connections allowed for transfer of *Listeria* from one agent to another, depending on link's directionality. Directed links represented one-way connections (termed "contact-links", consisting of "out-directed-links" on the sending agents and "in-directed-links" on the receiving agents) for mechanisms providing opportunities for *Listeria* transfer in a single direction (with agents sending *Listeria* performing “transfer”, and agents receiving performing “reception”), such as transfer belts and rollers. Undirected links (termed "proximity-links") represented repeated contact between two agents, transferring contamination with a certain frequency and regardless of direction. The models were constructed using observations from in-person visits to the modeled facilities by authors C.W.B.-N. and G.S. to conduct behavioral mapping (24) and to determine layout, key surfaces, water and traffic patterns, and connection pathways (Fig 2.1). The specifics of produce commodities packed in the two packinghouses cannot be provided; this was a condition for gaining access into the facilities and their data.

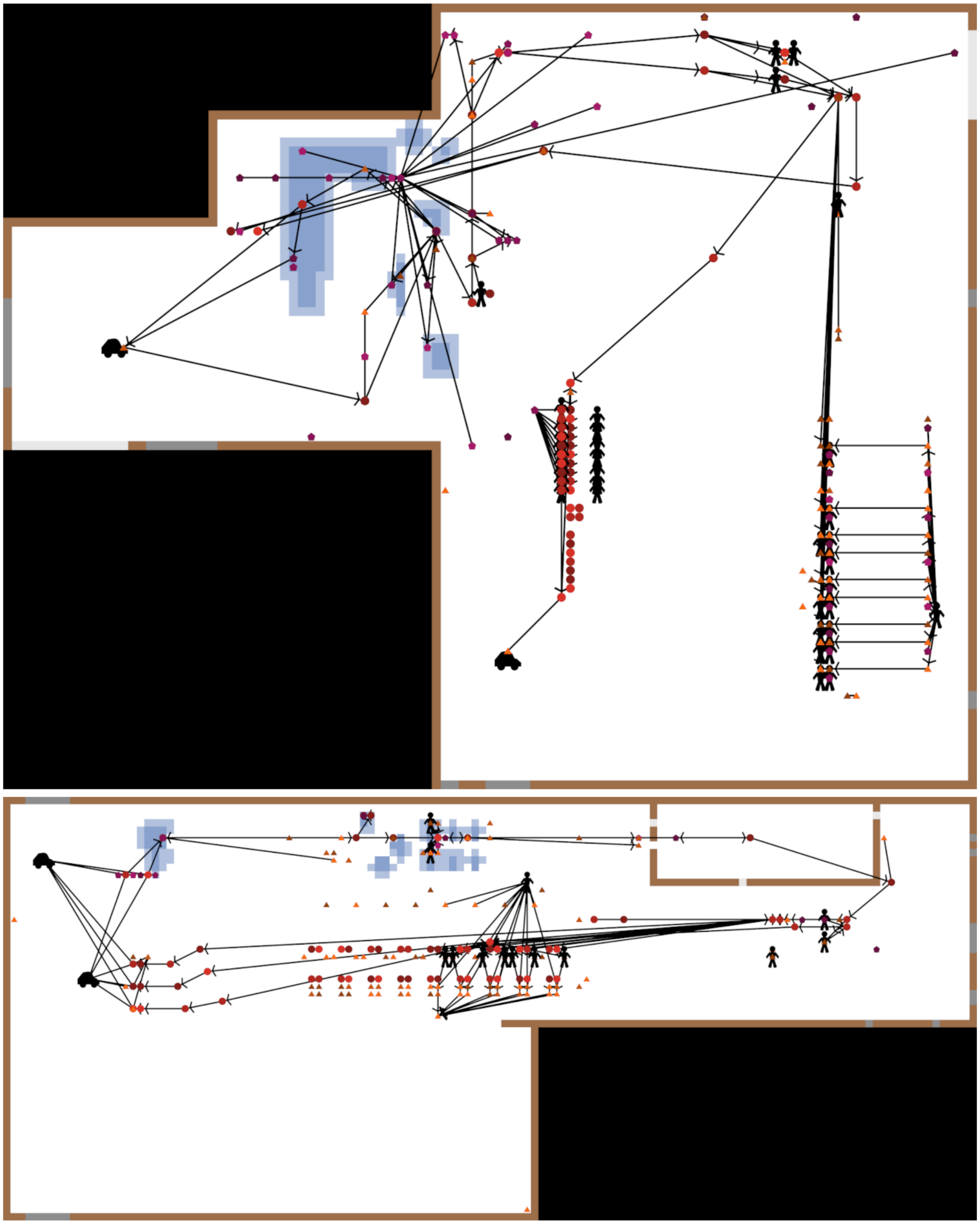

Fig 2.1 NetLogo views of Facilities A (upper panel) and B (lower panel) illustrating positions of and connections among agents and presence of water at a point in time during production.

Circles, triangles and pentagons represent equipment surfaces in zones 1, 2 and 3, respectively; agent water level is denoted by shape color darkness and is independent from floor conditions; employees and forklifts are denoted by specific icons (people and cars respectively); arrows represent the direction of directed agent links and lines without arrows represent undirected links; blue shaded areas represent water presence on the floor (darker colors representing puddles and lighter colors representing damp areas); brown patches denote wall-floor-junctures; grey patches denote doors, with dark grey patches being points of Zone 4 introduction; empty space is denoted by white patches; inactive space not represented by the model is denoted by black.

A baseline map in the form of text files was first constructed using numerical representation to establish size and grid scale for floor patches, as well as the location of structural components (wall-floor-junctures, open floor, ceiling, and doors). The surface area per patch was 2,500 cm^2, with Facility A consisting of 109 x 88 patches (9,592) and Facility B consisting of 130 x 56 patches (7,020). Additional maps were then created to represent water level (i.e., none, low, medium, and high) and traffic level (i.e., none, vehicle, low, medium, and high) on the floor for different phases of facility operation. A weekly schedule was also established in the form of a 7x24 csv file for each hour in a week, with each cell detailing the current event for a specific hour ("empty": no activity; "pre-op": pre-operations inspection with minimal staff; "production": standard operations with full staffing and activity; "clean": "Cleaning Only"/ "Cleaning & Sanitation" operations to remove *Listeria* from equipment) (Tables 2.S1-2.S5). The schedule was used not only to determine which traffic and water maps to load, but also defined the presence/absence of specific agents (employees and forklifts), as well as *Listeria* introduction processes over time.

Table 2.1 Agent characteristics by zone of agent-based models (Facilities A and B) representing two modeled packinghouses

	Facility A				Facility B			
	Zone 1[a]	Zone 2	Zone 3	Employees[b]	Zone 1[a]	Zone 2	Zone 3	Employees[b]
Number of Agents	57	68	63	36	74	122	16	13
Distance from floor (m)	0.90 [0.00, 2.02][c]	1.00 [0.00, 2.00]	0.00 [0.00, 1.20]	1.20 [0.88, 2.05]	1.0 [0.05, 1.80]	0.71 [0.00, 1.59]	0.0 [0.00, 1.15]	1.20 [1.20, 2.50]

Surface area (cm^2)	340.0 [240.0, 187785.8]	27,500.0 [145.4, 150995.2]	3,178.5 [340.0, 10259.7]	340.0 [340.0, 340.0]	11,250.0 [625.0, 214468.8]	2,500.0 [101.3, 43062.5]	2,725.0 [40.0 93281.3]	340 .0 [340.0, 340.0]
Number of out-directed links	0.0 [0.0, 3.0]	0.0 [0.0, 1.0]	0.0 [0.0, 0.0]	0.0 [0.0, 2.0]	1.0 [0.0, 3.0]	0.0 [0.0, 1.0]	0.0 [0.0, 0.1]	1.0 [1.0, 2.0]
Number of in-directed links	0.0 [0.0, 2.0]	0.5 [0.0, 1.0]	0.0 [0.0, 1. 0]	0.0 [0.0, 1.0]	1.0, [0.0, 1.0]	0.0 [0.0, 1.0]	0.0 [0.0, 2.5]	1.0 [1.0, 1.0]
Number of undirected links	1.0 [0.0, 5.0]	1.0 [0.0, 5.0]	1.0 [0.0, 2.0]	3.0 [0.0, 5.0]	0.0 [0.0, 3.0]	0.0 [0.0, 3.0]	1.0 [0.0, 5.2]	1.0 [0.0, 1.0]
Number (%) uncleanable	10 (11%)	20 (29%)	22 (35%)	0 (0%)	6 (8%)	94 (77%)	6 (38%)	0 (0%)
Equipment/ Employee Cleaning Schedule	Weekdays[d]	Weekdays[d]	Weekdays[d]	Upon leaving[e]	Weekly[f]	Weekly[f]	Weekly[f]	Upon leaving[e]

[a] Zone 1 agents and the summary of their attributes do not include employees. [b] Values listed specifically refer to a pair of human hands. [c] Values are given as median [5th-95th percentile] unless otherwise stated. [d] Cleaning & Sanitation: Weekdays (Monday-Friday). [e] All *Listeria* removed when employees leave production floor (Modeled are employees leaving the production floor for break and at the end of the shift). [f] "Cleaning Only": Weekly (Saturday); "Cleaning & Sanitation": Weekly (Monday).

Finally, input parameters (as either fixed values or probability distributions) were established from observations and information in the literature to describe *Listeria* growth, transmission, and reduction (Table 2.2; Tables 2.S6-2.S9). Data not available in literature sources was acquired from a web-based survey with industry and academic experts that was performed by Sullivan et al. (25) to specifically collect data needed for development of ABMs for fresh produce facilities. Briefly, this expert opinion survey was completed by six individuals (four from academia and two from produce industry backgrounds) with expertise on *Listeria* in food facilities. Each question addressed a specific parameter; survey results were summarized as a median, minimum, and maximum, which were used to develop distributions for each parameter (Table 2.2; Tables 2.S6 and 2.S9). Expert elicitation for the purpose of developing a novel modeling framework is considered beneficial because it permits rapid evaluation of the system and parameter uncertainty, and thus it allows prioritization of future data collection based on the results of sensitivity analysis (26). For model parameters represented as probability distributions, the parameter values across iterations 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.

To determine the degree of reduction in *Listeria* during cleaning for each agent and the timing of these operations, each facility was asked to provide information on the cleaning operations they apply during a regular week. These cleaning activities were modeled as two different levels of reduction: "Cleaning Only" (average of 0.5 $\log_{10}$ *Listeria* reduction) and "Cleaning & Sanitation" (average of 6 $\log_{10}$ *Listeria* reduction) (27).

Several assumptions were made in the model for simplicity: firstly, temperature was uniform and constant at 12°C within the facility and external weather conditions were not accounted for. Secondly, *Listeria* on the floor (i.e., patches) was not picked up by agents (i.e., modeled equipment surfaces and employee hands) due to a lack of data regarding frequency of occurrence and amount of *Listeria* transferred, as well as a lack of mechanics within the model to adequately judge if an agent's surface height is too far from the floor to become directly contaminated from the floor. Additionally, in the model employees were allocated to their working stations, however, their movement around the facility was represented through the traffic map and it was assumed they did not deviate from these patterns. This assumption was not expected to have affected the model prediction. Employees driving forklifts were not accounted for in the model because they do not contact the floor or agents in the model system. Finally, an agent's cleanability being switched from "uncleanable" to "cleanable" in scenarios simulating corrective actions was assumed to either represent (i) the inclusion of equipment that was not previously cleaned on a regular basis into facility's regular cleaning and sanitation operations schedule, (ii) modification or replacement of previously difficult to clean equipment to allow it to be fully cleaned during regular cleaning operations; thereby, in the model a previously uncleanable agent representing such equipment became cleanable.

Table 2.2 Baseline model input parameters, description, equation and distribution, summary values and sources for *Listeria* spp. introduction, growth, transmission, and reduction

Symbol	Description[a]	Equation/Distribution	Mean	5th-95th Percentile	Reference
P_z	Probability that *Listeria* spp. is introduced into the room via objects from Zone 4 per hour	$10^{\mathrm{Pert}(-2.3,-0.9,-0.6,4.8)}$	0.14	[0.02, 0.36]	(25)
N_z	Amount of *Listeria* spp. introduced to an agent or patch from Zone 4 (CFU) per occurrence	$10^{\mathrm{Pert}(0.0,1.9,3.3,4.2)}$	156	[6.04, 618.79]	(25)
R_d	Probability of a crate of incoming raw produce containing *Listeria*-contaminated produce on day d, for d = *Monday*, *Tuesday*, *Wednesday*,	$10^{\mathrm{Pert}(-2.3,-0.6,-0.6,5.4)}$	0.161	[5.82E-2, 0.243]	(25)

	Thursday, Friday				
N_R	Concentration of *Listeria* spp. per gram of contaminated raw produce (CFU/g)	Gamma(0.18,0.425)	0.42	[8.90E-8, 2.24]	(3)
α	Proportion of *Listeria* spp. transferred to a surface upon contact with a contaminated raw produce	$10^{Normal(-0.44,0.4)}$ for $\alpha < 1$, else $\alpha=1$	0.45	[0.08, 1.00]	(28)
P_r	Rate of random event occurrences that introduce *Listeria* spp. from outside the room per hour	$10^{Pert(-4.3,-0.9,-0.6,4.6)}$	0.07	[4.00E-3, 0.203]	(25)
N_r	Amount of *Listeria* spp. introduced per random event (CFU)	$10^{Pert(0.2,3.3,3.7,3.3)}$	1233	[42, 3829]	(25)
K	Environmental carrying capacity of *Listeria* spp. (CFU/ml)	-	1.00E8	-	(27)
GT	Generation time (hr) of *Listeria* spp. on environment surfaces at 12 °C	Uniform(16,217)	116.48	[26.05, 206.95]	(29)
μ	Maximum specific growth rate (hr^{-1}) of *Listeria* spp. on environment surfaces (12 °C)	Ln(2)/GT	0.01	[0.00, 0.03]	*calculated*
P_t	Probability that contact on floor from foot and equipment traffic is sufficient to spread *Listeria* spp. to adjacent patch	Pert(0.03,0.25,0.65,4)	0.28	[0.10, 0.48]	(30)

C_i	Contact rate between the contaminated patch and the adjacent patch given the traffic level I = veh, *high*, *low*, *neg*[b]	C_{veh}=120/patch/hr C_{high}=60/patch/hr C_{low}=12/patch/hr C_{neg}=0.2/patch/hr	-	-	*observed*
P_w	Probability that environmental *Listeria* spp. is transported to adjacent patches via (visible) water	Uniform(0.01,0.05)	0.03	[0.01, 0.05]	(18)
β	Probability of *Listeria* spp. transmission among patches via traffic and water	Uniform(0.0,0.05)	0.03	[3.00E-3, 4.80E-2]	(18)
P_f	Probability that a contaminated produce or organic debris falls to the floor during any given hour of production	Uniform(0.01,0.03)	0.2	[1.10E-2, 2.90E-2]	*observed*
P_c	Probability of a condensation transfer event given *Listeria* spp. is present	Uniform(0.0,0.02)	0.01	[1.00E-3, 1.90E-2]	(18)
θ_d	Log_{10} reduction of *Listeria* spp. from equipment during "Cleaning Only" on day d, for d=*Friday* within Facility A	Pert(-1.5,-0.5,0,4)	-0.58	[-0.78, -0.37]	(27)
η_d	Log_{10} reduction of *Listeria* spp. from equipment during "Cleaning & Sanitation" on day d, in Facility A d=Saturday; in Facility B for d=*Monday*,	Pert(-8,-6,-1.5,4)	-5.58	[-7.36, -3.47]	(27)

	Tuesday, Wednesday, Thursday, Friday				
γ	Probability that a cleanable agent was properly cleaned when “Cleaning & Sanitation” was performed	-	0.99	-	*assumed*[c]
δ	Probability that a cleanable agent was properly cleaned when “Cleaning Only” was performed	-	0.99	-	*assumed*[c]
R_i	Number of crates containing raw produce introduced per hour	-	5	-	*observed*
S_c	Amount of raw produce material in grams introduced per crate	-	362874	-	*provided by facility*
τ_{ij}	Probability of *Listeria* spp. transfer from *I* to *j* agent given contact, where *i*=*j*=Zone1, Zone 2, Zone 3, or Employee agent type [d]	$\tau_{ij}=10^{\mathrm{Normal(TC,STD)}}$ [e]	[f]	[f]	(28,31)

[a]All parameters correspond to an hourly time scale. [b]veh=vehicle, neg=negligible. [c]Values were assumed when data was not available from literature or expert opinion. [d]TC=mean transfer coefficient, STD=standard deviation of the transfer coefficient; full data in Supplemental Tables S2.7 and S2.8. [e]Full data in Supplemental Table S2.9

The models described above, and defined with parameters in Table 2.2 (and Tables S2.6-S2.9), were considered as the “baseline model” for Facilities A and B. All statistical analyses of data generated through model simulations were conducted in R

4.0.5 (32) using the ‘data.table’ package (33) to import large files. To aid interpretation and comparison of results from different sensitivity and scenario analyses, in each model iteration two primary outcomes of interest were recorded at Midday (12:00 pm) on Wednesday of the second simulation week (which in the model was coded as the last action before the mid-shift break). This timing for reporting of the model outcomes was selected to allow for the observation of employee contamination levels just prior to going on break while equipment-related data was collected, mimicking the sampling methods in the historical EM data used for validation. The two outcomes of interest were: (i) the prevalence of contaminated agents (*P_W*) and (ii) *Listeria* concentration on contaminated agents (*C_W*, CFU/cm^2). The outcome *P_W* was calculated by first estimating the prevalence of contaminated agents in one iteration (overall or in a specific subset of agents) and then to summarize prevalence across model iterations we used a boxplot; the median was recorded for comparisons. Similarly, the outcome *C_W* was summarized over agents (overall or in a specific subset of agents) and iterations using a boxplot; the median was recorded for comparisons.

Validation and Verification

Both models were validated using historical EM data collected from the respective facility and by recreating analogous *in silico* sampling scenarios that targeted the same equipment surfaces within the model. Historical EM data regarding *Listeria* presence throughout each facility was collected from a complementary study (34), in which Zone 2 and 3 surfaces were sampled using individually packaged sponges hydrated with 10 mL Dey-Engley neutralizing buffer. On a given day, sampling was performed by collecting 3-36 samples in Facility A, and 19-30 samples in Facility B; samples were collected 3-4 hours into a facility's production cycle and tested for the presence of *Listeria* spp. using the Food and Drug Administration "Bacteriological Analytical Manual method" (35). A simulated sampling routine was performed in each model using the sampling schedule and the number of samples used for collection of the historical data. Simulated sampling was weighted to favor sites that were historically more often sampled; the weight was calculated by dividing the individual agent's sampling probability by the sum of sampling probabilities of all agents in historical data:

$$Weighted\ P(x_i) = \frac{P(x_i)}{\sum_i^m P(x_i)} \qquad \text{Eq. (2.1)}$$

where x_i is an individual agent among a total of m agents and P stands for probability.

Simulated environmental sampling was interpreted with an assumed false negative rate of 10% if the agent's *Listeria* concentration was ≤10 CFU/cm^2, and 1% if the contamination level was between 11-100 CFU/cm^2. Samples from agents with a *Listeria* concentration over 100 CFU/cm^2 were assumed to have a zero false negative rate. Each model (Facility A and B) was used to run a 1,000-iteration BehaviorSpace experiment in NetLogo to compare the contamination status of agents representing historical sampling data. Validation data were evaluated graphically and using a Chi Square Test, or Fisher's Exact Test (if the number of samples in the group were too small for use of Chi Square test), to determine if there was a significant difference in the prevalence of positive samples between sampling results in historical data and the model simulation by shift, zone classification and wet/dry area type. The 95%

confidence intervals (95% CI) for the prevalence were estimated using the ‘Hmisc’ R package (36) using the Wilson score interval method.

Each model was additionally verified to be functioning correctly using NetLogo’s own debugging tool for code integrity. Model mechanics were tested using extreme scenarios and simplified models that only ran isolated parts of the original systems. Models for both facilities were also tested on alternate hardware to ensure they remained functional on other computer systems.

Sensitivity and Cluster Analysis

A 2-sided partial rank correlation coefficient (PRCC) evaluation using the ‘epiR’ R package (37) was used to perform a sensitivity analysis to identify relationships between the predicted agent contamination prevalence at Midday Week 2 of a randomly selected day from among the days when the facility underwent EM in historical data (Facility A: Monday, Tuesday, and Thursday; Facility B: Monday, Tuesday, and Wednesday) and specific input parameters. Coefficients were then filtered against a Bonferroni-corrected significance level ($p=0.05/46=0.0011$ and $p=0.05/45=0.0011$ for Facility A and B, respectively).

Cluster analysis was performed using factor analysis of mixed data (FAMD) or principle component analysis (PCA) (using the ‘factoextra’ and ‘FactoMineR’ R packages (38,39), respectively), to determine whether agents in a facility could be grouped, to inform corrective actions, by (i) agent attributes (i.e., Number of Incoming Links, Number of Outgoing Links, Number of Undirected Links, Agent Zone, Cleanability, Facility Area, Height, and Wet/Dry Area Type) and (ii) 21 agent contamination-related outcomes that were estimated across all iterations for each individual agent (Table 2.S11). In both methods (i.e., FAMD and PCA), the minimum number of dimensions to consider was determined using a prior PCA and cumulative percentage of variance explained with a cut-off of >=80% followed by inspection of the variables used. Correlation plots (40) were used to identify and select variables that primarily showed strong contribution to a single dimension, while variables with a weak contribution or that contributed more strongly to more than one dimension were discarded from the analysis. Finally, using the remaining variables (Table 2.S12), Hierarchical Clustering on Principal Components (HCPC) was performed with the number of clusters determined automatically except in the case of Facility A, where the PCA clustering was manually set to four to split a large cluster into subclusters to allow for easier interpretation.

Scenario Analysis

Scenario analyses evaluated the effect of several corrective actions that were created to simulate targeted control and cleaning strategies (Table 2.3). In Facility B, regular "Cleaning & Sanitation" of equipment is performed daily and thus these corrective actions were already embedded in the baseline model configuration of Facility B. However, Facility A performs both "Cleaning Only" and "Cleaning & Sanitation" procedures only once a week (Saturdays and Mondays, respectively), therefore scenario analysis for Facility A's risk-based corrective actions additionally tested both (i) daily "Cleaning Only" of equipment and (ii) daily "Cleaning & Sanitation". In scenarios involving modification of the Master Sanitization Schedule, agents previously designated as “uncleanable” could now be eligible to undergo cleaning, depending on their respective mean contamination probability.

Table 2.3 Corrective Action Scenarios and their virtual implementation within the Agent-Based Models

Scenario	Description	Computational implementation[a]	Scenario model-notation
Random Event Occurrence Reduction	The time until the next random introduction event to occur was extended by 25%, 50% or 75% from baseline	$P_r * 1.25$ $P_r * 1.50$ $P_r * 1.75$	PR_01 PR_02 PR_03
Random Load Reduction	The amount of *Listeria* introduced by random contamination events was reduced by 1, 2 or 3 $\log_{10}$.	$N_r * 0.1$ $N_r * 0.01$ $N_r * 0.001$	LR_01 LR_02 LR_03
Z4 Event Occurrence Reduction	The probability of a Zone 4 introduction event occurring in an hour in the baseline model was reduced by 25%, 50% or 75% or set to zero.	$P_z * 0.75$ $P_z * 0.50$ $P_z * 0.25$ $P_z * 0.00$	PZ_01 PZ_02 PZ_03 PZ_04
Z4 Load Reduction	The amount of *Listeria* introduced by Zone 4 contamination events was reduced by 1, 2 or 3 $\log_{10}$.	$N_z * 0.1$ $N_z * 0.01$ $N_z * 0.001$	LZ_01 LZ_02 LZ_03
Listeria Prevalence in Incoming Raw Produce Reduction	The baseline prevalence of product-borne *Listeria* arriving in the facility was reduced by 25%, 50% or 75% or set to zero. This simulated produce being treated prior to arriving in the packinghouse packing room.	$R_d * 0.75$ $R_d * 0.50$ $R_d * 0.25$ $R_d * 0.00$	EC_01 EC_02 EC_03 EC_04
Cleaning Effectiveness Improvement	The amount of *Listeria* removed during “Cleaning Only” and “Cleaning & Sanitation” was increased by 3 $\log_{10}$. This simulates the usage of more powerful reduction techniques.	$\theta_d * 0.001$ or $\theta_d * 0.001$ & $\eta_d * 0.001$	MI_01
Weekend Deep Clean	All *Listeria* in the facility was removed from agents every Sunday.	*Listeria* concentration of affected agents set to zero at the time of the scheduled cleaning	MI_02
Enhanced Flume Water Treatment	The amount of *Listeria* on the flume agent in each model was reduced by 2 $\log_{10}$ during each hour of production; this simulates a wash water treatment that effectively delivers a 2 $\log_{10}$ reduction.	$Flume\ agent\ load * 0.01$	AI_01

Broad Model-based Master Sanitation Schedule Restructuring	Agent cleaning and sanitation schedules were reassigned according to mean contamination probability predicted in the model over the second week into: (i) weekly schedule (when predicted contamination probability was ≤32%), (ii) alternating days (33-65%), (iii) daily (≥66%). At the scheduled cleaning and sanitation, *Listeria* concentration on select agents was reduced by θ_d or η_d as appropriate. In the case of Facility A where a daily schedule did not previously exist, one was implemented using either "Cleaning Only" or "Cleaning & Sanitation". This simulates a "risk-based reorganization of the cleaning and sanitation schedule".	*Listeria* concentration of affected agents reduced by θ_d at the time of the scheduled cleaning (Facility A)	AI_02C1
		Listeria concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation (Facility A)	AI_02C2
		Listeria concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation (Facility B)	AI_02
Directed Model-based Master Sanitation Schedule Restructuring	Only agents predicted to have a mean contamination probability ≥66% were scheduled for daily cleaning and sanitation, meaning that *Listeria* concentration on these agents was reduced by θ_d or η_d as appropriate on agents scheduled to be cleaned at that frequency; other agents were left with their original cleaning and sanitation scheduling. In the case of Facility A where a daily schedule did not previously exist, one was implemented using either "Cleaning Only" or "Cleaning & Sanitation". This simulates a "partial reorganization of the cleaning and sanitation schedule of surfaces determined to be most at risk of contamination".	*Listeria* concentration of affected agents reduced by θ_d at the time of the scheduled cleaning (Facility A)	AI_03C1
		Listeria concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation (Facility A)	AI_03C2
		Listeria concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation (Facility B)	AI_03
Transmission Pathways Modification Corrective Action	Links between specific agents were severed to represent physical isolation between them. In Facility A the interconnected drain system was compartmentalized so that agents could only receive *Listeria* (but not spread it further) while in Facility B each forklift was assigned to a single separate area.	Modified links defined at beginning of scenario	AI_04
Combined Corrective Action 01	Facility A ran scenarios EC_02 and AI_02C2 simultaneously, while Facility B ran scenarios EC_02 and AI_03. This simulated the simultaneous application of (i)	$R_d * 0.50$; *Listeria* concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation	CI_01

	reduced *Listeria* prevalence in incoming produce and (ii) the most effective schedule-based corrective action for each facility.		
Combined Corrective Action 02	EC_02 and AI_04 were applied in the model simultaneously, this simulated the simultaneous application of (i) reduced *Listeria* prevalence in incoming produce and (ii) agent compartmentalization.	$R_d * 0.50$; Modified links defined at beginning of scenario	CI_02
Combined Corrective Action 03	Facility A ran scenarios AI_02C2 and AI_04 simultaneously, while Facility B ran scenarios AI_03 and AI_04. This simulated the simultaneous application of (i) each model's most effective schedule-based corrective action and (ii) agent compartmentalization.	*Listeria* concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation; Modified links defined at beginning of scenario	CI_03

[a] Parameter notations are defined in Table 2.2.

Each corrective action scenario (54 in total: Facility A: 28, Facility B: 26) was evaluated by running 1,000 iterations. Scenario analyses simulations used the same fixed seed as in the baseline model to assure a fair (counterfactual) comparison among scenarios and between each scenario and the baseline model. Efficacy of a corrective action was evaluated by comparing the prevalence of contaminated agents, separately for wet and dry area, in counterfactual iterations of the baseline model and the model with a corrective action implemented using Eq. 2.2. The efficacy over all iterations, summarized with the median and interquartile range (IQR) statistics, was defined as:

$$Efficacy = (1 - \frac{Pca+c}{Pb+c}) \times 100 \qquad \text{Eq. (2.2)}$$

where *Pb* stands for prevalence in the baseline model iteration and *Pca* stands for prevalence in the corresponding iteration of the model with a corrective action. Constant *c* is a correction factor corresponding to 0.5/m (where *m* is the total number of agents of the area type in the model (i.e., Facility A: dry agents=171, wet agents=53; Facility B: dry agents=176 and wet agents=49); this correction factor was applied to be able to calculate the efficacy in iterations where prevalence in the baseline model was zero.

The estimate of efficacy of a corrective action provides useful information about the relative change in prevalence of contamination between the compared scenario and the baseline but it does not assess the magnitude of contamination for each scenario. Thus, the distribution of both predicted prevalence of contamination and concentration of *Listeria* on contaminated agents over all iterations were compared between the baseline and promising corrective action scenarios (identified based on sensitivity and efficacy analysis). The comparisons were presented graphically as boxplots for each scenario by wet and dry areas. For ease of interpretation, we estimated the difference between the median prevalence (expressed as percentage point (pp) difference) and between median concentration (expressed as $\log_{10}$ CFU/cm^2) for the corrective action scenario and the baseline. Data files and code used to build the two agent-based models using NetLogo, as well as data files and R code relevant to the cluster analysis, scenario analysis, and sensitivity analysis, are available on the GitHub repository: https://github.com/IvanekLab/CPS_ABM.

RESULTS

Validation

The baseline models were validated with historical data for Facilities A and B at both whole-model and area-specific levels (Fig 2.2; Table S2.10). All comparisons indicated lack of statistically significant differences between agent contamination prevalence observed in historical data and prevalence obtained with simulated environmental sampling.

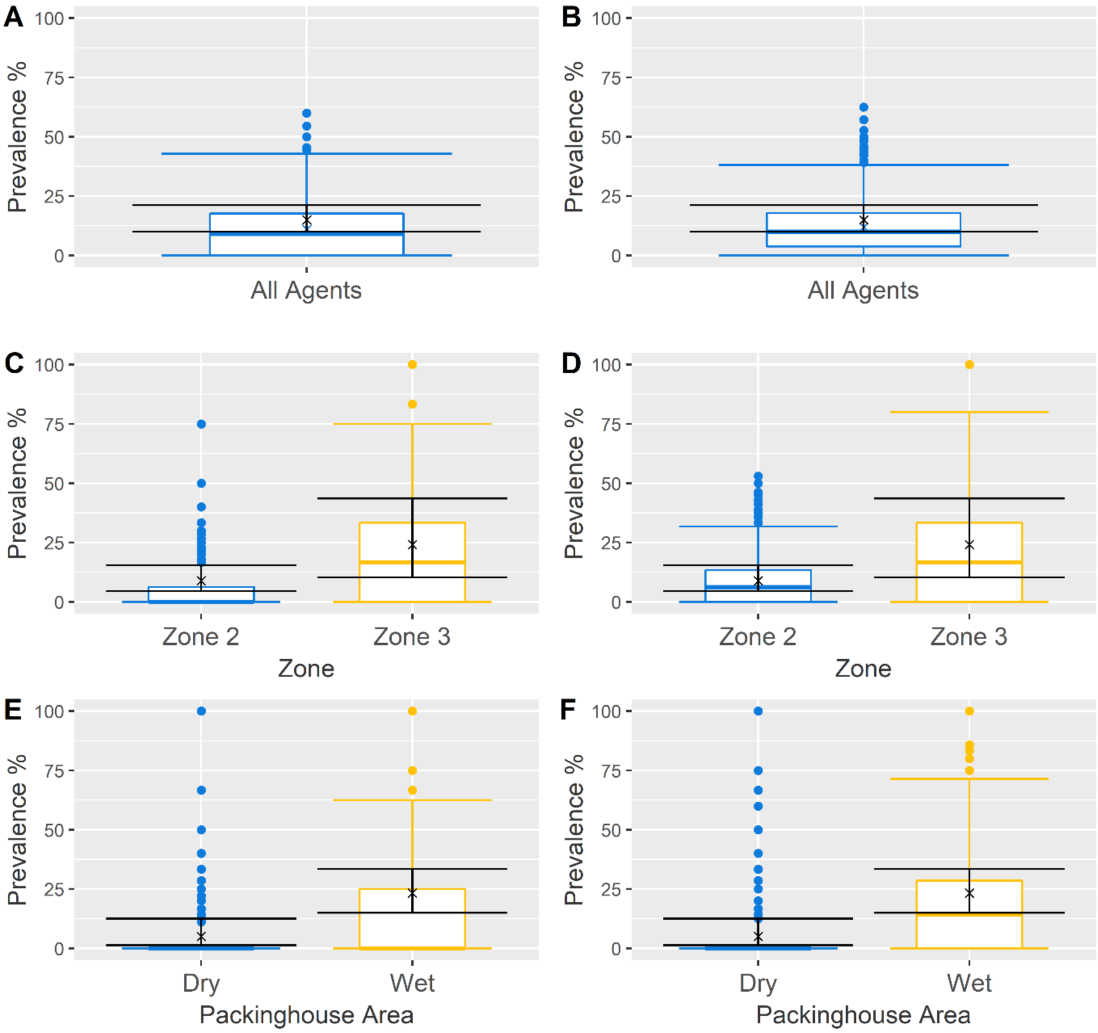

Fig 2.2 Graphical comparison of baseline Facilities A and B using historical data at midday against simulated sampling results

Validation groupings investigated included (i) all agents (panels A and B), (ii) Zone category (panels C and D), and (iii) presence of water in the area (panels E and F). Lack of significant differences between historical (black, covering the mean (denoted with x) and 95% confidence intervals for contamination prevalence) and simulated sampling (colored, covering the mean (denoted with x), median, interquartile range, 95% confidence intervals and any outliers for contamination prevalence) groups indicated the model's behavior could be considered representative of its respective facility.

Predicted Listeria prevalence and concentration in wet versus dry areas

The Facility A wet areas had median agent contamination prevalence of 25.9% and 23.1% within the Loading area and Cleaning area (i.e., the model’s “wet” area), respectively (Fig 2.3.A), while the Facility B Loading area and Cleaning area had respective median prevalence of 42.9% and 21.4% (Fig 2.3.B). “Dry” areas (i.e., combination of remaining facility areas not in proximity to water) had a lower prevalence of *Listeria* positive agents, with Facility A having medians of 6.3%, 4.1%, 1.7%, 11.1% and 7.7% for the Sorting, Tray Packing, Bag Packing, Reject and Other areas, respectively (Fig 2.3.A). Facility B did not feature an active Bag Packing area, but all its remaining dry areas except the Reject area had medians of 0%; the Reject area median prevalence was 26.1% (Fig 2.3.B). Within each area, the concentration of *Listeria* on contaminated agents was recorded and analyzed. Figs 2.3.C and 2.3.D show the low concentrations (in $\log_{10}$ CFU/cm^2) for Facilities A and B respectively, across all wet and dry areas in the modeled facilities. Each area group contained a combination of agents belonging to Zone 1-3. Visual evaluation of agents grouped by hygienic Zone revealed an overall higher prevalence and concentration in Zone 3 agents compared to Zones 1 and 2 (Fig S2.1).

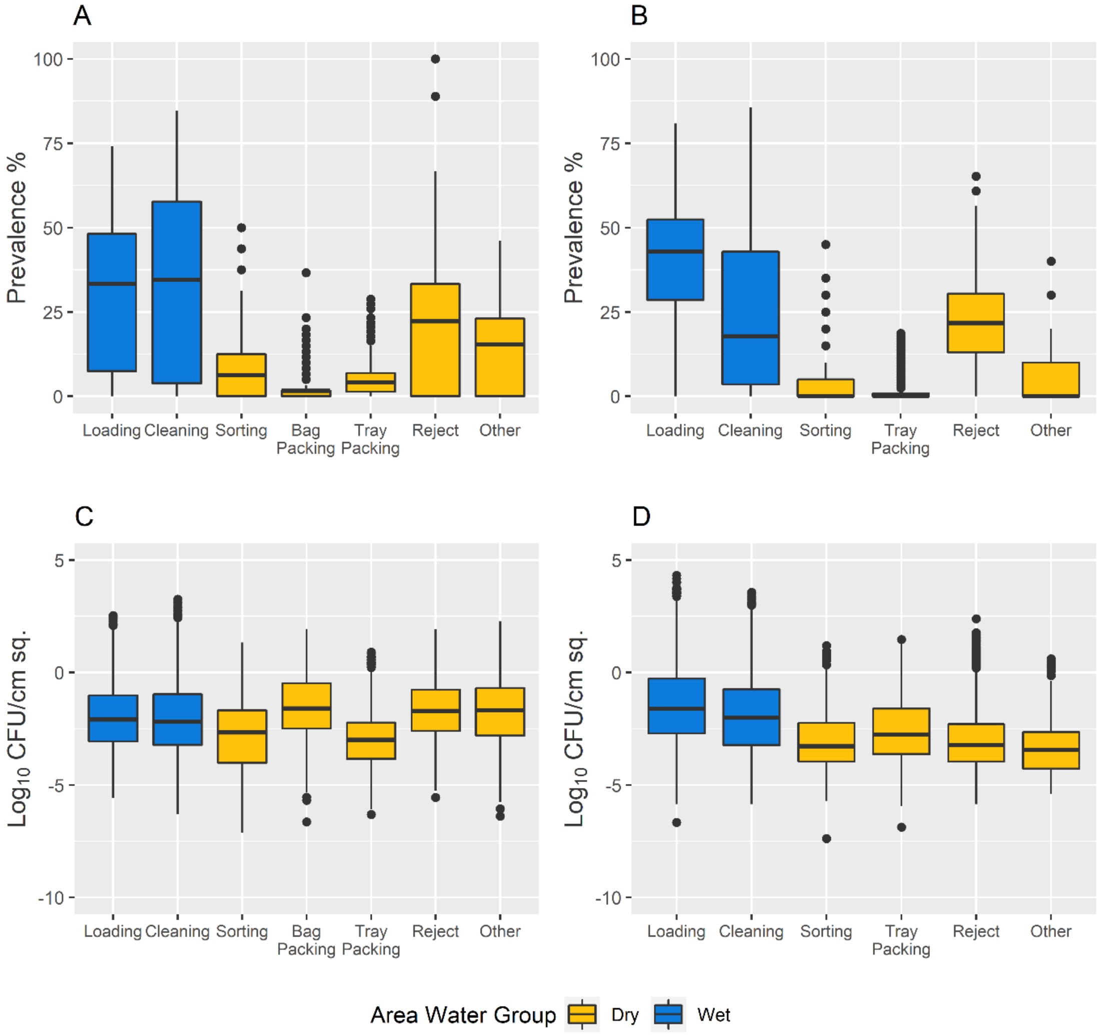

Fig 2.3 Boxplots describing *Listeria* contamination prevalence and concentration on contaminated agents on Wednesday at Midday for Facility A and B baseline conditions

Prevalence of *Listeria* contamination within each area of Facility A (panel A) and Facility B (panel B). Both facilities show higher prevalence in wet areas (blue) than dry areas (yellow), except for the Reject area. Log$_{10}$ concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D) with median concentrations listed showing low level of contamination.

Sensitivity Analysis

The effects of model input parameters on *P_W*, analyzed using PRCC, are depicted in Fig 2.4. The most influential parameters were the concentration of *Listeria* spp. per gram of contaminated raw produce (N_R) and the probability of contact between Zone 1 agents (P_{11}). In Facility A the probability of *Listeria* transfer between Zone 3 agents given contact (τ_{33}) was negatively correlated with prevalence of contaminated agents, while the transfer probability between Zone 2 agents and Zone 1 agents (τ_{21}) was negatively correlated in Facility B (Fig 2.4.B). In Facility A the negatively correlated parameter involved equipment that was connected to drainage systems within the facility (as well as interconnected drains); *Listeria* in the drainage system could not re-contaminate other agents and instead would show die off, which led to the negative correlation.

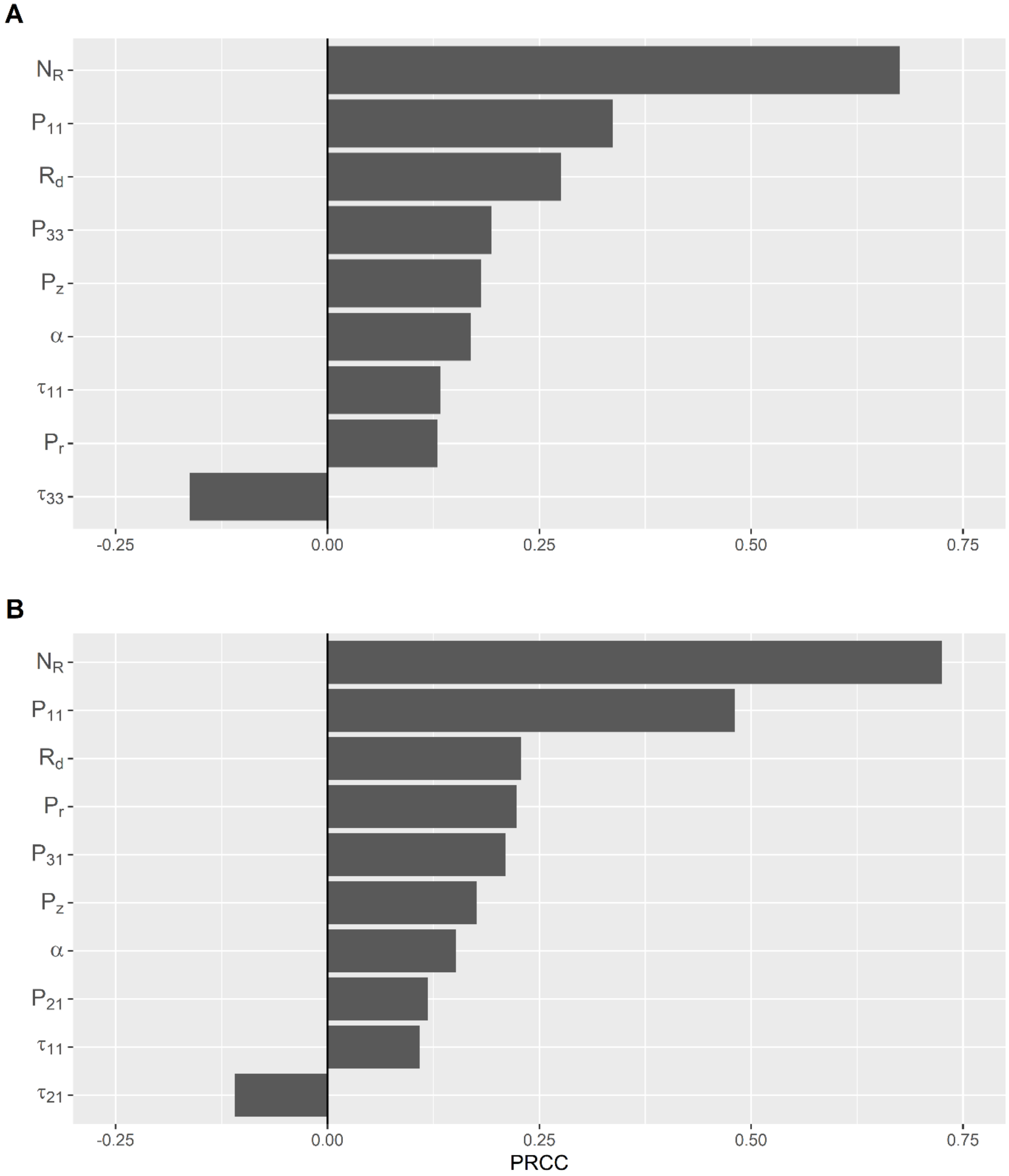

Fig 2.4 Sensitivity plots of significant model input parameters against prevalence of *Listeria* contaminated agents at midday Wednesday of the second week of simulation for each facility

Significant partial rank correlation coefficient (PRCC) values were determined using Bonferroni correction according to the number of parameters evaluated in Facilities A and B, respectively. (N_R: Concentration of *Listeria* spp. per gram of contaminated raw produce (CFU/g); P_{ij}: Probability of contact from contaminated surface in Zone *i* to another surface in Zone *j*, where *i*=*j*=Zone1, Zone 2, Zone 3 or Zone 4; P_r: Rate of random event occurrences that introduces *Listeria* spp. from outside the room per hour; P_z: Probability that *Listeria* spp. is introduced into the room via objects from

Zone 4 per hour; R_d: Prevalence of *Listeria* spp. in produce on day d, for d = *Monday, Tuesday, Wednesday, Thursday, Friday*; α: Proportion of *Listeria* spp. transferred to a surface upon contact with a contaminated raw produce; τ_{ij}: Probability of *Listeria* spp. transfer from *i* to *j* agent given contact, where *i*=*j*=Zone1, Zone 2, Zone 3, or Employee agent type.)

Cluster Analysis

A cluster analysis of all agents was conducted with FAMD and PCA methods (Tables S2.13 and S2.14) to identify a specific cluster of agents as targets for enhanced cleaning and sanitation. Targeting enhanced cleaning and sanitation to the cluster with the highest probability of contamination in each model's respective FAMD and PCA results did not result in any meaningful improvement to facility outcomes. This is due to the fact that these clusters were typically composed of "downstream" agents that were not likely to control the spread of contamination to the rest of the facility, but instead behave more like "sinks" (41) that may potentially serve as indicators of contamination presence in a facility (Fig S2.2).

Scenario Analysis

Comparison of modeled corrective actions with the baseline model allowed for evaluation of the efficacy of each corrective action and provided data for prioritizing strategies for implementation (Fig 2.5-2.6; Table S2.15). Based on efficacy comparison and sensitivity analysis results, Random Event Occurrence Reduction (PR), Random Load Reduction (LR), Z4 Event Occurrence Reduction (PZ) and Z4 Load Reduction (LZ) corrective actions were deemed ineffective and were excluded from further analysis (Fig S2.3-S2.6).

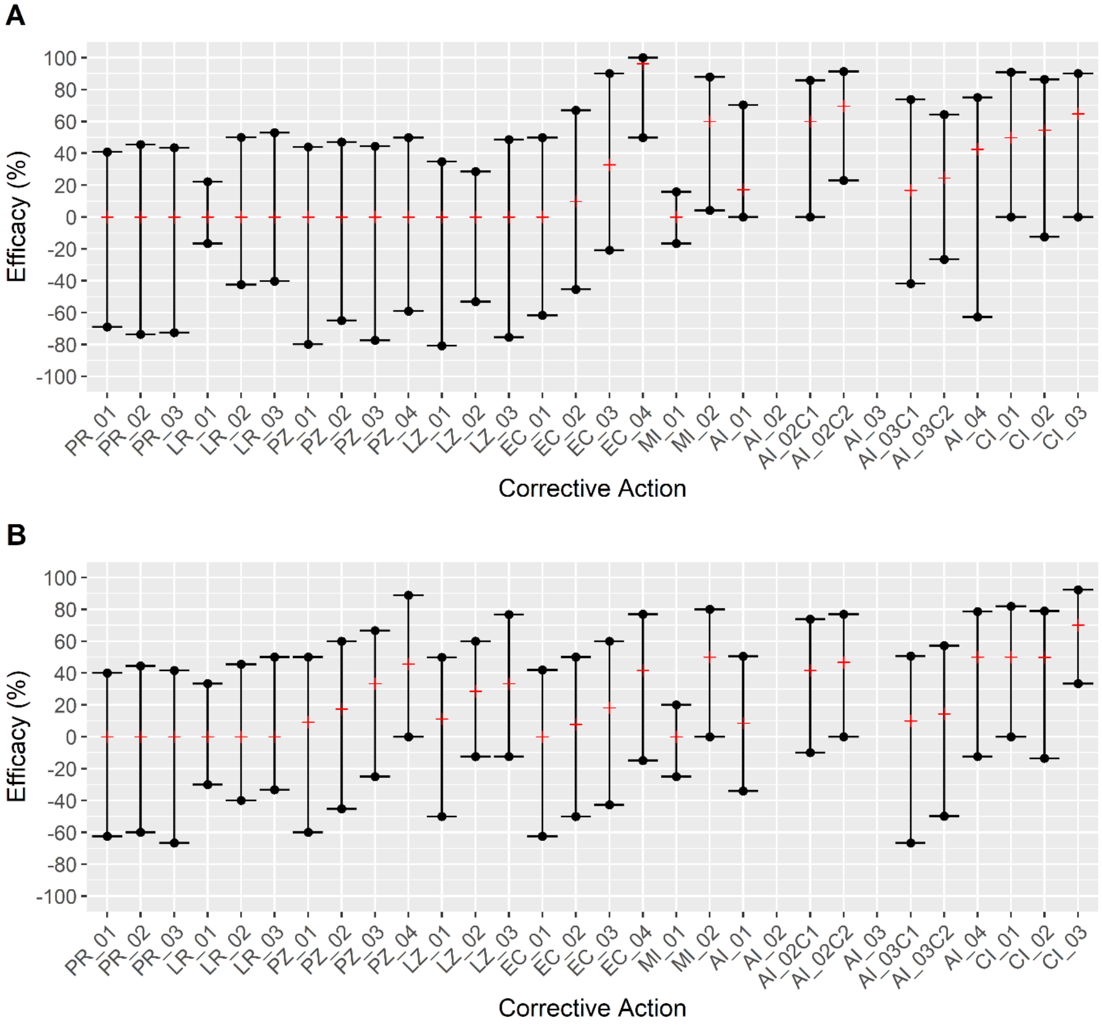

Fig 2.5 Comparison of corrective action efficacy against baseline conditions in Facility A

Efficacy was calculated using eq. 2.2 for each area (i.e., “wet” and “dry”) within a model for each applicable corrective action and displayed by median efficacy (red line marker) and interquartile range (black dot crossed by line marker). Positive efficacy indicated a lower *Listeria* prevalence in the model with a corrective action compared to the baseline model and thus effectiveness of the corrective action, while zero or negative efficacy indicated that the corrective action is predicted to not be able to reduce the agent contamination prevalence. Panel A: Facility A Wet area. Panel B: Facility A Dry area. PR_01-PR_03: Random Event Occurrence Reduction (125%; 150%; 175% event delay from baseline respectively). LR_01-LR_03: Random Load Reduction (1-3 Log_{10}, respectively). PZ_01-PZ_04: Z4 Event Occurrence Reduction (25%; 50%; 75%; 100% reduction from baseline respectively). LZ_01-LZ_03: Z4 Load Reduction (1-3 Log_{10}, respectively). EC_01-EC_04: Reduction of *Listeria* Prevalence in incoming produce (25%; 50%; 75%; 100% reduction from baseline, respectively). MI_01: Cleaning Effectiveness Improvement (increased *Listeria* removed during reduction events increased by 3 $\log_{10}$). MI_02: Weekend Deep Clean (Removal of *Listeria* from all agents every Sunday). AI_01: Enhanced Flume Water Treatment (2 $\log_{10}$ removal of *Listeria* in flume agent per hour of production). AI_02/AI_02C1/AI_02C2: Broad Model-based Master Sanitization Restructuring (Agent cleaning and sanitation schedules were fully reassigned according to a mean contamination probability; Facility A was given a daily schedule for both “Cleaning Only” and “Cleaning & Sanitation” respectively). AI_03/AI_03C1/AI_03C2: Directed Model-based Master Sanitization Schedule Restructuring (Sanitization of agents with a mean contamination probability ≥66% was set to a daily frequency; Facility A was given a daily schedule for both “Cleaning Only” and “Cleaning & Sanitation” respectively). AI_04: Transmission Pathways Modification Corrective Action (Drain compartmentalization). CI_01: EC_02 and AI_02C2 were applied simultaneously. CI_02: EC_02 and AI_04 were applied simultaneously. CI_03: AI_02C2 and AI_04 were applied simultaneously.

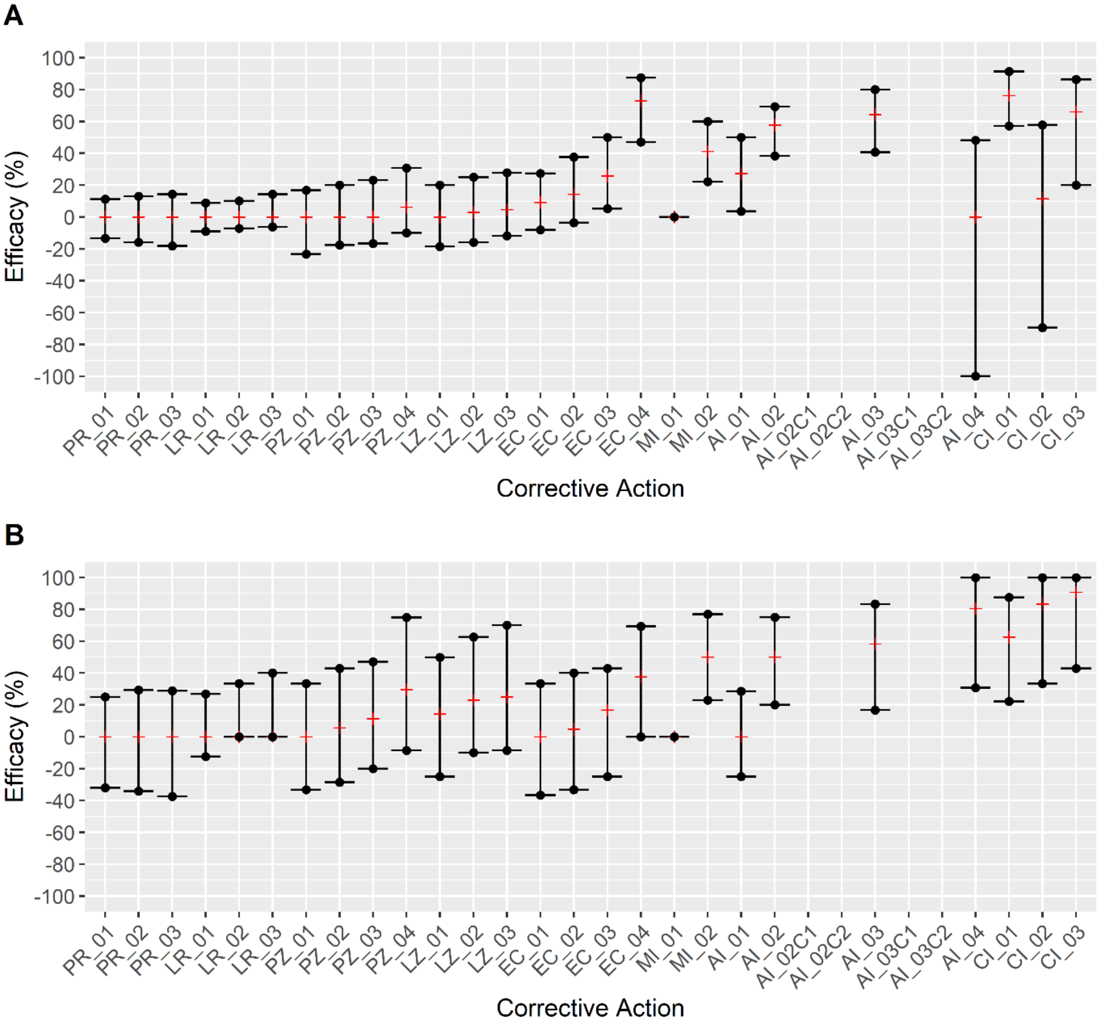

Fig 2.6 Comparison of corrective action efficacy against baseline conditions in Facility B

Efficacy was calculated using eq. 2.2 for each area (i.e., “wet” and “dry”) within a model for each applicable corrective action and displayed by median efficacy and interquartile range. Positive efficacy indicated a lower *Listeria* prevalence in the model with a corrective action compared to the baseline model and thus effectiveness of the corrective action, while zero or negative efficacy indicated that the corrective action is predicted to not be able to reduce the agent contamination prevalence. Panel A: Facility B Wet area. Panel B: Facility B Dry area. PR_01-PR_03: Random Event Occurrence Reduction (125%; 150%; 175% event delay from baseline respectively). LR_01-LR_03: Random Load Reduction (1-3 $\log_{10}$, respectively). PZ_01-PZ_04: Z4 Event Occurrence Reduction (25%; 50%; 75%; 100% reduction from baseline respectively). LZ_01-LZ_03: Z4 Load Reduction (1-3 $\log_{10}$, respectively). EC_01-EC_04: Reduction of *Listeria* Prevalence in incoming produce (25%; 50%; 75%; 100% reduction from baseline, respectively). MI_01: Cleaning Effectiveness Improvement (increased *Listeria* removed during reduction events increased by 3 $\log_{10}$). MI_02: Weekend Deep Clean (Removal of *Listeria* from all agents every Sunday). AI_01: Enhanced Flume Water Treatment (2 $\log_{10}$ removal of *Listeria* in flume agent per hour of production). AI_02/AI_02C1/AI_02C2: Broad Model-based Master Sanitization Restructuring (Agent cleaning and sanitation schedules were fully reassigned according to a mean contamination probability). AI_03/AI_03C1/AI_03C2: Directed Model-based Master Sanitization Schedule Restructuring (Sanitization of agents with a mean contamination probability ≥66% was set to a daily frequency). AI_04: Transmission Pathways Modification Corrective Action (Separation of forklift area assignment). CI_01: EC_02 and AI_03 were applied simultaneously. CI_02: EC_02 and AI_04 were applied simultaneously. CI_03: AI_03 and AI_04 were applied simultaneously.

Modifying Prevalence of Listeria Contamination in Incoming Produce

Reducing the prevalence of *Listeria* on incoming raw produce (scenarios EC_01, EC_02, EC_03 and EC_04) showed a corresponding drop in median prevalence of contamination on agents in both wet and dry areas. Facility A showed a maximum reduction (at EC_04) of 32.07 pp and 2.34 pp (Fig 2.7.A) for wet and dry area, respectively. Median *Listeria* concentrations on positive agents per area decreased by 0.57 $\log_{10}$ CFU/cm^2 and 0.52 $\log_{10}$ CFU/cm^2, respectively (Fig 2.7.C).

The maximum impact of reducing contamination prevalence on incoming raw produce in Facility B led to a reduction of 22.45 pp in median contamination prevalence for the wet area, and a median prevalence reduction of 1.71 pp in the dry area (Fig 2.7.B). Wet area *Listeria* concentrations on positive agents also showed a substantial decrease in predicted median (1.17 $\log_{10}$ CFU/cm^2; Fig 2.7.D), while predicted median *Listeria* concentrations in dry areas only were reduced by 0.20 $\log_{10}$ CFU/cm^2.

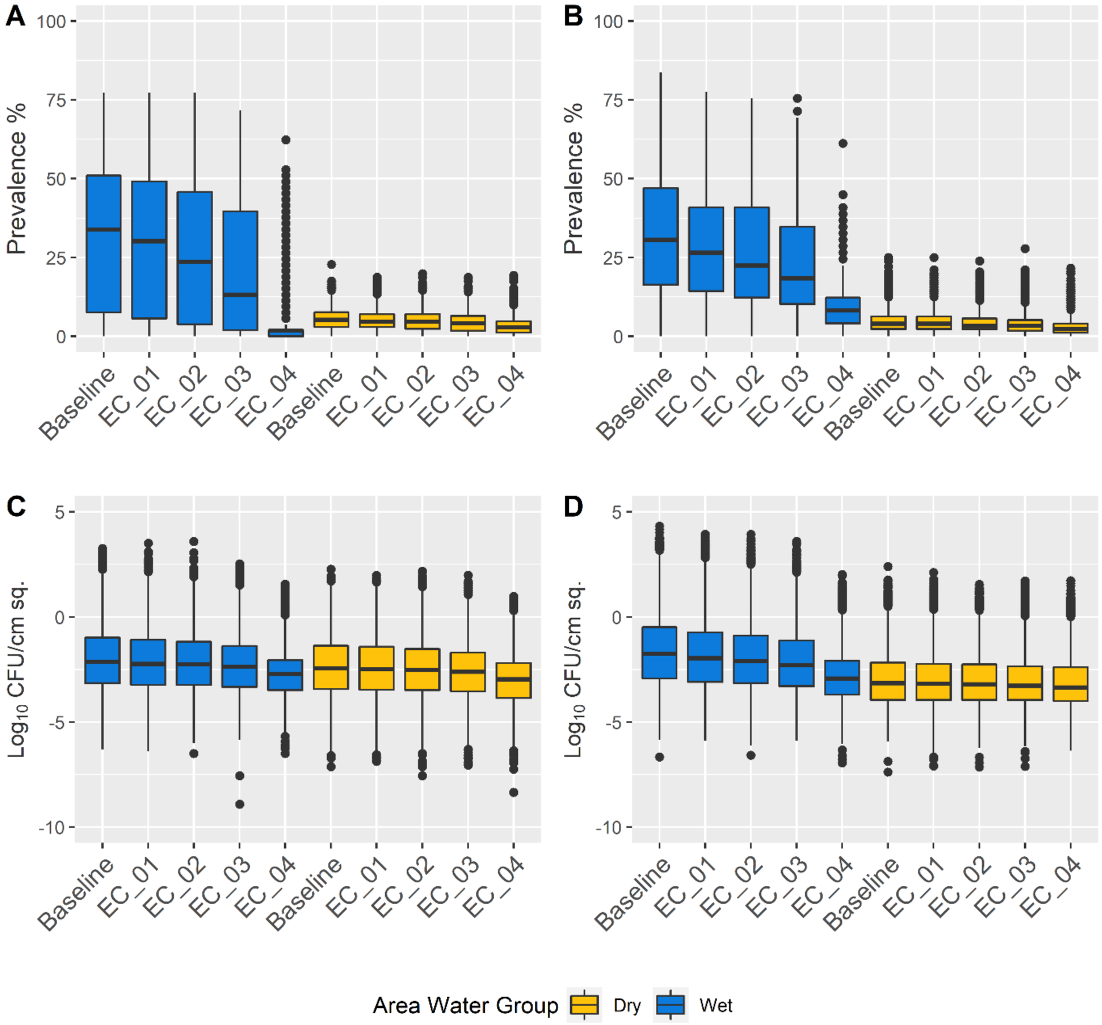

Fig 2.7 Boxplots describing the effects of reducing prevalence in incoming produce on both wet (blue) and dry (yellow) area agents
Listeria prevalence in incoming produce was reduced by multiplying the baseline *Listeria* prevalence by the factors of 0.75, 0.5, 0.25 and 0 (scenarios EC_01, EC_02, EC_03, and EC_04, respectively); this simulates produce being treated prior to arriving in the packinghouse packing room. A: Facility A *Listeria* contamination prevalence of all agents in wet and dry areas. B: Facility B *Listeria* contamination prevalence of all agents in wet and dry areas. C: Facility A *Listeria* $\log_{10}$ concentrations on all positive agents in wet and dry areas. D: Facility B *Listeria* $\log_{10}$ concentrations on all positive agents in wet and dry areas.

Enhanced Cleaning and Sanitation Strategies

Improving the effectiveness of *Listeria* removal actions ("Cleaning Only" and "Cleaning & Sanitation"; scenario MI_01) by 3 $\log_{10}$ showed no meaningful changes in Facility A or Facility B's median *Listeria* prevalence or concentration for either wet or dry areas. Weekend deep cleaning (scenario MI_02) however led to considerable reduction of median prevalence in both models. Facility A wet area prevalence decreased by 24.53 pp (Fig 2.8.A) and Facility B wet area prevalence decreased by 14.28 pp (Fig 2.8.B).

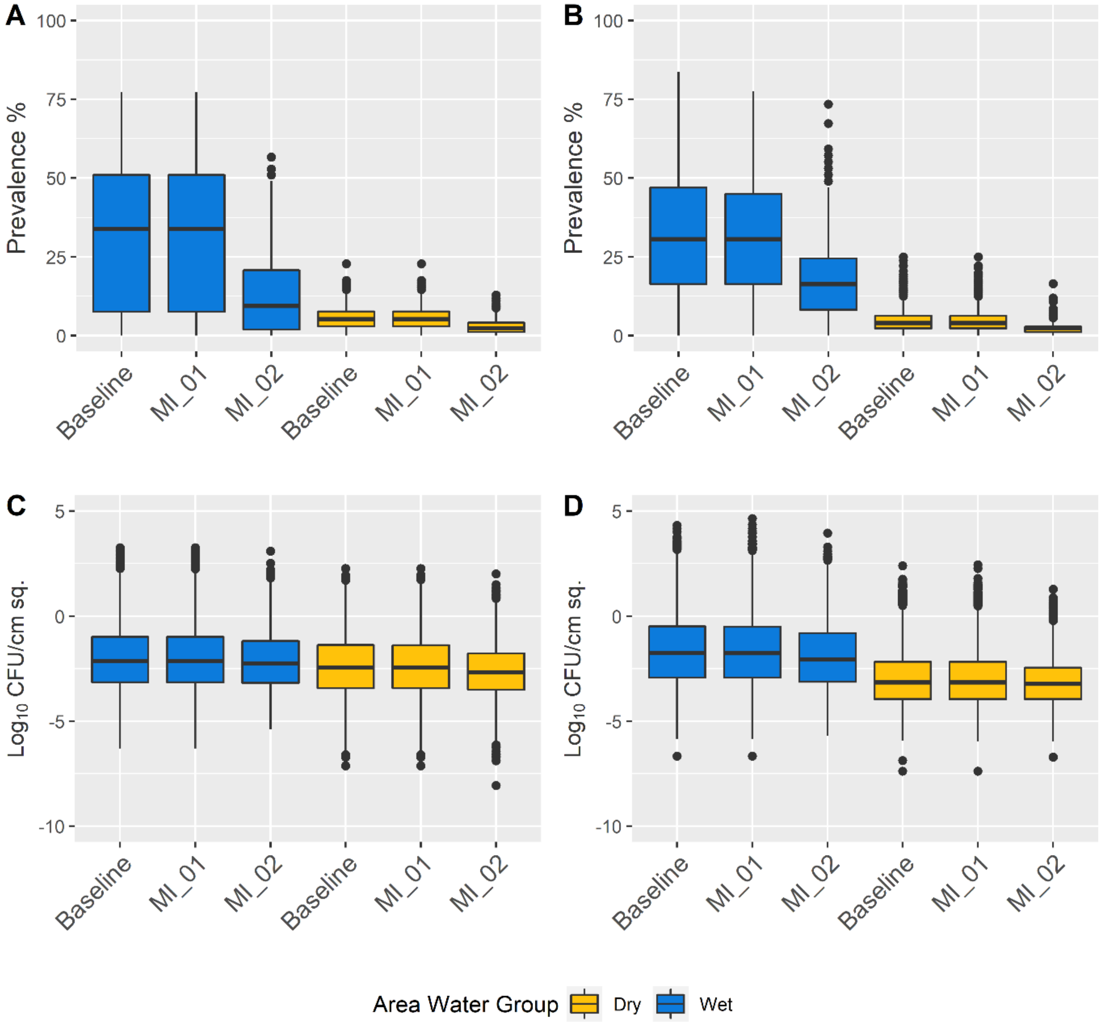

Fig 2.8 Boxplots describing the effects of increasing *Listeria* removal by 3 log$_{10}$ (scenario MI_01) or performing weekend deep cleaning (scenario M1_02) on both wet (blue) and dry (yellow) area agents
Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). Log$_{10}$ concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D). (MI_01: Cleaning Effectiveness Improvement (*Listeria* removal during reduction events increased by 3 log$_{10}$); MI_02: Weekend Deep Clean (Removal of all *Listeria* from all agents every Sunday))

Agent-Targeted Corrective Actions

In Facility A the enhanced flume water treatment (scenario AI_01) and risk-based (scenarios AI_02, AI_02C2, AI_03C2) “Cleaning Only”/ “Cleaning &

Sanitation" corrective actions showed the best performance in reducing both facility-wide median prevalence of contaminated agents and concentrations on contaminated agents; risk-based corrective actions are activities that target agents with higher probabilities of becoming contaminated according to the baseline model. Of the risk-based corrective actions applied, AI_02C2 had the largest impact in the wet areas (Fig 2.9.A), producing a median decrease in prevalence of contaminated agents against the baseline of 28.30 pp and a median decrease in concentration of 0.36 $\log_{10}$ CFU/cm^2. In contrast, AI_03 was the most effective intervention in Facility B's wet area, producing a median prevalence decrease of 22.45 pp (Fig 2.9.B) and median concentration decrease of 0.39 $\log_{10}$ CFU/cm^2 (Fig 2.9.D).

The "Transmission Pathways Modification Corrective Action" (AI_04) was applied to each model by eliminating connection links; the specific connections were chosen to have minimal impact to facility function and a high likelihood of implementation. This compartmentalization seeks to limit the spread of *Listeria* moving between zones (i.e., non-FCS-to-FCS transmission) by reducing the number of connections for strategically chosen agents. For example, in Facility A, the network of indoor square and trench drains was remodeled as isolated agents to simulate the introduction of anti-backflow valves within the system. This intervention reduced the Facility A wet area *Listeria* prevalence by 15.09 pp, concentration by 0.13 CFU/cm^2, dry area prevalence by 2.92 pp and the concentration by 0.70 $\log_{10}$ CFU/cm^2. In Facility B, the AI_04 corrective action involved assigning a single forklift to the Loading area operations, and one to its Reject area, rather than allowing both forklifts to interact with both areas. While this change in traffic patterns (i.e., connections) was effective for reducing the likelihood of *Listeria* contamination in the Facility B dry area, producing a median prevalence reduction of 3.41 pp, the amount of contamination on contaminated agents slightly increased by 0.11 $\log_{10}$ CFU/cm^2. As the corrective action was tailored to facility, it is unsurprising that the results of AI_04 differed between models. Compartmentalizing Facility A's drains prevented spread between wet and dry areas of the model, thus producing a facility-wide reduction in prevalence and concentration (Fig 2.9.A), while isolating Facility B's forklifts only impacted prevalence in dry areas.

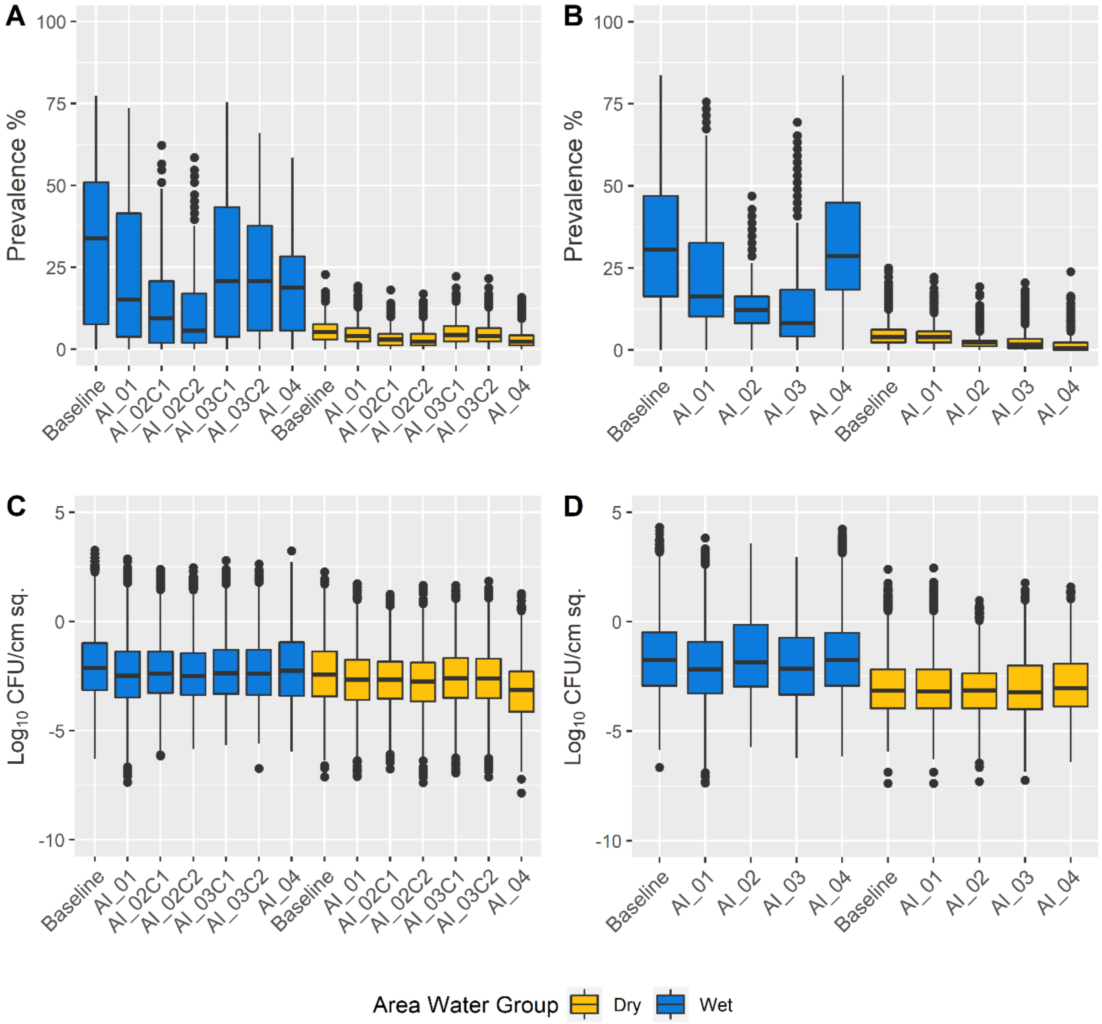

Fig 2.9 Boxplots describing the effects of various agent-specific corrective actions (scenarios AI_01, AI_02/AI_02C1/AI_02C2, AI_03/AI_03C1/AI_03C2, AI_04) on both wet (blue) and dry (yellow) area agents
Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). Log_{10} concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D). (AI_01: Enhanced Flume Water Treatment (2 $\log_{10}$ removal of *Listeria* in flume agent per hour of production); AI_02/AI_02C1/AI_02C2: Broad Model-based Master Sanitization Schedule Restructuring (Agent cleaning and sanitation schedules were fully reassigned according to mean contamination probability; Facility A was given a daily schedule for both "Cleaning Only" and "Cleaning & Sanitation" respectively); AI_03/AI_03C1/AI_03C2: Directed Model-based Master Sanitization Schedule Restructuring (Sanitization of agents with a mean predicted contamination probability ≥66% in the baseline model was set to daily cleaning; Facility A was given a daily schedule for both "Cleaning Only" and "Cleaning & Sanitation" respectively); AI_04: Transmission Pathways Modification Corrective Action (Facility A: Drain compartmentalization; Facility B: Separation of forklift area assignment))

Combined Corrective Actions

Of the three combined corrective actions, combinations CI_01 and CI_03 were most effective in reducing *Listeria* prevalence in Facility A wet areas (Fig 2.10.A; reduction of 24.53 pp for both); combination CI_01 decreased median concentration in the wet area by 0.44 $\log_{10}$ CFU/cm^2. For the dry areas, Scenario CI_03 showed the greatest reduction and efficacy with a median concentration decrease of 0.59 $\log_{10}$ CFU/cm^2 in the dry area (Fig 2.10.C).

In Facility B wet areas, CI_01 was the most effective correction action with a prevalence reduction of 24.49 pp (Fig 2.10.B). Median concentration among contaminated wet areas dropped by 0.66 $\log_{10}$ CFU/cm^2 (Fig 2.10.D). CI_02 and CI_03 were more effective in the dry areas with a decrease in prevalence of 3.41 pp for both; median concentration among contaminated dry areas increased by 0.08 and 0.18 $\log_{10}$ CFU/cm^2, respectively (Fig 2.10.D).

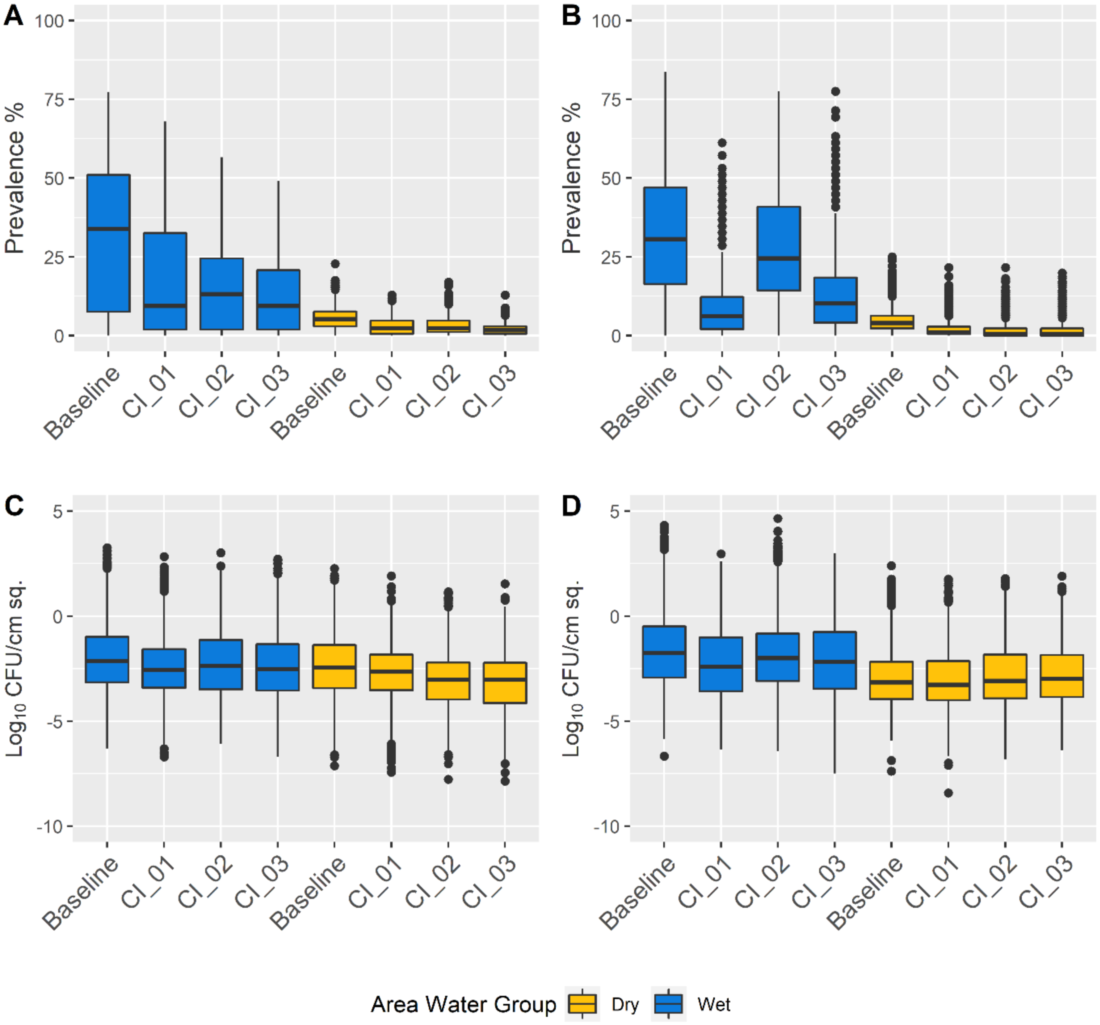

Fig 2.10 Boxplots describing the effects of the combined synergistic effect of simultaneous actions (scenarios CI_01, CI_02, CI_03) on both wet (blue) and dry (yellow) area agents

Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). $\log_{10}$ concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D). (CI_01: 50% Reduction of *Listeria* Prevalence in Incoming Produce and Broad Model-based Master Sanitization Schedule Restructuring were applied simultaneously in Facility A; 50% Reduction of *Listeria* Prevalence in Incoming Produce and Directed Model-based Master Sanitization Schedule Restructuring were applied simultaneously to Facility B; CI_02: 50% Reduction of *Listeria* Prevalence in Incoming Produce and Transmission Pathways Modification Corrective Action (Facility A: Drain compartmentalization; Facility B: Separation of forklift area assignment) were applied simultaneously to both; CI_03: Broad Model-based Master Sanitization Schedule Restructuring and Transmission Pathways Modification Corrective Action (Facility A: Drain compartmentalization; Facility B: Separation of forklift area assignment) were applied simultaneously in Facility A; Directed Model-based Master Sanitization Schedule Restructuring and Transmission Pathways Modification Corrective Action (Facility A: Drain compartmentalization; Facility B: Separation of forklift area assignment) were applied simultaneously to Facility B)

DISCUSSION

This study described the development of two ABMs of *Listeria* contamination dynamics in produce packinghouses, demonstrating their successful validation and characterization of baseline behaviors. Both facilities were functionally similar, receiving raw produce that is subsequently cleaned, sorted, and packed. However, the facilities differed in layout and specific food safety practices (e.g., frequency of "Cleaning Only" and "Cleaning & Sanitation" cleaning operations), which are important to consider when evaluating the risk of environmental *Listeria* contamination and mitigation strategies. Using the developed models, a range of corrective actions were tested, further demonstrating strengths of such ABMs as a decision support tool for industries. The most effective corrective actions in both models were: (i) reducing incoming *Listeria* on contaminated produce, (ii) simulation informed-informed modification of cleaning and sanitation strategies and (iii) eliminating specific agent-to-agent transmission pathways. While most of these corrective actions were more effective in the wet area of the respective facility than dry, eliminating specific transmission pathways (i.e., AI_04) was more effective in reducing the prevalence of Facility B's dry area.

Listeria Dynamics in Modeled Produce Packinghouses

Both models predicted elevated *Listeria* contamination within areas characterized as often containing a higher level of water, such as those areas involved in raw produce loading or cleaning operations. This predicted pattern of increased prevalence in areas containing higher levels of water agrees with findings regarding the water activity required for *L. monocytogenes* growth by Pietrysiak et al. and Farber et al. (42,43). However, the Reject area (which was classified as "dry") was also predicted to show elevated *Listeria* contamination prevalence compared to other Dry areas. While there may not have been water directly involved in this Reject area, wet or damaged produce was stored in large crates in this area for extended periods. This area contains "sink" sites that receive *Listeria*, but do not transfer it to another agent (sink sites were also described by Malley et al. (41)). Importantly, increased *Listeria* prevalence does not always rely on growth, it can simply reflect increased introduction without actual growth.

Dry areas showed lower contamination prevalence, but their closer proximity to the end of the product line presents a risk of contaminating finished produce. These areas are less likely to be contaminated by *Listeria* on incoming raw produce due to their distance from the loading area and lack of water in the area. This does not prevent them from being contaminated through alternative means (i.e., Zone 4 introduction) based on the facility's design. In this case, it is possible for other *Listeria* contamination routes to bypass other stages of a product line and reach finished produce more quickly. However, in these two models no Zone 2 or 3 surfaces were close enough to a Zone 4 introduction site to cause a large amount of *Listeria* contamination from it. Ultimately this is a facility-specific issue dependent on local layout and not mutually exclusive from introduction on contaminated raw produce.

Sensitivity analysis identified the concentration of *Listeria* spp. per gram of contaminated raw produce (N_R) and probability of contact from contaminated surface in Zone 1 to another surface in Zone 1 (P_{11}) as the two most influential factors in prevalence of *Listeria* contaminated agents in both facilities. Similar to previous reports (2,4), this suggests that if incoming produce is a primary source of *Listeria* introduced to the facilities, it would be able to rapidly spread among FCSs as mediated by P_{11} and subsequently contaminate finished product. In reality, it is difficult to trace the movement of *Listeria* spp. through a facility to such a fine degree. However, it is possible that even with a low contamination prevalence a sufficiently high volume of incoming raw produce may lead to introduction of an amount of *Listeria* that is likely to spread into the rest of the facility from the initial introduction site. Berrang et al. suggest a mechanism of *Listeria spp.* introduction like this may also occur in poultry processing plants (44).

Limiting Listeria Introduction into Produce Packinghouses

Corrective actions applied to the models were initially designed around targeting the three routes of *Listeria* introduction into a facility (i.e., contaminated raw produce, Zone 4, or random occurrences) to assess the effectiveness of preventing "exterior" *Listeria* entering the facility. Factors to consider in these corrective actions include: facility layout (especially agent proximity to potential Zone 4 introduction sites (44,45)), postharvest contamination status of raw produce, and employee movement patterns. Incoming raw produce (the primary route in both models) has also been identified as a key vehicle of introduction in other studies involving *L. monocytogenes* (44,46–48), reinforcing the findings of both the sensitivity analysis and route-based corrective action comparisons. Although controlling the prevalence of *Listeria* on incoming raw produce can be difficult given the abundance and randomness of external sources that can contaminate produce before reaching a packinghouse (49,50), stringent implementation and verification of Good Agricultural Practices (GAP) and other supply chain programs could represent one strategy to reduce *Listeria* prevalence on incoming raw produce.

Modifying Surface Cleanability and Listeria Harborage Capabilities

A second series of corrective actions were formed under the assumption that introduction could not be reduced, effectively targeting "interior" *Listeria* that has successfully entered the virtual facility. These measures involved enhancing or reorganizing existing measures used to reduce *Listeria*, such as increasing the effectiveness and frequency of sanitation events or changing in plant transmission routes (e.g., by restricting equipment such as forklifts to a specific room). While the exact implementation differed between modeled facilities, similar corrective actions have been historically implemented to control *Listeria* spread within food facilities, specifically in the forms of increased cleaning and sanitation frequency or replacing equipment with easier to clean versions, or by modifying equipment to eliminate niches (20) (both of which were implicitly and simplistically represented in the developed ABMs in corrective action scenarios that altered an agent’s cleanability, from uncleanable to cleanable).

The two types of cleaning and sanitation schedule restructuring evaluated were: (i) broad, where surfaces are eligible for cleaning and sanitation, with the mean contamination probability determining specific schedule frequency, and (ii) directed, where surfaces with a mean contamination probability ≥66% are rescheduled and subsequently cleaned and sanitized every day of the work week. A key difference in the *Listeria* contamination prevalence outcomes following restructuring of the cleaning and sanitation schedule between both facilities is due to differences in their initial schedules; with Facility A only performing a weekly cleaning and sanitation operation, and Facility B cleaning and sanitation at a daily frequency. As a result, introducing a higher frequency of cleaning and sanitation operations for more surfaces showed an improvement for Facility A regardless of whether the cleaning and sanitation scenario implemented is more (AI_02C2: “Cleaning & Sanitation”) or less (AI_02C1: “Cleaning Only”) comprehensive. This improvement was less effective in directed restructuring (AI_03C1/AI_03C2), as fewer surfaces were scheduled to undergo daily cleaning or cleaning and sanitation regardless of the method used. Conversely, Facility B showed the opposite pattern, with broad rescheduling producing smaller reductions in *Listeria* contamination prevalence than directed rescheduling. This is likely due to surfaces in Facility B that were initially cleaned and sanitized at a daily frequency being switched to a less frequent cleaning and sanitation operation due to a lower contamination probability under broad schedule restructuring. In contrast, directed schedule restructuring could only increase the frequency selected surfaces were cleaned and sanitized at. This highlights an important point that the predicted contamination probability of an agent (i.e., a surface that the agent represents) should not necessarily be the sole defining factor for determining the frequency of its cleaning and sanitation. Instead, schedule reorganization should be viewed with the context of prior conditions and surface-specific information (such as Zone, proximity to water and connectivity) to avoid deprioritizing key equipment surfaces. Tompkin (20) showed that the exact response to detecting contamination by industry can differ by the facility, food being handled, and equipment in question, but corrective actions typically will take into account various factors (such as existing cleaning and sanitation frequency, material composition and other relevant practices). In both models some of the most effective corrective actions similarly required situation-specific interpretation and analysis to be implemented; this methodology is reinforced by the number of corrective actions built with site-specific considerations detailed by Tompkins (20).

Several corrective action scenarios evaluated here (i.e., AI_02/AI_02C1/ AI_02C2 and AI_03/AI_03C1/AI_03C2) required information on the agent-specific contamination probability. This demonstrates a distinct advantage in ABM usage in providing supplementary *in silico* data to an EMP, as the data, such as a surface's predicted risk of being contaminated, can allow a facility to investigate and focus efforts on locations of higher predicted contamination risk. This may be of particular use in the event of a data scarcity in an EMP, which may be caused by insufficient coverage that cannot reliably detect *Listeria spp.* presence throughout a facility (51). Though a lack of data can be alleviated with intensive validation sampling (7), an

ABM may be a highly practical tool in directing efforts more quickly and efficiently, ultimately saving time and money (25). Furthermore, given the practical impossibility of a facility to assess multiple corrective actions in reality, an ABM can evaluate various corrective action scenarios and advise which are more likely to be useful.

Limiting Listeria Transmission Across Equipment Surfaces

Lastly, the third corrective action strategy, modifying existing surface transmission pathways, was functionally the most unique, as it wholly depended on the facility's preexisting layout and equipment structure. While it is relatively straightforward to take a single piece of equipment in isolation and determine potential risks during production, the interaction effects between multiple surfaces may be more difficult to assess. Good Manufacturing Practices (GMPs) (52) generally require compartmentalization within a facility to limit pathogen transmission (typically referred to as hygienic zoning) but employing an ABM can allow for a more extensive review of such control strategies in a relatively rapid timeframe. Though some transmission pathways cannot be removed or modified due to their critical functions (i.e., major belts or the flume system), there are several auxiliary equipment surfaces that may present a greater risk than initially considered due to elevated connectivity between them. These transmission pathways may allow for cross-contamination outside of typical FCS-to-FCS routes.

Transmission pathways were identified in both models, that were not critical to packinghouse operations but still allowed for *Listeria* movement between areas; subsequently they were modified to restrict transmission. The layout of Facility A's production line was predominantly designed as a one-way flow, but it featured a highly interconnected drainage system spanning multiple areas. In corrective action AI_04, the drain system in Facility A was modified to restrict drain cross-contamination, which was particularly effective in areas needing more drainage (i.e., wet areas). In practice, redesigning a facility's entire drainage system with anti-backflow valves would take time and effort to complete, and may be more practical when constructing a new packinghouse facility. Implementing AI_04 in Facility B was more straightforward, as the activity of two forklift agents operating between two interior areas allowed for frequent *Listeria* cross-contamination between the Reject and Loading areas. Limiting a single forklift to each area severed any direct contamination routes, leaving *Listeria* only able to follow the production line to reach the Reject area. This compartmentalization directly limited the spread of *Listeria* into other areas and demonstrates that a relatively simple corrective action can have widespread impact. In practice, reducing surface interconnectivity, may also be implemented through employee training or redesign of equipment to reduce or prevent cross-contamination (53).

Combined Corrective Actions Have Facility-Wide Impact on Listeria Harborage

As stated previously, a major advantage of an ABM is the ability to generate predictions specific to individual equipment surfaces and for specific simulated corrective actions, or their combinations, and subsequently direct efforts in more focused course. For both models, a useful metrics of measuring the performance of a corrective action was investigating its efficacy and comparing change in the *Listeria* contamination prevalence in wet and dry areas. The three combined corrective action scenarios (CI_01-CI_03) were selected from individual corrective action types that showed the highest *Listeria* contamination prevalence reductions in either area water group: reducing the *Listeria* prevalence on incoming raw produce, cleaning and sanitation schedule restructuring and transmission pathway modification. Of these options, a 50% reduction in *Listeria* prevalence on raw produce was chosen as a more plausible outcome than complete elimination of contamination on incoming raw produce, and each facility had its own best-performing respective cleaning and sanitation restructuring option chosen. These combined corrective actions could then be simulated and further analyzed themselves to determine the performance of using multiple corrective actions simultaneously. While this is largely similar to previous scenarios, combining corrective actions is an already suggested general strategy (54) and has the benefit of relying on multiple layers of defense. The selection and evaluation of which specific corrective actions to combine however, can be more robustly done with the additional performance data provided by an ABM's simulations.

Limitations and Future Directions

It should be noted that a fundamental limitation facing both models was the relatively small amount of historical data available, limiting the extent of validation. It is also possible that low historical prevalence in certain areas in the facility may make it more difficult to detect meaningful levels of improvement, given an already low baseline to begin with. Additionally, both models were simulated on a virtual timescale of two weeks, making for relatively short-term observations (though at the same time addressing the industry needs for decision support tools for short-term planning). Various interventions may have far more noticeable consequences to their facilities if observed for a longer period, which should be subject of a future investigation.

Future models should also include economic factors to assess the most appropriate interventions or combinations of interventions that should be implemented in a given facility. While the first reaction may be to implement as many separate corrections as possible for overlapping protection, each extra layer will incur additional costs (46). Instead, being able to identify potentially more cost-effective actions, such as employee training to reduce cross contamination (47) instead of equipment replacement, would allow for better decision-making that optimizes resource allocation. Furthermore, incorporating economic factors could allow for the model to estimate the overall cost to a facility of having to operate with more systemic issues, such as layout and drainage methods (48). A key takeaway that is applicable to any type of packinghouse or food processing facility attempting to combat ongoing *Listeria* contamination is that each facility should be treated uniquely and addressed with specifically designed corrective actions that have highest potential effectiveness (49). Models such those developed here could aid design of the facility specific corrective actions.

An additional direction for future development may include the use of ABMs in testing measures already implemented within a facility under hypothetical situations of an increased contamination risk. While the scenarios demonstrated here were to trial corrective actions, similar techniques could be used to test the impact of increased *Listeria* contamination (e.g., in incoming material) on facility and finished product contamination under the currently implemented procedures in a facility. This would expand the scope that these models can be applied to, allowing them to be used in both a diagnostic capacity for solving existing contamination issues, and to assist in pre-emptively assessing how well a facility would be able to reduce *Listeria* contamination risks in the event of a system failure.

CONCLUSIONS

Once established within a packinghouse, *Listeria* spp. has proven to be difficult to control, and decision support tools such as the ABM reported may be valuable in not only quantifying how contamination may move through a facility, but in finding effective options for combating it. With Facilities A and B, we have illustrated that ABMs can serve as highly adaptable tools in the field of food safety through their ability to replicate the unique components of individual produce packinghouses. From our ABM scenarios, targeting *Listeria* that is introduced through the primary contamination route (in this case contaminated incoming raw produce) was the most effective method in prevalence reduction, and may be generalizable to different facilities as its implementation does not depend on a facility's specific layout. However, assessing the effectiveness of this strategy relies on contamination data that currently are rarely available. Therefore, it may be more practical to focus on designing in-house corrective actions, such as increasing the frequency at which select surfaces are sanitized and employing measures to limit contamination spread between equipment surfaces, that account for facility-wide conditions and patterns. An element of particular note in this regard is the local presence of water within an area, as it has shown to affect *Listeria* growth and the performance of corrective actions within the specific area. The *in silico* data generated by ABMs has also shown to be useful for designing and evaluating additional corrective action scenarios. Combining contamination and corrective action results from an ABM with relevant economics data would further aid in determining the overall feasibility of implementing corrective actions specifically to individual facilities.

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CHAPTER 3

EXAMINING PATTERNS OF PERSISTENT *LISTERIA* CONTAMINATION IN PACKINGHOUSES USING AGENT-BASED MODELS

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Key words: agent-based model, *Listeria*, corrective actions, persistence, produce, packinghouse, decision support tool

ABSTRACT

Persistent contamination may occur in a packinghouse when *Listeria monocytogenes* successfully infiltrates the facility and reaches a harborage site, from where it is difficult to remove. This may threaten to contaminate post-harvest produce within the facility and ultimately endanger public health. Simulation-based decision tools can be used to predict which equipment surfaces may undergo persistent contamination and simulate potential corrective actions employed to prevent it. Thus, we developed long-term, fine-scale spatiotemporal agent-based models of *Listeria* contamination and persistence dynamics for two produce packinghouse facilities. Analysis of *Listeria* contamination predictions at an hourly level allowed for the distinct identification of persistent and transient *Listeria* contamination patterns on agents (representing equipment surfaces and employees). The testing of simulated corrective actions revealed that methods which involved risk-based sanitation were the most effective both long-term in reducing contamination persistence and in reducing the likelihood of occurrence of persistent contamination. This emphasizes that a generalized approach is unlikely to be particularly successful in long-term performance and suggests that a facility-specific sanitation schedule and hygienic design would be key in reducing persistence. Hourly *Listeria* contamination patterns also suggest that transient contamination may be accidentally mistaken for persistent contamination, depending on the frequency of environmental monitoring. Likewise, since concentrations of *Listeria* on most contaminated agents were predicted to be very low, there is also a possibility to mistake persistence for a transient contamination of surfaces or even missed contamination all together due to false negative results of

environmental monitoring for *Listeria* presence. Overall, these findings support that agent-based models may be of considerable assistance to facilities in a decision-support capacity, aiding in the identification of contamination patterns within individual packinghouses and assessing the viability of specific corrective actions.

INTRODUCTION

Within a food distribution chain there are several steps following harvest before foods (such as fresh produce) reach the consumer. Facilities such as packinghouses, which may receive produce from several different suppliers and similarly distribute to several destinations, are key points in this chain that risk spreading microbial hazards if contaminated. One such concern is *Listeria monocytogenes*, a gram-positive bacterium (1) of particular concern due to its ability to persist in a number of potential harborage sites within food handling equipment, as well as the presence of environmental conditions in these facilities favoring bacterial growth (2,3). Infection with this foodborne pathogen, listeriosis, can exhibit itself as more nondescript nausea, vomiting, fever and diarrhea in milder cases, or develop into meningitis, encephalitis, septicemia, abortion and death in more severe cases (1). Listeriosis has a relatively low incidence rate (0.1-10 cases per million per annum. depending on the specific country (4)), but its case-fatality rate can lie between 20% to 30% (5). Historically *L. monocytogenes* has been linked to a number of food-borne outbreaks including those linked to packinghouse facilities (6–8). As mentioned, the potentially large number of sites capable of harboring *Listeria* spp. in these food production environments may lead to persistent contamination of the environmental sites, which may provide the pathogen opportunity to contaminate food. Thus, removal of *L. monocytes* from a food production facility is an absolute necessity in ensuring food safety (1,9) and preventing further cross-contamination (2). The exact methods involved in successful sanitation will depend on a facility’s purpose, layout and equipment in use, as some may feature component designs that are more difficult to reliably sanitize (e.g., equipment casings with gaps that may allow moisture inside or equipment with internal components that are difficult to reach during regular sanitation) (2,10). Controlling contamination within a facility requires awareness of potential routes of contamination, be they external (e.g., incoming contaminated produce, employees arriving within the facility or random events like water dripping from the ceiling) (11) or internal (e.g., spreading from a contaminated surface through surface-to-surface contact, or via water) (12). When contamination is present within a facility, it can be due to two different patterns: (i) transient, where equipment is regularly contaminated by another source (external routes directly, or due to surface-to-surface transmission) but is also summarily removed; and (ii) persistent, where contamination is not properly removed from the surface (potentially due to the presence of a harborage site, or niche, that prevents surface sanitation during routine cleaning & sanitation (10)). While each ultimately results in the contamination of equipment and potentially the rest of the facility, presenting the public health risk, these two different patterns of contamination should be controlled in distinct methods: transient contamination may be unavoidable due to facility functions, but is also already regularly removed as part of the standard sanitation practices, whereas persistent contamination either requires a modification of sanitation practices, niche removal or sufficiently isolating the surface to prevent pathogen spread from it. Mistaking one pattern for another (i.e., assuming a transient contamination event to be persistent or vice versa) may result in an ineffective sanitation countermeasure, requiring a better understanding of how *L. monocytogenes* contamination moves through a facility. Practically, it is difficult to identify which pattern is affecting which surfaces within a facility due to the complexity in reliably tracing pathogen movement at a fine temporal resolution, as well as the fact that a surface undergoing persistent contamination can induce transient contamination in surrounding surfaces as well. Both phenomena are also capable of occurring simultaneously (i.e., a surface already undergoing persistent contamination can be repeatedly exposed by transient contamination as well), further obfuscating the root contamination cause for individual surfaces. The presence of *Listeria* spp. contamination (serving as an indicator of *L. monocytogenes*) can be detected via environmental monitoring programs (EMPs), allowing a facility to maintain internal surveillance and monitor the effectiveness of ongoing contamination countermeasures (13). However, how reliable EMPs are in revealing the contamination patterns in a food facility is not well understood. This may be further complicated by the usage of different criteria in defining persistence through EMPs used in different facilities, as Ferreira et al. noted limits in the discriminatory abilities of some phenotypic or genotypic subtyping methods used to determine persistence (9).

Tracing an issue as physically small as *Listeria* spp. contamination requires a functioning EMP for reliable detection, however, the complex nature of produce packinghouses or food processing facilities may make it difficult to reliably monitor every surface. Simulation model-based decision support tools can be used to further enhance safety protocols such as EMPs. One such tool is an agent-based model (ABM), a computational model designed to simulate the large number of heterogenous and autonomous parts that make up a produce packinghouse. In an ABM, these components are termed “agents”, and can interact with both themselves, and the environment of the model, depending on each agent's individual configuration. Consequently, this allows an ABM a very fine level of virtual observation (i.e., prediction) as every agent can be individually monitored. This form of *in silico* simulation can provide insight into pathogen movement, scrutinize existing EMPs, and determine the effectiveness of potential corrective actions to a facility (14–16).

The persistent presence of *Listeria* spp. within a food processing facility or produce packinghouse despite sanitation measures represents an ongoing risk of re-contamination to both equipment and any food being processed, though it is notably more difficult to define contamination as persistent solely through EMP (2). The primary objective of this study was to develop an ABM and analysis method to track persistence of *Listeria* spp. within a food production environment, using two different fresh produce packinghouses as model systems: said persistence within these models was evaluated at a fine temporal and spatial scale. The secondary objective was to use the developed method to evaluate potential corrective actions for controlling *Listeria* spp. in a produce packinghouse with regards to their effectiveness in combating persistent contamination.

METHODS

Model Construction

Previously created and validated ABMs of two produce packinghouses (models "Facility A" and "Facility B") (Chapter 2), were modified in NetLogo 6.2.0 (17) for the purpose of this study. These models have originally been built to represent the primary operations room of packinghouses. They have been constructed based on in-person visits to the modeled facilities to conduct behavioral mapping (18) of employee activities, determine the overall facility layout, key surfaces to be represented as agents in the model, water presence and patterns, traffic patterns, agent-to-agent connections, and event scheduling (Tables S2.1-S2.5). Briefly, the agents in the modeled facilities are grouped into functional areas as follows:

(i) Loading: produce is brought into the facility by forklift and loaded into a flume system

(ii) Cleaning: produce undergoes culling, brushing and waxing

(iii) Sorting: produce is sorted by size, color, and weight to determine the next destination

(iv) Bag-Packing/Tray-Packing: produce is hand-packed into paper bags/trays respectively

(v) Reject: produce that does not meet set criteria during sorting is rejected

(vi) Other: a collection of agents that do not fit the previous groups

Agents within the Loading and Cleaning areas are together classified as being in the "wet" area of the model, due to a close proximity to water in both facilities, while the remaining agents are classified under the "dry" area. Each agent also has its own unique fixed and time-varying attributes (Table 3.1) and is connected to the other agents via specific links to represent the surface-to-surface contact structure. Agents can be connected either via: (i) "Directed" one-way links and (ii) "Undirected" two-way links, with "directed" links representing single direction movement in the facility such as belts and rollers, and "undirected" links representing surfaces capable of repeated proximity contact. During one-way transmission, the agent "sending" *Listeria* to another agent is referred to as performing “transfer”, while the agent receiving *Listeria* performs “reception”. Input parameters (Tables 2.2 and S2.6-S2.9) are also established as either fixed values or probability distributions using observation from in-person visits, literature, and academic and industry experts. Parameters in the models that are represented as probability distributions are controlled by an independent global random seed to ensure comparability across scenarios. A second local seed is used within scenarios to ensure repeatability. Time in both models is represented as one-hour time steps (ticks). Both models previously have been validated by comparing their respective facility’s historical sampling data to the predictions from simulated EMP (Chapter 2).

Table 3.1 Fixed and time-varying attributes of an agent within the model

Name	Description
Fixed agent attributes	
Position (x,y)	Position in a 2D plane
Height (cm)	Distance from floor
Zone Category (1-3)	Category as per proximity to food products (Zone 1: Food-Contact Surfaces (FCS); Zone 2: Non-Food-Contact Surfaces (NFCS) in close proximity to food and FCS; Zone 3: NFCS not in close proximity to food or FCS (19,20))
Cleanability (Cleanable/Uncleanable)	‘Uncleanable’ if the agent’s design characteristics or location are such that routine sanitation cannot remove *Listeria* once the agent becomes contaminated (e.g., if *Listeria* enters gaps in the equipment surface where disinfectant cannot penetrate), ‘Cleanable’ otherwise
Cleaning Frequency (Alternating Days /Weekly/Daily)	How frequently can the agent undergo sanitation?
Surface Area (cm^2)	Actual surface area of the surface represented by the agent
Time varying agent attributes	
Listeria quantity (CFU and CFU/cm^2)	Absolute *Listeria* quantity (CFU) and concentration (CFU/cm^2) on an agent (calculated using respective CFU and surface area)
Contamination Event Counts	Frequency of (i) contamination from raw incoming food material; (ii) contamination from random introduction occurrences that could affect anywhere in the facility; (iii) contamination from “Zone 4” (outside the model) (19,20); (iv) “Transfer” to another agents; (v) “Reception” from another agent
Agent Water Level (1-3)	Amount of water on the agent consisting of three levels: (1: no water; 2: damp to the touch; 3: visible water on agent)
Niche formation events	Niche formation: *Listeria* has spread onto an “uncleanable” agent

In the current study, the above-described models were modified for the purpose of observing *Listeria* spp. persistence patterns in the produce packing environments. Specifically, the models were modified in NetLogo to run for a period of eight virtual weeks and to track each agent's *Listeria* concentration per hour to allow for the observation of contamination persistence. Additionally, each model's simulated EMP was expanded to be a weekly reoccurrence of simulated sampling to detect *Listeria* contamination in the facility for the duration of the simulation, simulating ongoing surveillance by the facility. This represented the baseline model setup where we modeled only the control strategies that are routinely implemented in each of the two facilities (i.e., Facility A performed weekly cleaning of all surfaces on Saturday, followed by a weekly sanitation of all surfaces on Monday; Facility B performed daily sanitation for eligible surfaces during the work week, and every week sanitized all surfaces on Saturday). Alternative scenarios were modeled to evaluate contamination persistence dynamics and effectiveness of different potential strategies to control *Listeria* persistence in a facility. The parameters of specific interest to represent these alternative scenarios are: prevalence of *Listeria* contamination on incoming produce (R_d), Zone 4 event probability (P_z) and quantity (CFU) of *Listeria* introduced during it (N_z), and Random event probability (P_r) and quantity introduced during it (N_r); these parameters are defined in Table 3.2 and shown along with their values representing the model baseline setup (definitions and values of the remaining model parameters can be found in Table S2.6). Finally, a novel analysis method was developed in R 4.0.5 software (21) to evaluate *Listeria* persistence while handling the large volume of data produced by the simulation model: each of the 224 and 225 agents (a) modeled for Facilities A and B, respectively, observed at each hour (h) of the 8 week-long model simulation and repeated over 100 model iterations (i) (method further described in the Model analysis section). This structure of produced simulation data (consisting of 224 x 1343 x 100 and 225 x 1343 x 100 datapoints for each respective model) was evaluated and compared across the baseline and scenarios that evaluated different control strategies. The decision to simulate each model over 100 iterations was made in an effort to balance the need to explore the parameter space and agent interactions while at the same time being able to manipulate and analyze in R the large volume of produced simulation data. Data files and code used to build the two agent-based models using NetLogo, as well as data files and R code relevant to the scenario analysis, and persistence analysis, are available on the GitHub repository: https://github.com/IvanekLab/CPS_Persistence_Models.

Table 3.2 Baseline specific model input parameters, description, equation and distribution, summary values and sources for *Listeria* spp. introduction, growth, transmission, and reduction.

Symbol	Description	Equation/Distribution	Mean	5th-95th Percentile	Reference
R_d	Probability of a crate of incoming raw produce containing *Listeria*-contaminated produce on day d, for d = *Monday*, *Tuesday*, *Wednesday*, *Thursday*, *Friday*	$10^{\mathrm{Pert}(-2.3,-0.6,-0.6,5.4)}$	0.161	[5.82E-2, 0.243]	(22)
P_z	Probability that *Listeria* spp. is introduced into the room via objects from Zone 4 per hour	$10^{\mathrm{Pert}(-2.3,-0.9,-0.6,4.8)}$	0.14	[0.02, 0.36]	(22)
N_z	Amount of *Listeria* spp. introduced to an	$10^{\mathrm{Pert}(0.0,1.9,3.3,4.2)}$	156	[6.04, 618.79]	(22)

	agent or patch from Zone 4 (CFU) per occurrence				
P_r	Rate of random event occurrences that introduce *Listeria* spp. from outside the room per hour	$10^{\mathrm{Pert}(-4.3,-0.9,-0.6,4.6)}$	0.07	[4.00E-3, 0.203]	(22)
N_r	Amount of *Listeria* spp. introduced per random event (CFU)	$10^{\mathrm{Pert}(0.2,3.3,3.7,3.3)}$	1233	[42, 3829]	(22)
η_d	Log$_{10}$ reduction of *Listeria* spp. from equipment during "Cleaning & Sanitation" on day d, in Facility A d=Saturday; in Facility B for d=*Monday, Tuesday, Wednesday, Thursday, Friday*	Pert(-8,-6,-1.5,4)	-5.58	[-7.36, -3.47]	(23)

Model Analysis

Two main outcomes of interest were used for analysis (Table 3.3). The first was the concentration of *Listeria* on each agent at every hour of the simulation across multiple iterations ($C\_T_{a,h,i}$ where subscripts are a=agent, h=simulation hour and i=iteration), which was used to observe concentration values on each agent over the course of the entire simulation (i.e., at each hour of the model h=[1, ..., 1344]). To describe how contamination concentration of an agent varies over time and from iteration to iteration, this outcome was summarized for each agent and hour across model iterations using heatmaps; for comparison purposes, a median over model iterations in which the agent was contaminated was also calculated (C_T_{a,h_M}). Hourly $C\_T_{a,h,i}$ was converted into a binary variable to serve as the second main outcome of interest: an agent's contamination status (1=contaminated, 0=not-contaminated) over time and across iterations, $L\_T_{a,h,i}$. To describe how contamination probability varies from iteration to iteration, this outcome was summarized by calculating the proportion of iterations in which the agent a was contaminated at time h (L_T_{a,h_P}).

Two secondary outcomes of interest were established to describe the results of the simulated EMP, which occurred at every week (w) Midday 12:00 pm, Wednesday, i.e., at hours h=(85, 253, 421, …, 1261) corresponding to weeks w=(1 2, 3, …, 8), respectively. Thus, similarly to the primary outcomes $C\_T_{a,h,i}$ and $L\_T_{a,h,i}$, here the interest was in capturing the contamination concentration and status of an agent at simulated weekly environmental *Listeria* monitoring events for each week and iteration (i.e., $C\_T_{a,w,i}$ and $L\_T_{a,w,i}$) and the corresponding median concentration and the probability of agent contamination over all iterations at a given week (i.e., C_T_{a,w_M} and L_T_{a,w_P}). It should be noted that $C\_T_{a,w,i}$ and $L\_T_{a,w,i}$ as well C_T_{a,w_M} and L_T_{a,w_P} are subsets of the primary outcomes and their associated summary statistics, just evaluated at specific weekly intervals as opposed to hourly. For comparison purposes these weekly outcomes were further summarized separately for different sub-groups of agents in a facility at a given week and over all agents separately at each week of model simulation. Also, for ease of interpretation of boxplot comparisons, we estimated the difference between the median prevalences (expressed as percentage point (pp) difference) and between median concentrations (expressed as log10 CFU/cm^2) predicted for the corrective action scenario and the baseline.

Table 3.3 Primary outcomes of interest, their definitions and the associated summery statistics calculated over all model iterations

Notation	Name	Definition	Associated summary statistic
$C\_T_{a,h,i}$	Hourly agent contamination concentration	Concentration recorded for agent (a) per hour (h) and iteration (i)	Median ($\log_{10}$ transformed) concentration of *Listeria* on agent (a) per hour (h) was calculated over all model iterations in which the agent was predicted to be contaminated (C_T_{a,h_M})
$L\_T_{a,h,i}$	Hourly agent contamination status	$C\_T_{a,h,i}$ was converted into a binary equivalent (with 1 for contaminated and 0 for non-contaminated) for agent (a) per hour (h) and iteration (i)	Probability of contamination for each agent (a) per hour (h) was calculated by dividing the number of iterations in which the agent was predicted to be contaminated by the total number of iterations, 100 (L_T_{a,h_P})

Persistence Definition and Calculation

An agent was classified as having undergone persistent contamination in an iteration if it remained contaminated for a duration of time ($D_{c\_a,i}$, where subscript a=agent, i=iteration) longer than the period of time between two consecutive sanitation events. Thus, the time taken before an agent can be classified as persistently contaminated is dependent on the agent’s sanitation schedule. For example, an agent sanitized daily was required to be contaminated for more than 48 hours to reach the persistency status, while an agent sanitized on a weekly basis was required to be contaminated for more than 336 hours (14 days) to be considered persistently contaminated. Agents representing locations or surfaces with design characteristics that deemed them “uncleanable” once contaminated were also classified as being persistently contaminated after 14 days. This approach was used because “uncleanable” agents largely represented surfaces that were never subjected to routine sanitation in the modeled facilities. Any durations shorter than an agent’s persistency threshold were classified as transient contamination. Both these terms are based on hourly contamination data (i.e., $C\_T_{a,h,i}$ and $L\_T_{a,h,I}$) and represent the "truth" of the simulation; however, persistence is typically observed in the real world through sampling occurrences as part of the EMP, e.g., weekly (i.e., $L\_T_{a,w,i}$). In this instance, this periodic observation may result in the detection of "apparent persistence" if two sequential weekly monitoring occurrences test positive for a specific agent; importantly, the inferred persistence status is termed "apparent" because the true contamination status during the whole period of time could not be observed (Table 3.4).

Table 3.4 Definition of contamination pattern terminology

Name	Description	Model Implementation
Persistent	Contamination remains present on an agent following the completion of two sanitation events. (According to hourly contamination data)	$D_{c\_a,i}>48$ (For agents with daily sanitation) $D_{c\_a,i}>336$ (For agents with weekly sanitation)
Transient	Contamination is removed from an agent during sanitation but is re-contaminated at a later point. (According to hourly contamination data)	$D_{c\_a,i}\leq 48$ (For agents with daily sanitation) $D_{c\_a,i}\leq 336$ (For agents with weekly sanitation)
Apparent Persistence	Contamination is repeatedly detected weekly during environmental monitoring events on an agent following the completion of two sanitation events, but the true contamination status between the two monitoring events is unknown	$C\_T_{a,w,i}>0$ & $C\_T_{a,w+1,i}>0$

$D_{c\_a,i}$ = Duration of time in hours an agent a is contaminated in iteration i; $C\_T_{a,w,i}$ = concentration of *Listeria* on an agent at an hour of the simulation which corresponds to sequential environmental monitoring time points in a week (w) and the subsequent week (w+1)

Scenario Analysis

Once the baseline conditions were evaluated, a number of alternative scenarios were designed to investigate persistence behavior within the model (Table 3.5: XI_01, XI_02) or as potential corrective actions to combat *Listeria* persistence (Table 3.5: EC_01-AI_04). Each scenario was run for 100 iterations and observed the same primary and secondary outcomes of interest as the baseline.

Table 3.5 Persistence Analysis and Corrective Action Scenarios and their virtual implementation within the ABMs

Scenario	Description	Computational implementation[a]	Scenario model-notation
No Niches	All uncleanable agents were modified to be cleanable during weekly sanitation. This simulated a perfect hypothetical facility where no location is capable of harboring persistent *Listeria*.	Modified agents defined at beginning of scenario	XI_01
No Outside Introduction + Random single Dirty Agent	All regular contamination method quantities (*Listeria* Prevalence in Incoming Produce, Random & Zone 4) were set to zero and a single uncleanable agent was randomly selected to be contaminated at the start of the simulation and contaminated with N_r CFU. This simulated the specific effect of a single contaminated equipment surface, representing a contamination niche, being contaminated at the start of the simulation.	$R_d * 0.00$ & $N_r * 0.00$ & $N_z * 0.00$; randomly selected uncleanable agent was contaminated at setup by a random amount of *Listeria* set by N_r at the simulation start	XI_02
Listeria Prevalence in Incoming Raw Produce Reduction	The baseline prevalence of product-borne *Listeria* arriving in the facility was reduced by 25%, 50% or 75% or set to zero. This simulated produce being treated prior to arriving in the packinghouse packing room.	$R_d * 0.75$ $R_d * 0.50$ $R_d * 0.25$ $R_d * 0.00$	EC_01 EC_02 EC_03 EC_04

Broad Model-based Master Sanitation Schedule Restructuring	Agent cleaning and sanitation schedules were reassigned according to mean contamination probability predicted in the model over the second week into: (i) weekly schedule (when predicted contamination probability was ≤32%), (ii) alternating days (33-65%), (iii) daily (≥66%). At the scheduled cleaning and sanitation, *Listeria* concentration on select agents was reduced by θ_d or η_d as appropriate. In the case of Facility A where a daily schedule did not previously exist, one was implemented using either "Cleaning Only" or "Cleaning & Sanitation". This simulates a "risk-based reorganization of the cleaning and sanitation schedule".	*Listeria* concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation	AI_02
Directed Model-based Master Sanitation Schedule Restructuring	Only agents predicted to have a mean contamination probability ≥66% were scheduled for daily cleaning and sanitation, meaning that *Listeria* concentration on these agents was reduced by θ_d or η_d as appropriate on agents scheduled to be cleaned at that frequency; other agents were left with their original cleaning and sanitation scheduling. In the case of Facility A where a daily schedule did not previously exist, one was implemented using either "Cleaning Only" or "Cleaning & Sanitation". This simulates a "partial reorganization of the cleaning and sanitation schedule of surfaces determined to be most at risk of contamination".	*Listeria* concentration of affected agents reduced by η_d at the time of the scheduled cleaning and sanitation	AI_03
Transmission Pathways Modification Corrective Action	Links between specific agents were severed to represent physical isolation between them. In Facility A the interconnected drain system was compartmentalized so that agents could only receive	Modified links defined at beginning of scenario	AI_04

	Listeria (but not spread it further) while in Facility B each forklift was assigned to a single separate area.		

[a]Model parameters are defined in Table 3.2.

RESULTS

Model-Predicted Agent Contamination Over 8 Weeks Under the Baseline Model Setup with Standard Sanitation and no Corrective Actions

Facility A predicted a sharp increase between weeks 1-2 in median wet area *Listeria* contamination prevalence (28.3 percentage points (pp)) before increasing at a slower rate, reaching a maximum of 56.6%. In contrast, median dry *Listeria* contamination prevalence had a flatter slope and a maximum of 8.8% (Fig 3.1.A). Facility B showed a similar increase pattern, but with a flatter slope and lower maximum *Listeria* contamination prevalence of 40.8% in wet areas, and 6.25% in dry (Fig 3.1.B). Median *Listeria* concentration on contaminated agents in Facility A's wet area showed a maximum increase of 0.93 $\log_{10}$ CFU/cm^2 by week 8, and the dry area's median reached a difference of 0.45 $\log_{10}$ CFU/cm^2 (Fig 3.1.C). Facility B showed a similar higher concentration change on contaminated agents in the wet area, with a maximum increase of 0.78 $\log_{10}$ CFU/cm^2 by week 8 and a lower change in dry area, only producing a change of 0.09 $\log_{10}$ CFU/cm^2 between weeks 1 and 8 (Fig 3.1.D).

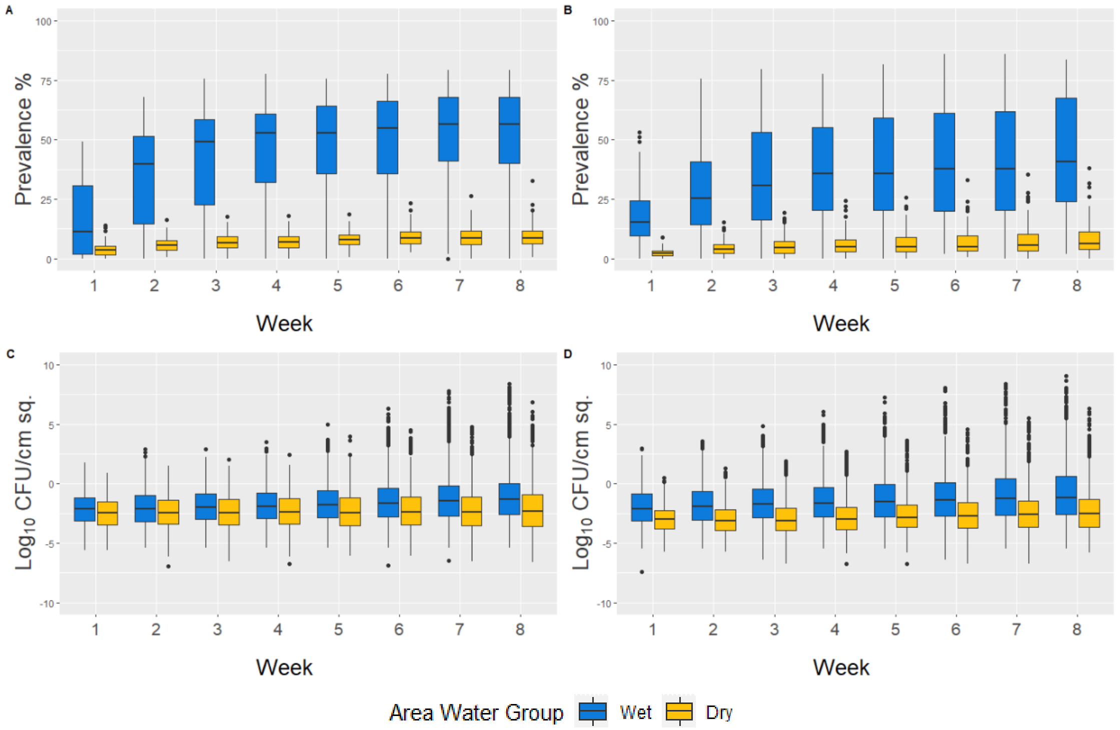


Fig 3.1 Boxplots describing *Listeria* contamination prevalence and concentration on contaminated agents on both wet (blue) and dry (yellow) area on Wednesday at midday for Facility A and B baseline conditions over eight weeks
A: Facility A *Listeria* contamination prevalence of all agents in wet and dry areas. B: Facility B *Listeria* contamination prevalence of all agents in wet and dry areas. C: Facility A *Listeria* $\log_{10}$ concentrations on all positive agents in wet and dry areas. D: Facility B *Listeria* $\log_{10}$ concentrations on all positive agents in wet and dry areas.

Comparing changes in *Listeria* contamination in each functional area of the facility (i.e., Loading, Cleaning, Sorting, Tray-Packing, Bag-Packing, Reject and Other) between weeks 1 and 8 allowed for the observation of area behavior by the end of the simulation. In both models, *Listeria* contamination prevalence was shown to increase substantially in areas with equipment in proximity to water (i.e., Loading and Cleaning), while dry areas showed some increases in median *Listeria* contamination prevalence, but not as pronounced as the first two areas (Fig 3.2.A and 3.2.B). A notable spike within the dry areas was an increase in median *Listeria* contamination prevalence of both model's Reject areas. Similarly, median concentration on positive agents in wet areas of both models rose between weeks 1 and 8, while dry areas showed a less consistent behavior (Fig 3.2.C and 3.2.D).

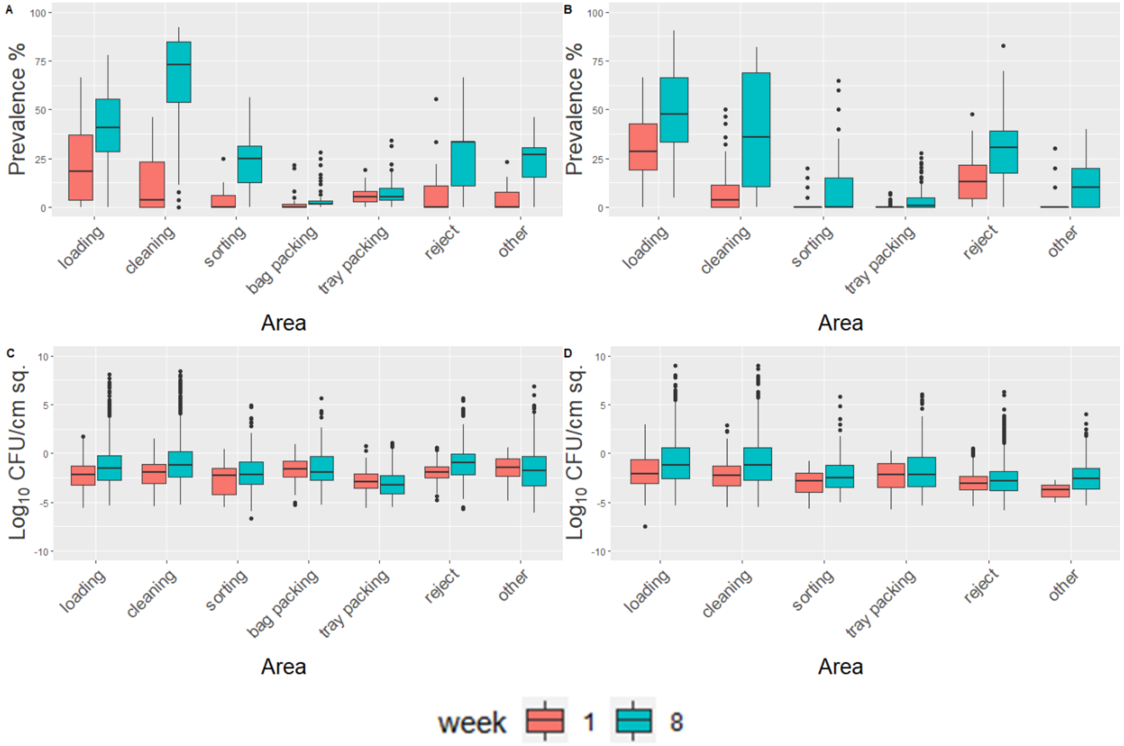


Fig 3.2 Boxplots describing *Listeria* contamination prevalence and concentration on contaminated agents on Wednesday at midday for Facility A and B baseline conditions in functional areas between weeks 1 (red) and 8 (blue)
Prevalence of *Listeria* contamination within each area of Facility A (panel A) and Facility B (panel B). All areas show an increase in *Listeria* contamination prevalence over time, except for packing-related areas. Log$_{10}$ concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D) with median concentrations showing minor increases over time for Loading and Cleaning areas, but otherwise no major changes.

Transient Versus Persistent Contamination

By tracking hourly *Listeria* concentration of each agent ($C\_T_{a,h,i}$) it was possible to observe an agent's *in silico* true contamination status over the course of the simulation. This also allowed the detection of apparent persistence occurrences (based on weekly environmental monitoring events) that in actuality may be transient contamination occurrences. Fig 3.3 shows the hourly contamination status of a single agent that undergoes daily sanitation and at no point demonstrates persistent contamination (with a single failed sanitation event early on). The near-constant removal and recontamination of *Listeria* on this agent when observed at an hourly level is fully indicative of transient contamination. However, if only observed during Wednesdays' EMP, the agent will consistently test positive with no breaks in contamination and would be mistaken for undergoing persistent contamination.

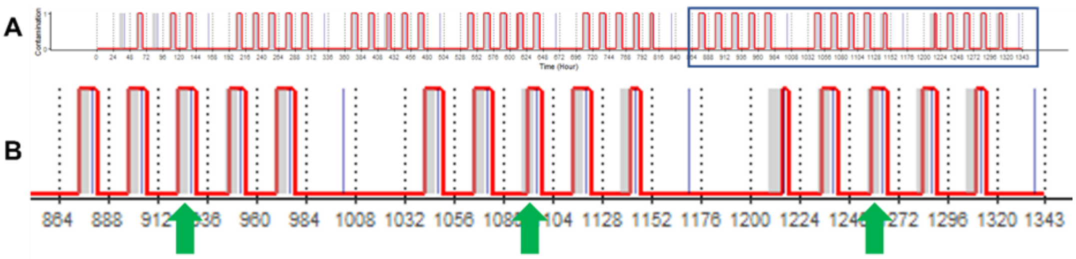
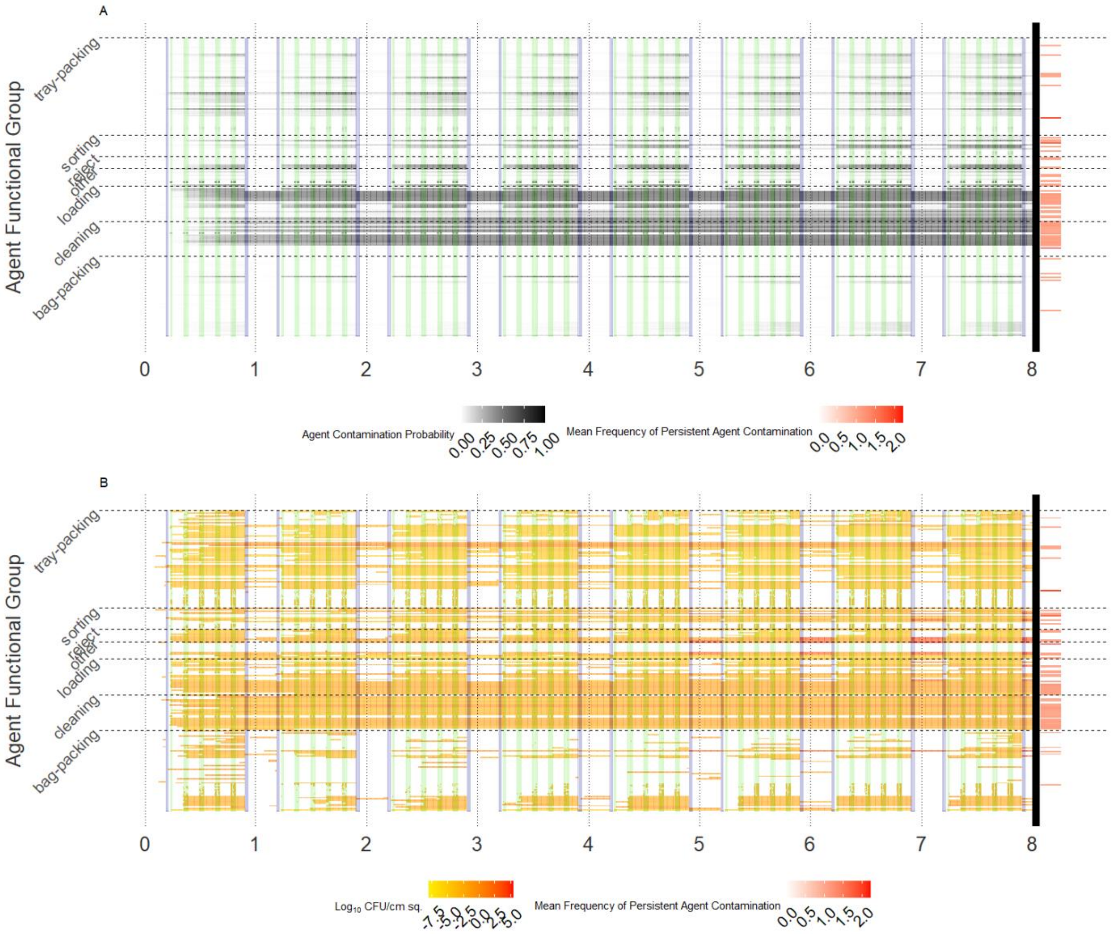


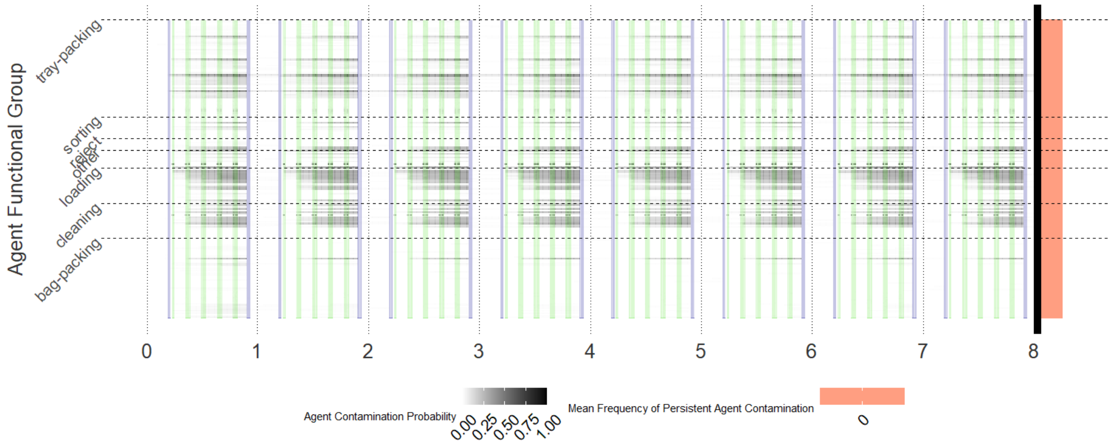
Fig 3.3 Hourly contamination status of an agent (agent 2: food-crate-02) in Facility B over a single iteration compared against the facility's event schedule
A single agent's contamination status (0=negative, 1=positive) was tracked on an hourly basis and compared to Facility events (grey=workday, blue=sanitation event, green arrows=Wednesday environmental monitoring during workdays). A: Full 0-1343 timeline. B: Zoomed segment of the last 3 weeks.

Persistence Behavior Analysis

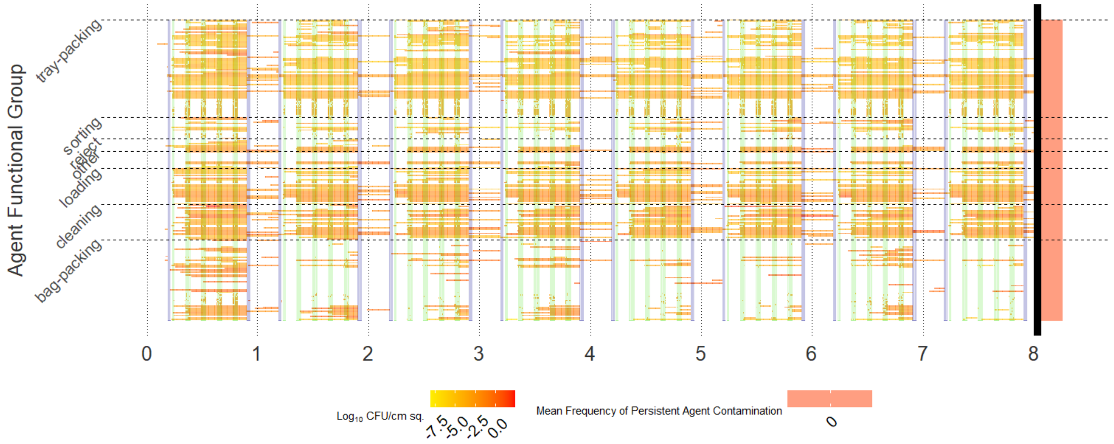
Listeria persistence behavior was observed using scenario XI_01 to prevent all niches, and XI_02 to prevent any external source of contamination from occurring albeit allowing a randomly assigned uncleanable agent as the source of contamination. With no niches capable of forming, both Facilities A and B showed no persistent formation in contamination patterns (Figs 3.4.C and 3.5.C respectively). While contamination did occur in both over the course of multiple days in XI_01, it was ultimately removed during weekly sanitation and therefore only maintained a transient status. Scenario XI_02 on the other hand, showed extremely reduced overall contamination (Figs 3.4.E and 3.5.E), but still showed signs of persistence occurring, only at lower probability compared to the baseline. In both XI_01 and XI_02, agents that did become contaminated showed either similar or lower $\log_{10}$ median contamination concentrations to their baseline scenario counterpart (Figs 3.4.B, 3.4.D, 3.4.F, 3.5.B, 3.5.D and 3.5.F).



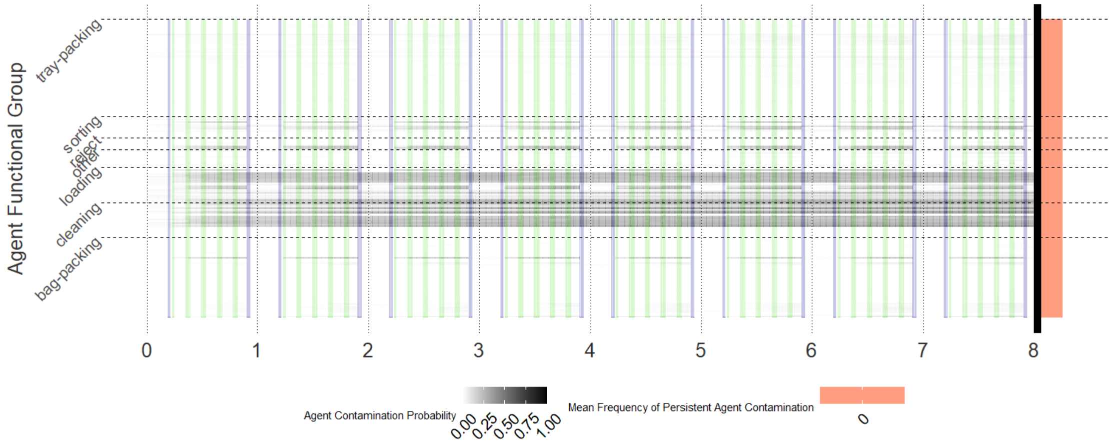
C


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

D


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-7.5
-5.0
-2.5
0.0
Mean Frequency of Persistent Agent Contamination
0

E


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

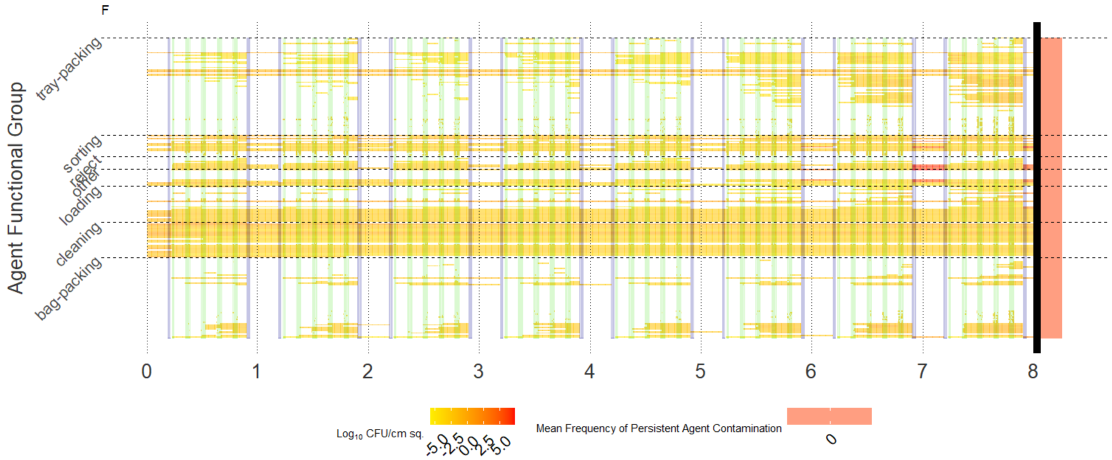
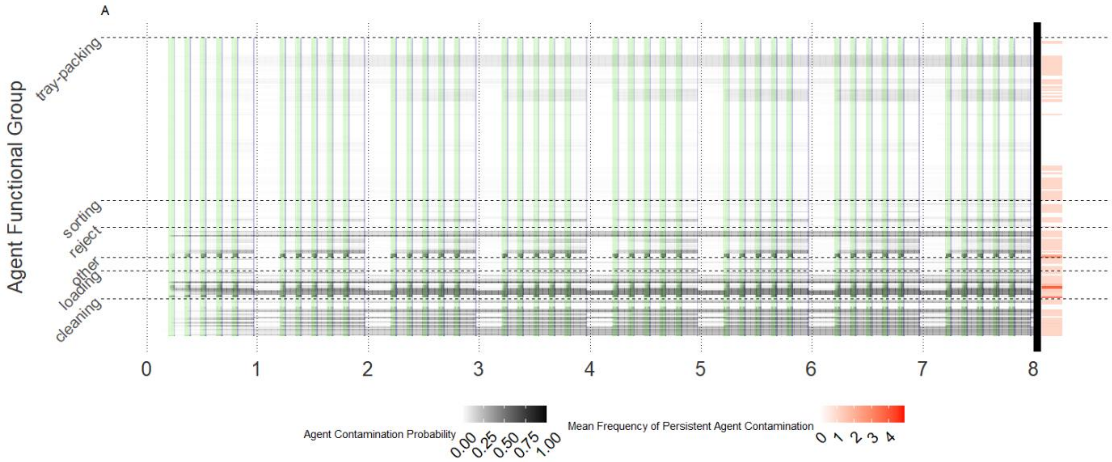
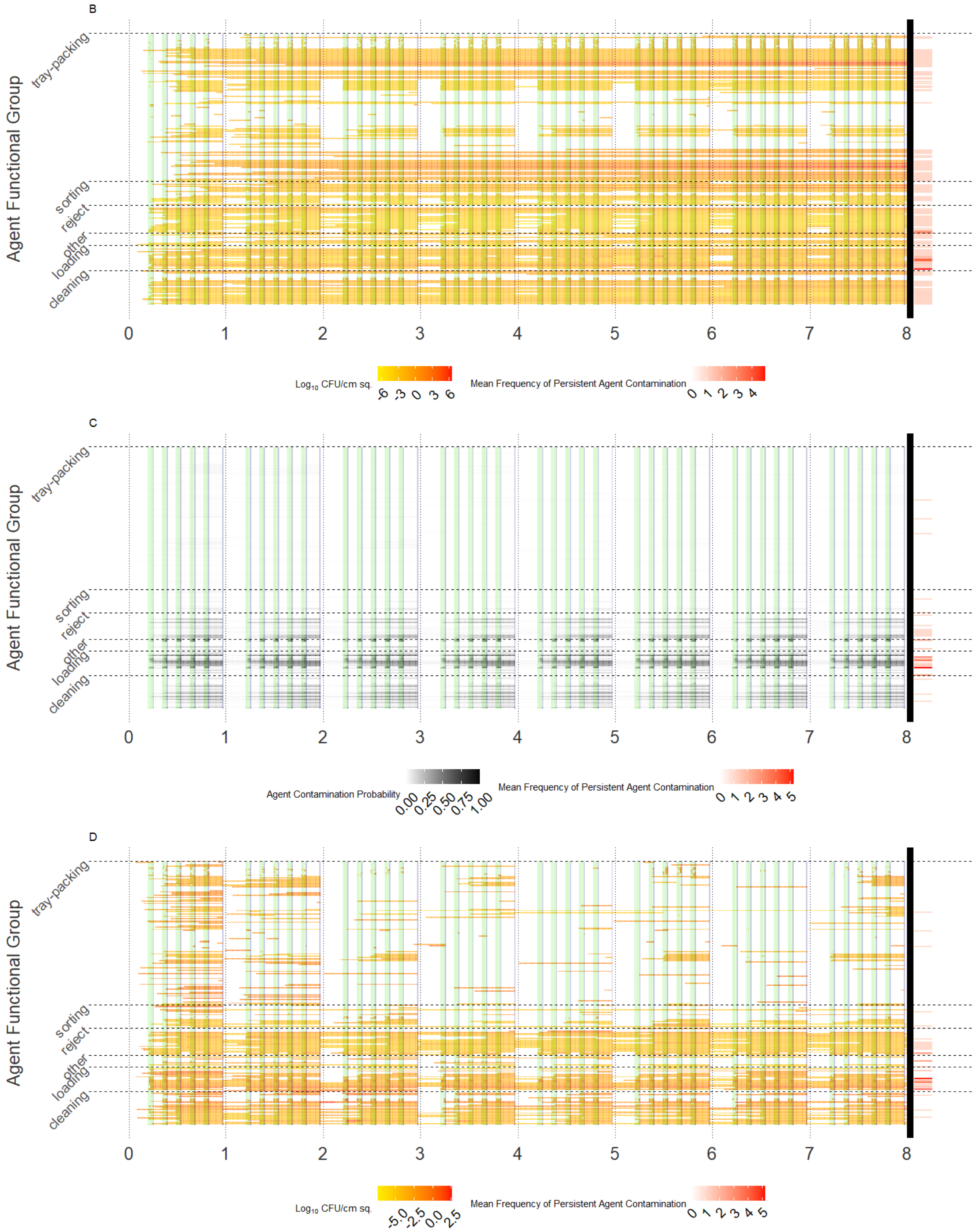


Fig 3.4 Heatmaps describing the hourly contamination probability of all agents, median $\log_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility A in baseline and persistence investigation scenarios (XI_01/XI_02)
(Green: work shift; Blue: sanitation; White: means no contamination). A: Contamination probability in Facility A baseline. B: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility A baseline. C: Contamination probability in Facility A XI_01. D: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility A XI_01. E: Contamination probability in Facility A XI_02. F: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility A XI_02.
(XI_01: No Niches; XI_02: No Outside Introduction + Random Single Dirty Agent from each model's uncleanable agents)




B
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-6
-3
0
3
6
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4
C
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4
5
D
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-5.0
-2.5
0.0
2.5
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4
5

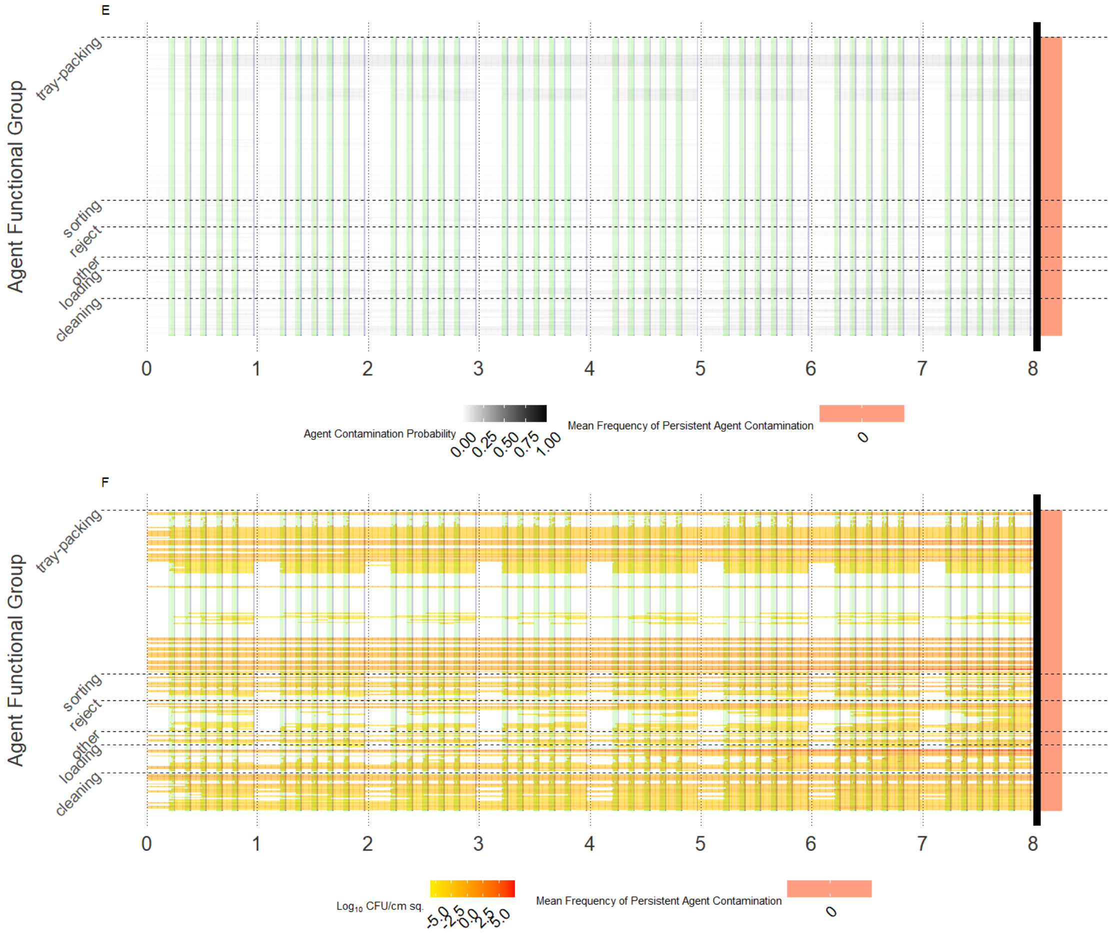


Fig 3.5 Heatmaps describing the hourly contamination probability of all agents, median $\log_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility B in baseline and persistence investigation scenarios (XI_01/XI_02)
(Green: work shift; Blue: sanitation; White: means no contamination). A: Contamination probability in Facility B baseline. B: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility B baseline. C: Contamination probability in Facility B XI_01. D: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility B XI_01. E: Contamination probability in Facility B XI_02. F: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) in Facility B XI_02.
(XI_01: No Niches; XI_02: No Outside Introduction + Random Single Dirty Agent from each model's uncleanable agents)

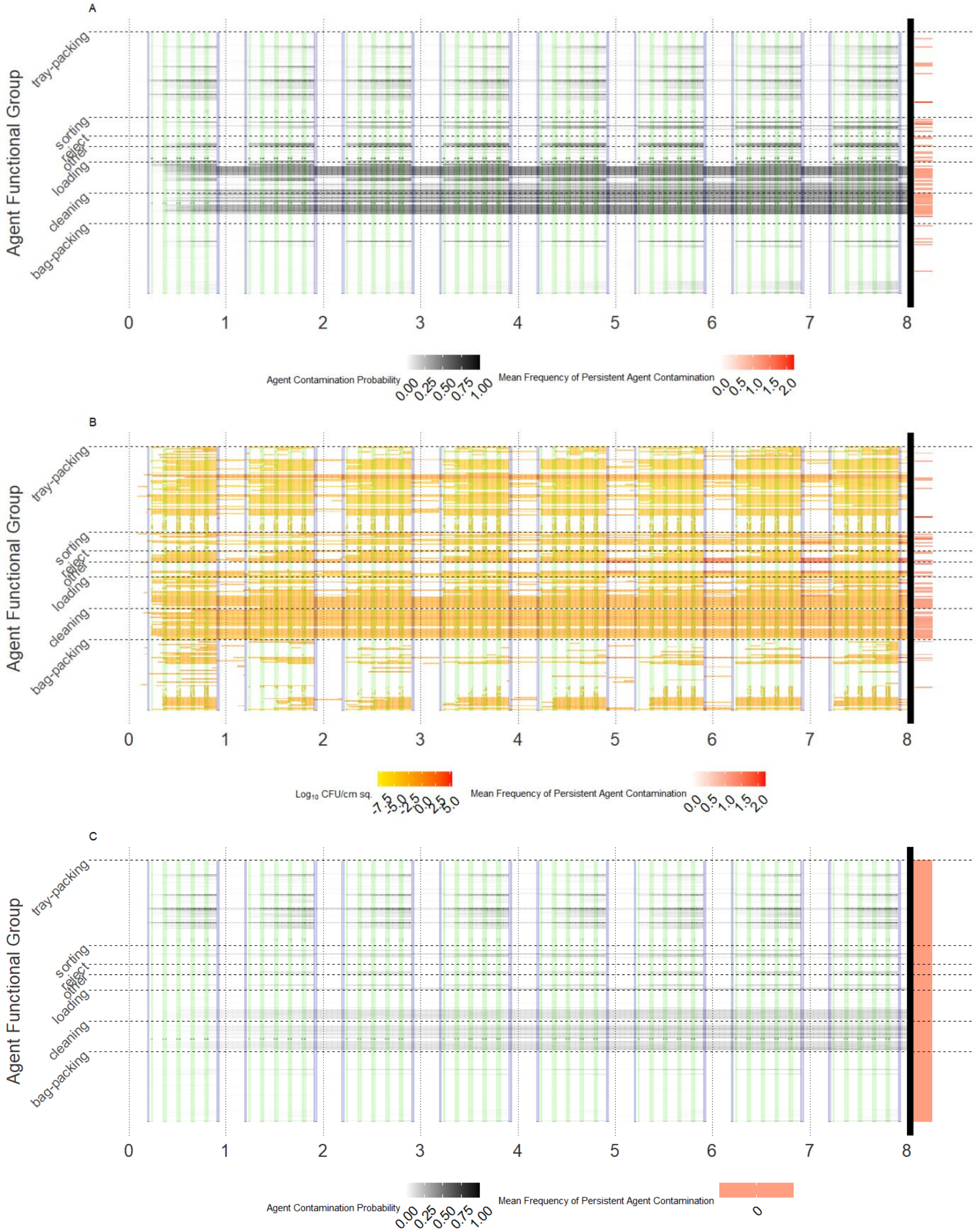
Scenario Analysis

By comparing the weekly median contamination prevalence of the model's wet and dry areas against the baseline, the options that demonstrated the best reduction in *Listeria* contamination prevalence could be selected for further analysis. Of the corrective actions employed, options that reduced *Listeria* contamination prevalence on incoming raw produce (scenarios EC_01-EC_04) or affected agent sanitation scheduling (AI_02-AI_03) where the most effective in reducing *Listeria* contamination prevalence in both wet and dry areas of Facilities A and B (Figs S3.1.A, S3.1.B, S3.2.A and S3.2.B). In both models, reducing *Listeria* prevalence on incoming raw produce showed a similar sloping pattern to the baseline but started at a lower point (except for EC_04, which either showed a very small or no slope over time). Manipulation of event probability or the quantity of *Listeria* introduced in Zone 4 (PZ_01-PZ_04 and LZ_01-LZ_03 respectively) and Random events (PR_01-PR_04 and LR_01-LR_03 respectively) showed no major improvements over time (not shown here). Corrective action AI_04 was not consistent between Facilities A and B, given this corrective action's implementation was specific to each model.

Likelihood of Persistence Behavior in Corrective Actions

Both models showed a combination of highly likely transient and highly likely persistent *Listeria* contamination patterns on their surfaces, depending on the agent in question. Furthermore, scenarios EC_01-EC_03 showed no changes to persistence patterns within either model (Figs S3.3 and S3.4) and instead showed an overall decrease in *Listeria* contamination probability of all agents at all hours. Scenario

EC_04 showed an overall reduction in probability of all persistence occurring and a sharp decrease in *Listeria* contamination concentration (on contaminated agents), but no change to the actual patterns in question (Figs 3.6.C, 3.6.D and 3.7.C, 3.7.D). The pattern of median *Listeria* concentration of contaminated agents did not show any notable changes, however, a decrease in maximum median *Listeria* concentration did occur in Facility A as *Listeria* prevalence on incoming raw produce was reduced. Scenarios AI_02 and AI_03 on the other hand showed a notable change to persistence patterns in both models (Figs 3.8 and 3.9). Of the two corrective actions, AI_02 showed the highest reduction in likelihood of persistence events compared to its respective baseline scenario (Figs 3.8.C and 3.9.C). AI_03 (Figs 3.8.E and 3.8.F) was notably less effective in Facility A as only a small number of agents were calculated to have a mean contamination probability ≥66%, leading to fewer agents that were switched to a more frequent daily sanitation schedule. In both AI_02 and AI_03, agents that did become contaminated showed either similar or lower $\log_{10}$ median *Listeria* contamination concentration values to their baseline scenario counterparts, with the maximum median *Listeria* concentration dropping in both (Figs 3.8.B, 3.8.D, 3.9.B and 3.9.D).


A
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
2
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4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0.0
0.5
1.0
1.5
2.0
B
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
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3
4
5
6
7
8
Log$_{10}$ CFU/cm sq.
-7.5
-5.0
-2.5
0.0
2.5
5.0
Mean Frequency of Persistent Agent Contamination
0.0
0.5
1.0
1.5
2.0
C
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
2
3
4
5
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7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

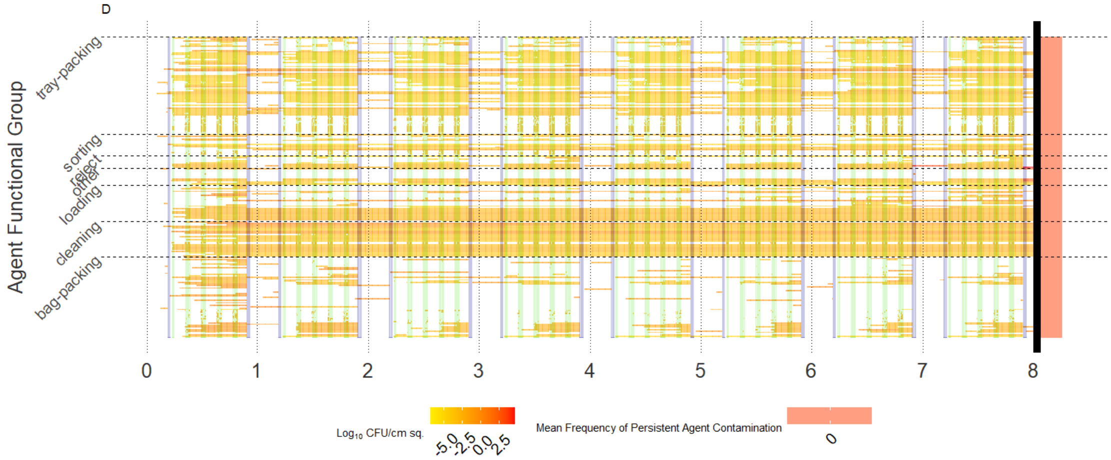
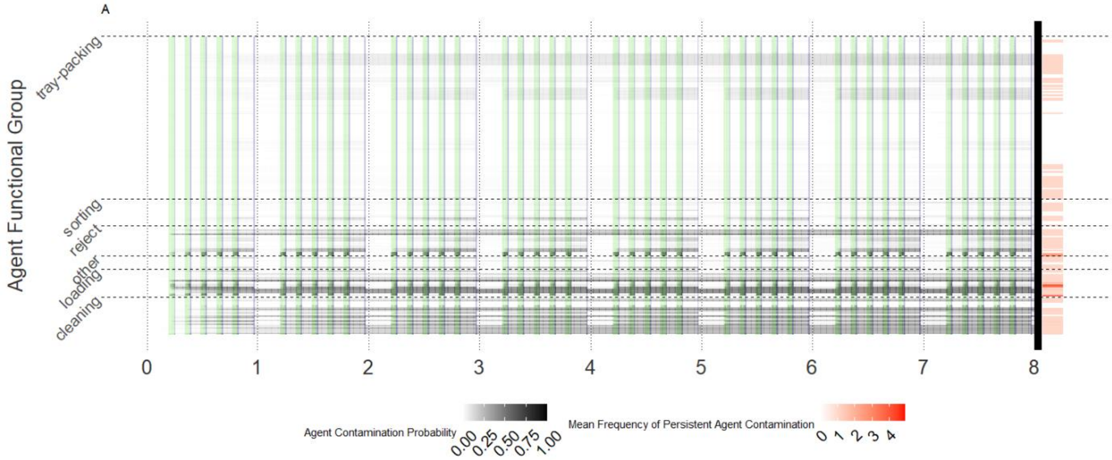


Fig 3.6 Heatmaps describing the hourly contamination probability of all agents, median log$_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility A in baseline and *Listeria* prevalence on incoming raw produce reduction corrective action scenario (EC_04) (Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility A baseline. B: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A baseline. C: *Listeria* Contamination probability in Facility A EC_04. D: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A EC_04.
(EC_04: *Listeria* Prevalence on Incoming Raw Produce reduced to 0%)



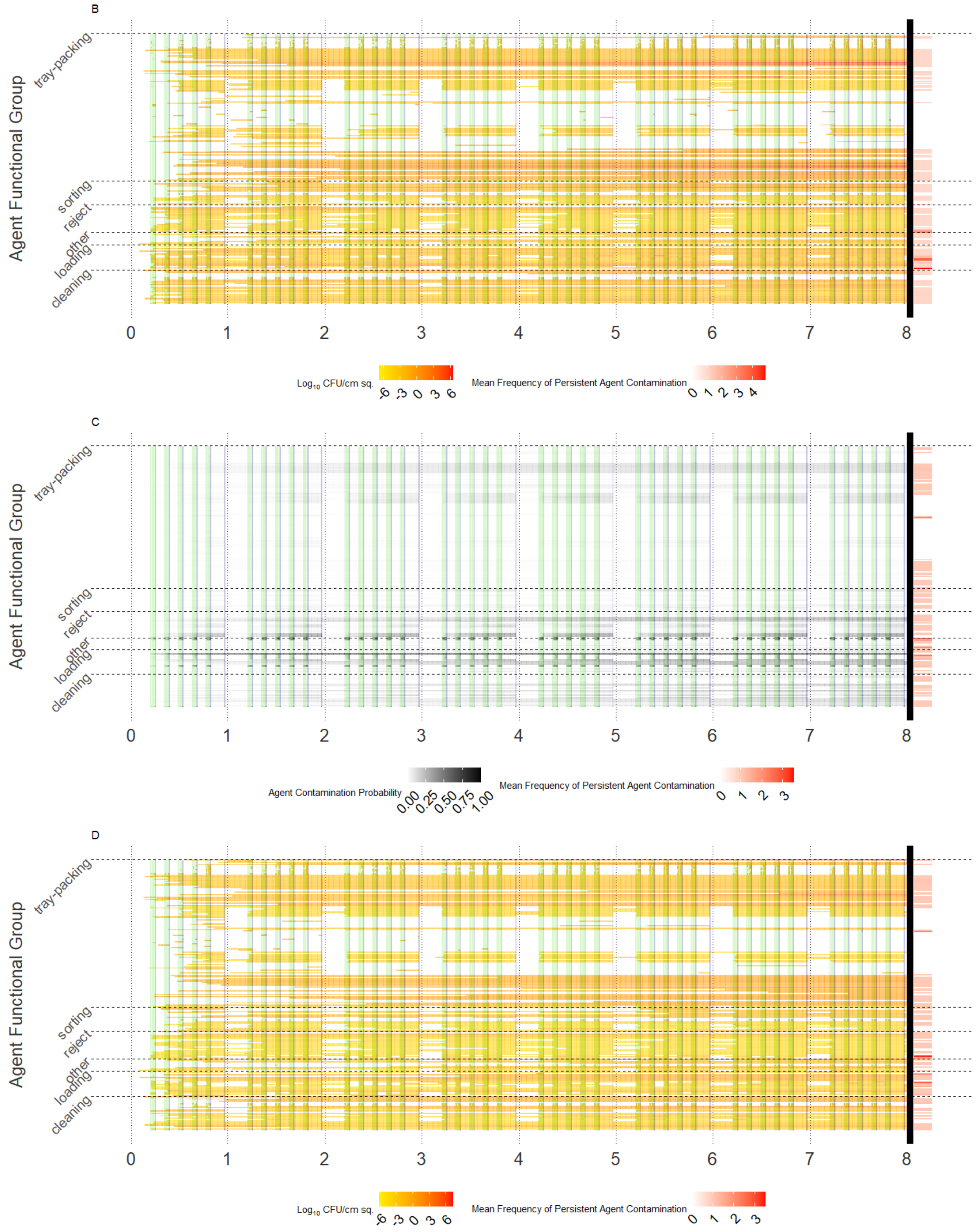
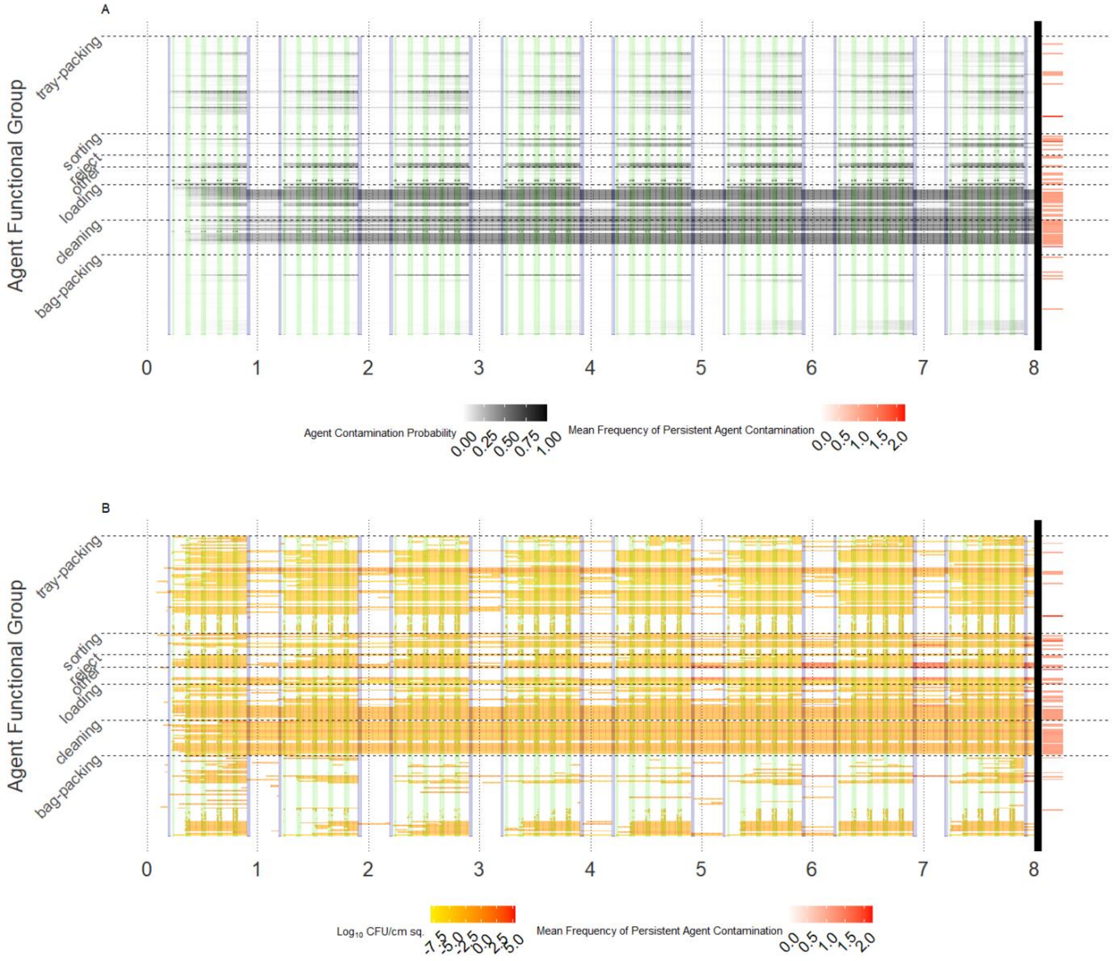
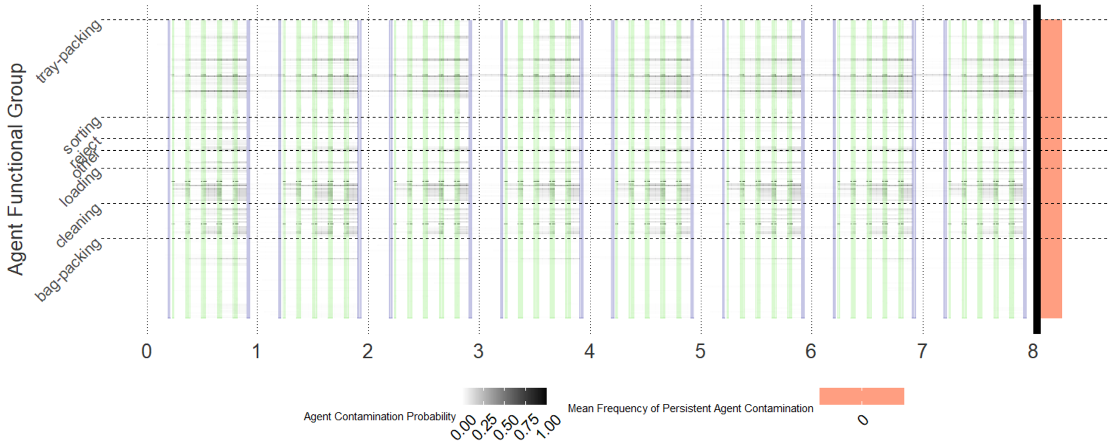


Fig 3.7 Heatmaps describing the hourly contamination probability of all agents, median $\log_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility B in baseline and *Listeria* prevalence on incoming raw produce reduction corrective action scenario (EC_04) (Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility A baseline. B: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B baseline. C: *Listeria*

Contamination probability in Facility B EC_04. D: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B EC_04. (EC_04: *Listeria* Prevalence on Incoming Raw Produce reduced to 0%)



C


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
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5
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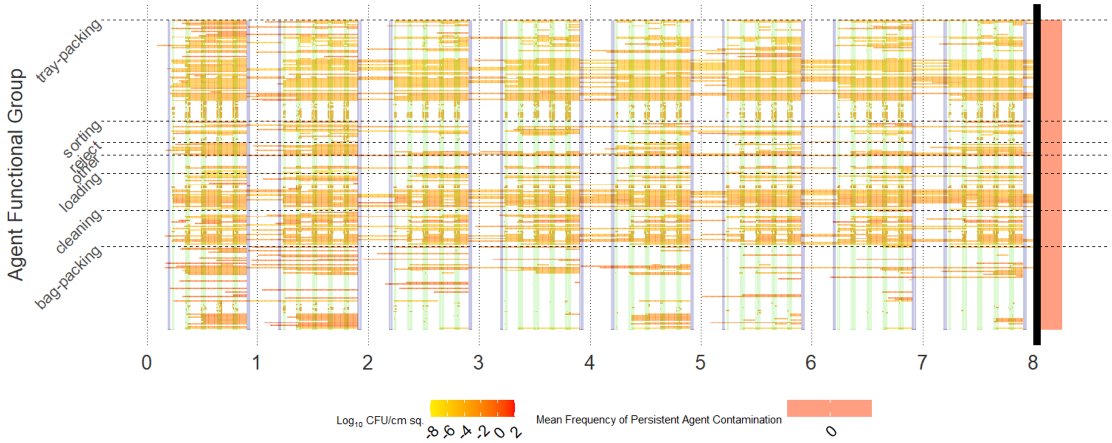
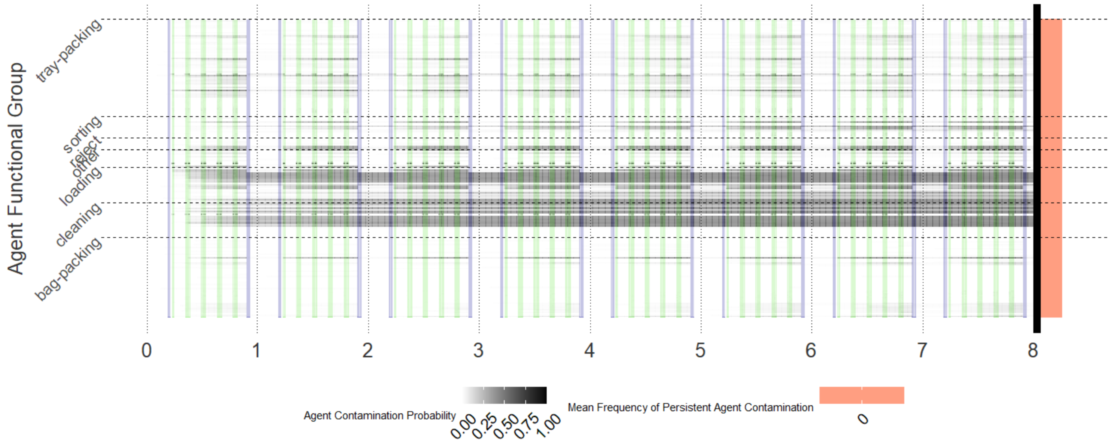
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

D


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-8
-6
-4
-2
0
2
Mean Frequency of Persistent Agent Contamination
0

E


Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
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3
4
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7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

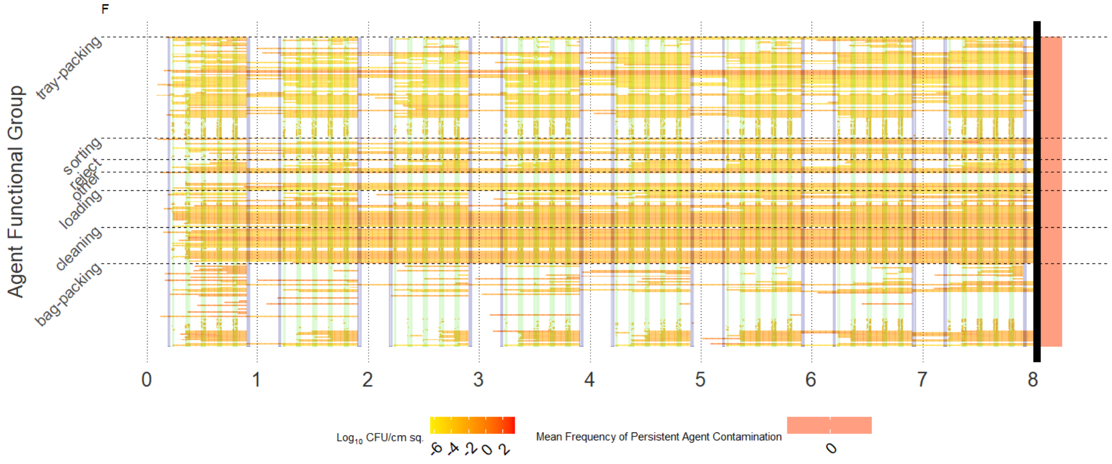
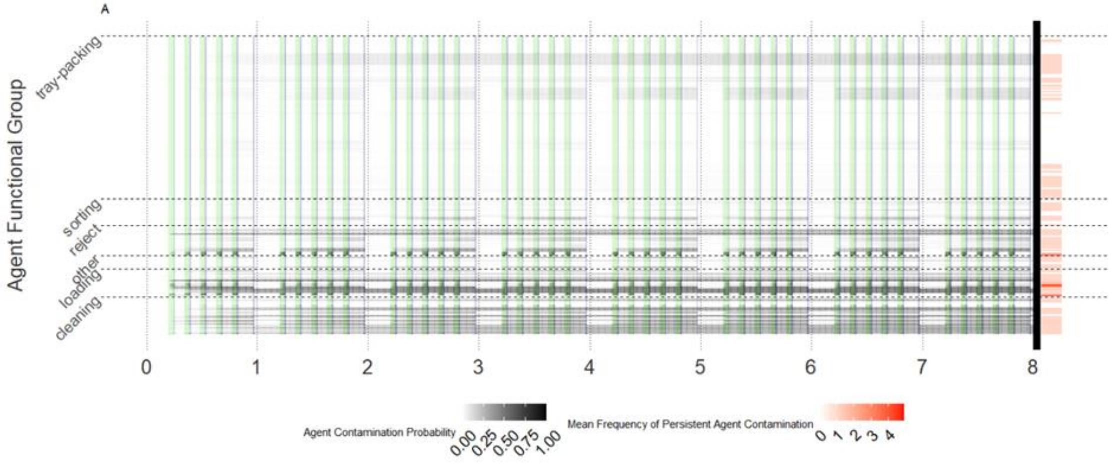
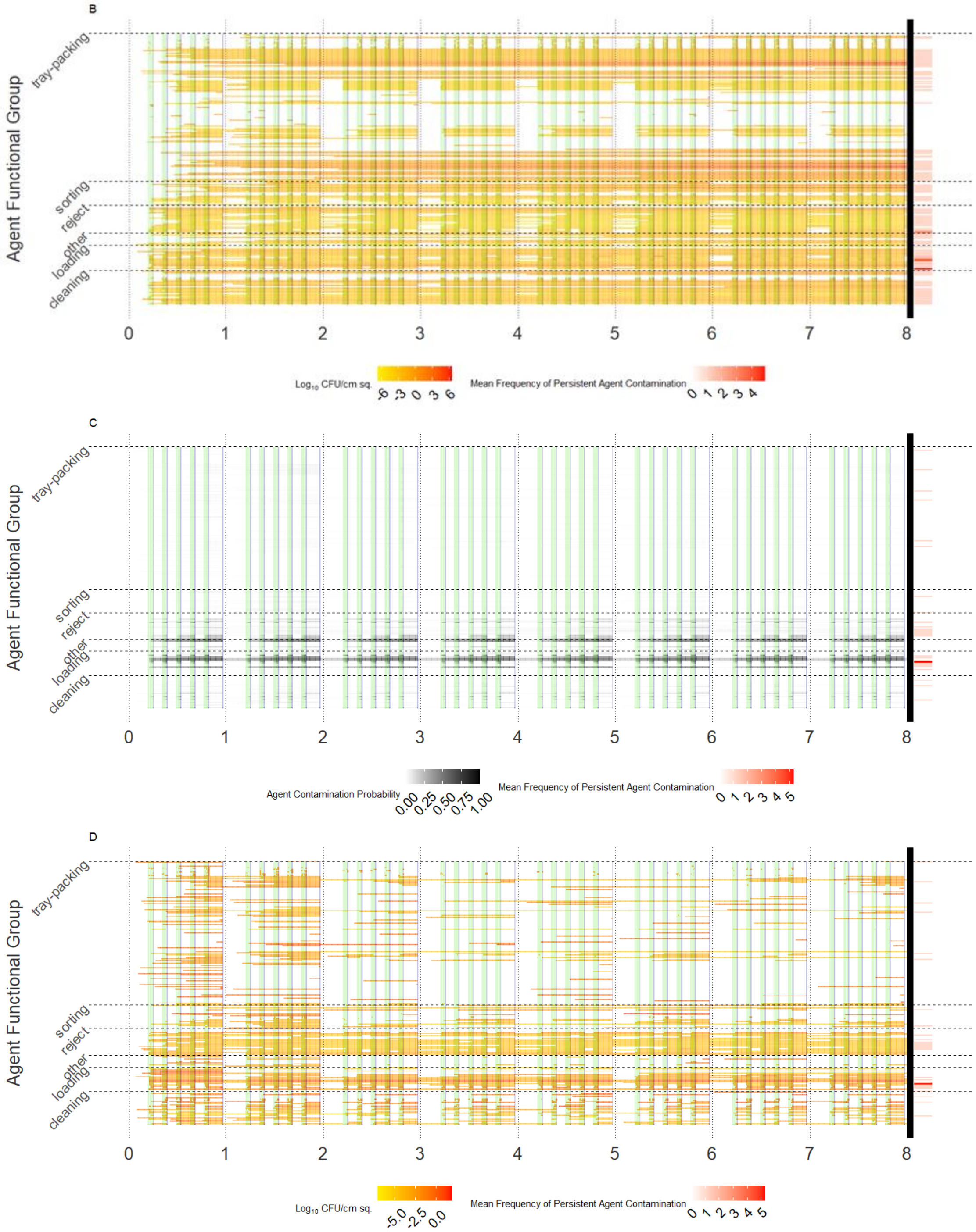


Fig 3.8 Heatmaps describing the hourly contamination probability of all agents, median log$_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility A in baseline and agent-specific corrective actions (AI_02/AI_03)
(Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility A baseline. B: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A baseline. C: *Listeria* Contamination probability in Facility A AI_02. D: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A AI_02. E: *Listeria* Contamination probability in Facility A AI_03. F: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A AI_03.
(AI_02: Broad Model-based Master Sanitation Schedule Restructuring (Agent sanitation schedules were fully reassigned according to mean predicted contamination probability; Facility A was given a daily schedule for and sanitation; AI_03: Directed Model-based Master Sanitation Schedule Restructuring (Agents with a mean predicted contamination probability ≥66% were set to daily cleaning & sanitation))




B
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-6 -3 0 3 6
Mean Frequency of Persistent Agent Contamination
0 1 2 3 4
C
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00 0.25 0.50 0.75 1.00
Mean Frequency of Persistent Agent Contamination
0 1 2 3 4 5
D
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-5.0 -2.5 0.0
Mean Frequency of Persistent Agent Contamination
0 1 2 3 4 5

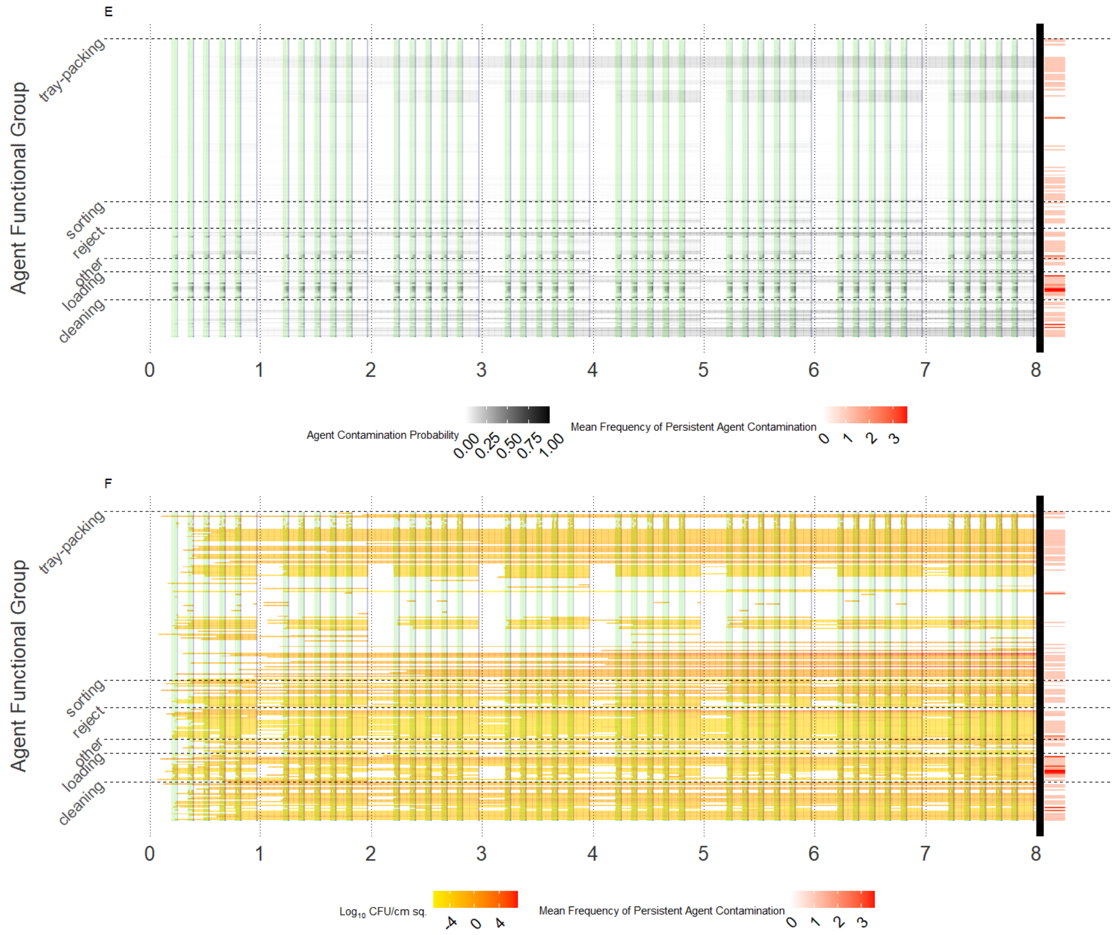


Fig 3.9 Heatmaps describing the hourly contamination probability of all agents, median $\log_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility B in baseline and agent-specific corrective actions (AI_02/AI_03)

(Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility B baseline. B: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B baseline. C: *Listeria* Contamination probability in Facility B AI_02. D: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B AI_02. E: *Listeria* Contamination probability in Facility B AI_03. F: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B AI_03.

(AI_02: Broad Model-based Master Sanitation Schedule Restructuring (Agent sanitation schedules were fully reassigned according to mean predicted contamination probability; AI_03: Directed Model-based Master Sanitation Schedule Restructuring (Agents with a mean predicted contamination probability ≥66% were set to daily cleaning & sanitation))

DISCUSSION

This study described the adaptation of existing ABMs of *Listeria* contamination dynamics in two different fresh produce packinghouses to identify and monitor *Listeria* persistence in the environment of the modeled facilities at a fine temporal and spatial scale. This allowed for the observation and establishment of *Listeria* contamination persistence patterns during the models' initial baseline conditions. Furthermore, this also allowed for the measuring the effectiveness of corrective actions on persistence patterns, rather than at isolated time points within the model, which simulates information about contamination obtained through the EMP.

Baseline Listeria Contamination Prevalence and Positive Agent Concentrations Increase in Areas Close to Water

While each facility performed similar roles in their receiving, cleaning, sorting, and produce packing functional areas, the specific pieces of equipment involved, and their sanitation measures differed drastically between the two facilities. Consequently, persistence patterns that were discovered within each model are specific to the facility they are based on and comparing one model to another is a limited avenue to follow. Nevertheless, regarding data that can be more easily summarized (i.e., *Listeria* contamination prevalence of areas close to water and *Listeria* concentration on positive agents in those areas) with both models showing an increase in *Listeria* contamination prevalence over time (which is higher in high-water areas than in dry areas), but with very little change in concentration. This increase in contamination prevalence in the wet areas is expected given the increased moisture can spread

Listeria across an area's surfaces and allows for more bacteria growth (3,24). However, as sanitation is modeled as a fixed process rather than being informed by EMP results, this growth pattern should be interpreted as a worst-case scenario for the modeled facility.

Hourly Observation Allows for Determination of Persistence Patterns and Scrutinization of Environmental Monitoring

The developed models provide the ability to monitor persistence patterns more thoroughly than beyond time points when EMPs occur. An already immediate observation is how easily it is to potentially misidentify transient contamination for persistent. That is because, though it is an integral part of a facility's safety practices, the EMP is not performed hourly and therefore will be unable to produce a "high resolution" timeline of results; additionally an EMP typically has spatially limited coverage (25). As part of the EMP, the state of the surface sampled must be inferred from samples that may have been taken at two very distant points in time, making it difficult to be certain as to what happened between sampling points. However, the analysis of hourly model predictions could be directly overlaid with EM results to determine the likely contamination status between samples collected as part of the EMP, allowing for clarification of the "apparent" patterns suggested by the results from the EMP. This highlights a potential risk of contamination pattern misidentification EM may produce if performed at a relatively low frequency. Furthermore, the outcome of an EMP may also be highly influenced by its timing and the current or ongoing events within a facility: sampling agents during or shortly after work shifts are more likely to produce positive results, while sampling during or after sanitation is more likely to produce negative results. While the aim of models developed here was not to redesign EMPs, these models demonstrate that environmental monitoring may benefit from fine-scale spatiotemporal modeling to serve as a decision support tool to help inform the full series of possible events between sampling events. Additionally, while the consistently low contamination concentrations observed in the model are no issue to observe virtually, in practice low levels of *Listeria* contamination on surfaces are difficult to reliably detect. For example, in Zoellner et al, it was assumed that there is a 10% probability of a false negative environmental monitoring test result if contamination concentration is ≤ 1 $\log_{10}$ CFU/cm^2. In these instances, EMP results may instead produce “apparent transience” (if two sequential weekly monitoring occurrences do not both test positive for a specific agent), where a persistent *Listeria* contamination is not detected because low contamination concentration produces false negative results. Again, as before, a decision support tool may be able to provide much-needed context to EMP results, allowing for some degree of outcome clarification.

Short-Term Corrective Actions Are Not Guaranteed to Have Long-Term Success

Using the developed models to monitor *Listeria* contamination persistence also provided key insight into potential corrective actions, especially further analysis of those that showed short-term improvement to model-wide or area-specific *Listeria* contamination prevalence (Chapter 2). Though incoming contaminated food may be a key component in causing facility-wide contamination (8,11,26,27), both models show that reducing *Listeria* prevalence on incoming raw produce (EC_01-EC_04) does not change the overall persistence patterns, only reduces the overall likelihood of them occurring. In these scenarios positive agent *Listeria* contamination concentration remained largely unchanged, except when preventing raw food-based contamination outright (EC_04). This reduction to contamination probability is an effective short-term solution, which however, may still reach the same contamination prevalence as the baseline given enough time, effectively only resulting in "buying time". Additionally, as expected, prevention of all outside contamination routes was shown to be incapable of preventing contamination from spreading from an already established harborage site within the facility.

Hygienic Design Can Reduce Listeria Contamination Persistence

Alternative mitigation strategies that specifically modify agent attributes (i.e., values or behavioral rules that directly affect agents themselves) instead of altering incoming contamination routes (contaminated food, Zone 4 or random events) show improvement in reducing the likelihood of occurrence of *Listeria* contamination persistence patterns. Two strategies that involve agent attributes that could be implemented are: (i) changing uncleanable to cleanable and (ii) changing sanitation frequency (10). A facility that requires a more extensive redesign of both surfaces and its sanitation schedule may require a substantial amount of time and money to fully complete it. However, even changing a limited number of these surfaces from "uncleanable" to "cleanable" (i.e., AI_03, which prioritizes only targeting the most at-risk agents with the highest mean contamination probability group of ≥66%) can be incredibly impactful if the surfaces selected are the largest contributors to persistent contamination. A key point regarding these corrective actions is that they are designed specifically to the facility in question and cannot be easily copied as-is from one to another.

Transient Contamination Will Still Occur Regardless of the Persistence Conditions

The more niches (i.e., agents deemed uncleanable that became contaminated) that can be eliminated from a facility, the fewer instances of persistent contamination will occur. Crucially however, the elimination of niches will not prevent outright contamination, but instead more of it will be able to be removed under regular sanitation events (i.e., resulting in transient contamination as opposed to persistent contamination) and consequently contamination that would have spread from these niches may be prevented (28,29). In this instance, environmental monitoring must still be performed with spatiotemporal awareness of the facility, as apparent persistence can still be easily concluded from a fully transient contamination pattern. Models such as these developed here may not only be useful in assessing the performance of corrective actions with regards to long-term simulation and persistence behavior (which is fundamentally influenced by the presence of niches), but also could advise facilities on the specific behavior of surfaces targeted for environmental monitoring to reduce the likelihood of misidentification. This may show facilities that some occurrences of contamination are ultimately not caused by niches within the system but can be controlled by regular sanitation measures and may have to be considered an unavoidable hazard that requires control.

Additional Data Sources Will be of High Utility in Future Models Performing Hourly Observation

As these models are designed for hourly observation, they would benefit considerably from the addition of data sources that can be applied on daily or even hourly levels. Most notably, this would include the integration of facility-specific economic data associated to both the cost of corrective actions, as well as that relating to sanitation events. Currently a cost analysis may be limited to the overall costs of a potential corrective action, limiting how detailed the comparison between corrective actions may be. Instead, being able to directly use sanitation-related costs could allow future models to prioritize which agents need to be part of the sanitation schedule (and at what frequency) by comparing an agent's sanitation costs against its predicted reduction in *Listeria* contamination risk and potential change in persistence patterns. Thus, it may be possible to perform a cost-benefit analysis for each agent's sanitation behavior in the ABM to provide a more detailed analysis of the facility as a whole.

Additionally, certain corrective actions were performed using mean contamination probabilities per agent (i.e., AI_02 and AI_03). These would benefit from the more intricate hourly monitoring dataset produced by these persistence models, as agents could be modified based on a more specific contamination probability, as well as based on contamination persistence pattern occurrences.

Limitations

The most notable limitation in both developed models is the usage of a singular nondescript strain of *Listeria* to represent any strain of any *Listeria* spp. growth, consequently no new strains could emerge within the facility or from within niches over time. In reality, it is possible for multiple strains to be introduced into or circulate within packinghouses or processing facilities (30,31). This simplification occludes any potential interaction that could occur between multiple strains within a single facility (or could interfere with the investigator's ability to interpret contamination patterns when multiple strains are present). This level of complexity may be beyond the scope of this type of ABMs, requiring data on the dynamics of multiple *Listeria* strains interacting. Addressing this may be possible with sufficient data to establish a simplified multi-strain system within a model. Another limitation of the developed models is that the identified persistence patterns cannot be validated in practice. That is because of the inherent limitations of information from EMP (or any other investigation of contamination presence in food production environments), which cannot be feasibly conducted at the temporal and special resolution necessary for a reliable determination of the true persistence patterns, which was the reason for development of ABMs described here in the first place. In fact, ABMs are praised for their ability to provide insights into the modeled systems that would not be possible a priori (32).

An additional limitation to consider is computational: due to the length of the simulation duration, running multiple iterations on a conventional desktop takes a considerable amount of time, thus limiting the number of iterations that can be produced in a reasonable amount to time to 100. Though more iterations could be produced, the size of the data files produced will increase with the number of iterations, requiring more RAM to be imported into R and analyzed. Both of these issues can be alleviated with a more powerful machine, allowing for more CPU cores to be used in simulation parallel processing, and providing more RAM for modeling and data handling.

CONCLUSION

Persistence of *Listeria* spp. contamination is a notably difficult phenomena to accurately measure and highlights a vital aspect of food safety where an ABM may be of a valuable aid as a decision support tool. If built using documented historical contamination data, ABMs can serve as powerful decision support tools in this setting, able to simulate the complex interconnectivity between equipment surfaces in a packinghouse facility to predict which contamination patterns are most likely to occur. While expanding an EMP to be used on a more frequent basis may be expensive, a decision support tool can augment existing programs to provide valuable information not available from environmental monitoring alone. The hourly prediction of contamination these models can provide has shown to be key in elucidating likely persistence patterns at model baseline conditions, and in evaluating possible corrective actions. Furthermore, the establishment of a “high resolution” baseline dataset is crucial in comparing corrective actions as their perceived effectiveness may vary depending on the moment of measurement, while an hourly simulation can provide a more complete understanding. Being able to identify which contamination pattern an agent is more likely to be prone to, can also streamline corrective actions, as persistent contamination patterns may require a greater degree of intervention than transient contamination. When designing corrections to counter persistence occurrence, corrective actions that are oriented around reducing the likelihood of outside contamination from occurring will be less effective than those that target surfaces within the facility itself. Even a partial modification of agents prone to developing persistent behavior can induce considerable improvements if appropriately selected.

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CHAPTER 4

CONCLUSIONS

Agent-based models are complex tools, but as shown, capable of a sophisticated degree of analysis support when it comes to their application in food safety-oriented decision-making. With sufficient input data, a model can provide a prediction of the state of surfaces beyond the scope of an environmental monitoring program (EMP), a fine spatiotemporal scale for all individual agents, and allows it to highlight surfaces outside EMP coverage that may require additional investigation. Thus, the overall aim of this work was to construct, validate and analyze agent-based models (ABMs) of *Listeria* spp. dynamics within two packinghouses for both short- and long-term investigation. These ABMs were specifically constructed to test the effectiveness of potential corrective actions to reduce the prevalence of agents contaminated with *Listeria* and the concentration of *Listeria* on contaminated agents.

In the first study, we constructed two models based on existing packinghouse facilities, and validated each one with its respective historical EMP data. This allowed for the investigation into modeled *Listeria* dynamics for a short timeframe (2-weeks), for short-term corrective action response. An immediate finding was that despite the structural and organizational differences between both facilities (and their respective models), was the presence of several key commonalities in contamination pattern behavior. Chiefly, both models predicted an elevated *Listeria* contamination prevalence in functional areas in proximity to water (i.e., produce loading and cleaning operations), while those of the dry areas were typically lower (apart from the Reject area). The *Listeria* concentration of contaminated agents, however, was similar (and generally low) across all areas in both models. This does not mean the dry area should be ignored due to its lower contamination prevalence, rather, its proximity to finished products presents a real concern in cross-contamination dynamics. Furthermore, of the corrective actions simulated in the two-week models, the ones most effective in reducing contamination prevalence were found to (i) target the *Listeria* prevalence in incoming produce crates, (ii) reorganized the sanitation schedule based on mean contamination probability or (iii) modified specific agent-to-agent connection links. Though all three corrective actions produced reductions in contamination prevalence, it is difficult to gauge which are more feasible for an actual facility without economic data, which may further guide decisions when it comes to choosing preferred corrective actions.

In the second study, we adapted the previously created models for long-term hourly monitoring of agent *Listeria* concentration and created analyses to investigate these new data sources. General contamination dynamics did not change their overall contamination prevalence patterns, with areas closer to water still having higher contamination prevalences at the end of eight weeks. However, the key development in these modified models was the ability to observe contamination agent-specific persistence patterns from the hourly contamination predictions. Not only did both facility models show how easy it was for EMPs to accidentally mistake transient contamination for persistence due to low sampling frequency (which could potentially be evaluated against an ABM to help determine the likelihood of detecting persistent contamination), but corrective actions could now be monitored (*in silico*) far more frequently to determine their effectiveness. The three corrective action strategies investigated beforehand were shown to have notably different performance in long-term simulations. Unlike previously, reducing *Listeria* prevalence in incoming produce crates was shown to be far less effective in reducing contamination prevalence or persistence occurrence unless all *Listeria* could be removed from incoming crates. Modifying agent connections was similarly unreliable for long-term corrective actions, and possibly requires more extensive implementation to be effective over this period of time. Instead, changing the sanitation schedule and hygienic design within each facility was the most effective option in reducing long-term contamination prevalence, maximum median *Listeria* concentration on positive agents and the likelihood of persistence occurrences. As before, this would highly benefit from additional economic data, which could now be applied on an hourly level under the new monitoring system. Ultimately, an ABM that captures the sanitation frequency, duration, and the relevant costs per agent (representing a surface) in the facility, could provide recommendations on facility hygienic design on a surface-by-surface level. This facility-specific level of information could be used to supplement existing guidelines that are not able to be specific enough, as they are written for general facility types.

Furthermore, from these specific ABMs we can see that both facilities can reduce the contamination prevalence in all areas in the short-term by reducing the amount of *Listeria* introduced on the main introductory route (i.e., contaminated produce). However, this corrective action is predicted to only have effectiveness for a short period of time before *Listeria* contamination prevalence in facility areas reach the same levels as the baseline. Instead, solutions with a longer effectiveness are data-informed: involving the specific targeting of agents (and the surfaces they represent) that are predicted to be at highest risk of contamination. These corrective actions will be dependent on pre-existing sanitation operations and may require a facility to modify a number of surfaces to be more reliably sanitized during regular operations. Lastly, improving compartmentalization practices and design (either through training or physical modifications) throughout a facility will reduce cross-contamination. The scope of the predicted reduction in contamination prevalence will depend on where the corrective action is performed, with some modifications producing a reduction in only a single area and others across multiple facilities areas. In cases of the former this may not be an issue per se, as a corrective action does not necessarily need to be effective in all areas of the facility to be deemed successful.

Limitations facing ABMs can be broken down into two types: data limitations and performance limitations. A fundamental data limitation in using any ABM is the availability of historical data to validate the model, with more historical data being able to provide a more extensive validation. Additionally, areas of a facility that have low historical prevalence may set a low baseline and make it difficult to measure any improvements in reducing contamination prevalence within an area. Long-term models are also limited by a more restrictive computational overhead due to the longer duration of the simulation, requiring more powerful hardware to both generate more iterations and to analyze the large amount of data produced afterwards. These issues can be reduced through the use of machines with a greater number of CPU cores and RAM.

Overall, these studies show that ABMs can become extremely valuable tools in food safety, providing enhanced decision-making capabilities for facilities. The scope of an ABM’s facility is mostly limited by what data it has access to, as any number of potential corrective actions can be simulated for a specific facility as needed. This work focused specifically on fresh produce packinghouses to provide insight as to the variability in corrective action results between facilities, as well as to demonstrate the risks of persistence misidentification. Furthermore, the approaches used here may provide information for future studies and other food safety-specific models in this or similar settings.

APPENDIX A

Parameter Calculation

Concentration of Listeria spp. per gram of contaminated raw produce (CFU/g) (NR) was calculated by converting data from Chen et al.'s (1) Table 1 into CFU/g using total CFU/fruit and the average fruit mass from recorded minimum and maximum (136g). A gamma distribution was then constructed to match calculated minimum, maximum, median, and mean average values as closely as possible.

Model Schedules

Table S2.1 Facility A baseline operations event schedule

Day/Hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sunday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM
Monday	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	PO	EM	PD	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM
Tuesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM
Wednesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM
Thursday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM
Friday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM
Saturday	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	CL	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM

EM: empty, CL: cleaning, PO: pre-op, PD: production.

Table S2.2 Facility B baseline operations event schedule

Day/Hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sunday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM
Monday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM
Tuesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM
Wednesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM
Thursday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM
Friday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM
Saturday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	CL	CL	EM	EM	EM	EM	EM

EM: empty, CL: cleaning, PO: pre-op, PD: production.

Table S2.3 Facility A operations event schedule with daily cleaning (AI_02C1/AI_02C2/ AI_03C1/AI_03C2)

Day/Hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sunday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM
Monday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	PO	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Tuesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Wednesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Thursday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Friday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Saturday	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	CL	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM

EM: empty, CL: cleaning, PO: pre-op, PD: production.

Table S2.4 Facility A operations event schedule with Sunday deep cleaning (MI_02)

Day/Hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sunday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	EM	EM	EM	EM	EM	EM
Monday	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	PO	EM	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM	EM
Tuesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM	EM
Wednesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM	EM
Thursday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM	EM
Friday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	EM	EM	EM	EM	EM	EM	EM	EM
Saturday	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	CL	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM

EM: empty, CL: cleaning, PO: pre-op, PD: production.

Table S2.5 Facility B operations event schedule with Sunday deep cleaning (MI_02)

Day/Hour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sunday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	CL	CL	CL	CL	CL	EM	EM	EM	EM	EM	EM
Monday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Tuesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Wednesday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Thursday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Friday	EM	EM	EM	EM	EM	EM	EM	EM	PO	PD	PD	PD	EM	PD	PD	PD	CL	CL	EM	EM	EM	EM	EM	EM
Saturday	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	CL	CL	EM	EM	EM	EM	EM	EM

EM: empty, CL: cleaning, PO: pre-op, PD: production.

Model Specifications

Table S2.6 Probability of agent contact description, equation and distribution, summary values and sources

Symbol	Description	Equation/Distribution	Mean	5th-9th Percentile	Reference
P_{11}	Probability of contact from contaminated surface in Zone 1 to another surface in Zone 1	Pert(0,0.1,0.9,4)	0.22	[0.03, 0.5]	(25)
P_{12}	Probability of contact from contaminated surface in Zone 1 to another surface in Zone 2	Pert(0.001,0.2,0.8,4)	0.27	[0.06, 0.53]	(25)
P_{13}	Probability of contact from contaminated surface in Zone 1 to another surface in Zone 3	Pert(0.001,0.15,0.85,4)	0.24	[0.04, 0.51]	(25)
P_{14}	Probability of contact from contaminated surface in Zone 1 to another surface in Zone 4	Pert(0.001,0.1,0.8,4)	0.20	[0.03, 0.45]	(25)

P_{21}	Probability of contact from contaminated surface in Zone 2 to another surface in Zone 1	Pert(0,0.2,0.95,4)	0.29	[0.06, 0.6]	(25)
P_{22}	Probability of contact from contaminated surface in Zone 2 to another surface in Zone 2	Pert(0.00005,0.15,0.7,4)	0.22	[0.04, 0.44]	(25)
P_{23}	Probability of contact from contaminated surface in Zone 2 to another surface in Zone 3	Pert(0.001,0.2,0.85,4)	0.28	[0.06, 0.55]	(25)
P_{24}	Probability of contact from contaminated surface in Zone 2 to another surface in Zone 4	Pert(0.05,10,80,4)	0.20	[0.03, 0.45]	(25)
P_{31}	Probability of contact from contaminated surface in Zone 3 to another surface in Zone 1	Pert(0,0.2,0.9,4)	0.16	[0.01, 0.43]	(25)
P_{32}	Probability of contact from contaminated surface in Zone 3 to another surface in Zone 2	Pert(0,0.035,0.9,4)	0.17	[0.02, 0.44]	(25)
P_{33}	Probability of contact from contaminated surface in Zone 3 to another surface in Zone 3	Pert(0.0002,0.125,0.85,4)	0.23	[0.04, 0.49]	(25)
P_{34}	Probability of contact from contaminated surface in Zone 3 to another surface in Zone 4	Pert(0.0001,0.02,0.6,4)	0.11	[0.01, 0.29]	(25)
P_{41}	Probability of contact from contaminated surface in Zone 4 to another surface in Zone 1	Pert(0,0.1,0.95,4)	0.23	[0.03, 0.52]	(25)

P_{42}	Probability of contact from contaminated surface in Zone 4 to another surface in Zone 2	Pert(0.0001,0.1,0.8,4)	0.20	[0.03, 0.45]	(25)
P_{43}	Probability of contact from contaminated surface in Zone 4 to another surface in Zone 3	Pert(0,0.1,0.9,4)	0.22	[0.03, 0.5]	(25)
P_{44}	Probability of contact from contaminated surface in Zone 4 to another surface in Zone 4	Pert(0,0.05,0.4,4)	0.10	[0.01, 0.23]	(25)

Table S2.7 Transfer Coefficient (TC) matrix based on Zone or employee type

	1	2	3	Employee (e)
1	-1.51	-3.68	-0.28	-1.97
2	-1.51	-1.51	-3.53	-1.97
3	-0.31	-0.31	-0.31	-0.82
e	-1.97	-1.97	-1.69	-3.43

Transfer Coefficient was selected based on a combination of the sender (i: horizontal) and receiver (j: vertical) (28,64)

Table S2.8 Standard Deviation (STD) matrix based on Zone or employee type

	1	2	3	Employee (e)
1	0.2	0.2	0.2	0.87
2	0.2	0.2	0.2	0.87
3	0.2	0.2	0.2	0.87
e	0.87	0.87	0.87	0.79

Standard Deviation was selected based on a combination of the sender (i: horizontal) and receiver (j: vertical) (28,64)

Table S2.9 Mean and 5th-95th percentiles of probability of *Listeria* spp. transfer between agent types given contact

	1	2	3	Employee (e)
1	$3.44E-02$ $[1.45E-02, 6.59E-02]$	$3.44E-02$ $[1.45E-02, 6.59E-02]$	$5.45E-01$ $[2.30E-01, 1.04E+00]$	$7.97E-02$ $[3.97E-04, 2.89E-01]$
2	$2.32E-04$ $[9.80E-05, 4.46E-04]$	$3.44E-02$ $[1.45E-02, 6.59E-02]$	$5.45E-01$ $[2.30E-01, 1.04E+00]$	$7.96E-02$ $[3.97E-04, 2.89E-01]$
3	$5.83E-01$ $[2.46E-01, 1.12E+00]$	$3.28E-04$ $[1.38E-04, 6.29E-04]$	$5.45E-01$ $[2.30E-01, 1.04E+00]$	$1.52E-01$ $[7.56E-04, 5.52E-01]$
e	$7.98E-02$ $[3.97E-04, 2.89E-01]$	$7.98E-02$ $[3.97E-04, 2.89E-01]$	$1.13E+00$ $[5.61E-03, 4.09E+00]$	$1.95E-3$ $[1.86E-05, 7.39E-03]$

Transfer coefficient (TC) (Table S7) and standard deviation (STD) (Table 2.S8) were selected based on agent type, then calculated via $10^{Normal(TC,STD)}$. (28,64)

Validation

Table S2.10. Comparison of results of Historical and Simulated Sampling and testing for *Listeria* contamination in Facilities A and B.

	Historical Sampling			Simulated Sampling	p-value
	Total	Positive	Mean Prevalence (%, 95% CI)[a]	Mean Prevalence (%, 95% CI)	
Facility A					
All Samples	102	16	16 (10-24)	11 (6-19)	0.33[b]
Wet Agents	56	14	25 (16-38)	15 (8-27)	0.19[c]
Dry Agents	46	2	4 (1-15)	7 (2-18)	1.00[c]
Zone 2	55	4	7 (3-17)	4 (1-13)	0.68[c]
Zone 3	43	12	28 (17-43)	20 (11-34)	0.38[b]
Facility B					
All Samples	174	26	15 (10-21)	12 (8-18)	0.42[b]
Wet Agents	90	21	23 (16-33)	18 (12-28)	0.41[b]
Dry Agents	79	4	5 (2-12)	9 (4-17)	0.36[b]
Zone 2	123	11	9 (5-15)	9 (5-15)	0.96[b]
Zone 3	29	7	24 (12-42)	20 (9-38)	0.71[b]

[a]95% Confidence interval based on the Wilson score interval method. [b]Chi Square Test analysis. [c]Fisher's Exact Test analysis.

Predicted Listeria prevalence and concentration in Zones 1-3

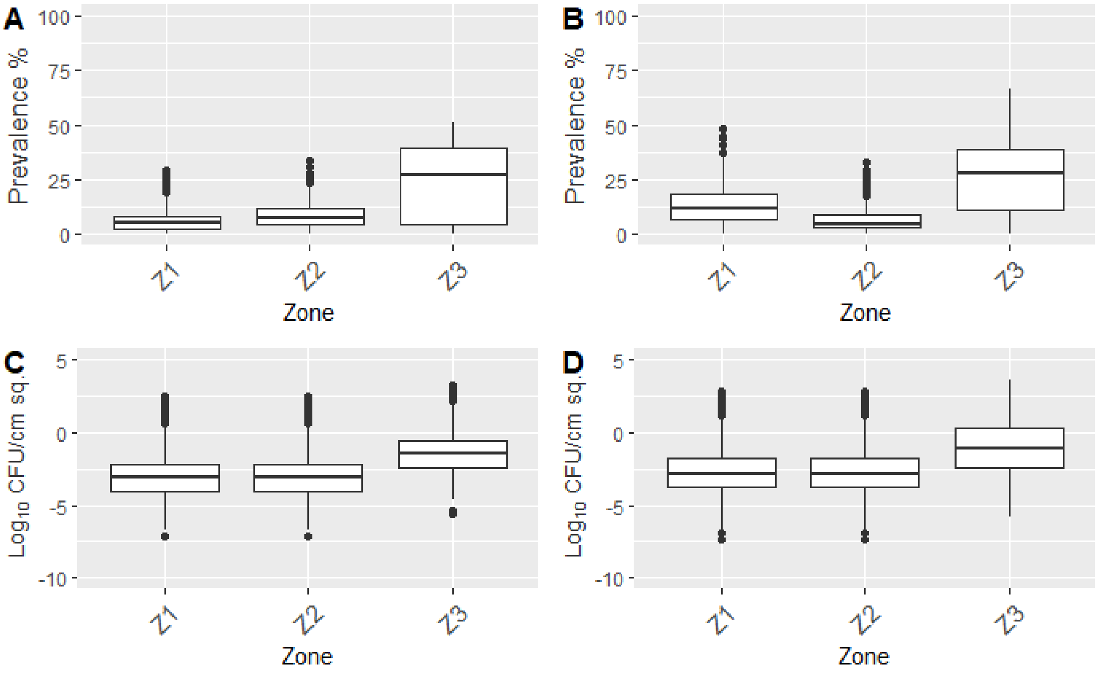

Fig S2.1. Boxplots describing *Listeria* contamination prevalence and concentration on contaminated agents on Wednesday at Midday for Facility A and B baseline conditions by Zone group.
Prevalence of *Listeria* contamination within each area of Facility A (panel A) and Facility B (panel B). Both facilities show higher prevalence in Zone 3 than Zones 1 and 2. Log_{10} concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each Zone group of Facility A (panel C) and Facility B (panel D) with median concentrations listed showing low level of contamination.

Cluster Analysis

Using selected secondary outcomes (Supplemental Tables S11 and S12), a cluster analysis of all agents was conducted with FAMD and PCA methods (Supplemental Tables S13 and S14) to identify agents clusters for targeted corrective actions to determine if these identification methods could produce a viable intervention strategy. FAMD analysis of agent attributes in both models identified a respective cluster with higher mean probability of contamination and concentrations in each model (Facility A: cluster 1, Facility B: cluster 2; Table S3). The characteristics of these clusters differed, with Facility A cluster 1 primarily composed of Zone 3 agents and having the highest number of contacts and transfers from other contaminated agents, while Facility B cluster 2 consisted of Zone 2 agents with the lowest number of connection-based contamination events. Finding a single cluster to target for corrective actions with PCA clustering of agent contamination outcomes required additional steps: with Facility A having three clusters of high contamination probabilities and longer mean time contaminated (clusters 2, 3 and 4), but only the latter showing higher concentrations. Cluster 4 only consisted of two similar agents, whereas clusters 2 and 3 were composed of 22 and 24 agents, respectively. A key difference between clusters 2 and 3 however, was that each consisted of solely either cleanable (cluster 2) or uncleanable (cluster 3) agents, resulting in higher likelihood of niches among cluster 3 agents. Facility B demonstrated a similar clustering pattern between its PCA clusters 2 and 3, both of which also showed high contamination probabilities and mean time contaminated, but with cluster 3 showing far higher concentrations. Like in A, B's cluster 3 was extremely small, consisting of only one agent, while cluster 2 contained 25 agents. An additional similarity between cluster 2 in Facilities A and B was the elevated number of niches established in B's 2nd cluster. Furthermore, the "high-niche clusters" identified in both models also contained the highest mean number of undirected agent links, along with higher numbers of incoming and outgoing links (though Facility A's 2nd cluster was not the highest in this regard).

Table S2.11 Secondary outcomes of interest, calculated over all model iterations for each individual agent, and their definitions

Notation	Definition
D_T_i	Average of total times contaminated for agent i^1 (h, per 2 wk simulation)
D_L_i	Average of longest durations consecutively contaminated for agent i^1 (h, per 2 wk simulation)
N_P_i	Average of the numbers of niches established for agent i^1 (per 2 wk simulation)
N_PT_i	Average of the numbers of temporary niches established for agent i (per 2 wk simulation)
Ce_R_i	Average of the numbers of reception events for agent i^1 (per 2 wk simulation)
Ce_T_i	Average of the numbers of transfer events for agent i^1 (per 2 wk simulation)
Ce_F_i	Average of the numbers of food-based contamination events for agent i^1 (per 2 wk simulation)
Ce_C_i	Average of the numbers of random (chance) contamination events for agent i (per 2 wk simulation)
Ce_Z4_i	Average of the numbers of Zone 4 contamination events for agent i^1 (per 2 wk simulation)
C_i	Average of the concentrations at the end of simulation for agent i^1 (CFU/cm^2)
P_i	Probability of contamination at the end of simulation for agent i^1
$C_{d,i}$	Average of the concentrations at 2nd week midday on day d^2 for agent i^1 (CFU/cm^2)
$P_{d,i}$	Probability of contamination at 2nd week midday on day d^2 for agent i^1

[1]i: agent is 1-224 (Facility A) or 1-225 (Facility B). [2]d: day is Monday, Tuesday, Wednesday, Thursday, or Friday. Notation: h=hour, wk=week.

Table S2.12 List of agent attributes and agent contamination-related outcomes selected for cluster analysis usage.

	Agent attributes	Agent contamination-related outcomes[1]
Facility A	Number of Incoming Links Agent Zone Cleanability	N_P_i[2] N_PT_i Ce_T_i $C_{d,i}$ P_i
Facility B	Number of Incoming links Number of Outgoing links Number of Undirected Links Cleanability	D_T_i[3] D_L_i N_P_i N_PT_i Ce_R_i Ce_T_i $C_{d,i}$ P_i

[1] Outcomes defined in Table S2.11. [2] i: agent is 1-224. [3] i: agent is 1-225.

Table S2.13 FAMD-based Cluster Analysis

Attributes	Facility A (n=224)			Facility B (n=225)			
	A-I	A-II	A-III	B-I	B-II	B-III	B-IV
Number of agents	72	68	84	100	93	28	4
Zone 1	9	0	84	3	68	11	3
Zone 2	0	68	0	91	16	14	1
Zone 3	63	0	0	6	9	3	0
Representative Agent(s)	fans, fans-tray, rot-bin, drain-drying-01, guard-plate-return-empty	tray-belt-02, tray-belt-05, tray-belt-06, tray-belt-07, tray-belt-08	bag-feed-belt, bagging-station-04, bagging-station-05, bagging-station-06, bagging-station-07	rot-trolley-track, box-sticker-printer-01, box-sticker-printer-02, box-sticker-printer-03, box-sticker-printer-04	tray-belt-02, small-produce-crate-feed, trench-drain-drying, npw-belt, crate-filler-01	produce-crate-03, produce-crate-04, produce-crate-05, dumper, flume-exterior	reject-belt-05, box-belt, rot-trolley, track-belt
	Cleanability						
Yes	41	48	83	0	93	23	3
No	31	20	1	100	0	5	1
Distance from floor (cm)[a]	36.06	87.51	87.76	51.85	99.92	94.97	75.00
Number of niches established[a]	1.12	0.12	0.15	0.55	0.00	1.56	1.87
Number of temporary	0.00	0.00	0.00	0.00	0.00	0.02	0.00

niches established[a]							
	Area of Packinghouse						
Wet	31	17	5	22	12	10	1
Dry	41	51	79	78	81	18	3
Number of incoming links[a]	0.26	0.51	0.52	0.22	0.47	0.29	7.0
Number of outgoing links[a]	0.17	0.22	0.85	0.16	0.53	0.89	3.0
Number of undirected links[a]	1.35	1.47	1.49	0.28	0.18	3.32	0.00
	Probability of contamination at mid-shift[a]						
Monday	0.16	0.03	0.02	0.06	0.04	0.28	0.16
Tuesday	0.22	0.07	0.03	0.07	0.06	0.32	0.18
Wednesday	0.24	0.09	0.04	0.07	0.08	0.33	0.19
Thursday	0.26	0.11	0.04	0.08	0.09	0.35	0.21
Friday	0.28	0.13	0.05	0.09	0.09	0.36	0.22
	Concentration at mid-shift[a] (CFU/cm^2)						
Monday	0.29	0.01	0.05	1.39	0.20	0.56	0.04
Tuesday	0.31	0.01	0.06	1.83	0.23	0.56	0.05
Wednesday	0.42	0.01	0.04	2.25	0.29	0.58	0.07
Thursday	0.47	0.01	0.05	3.22	0.33	0.61	0.10
Friday	0.72	0.01	0.05	4.56	0.34	0.69	0.14
	Probability of contamination at mid-shift Wednesday[a]						
Zone 1	0.31	N/A	0.04	0.15	0.07	0.53	0.22
Zone 2	N/A	0.09	N/A	0.05	0.01	0.25	0.10
Zone 3	0.23	N/A	N/A	0.39	0.27	0.01	N/A
Wet	0.39	0.18	0.31	0.25	0.34	0.46	0.57
Dry	0.13	0.07	0.02	0.03	0.04	0.26	0.07
	Concentration at mid-shift Wednesday[a] (CFU/cm^2)						
Zone 1	0.02	N/A	0.04	0.03	0.01	1.24	0.22
Zone 2	N/A	0.01	N/A	1.48	0.00	0.18	0.10
Zone 3	0.48	N/A	N/A	14.94	2.93	0.00	N/A
Wet	0.87	0.03	0.72	10.13	2.24	0.46	0.29
Dry	0.09	0.00	0.00	0.02	0.00	0.26	0.00
Contacts by contaminated agent[a] (per wk, via link)	13.89	6.20	3.88	3.39	3.94	41.01	28.68
Transfers by contaminated agent[a] (per wk, via link)	12.39	5.61	5.65	2.77	4.05	44.05	20.44
Time contaminated[a] (hrs)	52.46	20.71	5.35	17.26	14.08	60.04	44.85

[a] Mean of cluster

Table S2.14 PCA-based Cluster Analysis

Attributes	Facility A (n=224)			Facility B (n=225)			
	A-I	A-II	A-III	B-I	B-II	B-III	B-IV
Number of agents	177	21	84	100	93	28	4
Zone 1	84	2	84	3	68	11	3
Zone 2	56	10	0	91	16	14	1
Zone 3	37	9	0	6	9	3	0
Representative Agent(s)	employee-tray-04, employee-tray-03, employee-clean-01, outlet-13, sorting-platform-blue-bucket	drain-reject-02, drain-sorting-01, drain-drying-02, loader, tray-belt-11	bag-feed-belt, bagging-station-04, bagging-station-05, bagging-station-06, bagging-station-07	rot-trolley-track, box-sticker-printer-01, box-sticker-printer-02, box-sticker-printer-03, box-sticker-printer-04	tray-belt-02, small-produce-crate-feed, trench-drain-drying, npw-belt, crate-filler-01	produce-crate-03, produce-crate-04, produce-crate-05, dumper, flume-exterior	reject-belt-05, box-belt, rot-trolley, track-belt
	Cleanability						
Yes	151	21	83	0	93	23	3
No	26	0	1	100	0	5	1
Distance from floor (cm)a	77.19	49.05	87.76	51.85	99.92	94.97	75.00
Number of niches established[a]	0.02	0.00	0.15	0.55	0.00	1.56	1.87
Number of temporary niches established[a]	0.00	0.01	0.00	0.00	0.00	0.02	0.00
	Area of Packinghouse						
Wet	23	6	5	22	12	10	1
Dry	154	15	79	78	81	18	3
Number of incoming links[a]	0.40	0.48	0.52	0.22	0.47	0.29	7.0
Number of outgoing links[a]	0.36	0.62	0.85	0.16	0.53	0.89	3.0
Number of undirected links[a]	1.28	1.71	1.49	0.28	0.18	3.32	0.00
	Probability of contamination at mid-shift[a]						
Monday	0.01	0.12	0.02	0.06	0.04	0.28	0.16
Tuesday	0.01	0.34	0.03	0.07	0.06	0.32	0.18

Wednesday	0.02	0.44	0.04	0.07	0.08	0.33	0.19
Thursday	0.03	0.50	0.04	0.08	0.09	0.35	0.21
Friday	0.03	0.54	0.05	0.09	0.09	0.36	0.22
	Concentration at mid-shift[a] (CFU/cm^2)						
Monday	0.00	0.16	0.05	1.39	0.20	0.56	0.04
Tuesday	0.00	0.31	0.06	1.83	0.23	0.56	0.05
Wednesday	0.00	0.36	0.04	2.25	0.29	0.58	0.07
Thursday	0.00	0.38	0.05	3.22	0.33	0.61	0.10
Friday	0.00	0.49	0.05	4.56	0.34	0.69	0.14
	Probability of contamination at mid-shift Wednesday[a]						
Zone 1	0.02	0.54	0.04	0.15	0.07	0.53	0.22
Zone 2	0.03	0.36	N/A	0.05	0.01	0.25	0.10
Zone 3	0.01	0.50	N/A	0.39	0.27	0.01	N/A
Wet	0.06	0.45	0.31	0.25	0.34	0.46	0.57
Dry	0.01	0.43	0.02	0.03	0.04	0.26	0.07
	Concentration at mid-shift Wednesday[a] (CFU/cm^2)						
Zone 1	0.00	2.09	0.04	0.03	0.01	1.24	0.22
Zone 2	0.01	0.01	N/A	1.48	0.00	0.18	0.10
Zone 3	0.00	0.43	N/A	14.94	2.93	0.00	N/A
Wet	0.02	0.70	0.72	10.13	2.24	0.46	0.29
Dry	0.00	0.22	0.00	0.02	0.00	0.26	0.00
Contacts by contaminated agent[a] (per wk, via link)	2.16	24.76	3.88	3.39	3.94	41.01	28.68
Transfers by contaminated agent[a] (per wk, via link)	1.90	25.87	5.65	2.77	4.05	44.05	20.44
Time contaminated[a] (hrs)	4.79	85.39	5.35	17.26	14.08	60.04	44.85

[a] Mean of cluster

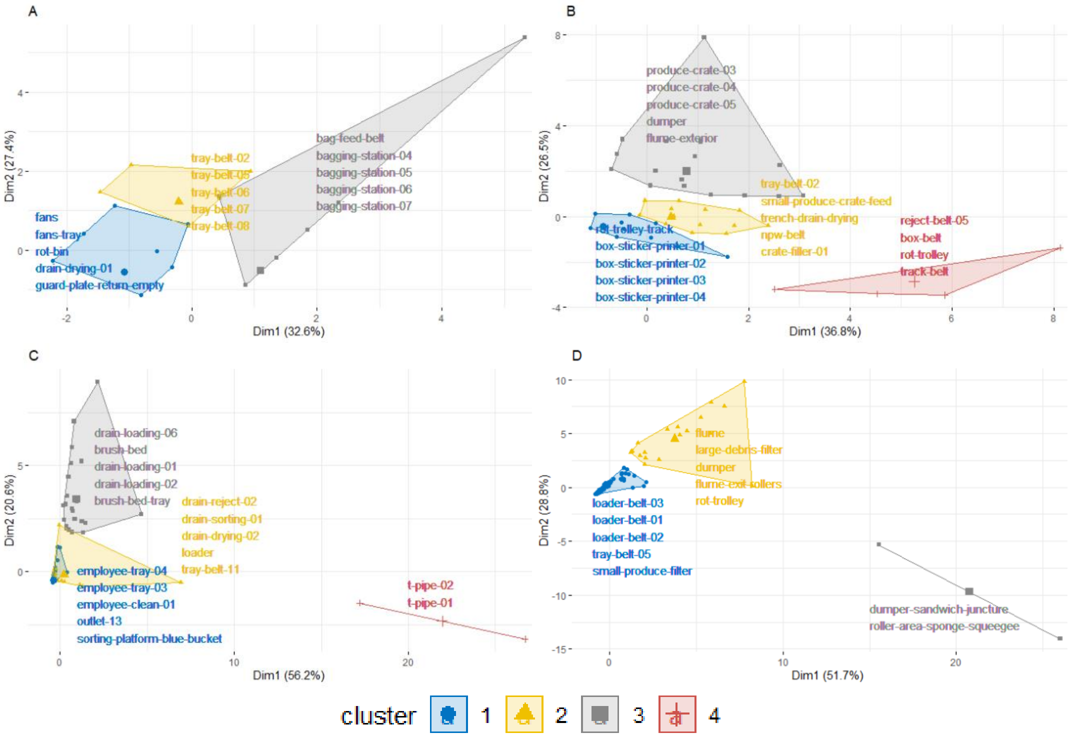

Fig S2.2 Visualization of Hierarchical Clustering of Principal Components (HCPC) Cluster Analysis of agent attributes (from Factor Analysis of Mixed Data (FAMD)) and agent contamination outcomes (from Principal Component Analysis (PCA)) results of both models captured at Wednesday midday during the 2nd simulated week A: Facility A HCPC of agent attributes. B: Facility B HCPC of agent attributes. C: Facility A HCPC of agent contamination outcomes. D: Facility B HCPC of agent contamination outcomes. FAMD evaluated agent cleanability, number of in- and out-directed links while PCA evaluated agent total probability of contamination, mean time contaminated, mean maximum consecutive time contaminated, mean number of contact and transfer events, mean number of niches and temporary niches established and mean concentrations at Midday for each workday. Named agents for each cluster are representative individuals of the total cluster and closest to their respective cluster's center.

HCPC identified a small cluster of two agents in Facility A that were predicted to have a mean concentration of approximately 2 $\log_{10}$ higher as compared to others at mid-shift (Supplemental Table S2.4). The specific agents identified were found to be part of the facility's drainage system that did not lead to further FCS surfaces and had a low number of connections to other agents. While this cluster was not an effective target for large-scale corrective actions, as these agents were too far from FCS equipment to affect *Listeria* introduced via incoming produce, it did serve to identify "downstream" agents that were at high risk of subsequent contamination. Facility B had a similar small cluster consisting of a dumper-sandwich-juncture (Supplemental Table S2.4); however, its connectivity was limited to only a single directed link. Unlike Facility A's micro-cluster, B's was more directly connected to FCS agents and could potentially be a harborage site that would allow *Listeria* to re-contaminate the rest of the system.

Scenario Analysis

Table S2.15. Comparisons of median corrective action efficacy against baseline conditions and corresponding interquartile range (IRQ)

Scenario model-notation	Median Efficacy (%) (25th percentile-75th percentile)			
	A		B	
	Wet	Dry	Wet	Dry
PR_01	0.0 (-124.9-57.6)	0.0 (-71.4-46.7)	0.0 (-11.1-8.6)	0.0 (-33.3-18.2)
PR_02	0.0 (-141.0-53.6)	0.0 (-80.0-45.1)	0.0 (-11.5-11.1)	0.0 (-33.3-26.7)
PR_03	0.0 (-134.6-58.7)	0.0 (-75.0-46.7)	0.0 (-13.3-10.7)	0.0 (-44.4-25.0)
LR_01	0.0 (-149.9-64.9)	0.0 (-70.3-47.4)	0.0 (-6.2-8.3)	0.0 (-11.1-26.7)
LR_02	0.0 (-99.5-65.6)	7.7 (-50.0-50.0)	0.0 (-7.1-8.2)	0.0 (0.0-35.3)
LR_03	0.0 (-99.8-66.0)	6.7 (-54.0-55.5)	0.0 (-5.6-11.1)	0.0 (0.0-41.2)
PZ_01	0.0 (-122.9-60.0)	9.5 (-64.4-50.6)	0.0 (-18.2-16.7)	0.0 (-33.3-33.3)
PZ_02	0.0 (-115.7-58.9)	16.7 (-57.4-57.1)	0.0 (-16.7-17.7)	7.4 (-28.9-40.0)
PZ_03	0.0 (-99.9-62.5)	28.6 (-28.6-69.2)	0.0 (-15.4-20.9)	15.1 (-21.0-49.9)
PZ_04	0.0 (-100.0-63.6)	44.4 (0.0-87.5)	5.6 (-11.8-25.3)	26.7 (-9.1-69.2)
LZ_01	0.0 (-222.0-45.1)	18.2 (-50.0-55.5)	0.0 (-14.3-18.2)	14.3 (-20.0-47.4)
LZ_02	0.0 (-122.8-52.9)	28.6 (-25.0-70.3)	3.8 (-11.8-20.0)	23.1 (-9.8-58.8)
LZ_03	0.0 (-145.4-53.6)	36.3 (-12.5-76.9)	5.4 (-11.5-25.0)	27.3 (-8.3-66.6)
EC_01	2.9 (-131.3-64.7)	0.0 (-66.6-49.9)	6.2 (-9.1-25.0)	4.6 (-33.3-33.3)
EC_02	12.5 (-86.1-81.8)	6.9 (-57.4-53.4)	12.8 (-2.8-33.3)	11.8 (-25.4-40.0)
EC_03	31.0 (-49.9-90.9)	17.2 (-54.8-55.5)	25.0 (4.9-47.1)	14.3 (-23.5-43.9)
EC_04	96.1 (49.9-100.0)	40.0 (-25.0-71.4)	73.1 (42.8-88.2)	33.3 (-10.0-66.6)
MI_01	0.0 (-100.0-52.0)	0.0 (-54.0-33.3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
MI_02	9.9 (-133.2-59.3)	8.3 (-62.5-50.0)	4.8 (-12.5-21.4)	6.3 (-33.3-35.3)

AI_01	29.3 (-54.0-84.3)	10.0 (-50.0-55.7)	27.1 (8.3-50.0)	0.0 (-20.0-28.8)
AI_02C1	18.2 (-149.7-58.2)	-30.0 (-149.7-58.2)	N/A	N/A
AI_02C2	52.8 (-33.3-86.6)	-9.3 (-119.9-42.8)	N/A	N/A
AI_02	N/A	N/A	50.0 (29.8-63.0)	21.7 (-25.0-50.0)
AI_03C1	0.0 (-149.9-55.5)	-45.8 (-149.8-14.3)	N/A	N/A
AI_03C2	0.0 (-203.0-45.5)	-33.3 (-137.4-22.2)	N/A	N/A
AI_03	N/A	N/A	64.1 (42.5-80.0)	50.0 (1.8-76.4)
AI_04	45.3 (-83.6-77.7)	42.8 (-18.2-75.0)	0.0 (-89.8-44.4)	77.3 (10.4-99.9)
CI_01	20.8 (-80.8-81.9)	36.8 (-14.3-74.9)	77.7 (57.1-91.6)	58.8 (12.3-83.3)
CI_02	50.0 (-49.9-87.0)	42.8 (-22.2-73.4)	13.6 (-66.6-53.3)	76.4 (15.1-99.9)
CI_03	45.3 (-88.1-77.8)	75.0 (37.2-91.6)	66.6 (21.2-85.7)	88.2 (26.2-99.9)

Random Probability Occurrence Reduction

Facility A did not project any changes to median prevalence in either area against the baseline beyond 1.88 pp decrease (Fig S2.3.A) following an increase in time to random contamination introduction by 150-250%. Median concentrations on contaminated agents showed negligible changes in both the wet and dry area (Fig S2.3.C).

Facility B also showed no change in wet or dry area median prevalence against the baseline model (Fig S2.3.B). Changes in median concentration (Fig S2.3.D) in the respective areas were negligible.

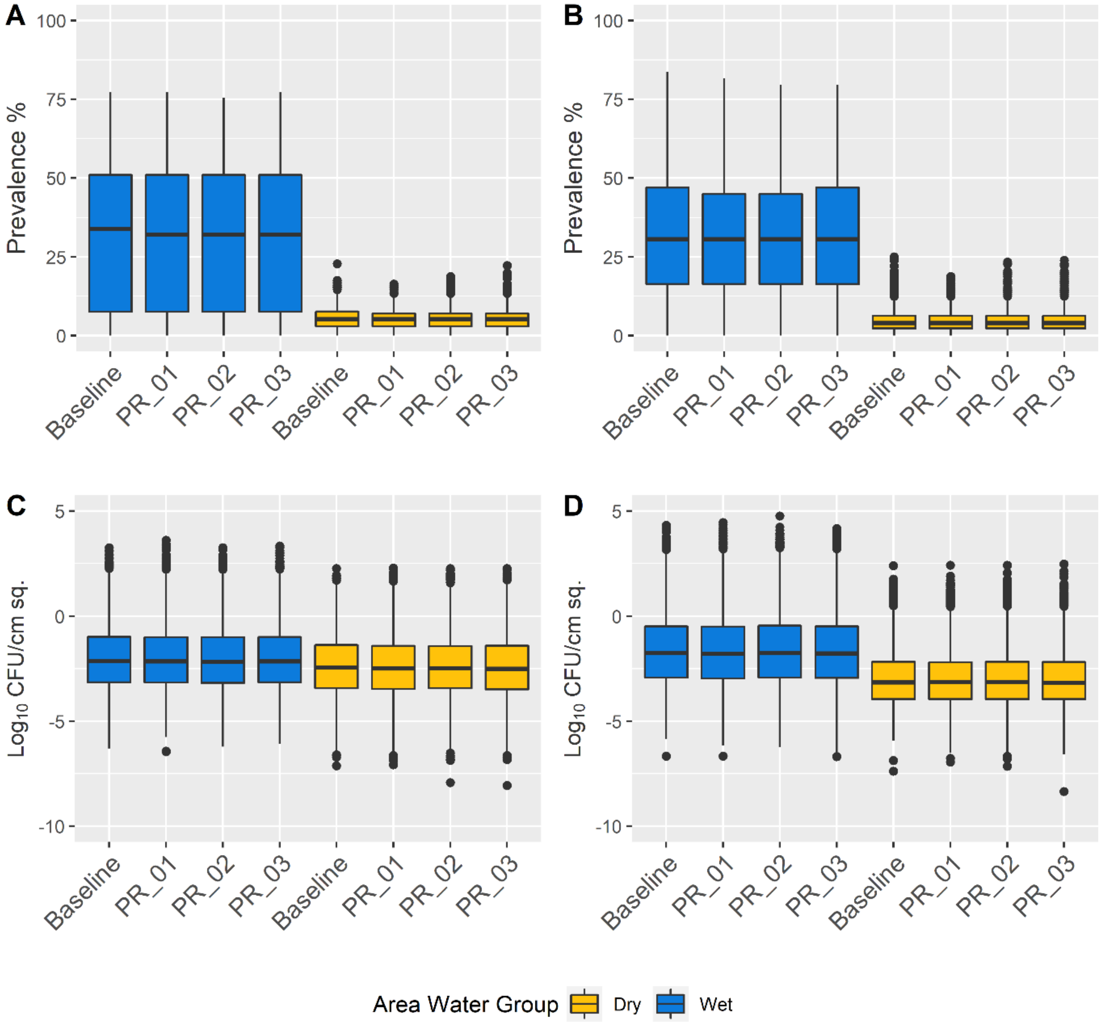

Fig S2.3 Boxplots describing the effects of reducing the rate of Random Event Occurrence by 25-75% from baseline on both wet (blue) and dry (yellow) area *Listeria* contamination prevalence and concentration in both models
Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). Log_{10} concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D).

Random Load Reduction

Facility A showed a small decrease in median prevalence across iterations of a simulation in both wet and dry areas against the baseline model (Fig S2.4.A): the wet area showed a maximum drop of 5.66 pp, while dry areas experienced a maximum drop of 0.58 pp. Median *Listeria* concentration (Fig S2.4.C) in wet areas showed almost no difference between scenarios (maximum drop of 0.04 $\log_{10}$ CFU/cm^2) and a similarly small decrease in dry areas of 0.07 $\log_{10}$ CFU/cm^2.

Facility B's (Fig S2.4.B) wet area median prevalence showed a decrease of 2.04 pp at the highest corrective action and dry area median prevalence dropped by 0.57 pp. Median concentration of *Listeria* on positive agents (Fig S2.4.D) in the wet area decreased by 0.05 $\log_{10}$ CFU/cm^2 and in the dry area by 0.20 $\log_{10}$ CFU/cm^2.

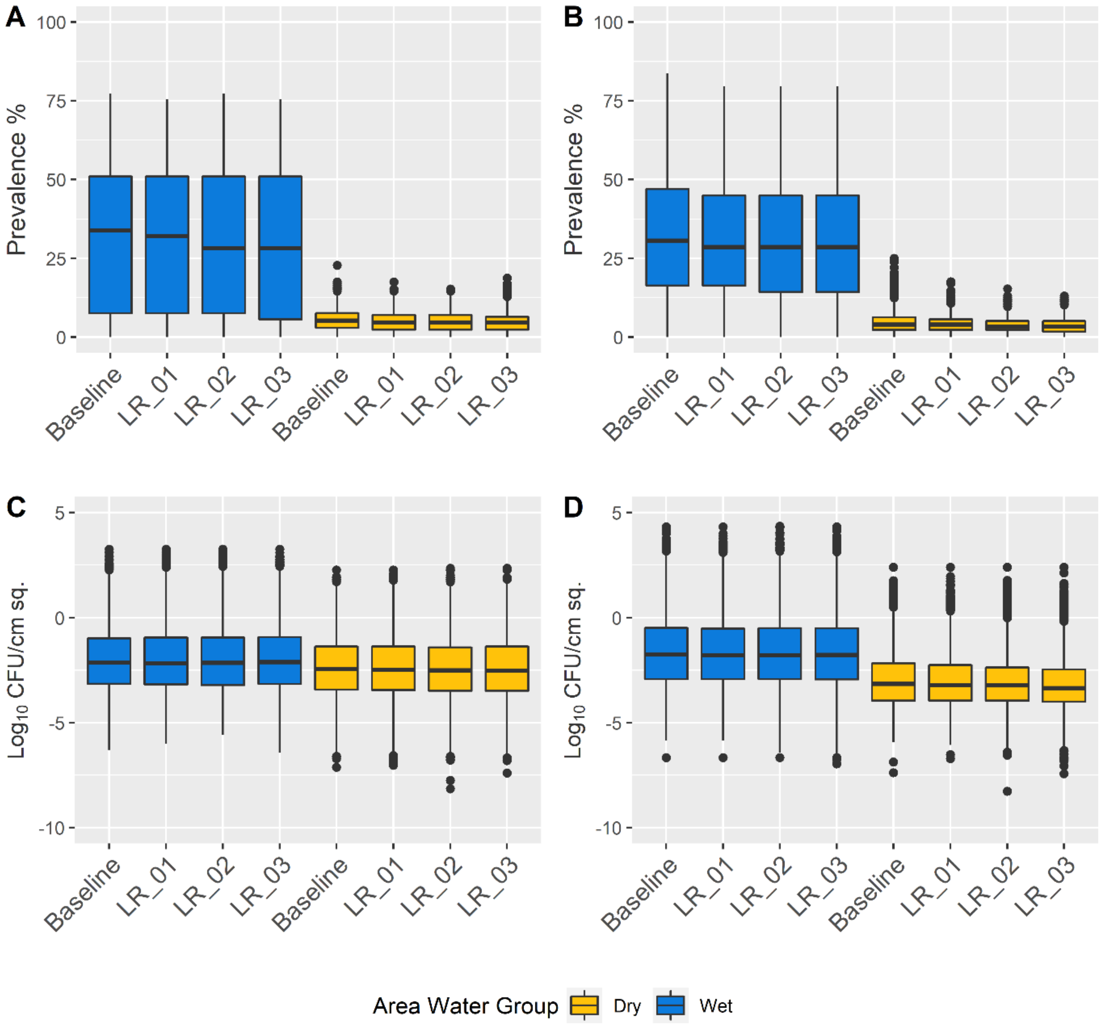

Fig S2.4 Boxplots describing the effects of Random Load reduction by 1-3 logs on both wet (blue) and dry (yellow) area *Listeria* contamination prevalence and concentration in both models

Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). Log_{10} concentrations CFU/cm^2 of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D).

Z4 Probability Event Reduction

In Facility A, median prevalence in wet and dry areas showed at most a decrease by 2.34 pp at 0% probability of Z4 (Fig S2.5.A). Median concentrations on positive agents in the wet area showed effectively negligible fluctuation (Fig S2.5.C), while the median of dry area concentrations increased with each corrective action, reaching a maximum increase of 0.66 $\log_{10}$ CFU/cm^2 from baseline median.

Facility B followed a similar pattern of minimal improvement, with median wet area prevalence decreasing by 2.04 pp at 0% Z4 event probability, and dry area prevalence decreasing by 1.14 pp (Fig S2.5.B). Median concentration on positive agents in wet areas did not change beyond minor fluctuations, while dry area median concentrations showed a small trend of increasing as probability decreased, culminating in a maximum difference of 0.10 $\log_{10}$ CFU/cm^2 from baseline at 0% (Fig S2.5.D).

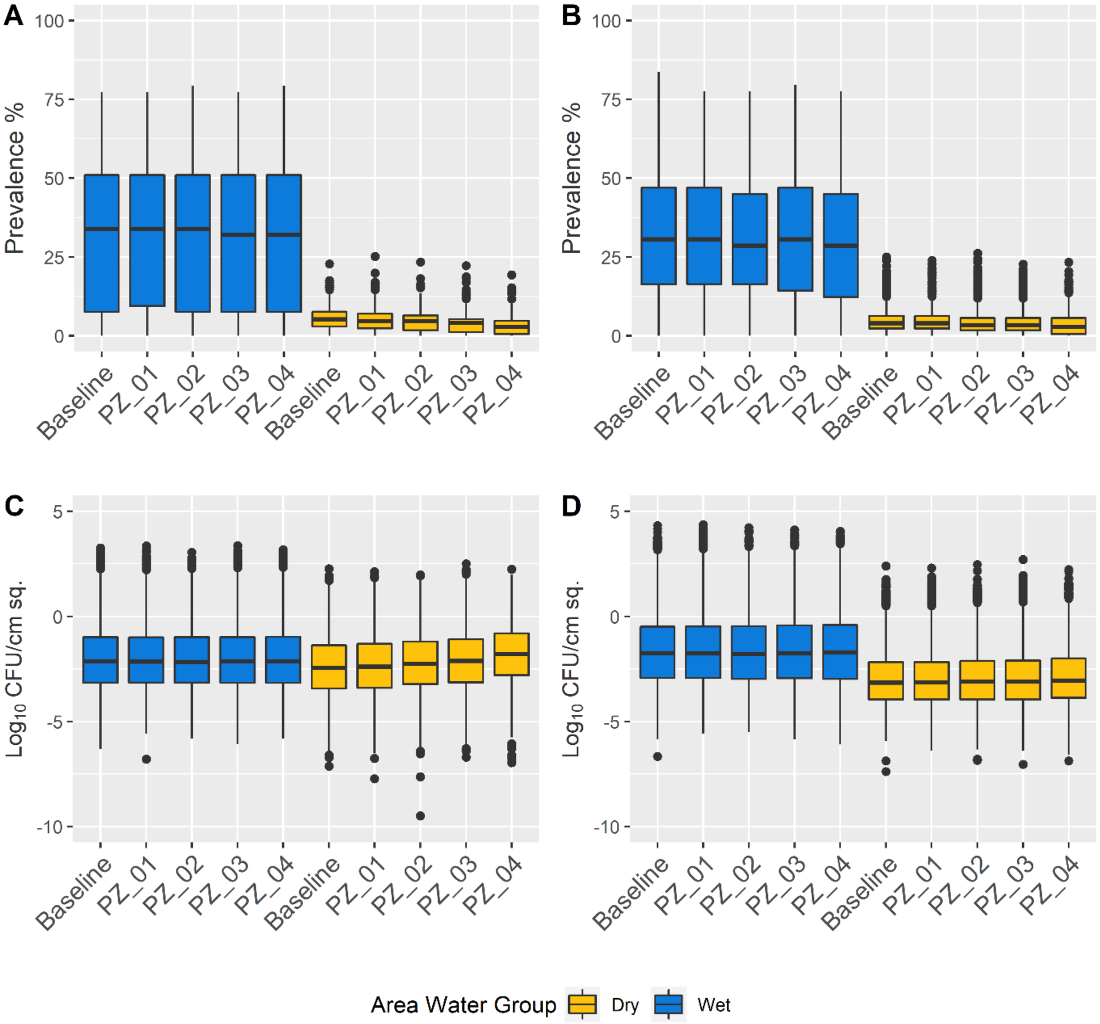

Fig S2.5 Boxplots describing the effects of reducing Z4 Event Occurrence from 100-0% in increments of 25% on both wet (blue) and dry (yellow) area contamination prevalence and concentration in both models.
Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B 1). Log$_{10}$ concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D).

Z4 Load Reduction

Facility A showed little-to-no-changes in area prevalence values against the baseline (Fig S2.6.A) beyond minor fluctuations and a minor decrease in dry area median, dropping by a maximum 0.63 pp at 3 Log Reduction. The median concentration of the wet area showed no major change against the baseline model (0.04 log$_{10}$ CFU/cm^2

decrease) while dry showed a slight increase in median concentration (0.36 $\log_{10}$ CFU/cm^2) at 3 Log Reduction (Fig S2.6.C).

Similarly, Facility B's largest log reduction produced no change to median prevalence in the wet area and a reduction of 1.14 pp in the dry area (Fig S2.6.B). Median concentration on positive agents in the wet area dropped by a maximum of 0.07 $\log_{10}$ CFU/cm^2 and dry by a maximum of 0.06 $\log_{10}$ CFU/cm^2 (Fig S2.6.D).

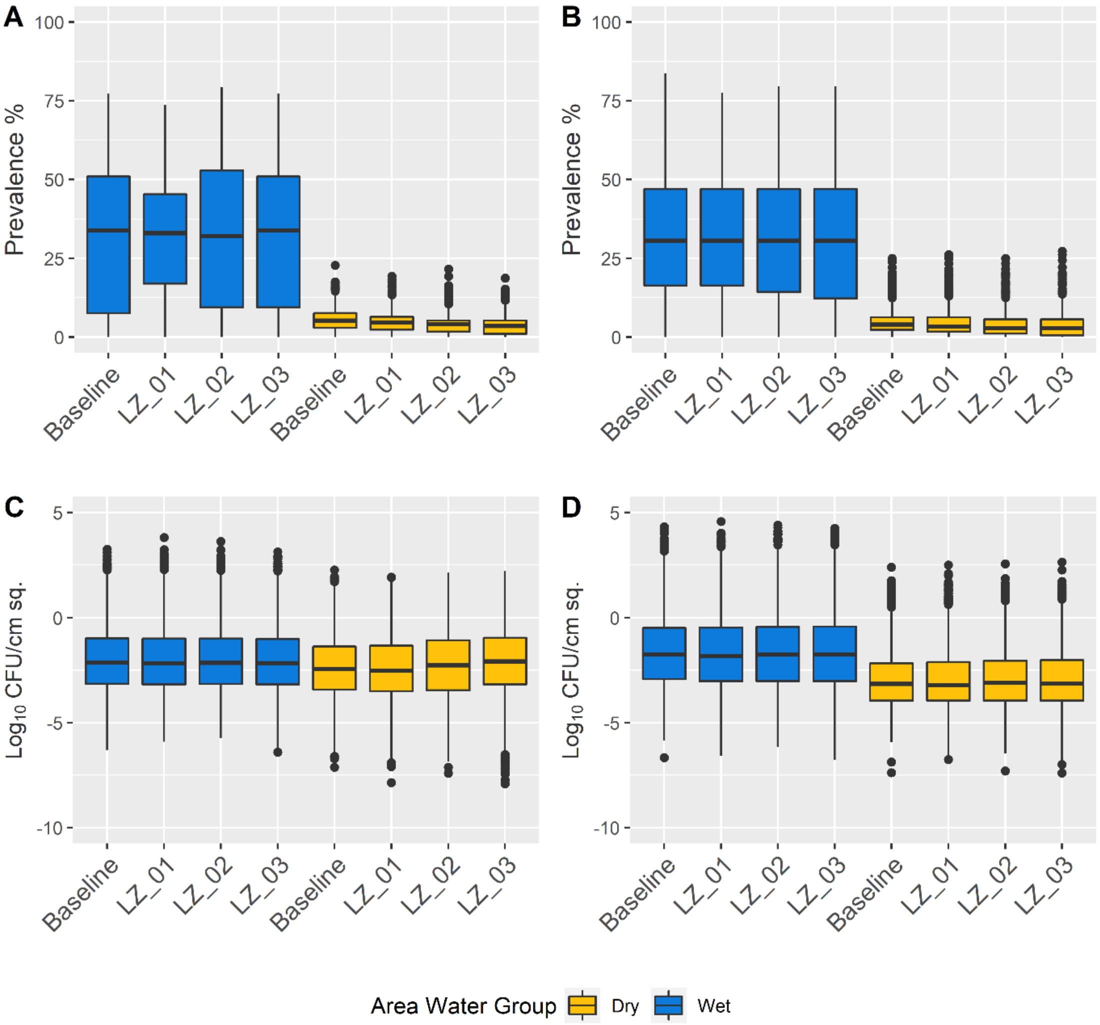

Fig S2.6 Boxplots describing the effects of Z4 Load reduction by 1-3 logs on both wet and dry area contamination prevalence and concentration in both models Prevalence of *Listeria* contamination of all agents within each area of Facility A (panel A) and Facility B (panel B). Log_{10} concentrations (CFU/cm^2) of *Listeria* on contaminated agents within each area of Facility A (panel C) and Facility B (panel D).

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APPENDIX B

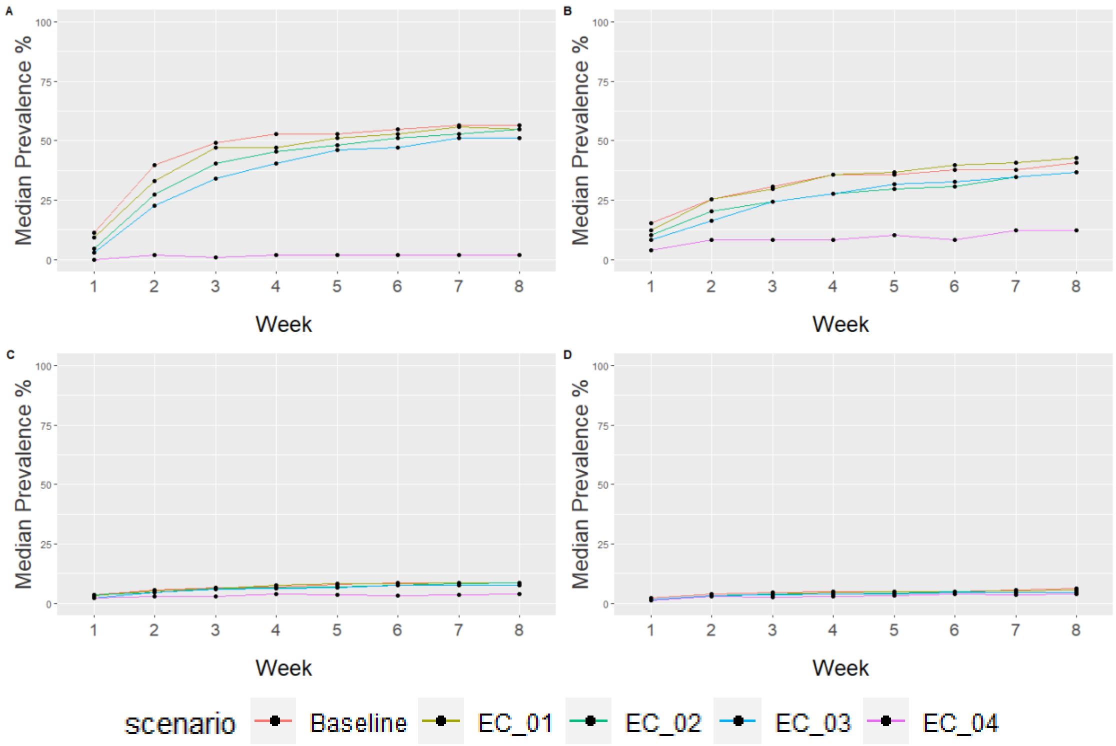


Fig S3.1 Median Prevalence of wet and dry agent areas of baseline scenarios compared against reducing *Listeria* prevalence in incoming produce crates corrective actions over eight weeks for Facilities A and B (EC_01, EC_02, EC_03 and EC_04) A: Facility A *Listeria* contamination prevalence of all agents in wet areas. B: Facility B *Listeria* contamination prevalence of all agents in wet areas. C: Facility A *Listeria* contamination prevalence of all agents in dry areas. D: Facility B *Listeria* contamination prevalence of all agents in dry areas.
Listeria prevalence in incoming produce crates in the model was reduced by multiplying the baseline *Listeria* prevalence by the factors of 0.75, 0.5, 0.25 and 0 (scenarios EC_01, EC_02, EC_03, and EC_04, respectively)

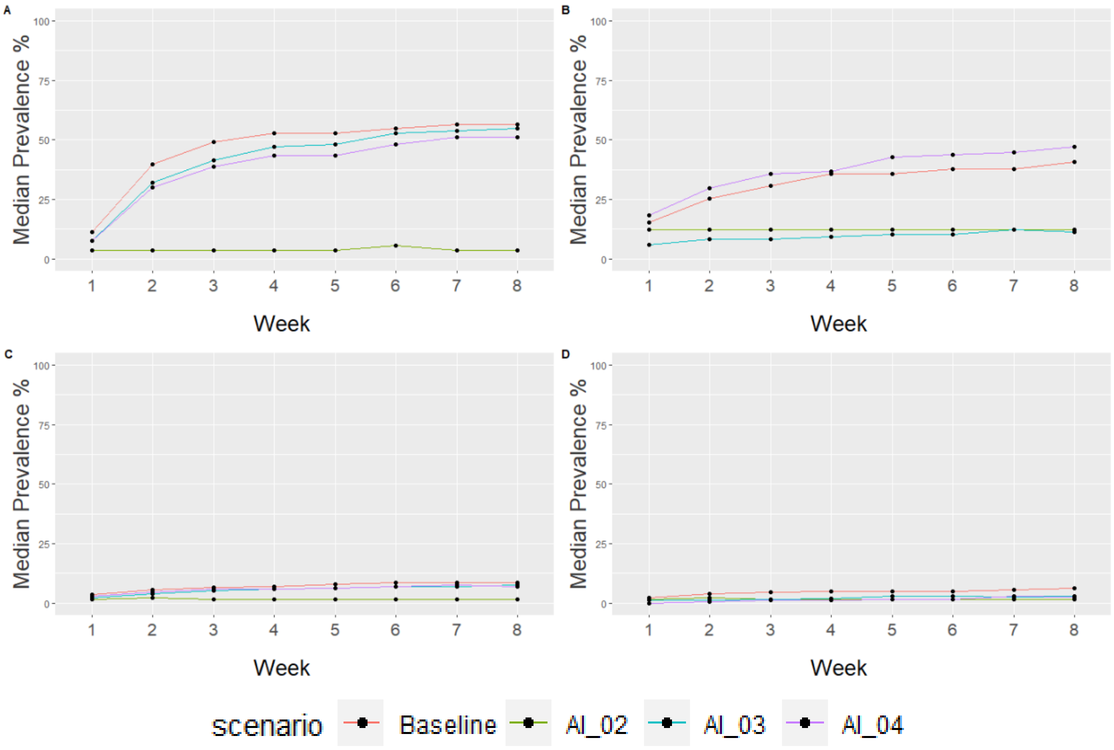
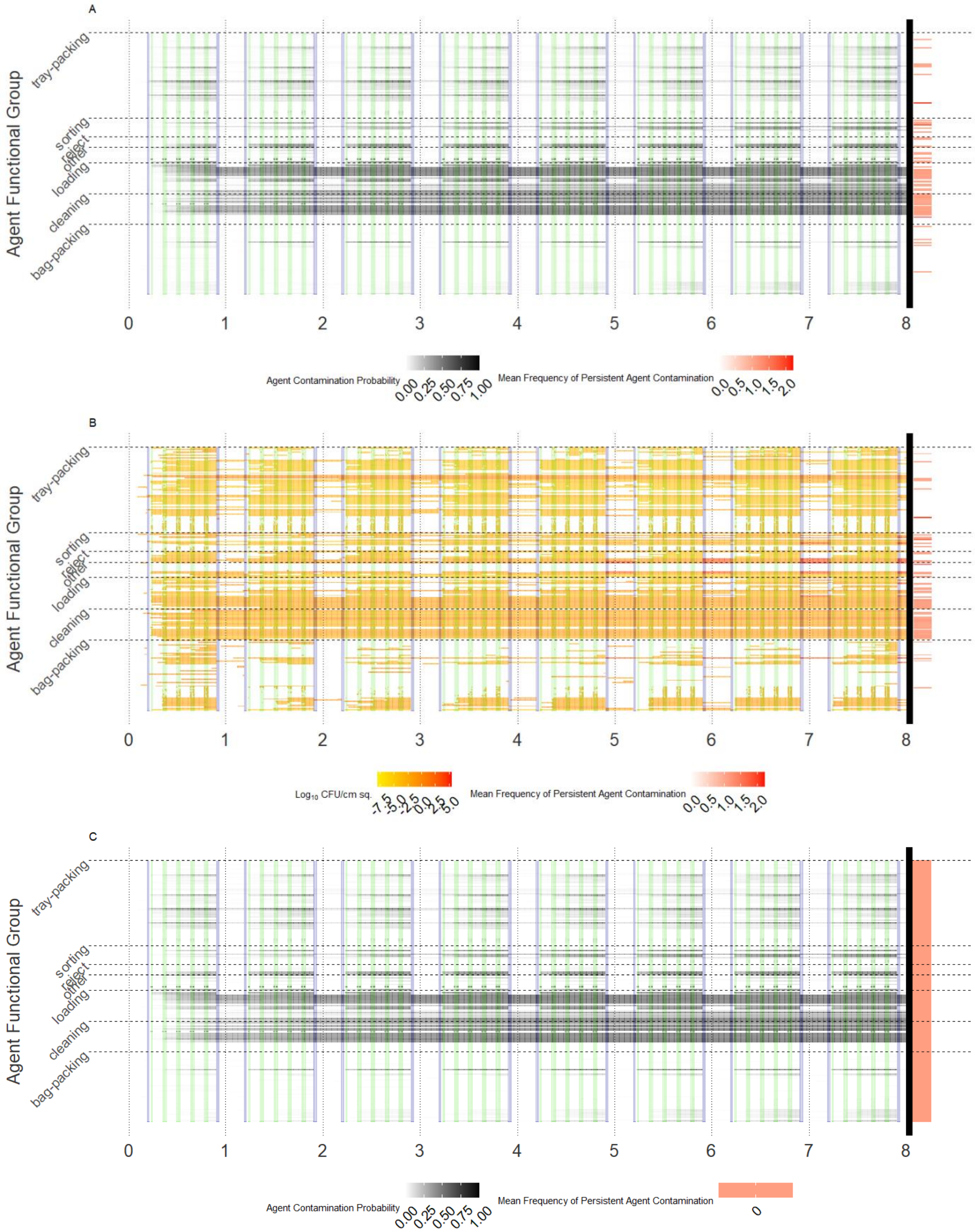


Fig S3.2 Median Prevalence of wet and dry agent areas of baseline scenarios compared against sanitation and agent connection-based corrective actions over eight weeks for Facilities A and B (AI_02, AI_03 and AI_04)

A: Facility A *Listeria* contamination prevalence of all agents in wet areas. B: Facility B *Listeria* contamination prevalence of all agents in wet areas. C: Facility A *Listeria* contamination prevalence of all agents in dry areas. D: Facility B *Listeria* contamination prevalence of all agents in dry areas.

(AI_02: Broad Model-based Master Sanitation Schedule Restructuring (Agent sanitation schedules were fully reassigned according to mean contamination probability; Facility B was given a daily schedule for and sanitation; AI_03: Directed Model-based Master Sanitation Schedule Restructuring (Sanitation of agents with a mean contamination probability ≥66% were set to daily cleaning; Facility B was given a daily schedule for sanitation))


A
Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0.0
0.5
1.0
1.5
2.0
B
Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-7.5
-5.0
-2.5
0.0
2.5
5.0
Mean Frequency of Persistent Agent Contamination
0.0
0.5
1.0
1.5
2.0
C
Agent Functional Group
tray-packing
sorting
reject
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0
1
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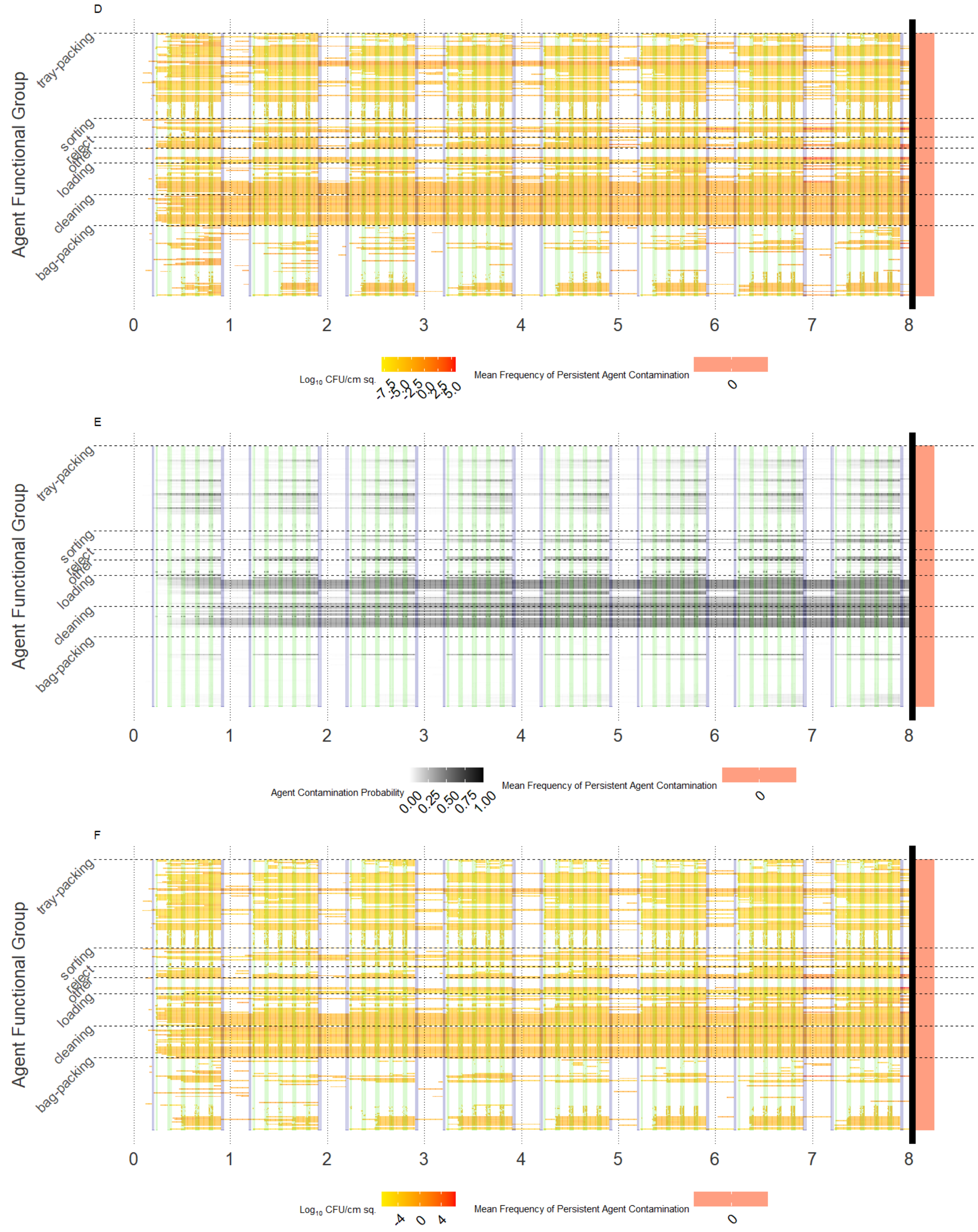
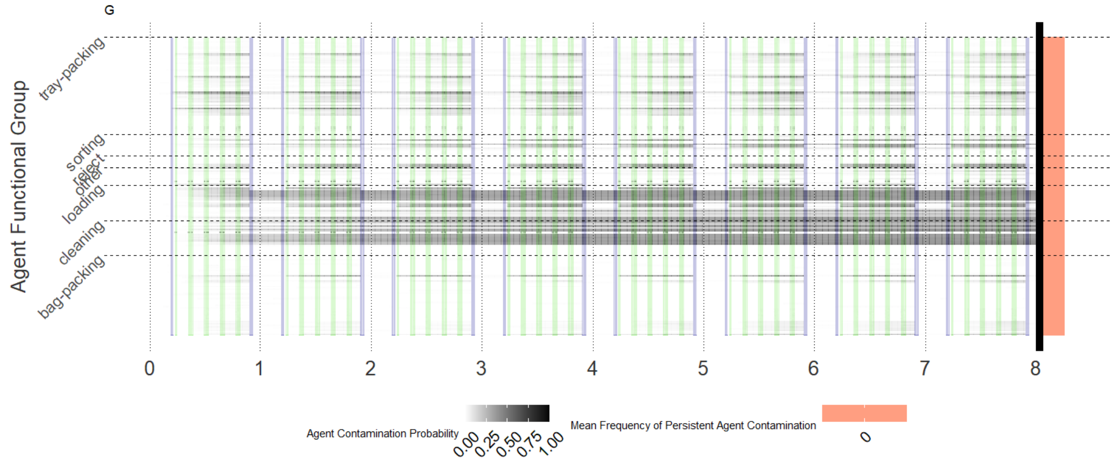
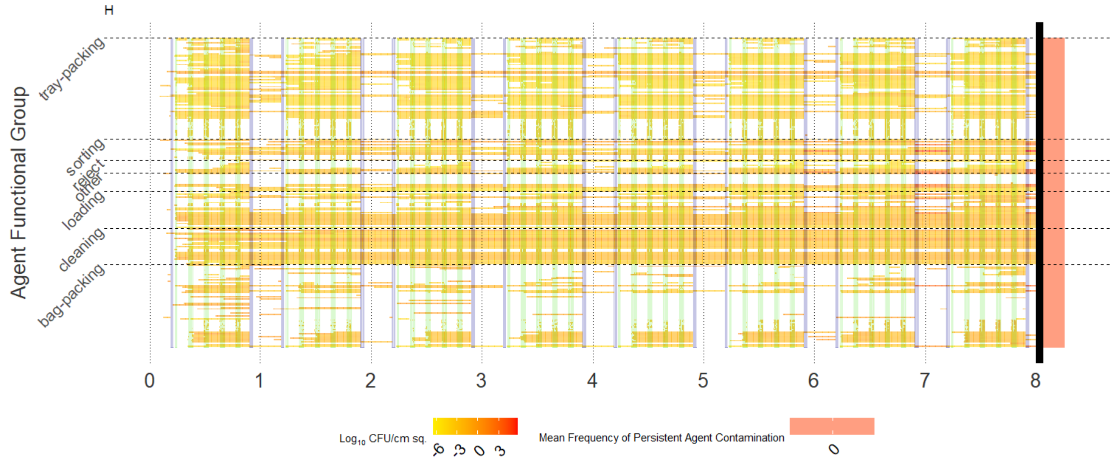
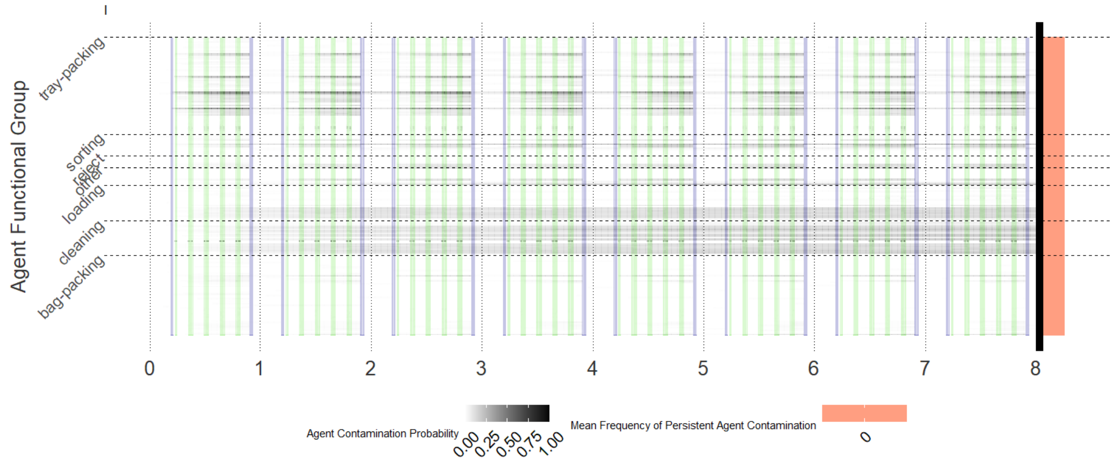
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0


D
Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-7.5
-5.0
-2.5
0.0
2.5
5.0
Mean Frequency of Persistent Agent Contamination
0
E
Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
F
Agent Functional Group
tray-packing
sorting
reject
offer
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-4
0
4
Mean Frequency of Persistent Agent Contamination
0


G
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0


H
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-6
-3
0
3
Mean Frequency of Persistent Agent Contamination
0


I
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
bag-packing
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0

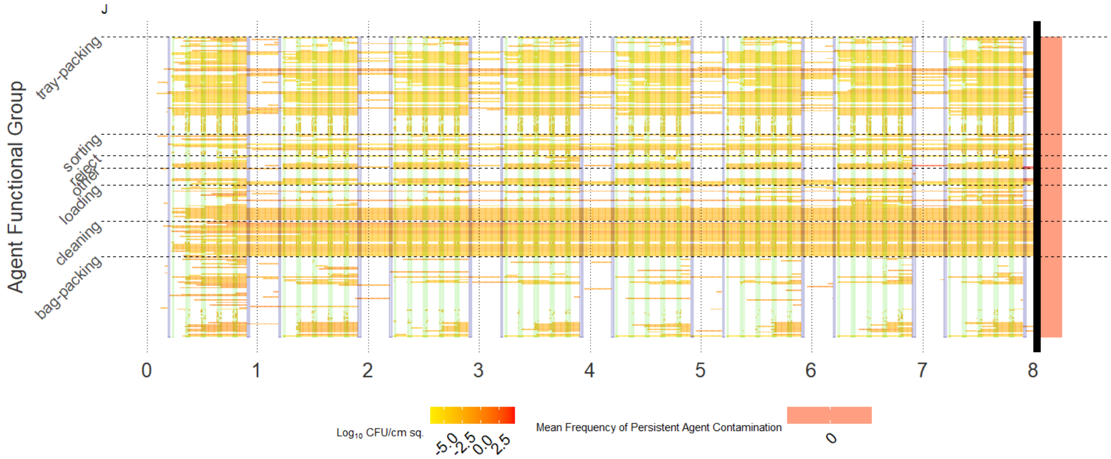
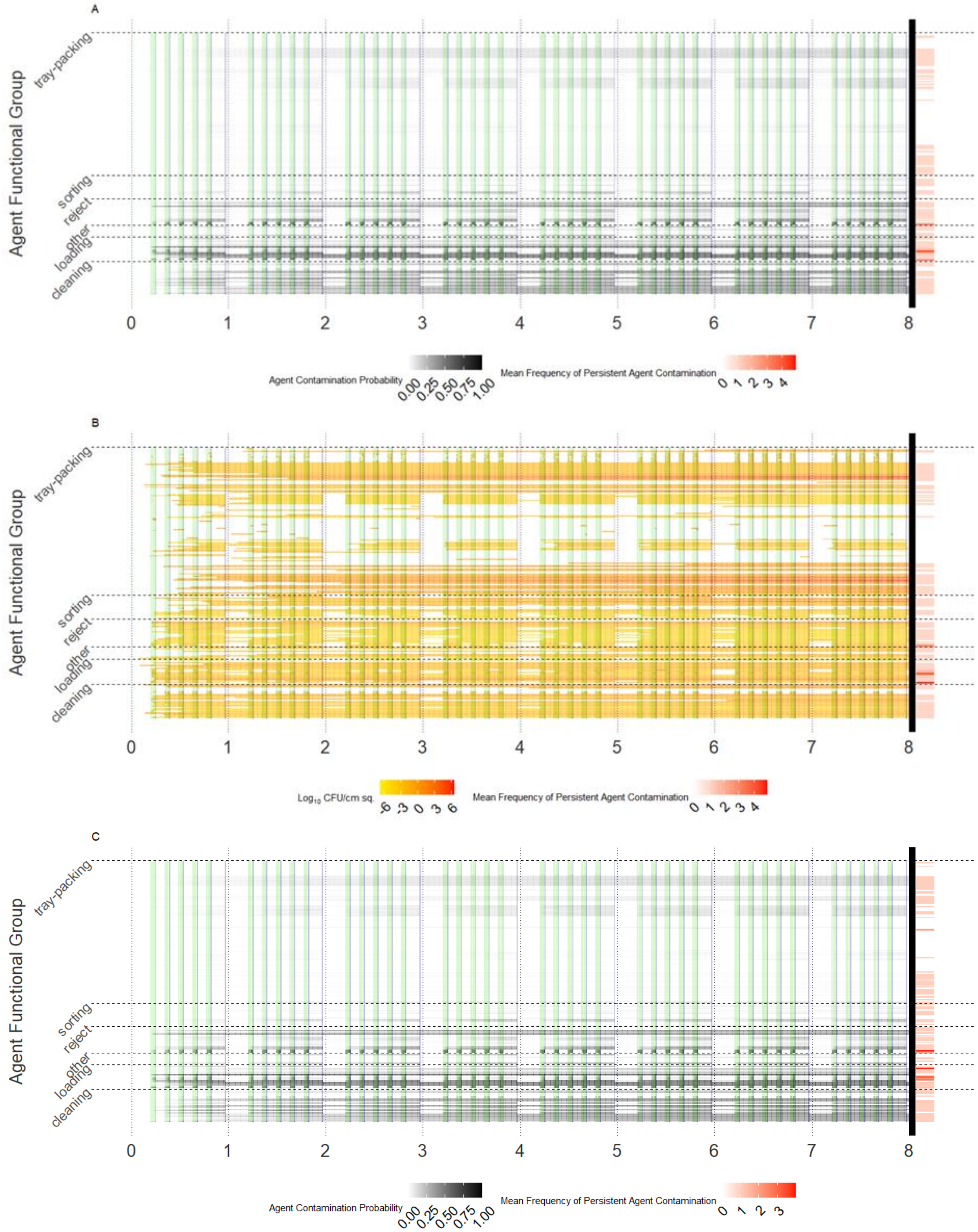
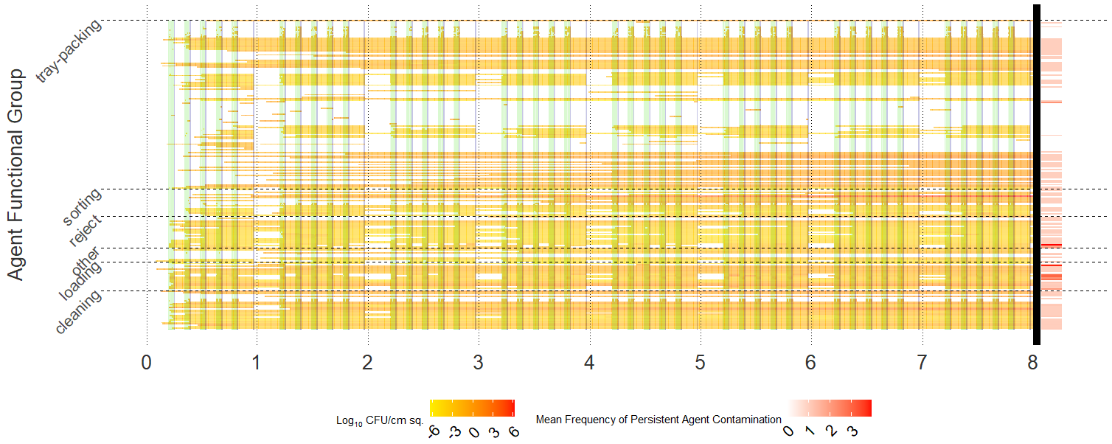


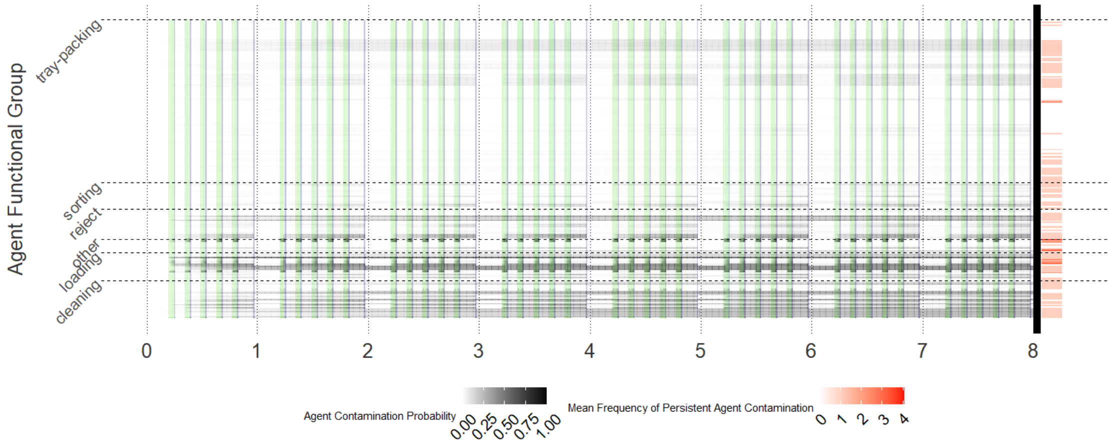
Fig S3.3 Heatmaps describing the hourly contamination probability of all agents, median $\log_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility A in baseline and *Listeria* prevalence on incoming raw produce reduction corrective action scenario (EC_01-EC_04)
(Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility A baseline. B: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A baseline. C: *Listeria* Contamination probability in Facility A EC_01. D: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A EC_01. E: *Listeria* Contamination probability in Facility A EC_02. F: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A EC_02. G: *Listeria* Contamination probability in Facility A EC_03.H: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A EC_03. I: *Listeria* Contamination probability in Facility A EC_03. J: Median $\log_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A EC_04.
(EC_01-EC_04: *Listeria* Prevalence on Incoming Raw Produce reduced to 75%, 50%, 25% and 0% respectively)


A
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4
B
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log10 CFU/cm sq.
-6
-3
0
3
6
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4
C
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3

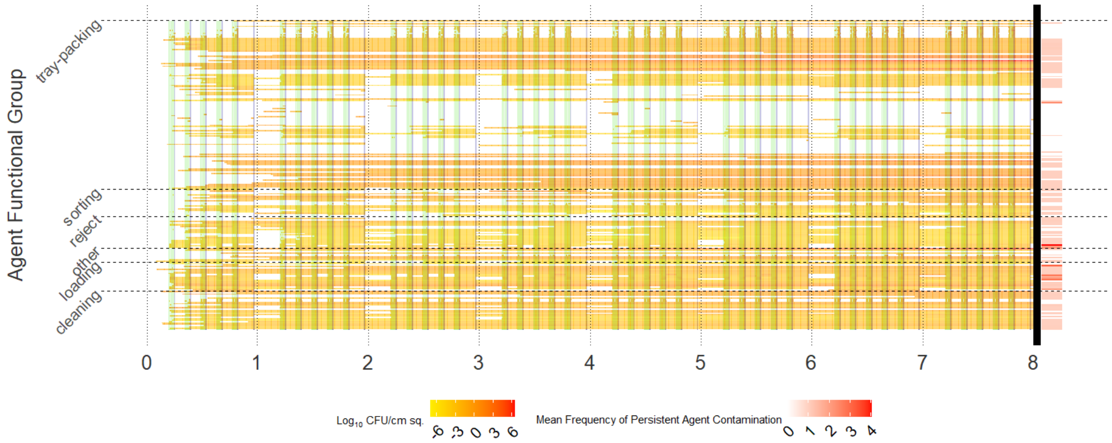
D


Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log₁₀ CFU/cm sq.
-6
-3
0
3
6
Mean Frequency of Persistent Agent Contamination
0
1
2
3

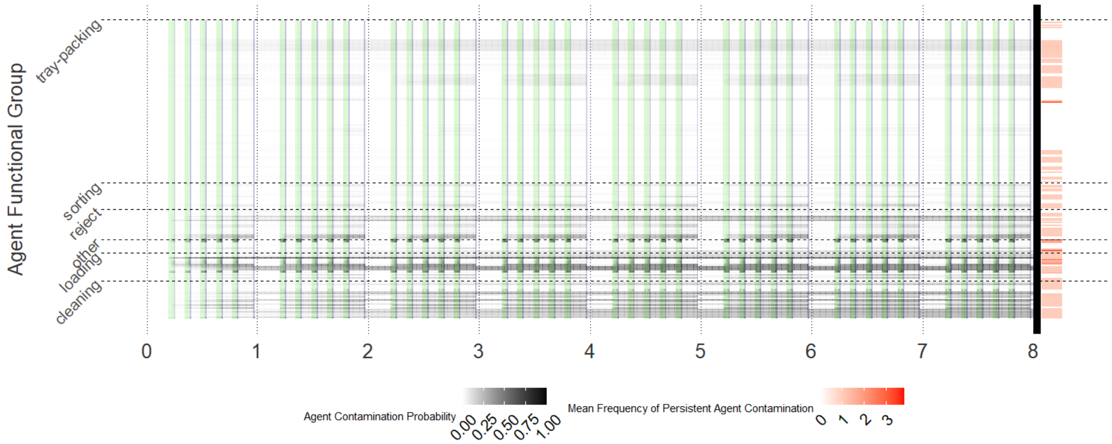
E


Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4

F

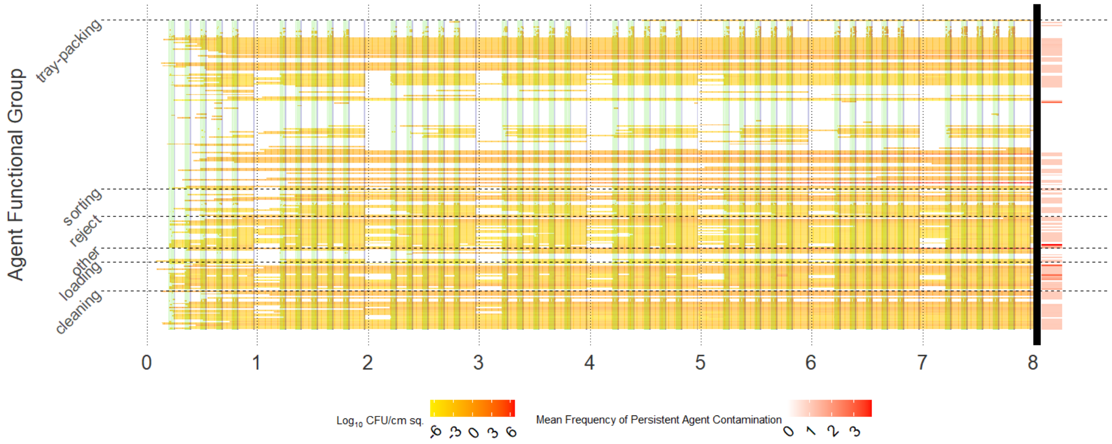

Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log₁₀ CFU/cm sq.
-6
-3
0
3
6
Mean Frequency of Persistent Agent Contamination
0
1
2
3
4

G



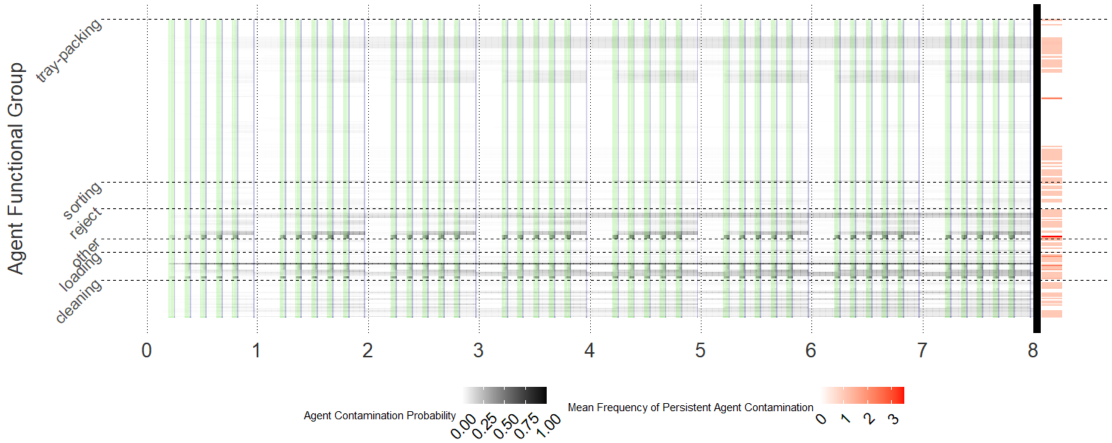
Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3

H



Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Log_{10} CFU/cm sq.
-6
-3
0
3
6
Mean Frequency of Persistent Agent Contamination
0
1
2
3

I



Agent Functional Group
tray-packing
sorting
reject
other
loading
cleaning
0
1
2
3
4
5
6
7
8
Agent Contamination Probability
0.00
0.25
0.50
0.75
1.00
Mean Frequency of Persistent Agent Contamination
0
1
2
3

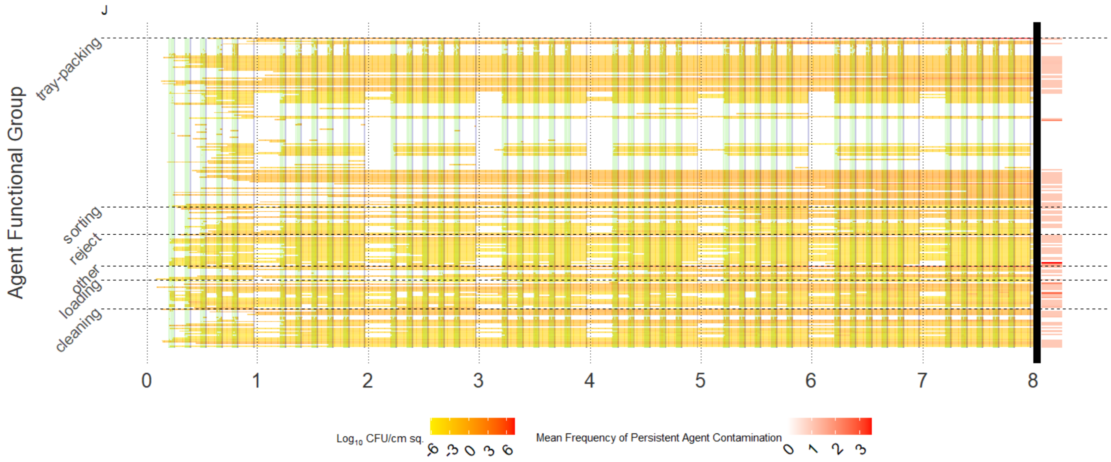


Fig 3.4 Heatmaps describing the hourly contamination probability of all agents, median log$_{10}$ *Listeria* concentration of positive agents over the simulation and mean frequency of persistent contamination for Facility B in baseline and *Listeria* prevalence on incoming raw produce reduction corrective action scenario (EC_01-EC_04)

(Green: work shift; Blue: sanitation; White: means no contamination). A: *Listeria* Contamination probability in Facility B baseline. B: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility A baseline. C: *Listeria* Contamination probability in Facility B EC_01. D: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B EC_01. E: *Listeria* Contamination probability in Facility B EC_02. F: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B EC_02. G: *Listeria* Contamination probability in Facility B EC_03.H: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B EC_03. I: *Listeria* Contamination probability in Facility B EC_03. J: Median log$_{10}$ *Listeria* concentrations (CFU/cm^2) of positive agents in Facility B EC_04.

(EC_01-EC_04: *Listeria* Prevalence on Incoming Raw Produce reduced to 75%, 50%, 25% and 0% respectively)