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***Listeria monocytogenes* prevalence varies more within fields than between fields or over time on conventionally farmed New York produce fields**

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Abstract

Past studies have shown that the on-farm distribution of *Listeria monocytogenes* is affected by environmental factors (e.g., weather). However, the majority of studies were conducted at large scales (e.g., across farms), while few studies examined drivers of *L. monocytogenes* prevalence at smaller scales (e.g., within a single field). This study was performed to address this knowledge gap by (i) tracking *L. monocytogenes* distribution in two fields on one farm over a growing season, and (ii) identifying factors associated with *L. monocytogenes* isolation from drag swab, soil, and agricultural water samples. Overall, *L. monocytogenes* was detected in 78% (21/27), 19% (7/36), and 8% (37/486) of water, drag swab, and soil samples, respectively. All isolates were characterized by pulsed-field gel electrophoresis (PFGE). Of the 43 PFGE types identified, 14 were isolated on multiple sampling visits and/or from multiple sample types, indicating persistence in or repeated introduction into the farm environment during the study. Our findings also suggest that *L. monocytogenes* prevalence, even at the small spatial scale studied here, (i) was not uniform, and (ii) varied more within fields than between fields or over time. This is illustrated by the fact that plot (in-field variation), field (between field variation), and sampling visit (time) accounted for 18%, 2% and 3% of variance in odds of isolating *L. monocytogenes*, respectively. Moreover, according to random forest analysis, water-related factors were among the top-ranked factors associated with *L. monocytogenes* isolation from all sample types. For example, the likelihood of isolating *L. monocytogenes* from drag and soil samples increased monotonically as rainfall increased. Overall, findings from this single-farm study suggests that mitigation strategies for *L. monocytogenes* in produce fields should focus on water-associated risk factors (e.g., rain, distance to water), and be tailored to specific high-risk in-field areas.

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Keywords

Listeria; PFGE; Precipitation; Produce; Prevalence; Spatiotemporal

Listeria monocytogenes is a saprophytic soil microbe capable of transitioning to an intracellular lifestyle following ingestion by humans in whom it is the causative agent of listeriosis (9, 11). Produce can be contaminated by *L. monocytogenes* at varying points throughout the supply chain, including preharvest, postharvest handling and processing (31). The Food Safety Modernization Act (FSMA) established the first federal produce safety standards for the growing, harvesting and packing of produce in the United States thereby increasing the need for scientific data to support development of science-based, on-farm produce safety practices (38). Specifically, understanding environmental factors that drive spatiotemporal variability in *L. monocytogenes* prevalence within a single farm is essential to develop intervention and control strategies that can be individualized to specific fields and sub-field areas.

Previous studies have examined the prevalence of *L. monocytogenes* in pre-harvest environments (8, 34, 45) and from surface water sources, including sources used for produce production (7, 19). For example, a study conducted on farms in Upstate New York isolated *L. monocytogenes* from 12% (128/1,056) of drag swab samples (representing soil surface content) collected from produce fields (45). Other studies, which collected from agricultural watersheds, isolated *L. monocytogenes* in 43% (604/1,405) of Moore swab samples (7), and in 10% (32/314) of surface water samples (19). Previous surveys in farm and natural environments found that the prevalence of *L. monocytogenes* was not uniform and was affected by multiple environmental factors (e.g., weather, adjacent environmental land use) (6, 17, 23). For instance, a cross-sectional study found an increased likelihood of isolating *L. monocytogenes* from fields near pastures and impervious surfaces compared to fields farther from pastures and impervious surfaces (33). However, the majority of these earlier studies focused on drivers of *L. monocytogenes* at the landscape-level (e.g., between farms, within large watersheds (19, 34)), and relatively few studies focused on factors associated with the likelihood of *L. monocytogenes* isolation within a single farm or field. As such, there is limited data on if *Listeria* is more likely to vary spatially or temporally at the scales that are most important for designing effective hazard mitigation strategies (i.e., field-level). This study was performed to address this knowledge gap and improve our understanding of *Listeria* ecology at small spatial scales. Specifically, this study (i) tracked changes in *L. monocytogenes* prevalence in two nearby fields on a single farm over one growing season, and (ii) identified environmental factors associated with *L. monocytogenes* isolation from multiple sample types (i.e., drag swab, soil, and agricultural water).

MATERIALS AND METHODS

Study Design and Sampling.

Spinach (Uni-Pack 151-F1) was planted in two approx. 0.2 ha fields at an experimental farm in Upstate New York in May 2013. The fields were approx. 400 m apart from each other. Each field was divided into 5 m × 5 m plots. Nine sampling events occurred between

planting (April) and harvest (June). During the collection of produce, soil, drag swab, and water samples gloves and boot covers were worn; gloves were changed between collection of each sample. At each sampling visit, a total of 54 soil samples were collected (i.e., one composite soil sample per plot), as well as one drag swab sample per field; one composite produce sample was collected from each plot beginning on the seventh sampling visit (i.e., 40 days post seeding). For each field, a drag swab pre-moistened with 30 ml of buffered *Listeria* enrichment broth (BLEB; Becton, Dickinson, Franklin Lakes, NJ), was dragged around the perimeter and from side-to-side in 1 m increments for approx. 10 min, covering an area of 0.2 ha per field and a linear distance of approx. 2,300 m. Composite soil and produce samples were collected using systematic random sampling. Briefly, each plot was divided into 1 m × 1 m subplots. For soil samples, five subplots were randomly selected, and 5 g of soil were collected from each subplot centroid (approx. 6 inches below the soil surface). All five 5 g samples were placed in the same sterile, Whirl-Pak bag. For produce samples, leaves were collected from 10 randomly selected plants per plot and placed in the same sterile, Whirl-Pak bag. Produce samples were collected 40 d after seeding (i.e., visits 7, 8 and 9) since plants were too small prior to this date to survive removal of one leaf. At each sampling visit, one water sample was collected from each of the following sites: (i) a roadside ditch that intersected Creek A (the source of irrigation water in the study) upstream of the irrigation intake, (ii) Creek A upstream of the ditch intersection, and (iii) the site along Creek A where the irrigation intake was located. Water samples were collected using an extendable sampling pole (Nasco, Fort Atkinson, WI) directly into sterile 250-mL jars. Overall, 711 samples were collected during this study (Table 1, Figure 1). All samples were stored on ice for transportation, followed by storage at 4°C and processed within 24 h.

Sample Processing, and *L. monocytogenes* Detection, Isolation and Subtyping.

For sample processing, drag swabs from each field were stomached for 2 min in Whirl-pak bags. Water samples were passed through a 0.45-µm filter unit (Nalgene, Rochester, NY). Each filter was then aseptically removed and transferred to a separate sterile Whirl-Pak bag. Filters and drag swabs were added to 90 ml of BLEB, while 225 mL of BLEB were added to Whirl-pak bags containing soil or produce samples. All enrichments were incubated at 30±2°C. *Listeria* selective enrichment supplement (Oxoid, Cambridge, United Kingdom) was added to all enrichments after 4 h. Following incubation for 24 and 48 h, 50 µl of enrichment was streaked onto modified Oxford agar (MOX; Becton, Dickinson, Franklin Lakes, NJ) and *L. monocytogenes* plating medium (LMPM; Biosynth International, Itasca, IL). MOX and LMPM plates were incubated for 48 h at 30 and 35±2°C, respectively. Presumptive *L. monocytogenes* colonies isolated from MOX plates were sub streaked onto LMPM plates, followed by incubation for 48 h at 35±2°C. Up to four *L. monocytogenes* colonies per sample were confirmed by PCR amplification of the partial *sigB* gene, followed by Sanger sequencing (Cornell University, Life Science Core Laboratories Center). In order to assign *sigB* allelic types (AT), sequences were referenced against an internal database (Food Safety Laboratory, Cornell University) as previously described (1, 22). Confirmed *L. monocytogenes* isolates were further subtyped by pulsed-field gel electrophoresis (PFGE), following the standard CDC PulseNet protocol using restriction enzymes *AscI* and *ApaI* (13). Gel images were normalized through a reference standard, *Salmonella* serovar Braenderup digested with *XbaI*. PFGE types were assigned to each isolate by comparison to

an internal PFGE reference database (Cornell Food Safety Lab; Ithaca, NY) using Bionumerics software (Applied Maths, Saint-Matins-Latem, Belgium), unweighted pair group-matching algorithm (UPGMA) and the Dice correlation coefficient with a maximum space tolerance of 1.5%. Isolate information is available through the Food Microbe Tracker database (<http://www.foodmicrobetracker.com>).

Spatiotemporal Data Acquisition and Visualization.

Meteorological and spatial data were obtained from publicly available databases (45, 47). Specifically, the proximity of each sampling site to impervious cover was calculated using the U. S. Geological Survey National Transportation Dataset (40) and the proximity of each sampling site to flowing water was calculated using the National Hydrography Dataset (39). Weather data was obtained from the Network for Environment and Weather Applications station for each sampling day, as well as the ten days preceding each sampling day (21). The frequency of *L. monocytogenes* positive soil samples was mapped using the *sf* and *ggplot* packages in R (28, 49).

Statistical Analysis.

A generalized linear mixed model followed by Tukey's HSD was used to determine if the likelihood of isolating *L. monocytogenes* differed significantly between sample types (3, 29). The Simpson's index of diversity for PFGE type for Field A, Field B, and the creek used for irrigation was calculated using the *vegan* package version 2.5–6 in R (24) with 95% confidence intervals (95% CI). In order to determine how the *L. monocytogenes* population changed over the course of the study, two separate mixed models were developed using the package *lme4* v.1.1–13 in R (3). The first model examined the number of novel PFGE types (a PFGE type that was not previously isolated from samples from a given field) isolated at each sampling visit. As such, the outcome of this model was number of novel types isolated from each field at each sampling visit. The second model examined how PFGE richness changed over time, and the outcome was total number of PFGE types isolated per field. Sampling visit was included as fixed effect while field was included as random effect in both models.

Random forest analysis (RFA) was performed to identify and rank factors associated with *L. monocytogenes* isolation from soil, drag swab and water samples using the *caret* (16) and *party* packages and controls suggested by the *party* package authors (37). The explanatory factors included in RFA included average air temperature, total rainfall and average daily solar radiation on the day of sample collection as well as 1, 2, and 3 days before sample collection, and averaged over the 0–5 and 0–10 days before sample collection. Two spatial factors (proximity of the sampled plot to impervious cover and to flowing water) and one study design variable (Field ID) were also included in the RFA where *L. monocytogenes* isolation from soil and drag samples was the outcome. Water source (i.e., roadside ditch, creek upstream of the ditch intersection, or irrigation pump) was included in the RFA where *L. monocytogenes* isolation from water samples was the outcome. For each RFA repeated 10-fold cross-validation was performed, and the classification accuracy of the RFA was calculated (35, 36). Since the number of soil and drag swab samples positive for *L. monocytogenes* were unbalanced, upsampling was performed (16). RFA results are

interpreted by quantifying conditional variable importance as previously described (VI; [35]), and visualized as bar graphs. To facilitate interpretation of the RFA, partial dependence plots (PDPs; [14]) were developed to graphically characterize the relationships between the 4 top-ranked factors in each RFA and likelihood of isolating *L. monocytogenes*. The y-axis of the PDP shows the marginal effect of a given factor on the RFA outcome after accounting for the effects of all other factors.

Random effects models were developed to compare the relative impact of spatial and temporal factors on the odds of isolating *L. monocytogenes*. Separate models were developed where the dependent variable was likelihood of *L. monocytogenes* isolation from (i) all samples, (ii) all terrestrial samples (soil, drag swab, produce), (iii) soil samples, and (iv) water samples. Models were used to quantify the percent of variance in odds of isolating *L. monocytogenes* that could be assigned to temporal factors and spatial factors. Temporal factors considered included sampling visit (as a factor with nine levels) and time since start of study (as a continuous variable based on number of days since the first sampling). Spatial factors considered were dependent on the model outcome; latitude and longitude were considered for all outcomes. Field and plot nested in field were considered when the dependent variable was likelihood of *L. monocytogenes* isolation from terrestrial (i.e., soil, drag swab and produce samples), or from soil samples. These analyses were not conducted on drag swab or produce data only due to the low prevalence of *L. monocytogenes* in drag swab and produce samples. Water sampling site was considered when the dependent variable was likelihood of *L. monocytogenes* isolation from water samples. Separate models were developed to examine the individual impact of spatial (latitude and longitude) and temporal (sampling visit and time since start of study) factors on variance. By comparing the percent variance accounted for in these four models, the relative contribution of space and time to the observed variation in *L. monocytogenes* isolation could be determined. More complex nested models that accounted for study design (e.g., field, plot, sample type), were also developed to (i) examine variance between and within fields, (ii) variance associated with ecological niche (i.e., sample type), and (iii) the relative gain associated with inclusion of additional parameters in the random effects model. The nested models developed included the following as random effect: (i) field, (ii) field, and plot nested in field, (iii) field, plot nested in field, and sample type, and (iv) field, plot nested in field, sample type, and sampling visit. Models were then compared using Akaike's Information Criteria for small sample sizes (AIC) to determine if a given factor combination best fit the data.

RESULTS AND DISCUSSION

The objective of this study was to track the *L. monocytogenes* distribution within two spinach fields over one growing season to identify factors that were associated with *L. monocytogenes* isolation from different sample types (i.e., drag swab, soil, produce and agricultural water). By intensively sampling a small area, this study allowed for identification of factors that affect *L. monocytogenes* at field-level, as opposed to landscape-level. This is particularly important since pre-harvest risk management decisions are more likely to be made at these smaller spatial scales (i.e., for individual fields as opposed to across multiple farms). Moreover, by conducting this research, we were able to determine if conclusions drawn from studies conducted at large scales, are reproducible at these smaller

scales and can be used to inform field-level or within field management decisions. While this study only sampled two fields, over one growing season in one region, and thus caution should be used in generalizing the findings reported here. However, the fact that certain factors (e.g., distance to water, rainfall) were consistently identified in previous studies conducted at larger scales (33, 45–47) and the study reported here provides confidence in the overall findings.

***L. monocytogenes* prevalence in environmental and produce samples.**

Overall, 10% (68/711) of samples were positive for *L. monocytogenes*. Specifically, 78% (21/27), 19% (7/36), 8% (37/486), and 2% (3/162) of water, drag swab, soil, and produce samples were *L. monocytogenes* positive, respectively (Table 1). The likelihood of isolating *L. monocytogenes* was significantly higher for water samples compared to all other sample types ($P < 0.001$) (Table 1; Fig. 1). When sample type was the only covariate in random effects models, the percent of variance in the odds of isolating *L. monocytogenes* accounted for by the model decreased from 52% when water samples were included in the dataset to 19% when water samples were excluded. This suggests that almost 33% of variance in the odds of isolating *L. monocytogenes* across all sample types collected in this study can be attributed to differences between water and terrestrial samples (Table 2). Additionally, the likelihood of isolating *L. monocytogenes* was significantly higher for drag swabs compared to soil and produce ($P = 0.021$) (Fig. 1). While these findings are consistent with previous studies conducted in the Northeast (33, 34, 46, 47), there are limited studies conducted in other regions that compared *L. monocytogenes* prevalence in drag, soil and produce samples. For example, a study that surveyed *L. monocytogenes* prevalence on five Northeastern produce farms between 2009 and 2011 found a significantly higher prevalence of *L. monocytogenes* in water samples (48%) compared to drag swabs (15%) and soil samples (16%) (33). Both the current and past studies (e.g., (25, 47)) reported a low prevalence of *Listeria* in produce samples (e.g., 0.6% [2/334], (47)) relative to the high prevalence of *Listeria* in the surrounding farm environment (e.g., soil, irrigation water). This may suggest that the transfer of *Listeria* to pre-harvest produce from environmental sources is a rare, stochastic process, or that *Listeria* rapidly dies off on produce following contamination. However, it is important to note that events and conditions immediately before harvest may affect both processes (e.g., irrigation, rain). Since past studies have shown that irrigation facilitates the transfer of foodborne pathogens (e.g., *E. coli*, *Salmonella*) from environmental sources to produce (2, 43), irrigation immediately before harvest may increase *Listeria* prevalence in produce samples. Additionally, while previous studies (46, 47) reported that irrigation can affect *Listeria* prevalence in soil, limited irrigation (e.g., immediately following seeding) occurred in the present study due to the prevalence of rain events. As such, irrigation was not included as a risk factor in the models reported here. While multiple studies have examined the transfer to and survival of *Salmonella* and pathogenic *E. coli* from environmental sources to preharvest produce (2, 5, 15, 26, 27, 43), specific research on how and when *Listeria* transfers and survives on pre-harvest produce from environmental sources is limited (12, 25, 47). Thus, additional research (including field studies that perform enumeration) are needed to further determine (i) why low *L. monocytogenes* prevalence is observed in produce samples, even in environments where the overall *L. monocytogenes* prevalence is high, and (ii) whether

certain factors (e.g., irrigation close to harvest) would facilitate transfer of *L. monocytogenes* from soil to produce and increase *L. monocytogenes* prevalence on produce.

Likelihood of *L. monocytogenes* isolation varies spatially within the farm environment and the composition of the *L. monocytogenes* population varies over time.

L. monocytogenes appeared to be more frequently isolated from plots along the northeastern edge of Field A compared to the interior or southeastern edge suggesting the distribution of *L. monocytogenes* was not uniform within the sampled fields (Fig 1). This is consistent with the random effect model built using all four sample types, which showed that sample type (~52% of variance) and spatial factors (~ 28–29% of variance) could explain more of the variance in odds of isolating *L. monocytogenes* than temporal factors (~1–2% of variance) (Table 2). Similarly, spatial factors explained up to 11% of variance in odds of *L. monocytogenes* isolation while temporal factors explained <1%, when the random effects model was built using just the soil data (Table 2). Overall, the random effects models suggest that the odds of isolating *L. monocytogenes* varied more across space than over time, and that *L. monocytogenes* prevalence was not uniform even at the small spatial scales studied here. This is illustrated by the fact that plot accounted for 18% of the variance in odds of *L. monocytogenes* isolation when the random effects model was built using all terrestrial data; terrestrial samples refers to all soil, drag swab, and produce samples. Since field only accounted for approx. 2% of the variance in odds of *L. monocytogenes* isolation, this suggests that the likelihood of *L. monocytogenes* isolation varied more within a field than between fields. As such, growers may need to tailor risk management strategies to address hotspots within fields (e.g., planting high-risk crops far away from streams, using buffers around fields). It is important to note, that even though spatial factors account for a greater percent of the variance in the likelihood of isolating *L. monocytogenes* than temporal factors, this only reflects temporal factors within a single nine-week growing season (i.e., from planting to harvest for a single crop), which is a timeframe highly relevant for managing *Listeria* risks. Indeed, past studies have reported seasonality in *Listeria* prevalence in agricultural and non-agricultural environments (30, 32, 33). Overall, our study is unique not only in that it has identified risk factors for *L. monocytogenes* prevalence at small scales (i.e., within fields), but also in its longitudinal nature since many past studies in produce farms were cross-sectional or conducted sampling over short periods [e.g., (4, 45, 46)].

In total, 86 *L. monocytogenes* isolates were obtained from the 68 *L. monocytogenes*-positive samples. Allelic typing and PFGE were performed on all *L. monocytogenes* isolates obtained in this study, if more than one isolate was obtained from a sample all isolates were subtyped. The 86 isolates represented eight ATs; 32 and 54 isolates were classified into lineage I and II, respectively. Over the course of the study, 20 PFGE types were identified in Field A, 15 PFGE types in Field B and 22 PFGE types in Water. The Simpson's Index of Diversity (D) was similar for Field A (D=0.93; confidence interval (CI)=0.91, 0.96), Field B (D=0.93; CI=0.90, 0.95) and Water (D=0.94; CI=0.91, 0.96). Of the 41 distinct PFGE types identified across all three sampling sides, 14 PFGE types were isolated on two or more sampling visits. For example, six PFGE types were isolated from soil, drag and water samples over multiple visits, including samples collected from both fields (Fig 2). Based on a linear mixed model, the PFGE type richness ($P<0.001$) and the number of novel PFGE

types ($P < 0.001$) was significantly different between the nine sampling events. This suggests temporal patterns in isolation of individual AT and PFGE types, despite the fact that temporal factors accounted for $< 4\%$ of variance in odds of isolating *L. monocytogenes*. The repeated isolation of several PFGE types during the course of the study, from different on-farm locations, and different sample types suggests persistence, repeated introduction and/or movement of *L. monocytogenes* into the farm environment from farm-adjacent environments or other areas on-farm. Determining the directionality of this movement (e.g., from Creek A to the field, from subsurface soil [soil samples] to soil surface [drag swabs]) is outside the scope of the current project and cannot be inferred from the data collected.

Our findings suggest that the prevalence of *L. monocytogenes* within the farm environment is not uniform over space and that the composition of the *L. monocytogenes* population varies over time. This study is consistent with the findings of past studies that examined the distribution of *L. monocytogenes* and other foodborne pathogens in preharvest environments (e.g., [33, 34, 47]). For example, a study that examined the spatial distribution of *L. monocytogenes* on four New York farms in 2014 found that the likelihood of isolating *L. monocytogenes* was significantly greater near ecotones (an area of transition between the field and adjacent non-agricultural areas) compared to areas far from ecotones (45). Due to the findings that *L. monocytogenes* was not uniformly distributed within preharvest environments, these past studies hypothesized that the risk of *L. monocytogenes* contamination of preharvest produce varied over space and time and identification of spatiotemporal factors (e.g., specific weather conditions, soil types) associated with an increased likelihood of pathogen isolation from preharvest environments could facilitate the development of science-based risk management strategies (e.g., planting high-risk crops far away from streams, using buffers between streams and fields) (33, 34, 47); overall, our findings support these previous studies' conclusions.

***L. monocytogenes* isolation associated with water-related factors.**

According to RFA, the top-ranked factors associated with the likelihood of isolating *L. monocytogenes* from (i) drag swabs were total rainfall 0–5 d before sample collection and avg. air temperature 2–3 d before sample collection, (ii) soil were distance to impervious cover and to water, and (iii) water were rainfall 0–1 d before sample collection and avg. air temperature 0–2 d before sample collection (Figs. 3–4). Hence, what could be described as “water-related” factors were among the top-ranked factors for all three sample types. According to the PDPs, the likelihood of *L. monocytogenes* isolation from soil samples increased as proximity to flowing water and to impervious cover increased (Fig. 3). Additionally, the likelihood of *L. monocytogenes* isolation from drag swabs and water samples increased as rainfall increased (Fig. 3). The finding of an increased likelihood of *L. monocytogenes* isolation after rainfall is consistent with past studies (20, 47), including a study that repeatedly sampled surface water sources used for produce irrigation and found that the likelihood of *L. monocytogenes* isolation was strongly correlated with total rainfall over the five days prior to sampling (10). Similarly, a study conducted in New York State showed that *L. monocytogenes* detection in soil samples was highest within 24 h of rainfall or irrigation (47). There are several potential mechanisms that might explain the association between rainfall and likelihood of *L. monocytogenes* isolation. For example, splash and run-

off during rain events may facilitate bacterial movement within the farm environment or from farm-adjacent environments (2, 41, 43, 44). A previous study that examined the transfer of rifampicin-resistant *E. coli* in simulated wildlife feces to preharvest produce via splash found that *E. coli* could transfer up to 3.4 m from feces (15). Rain may also alter the farm environment (e.g., by increasing soil moisture), thereby facilitating the growth of endemic *L. monocytogenes* (42, 47, 48). Indeed, past studies have shown that *L. monocytogenes* can grow, survive, and persist better in moist environments than in soils with less moisture content (6, 42, 48); this may also explain the negative association between the likelihood of *L. monocytogenes* isolation and distance to water, since areas near streams (e.g., floodplains) are likely to have higher soil moisture levels. While we did not collect data on soil moisture levels and cannot test this hypothesis, according to the USGS soil survey (<https://websoilsurvey.sc.egov.usda.gov/>) soil type and average moisture content on the sampled farm does change as one moves away from Creek A in a northeasterly direction towards Field B, which had significantly lower *Listeria* prevalence than Field A. Overall, our findings are consistent with those of past studies that also found associations between water-related factors and *L. monocytogenes* isolations from produce farm environments, suggesting that these associations are reproducible across studies conducted in different years, on different farms and in different regions (18).

Overall, the study reported here adds important new information due to the spatial scale at which it was conducted (i.e., field-level), and provides key data on *Listeria* ecology at small spatial scales. Such data is important since many management decisions will be made for individual fields and field areas as opposed to whole farms or across multiple farms (which is the scale used by previous *Listeria* studies conducted on Northeastern produce farms). Specifically, the study presented here assessed the distribution of *L. monocytogenes* within two fields over one growing season and found that *L. monocytogenes* varied more within individual fields than between fields. Thus, *Listeria* management strategies may need to be adapted sub-field areas. Moreover, this study showed that findings from previous studies conducted at larger spatial scales were reproducible even at the small scale studied here. This suggests that conclusions from these studies are reproducible across farms, spatial scales (e.g., landscape, farm, field) and time periods (46, 47), and may therefore be useful for managing *Listeria* risks at small spatial scales. For example, this and other studies found association between rainfall, proximity to streams, and *Listeria* isolation. Thus, planting high-risk crops (e.g., leafy greens) in low risk areas (e.g., far from streams) as previously proposed in these earlier studies (45, 47) may be a viable strategy grower can use to reduce contamination risks at the field level.

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HIGHLIGHTS

- *L. monocytogenes* (LM) prevalence varied more over space than time
- Water samples had a higher LM prevalence than soil samples
- Despite high LM prevalence in environmental samples, produce contamination was rare
- Rain and distance to water were positively associated with LM isolation
- Repeated isolation of PFGE types suggests persistence or repeated introduction

Figure 1.

The map shows the Upstate New York farm where the study was conducted. Both fields were approx. 0.2 ha in area and divided into 5 m × 5 m plots. Each plot is represented by one yellow circle; the size of each circle is proportional to the number of samples positive for *L. monocytogenes* (i.e., plots represented by larger circles had more positive samples). The three water sampling sites are represented by blue circles, whose size is also proportional to the number of *L. monocytogenes* positive samples.

Figure 2.
L. monocytogenes PFGE types isolated on multiple sampling dates in this study. Date of sample collection is shown at the top right of the figure. Terrestrial sample types are represented by different colors [soil (brown), drag swab (yellow), leaf (green)], while field is represented by different shapes [i.e., Field A (circles), from Field B (triangles)]. Water samples are represented by blue squares.

Figure 3.

Results of conditional random forest analysis (RFA) that identified factors associated with the likelihood of isolating *L. monocytogenes* from (A) drag swabs, (B) soil samples, and (C) water samples. The y-axis shows the factors ranked from most to least important. The x-axis shows normalized variable importance; a higher normalized variable importance (NVI; relative to other factors in the RFA) corresponds to a stronger association between likelihood of isolation and the given factor. A NVI = 0 indicates no association. BSC = before sample collection. SC = sample collection. IC=Impervious Cover.

Figure 4.

Partial dependence plots for the two top-ranked factors associated with *L. monocytogenes* isolation from (A) drag swabs, (B) soil samples, and (C) water samples according to conditional random forest analysis (see Fig. 3). The y-axis shows the factor's marginal effect on the likelihood of isolating *L. monocytogenes* and should be interpreted in terms of the relative impact on the likelihood of *L. monocytogenes* isolation (e.g., top panel in B shows that the likelihood of isolating *L. monocytogenes* from soil decreases monotonically as distance to impervious cover increases). The vertical gray lines along the x-axis show the distribution of the factor; the relationship between the factor and likelihood of *L. monocytogenes* isolation should only be interpreted for this range (e.g., the relationship between likelihood of *L. monocytogenes* and distance to impervious cover should only be interpreted when distance to impervious cover is between ~0 and 90 m).

Table 1:

Frequency of *L. monocytogenes* isolation from spinach leaf, drag swab, soil, and water samples.

Sample Type ^{ab}	Total No. of Samples Collected	No. of <i>L. monocytogenes</i> Positive Samples (Prevalence %)
Spinach Leaf^A		
Field A	84	3 (4)
Field B	78	0 (0)
Total	162	3 (2)
Drag Swab^B		
Field A	18	3 (17)
Field B	18	4 (22)
Total	36	7 (19)
Soil^{A B}		
Field A	252	25 (10)
Field B	234	12 (5)
Total	486	37 (8)
Water^C		
Ditch	9	5 (56)
Creek Site 1 at Road Crossing	9	7 (78)
Creek Site 2 at Irrigation Intake	9	9 (100)
Total	27	21 (78)

^aThe likelihood of *L. monocytogenes* isolation for sites with the same capital superscript letters was not significantly different according to Tukey's HSD.

^bSpinach, drag swab and soil samples were collected from two fields; Field A consisted of 28 plots and Field B consisted of 26 plots. Water samples were collected from 3 sites, including (i) a ditch that paralleled the road and drained into Creek A upstream of the irrigation intake, (ii) where the creek passed under a road upstream of the irrigation intake (Site 1), and (iii) at the irrigation intake (Site 2).

Table 2:

Percent variance in odds of isolating *L. monocytogenes* isolation that can be attributed to spatial and temporal factors.

Random Effects included in Each Model	Percent Variance (Akaike's Information Criteria) ^a			
	All Data ^a	All Terrestrial Data	Soil Data ^b	Water Data ^{a,b}
Field	- ^c	1.5% (345.7)	< 1% (265.1)	-
Field:Plot ^d	-	17.7% (342.0)	11.3% (266.2)	-
Field:Plot ^d , Sample Type	-	26.3% (340.4)	-	-
Field:Plot ^d , Sample Type, Sampling Visit	-	28.2% (341.8)	-	-
Latitude	28.8% (413.8)	15.0% (341.7)	11.3% (264.2)	17.9% (32.0)
Longitude	27.5% (415.0)	13.3% (342.6)	8% (265.0)	17.9% (32.0)
Sample Type	51.9% (376.9)	19.2% (340.5)	-	-
Sampling Visit	1.1% (452.3)	2.8% (345.8)	0% (265.7)	0% (32.6)
Time Since Start of Study	1.6% (452.0)	3.4% (345.3)	< 1% (265.7)	0% (32.6)

^aPercent variance in odds of isolating *L. monocytogenes* accounted for by inclusion of each set of random effects in the model. All data includes drag swab, produce, soil, and water data, while terrestrial data includes drag swab, produce, and soil data.

^bSince these models include data from only one sample type, sample type was not included as a random effect in the models.

^cThe symbol "-" indicates absence of model results since for water data plot could not be included as random effect.

^dPlot nested in field.