CPS 2013 RFP
FINAL PROJECT REPORT

Project Title

Validation of geospatial algorithms to predict the prevalence and persistence of pathogens in produce fields to improve GAPs

Project Period

January 1, 2014 – December 31, 2015 (extended to January 31, 2016)

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Objectives

The proposed research will use GIS modeling and statistical tools in combination with extensive field sampling to accomplish the following three objectives:

1. Implement geospatial algorithms to develop predictive maps identifying environmental reservoirs (i.e., well defined spatial areas) for Listeria monocytogenes on produce farms.

2. Independently validate each geospatial algorithm's power to predict areas that have a significantly higher prevalence of L. monocytogenes (i.e., high risk areas), as compared to areas identified as having significantly lower prevalence of L. monocytogenes by the algorithm (i.e., low risk areas).

3. Quantify the effect of precipitation on the frequency of L. monocytogenes detection in high and low risk areas identified by the geospatial algorithms and the risk of L. monocytogenes transfer to produce during or after precipitation events.

Funding for this project provided by the Center for Produce Safety through:
CDFA SCBGP grant # SCB13063

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Abstract

The risk of produce contamination can be reduced if contamination is minimized in the production environment, specifically during the growth and harvest stages. However, on-farm produce safety is complicated by the fact that each farm has a distinct combination of topography, land-use and weather. By understanding how these factors interact to affect the ecology and transmission of foodborne pathogens in produce production environments we can develop targeted approaches to on-farm risk management. Technological advancements, particularly in geographic information systems (GIS), have made the development of such targeted approaches possible. This project therefore included two major aims (*I)* to validate a previously developed geospatial model that predicts the likelihood of *L. monocytogenes* isolation for individual produce fields using remotely sensed land-use and topographical data (Objective 1 and 2), and (*II*) to validate a model that predicted that recent rain and irrigation events would increase the likelihood of *L. monocytogenes* detection in the produce pre-harvest environment (Objective 3). To address *Aim I*, a previously developed geospatial model was used to categorize produce fields for each of four enrolled farms into areas of high or low predicted *L. monocytogenes* prevalence based on the field's available water storage (AWS), and its proximity to water, impervious cover, and pasture. Drag swabs (n=1,056) were collected from plots assigned to each risk category. Logistic regression, which tested the ability of each rule to accurately predict *L. monocytogenes* prevalence, validated the rules based on water and pasture. Samples collected near water (odds ratio [OR] = 3.0) and pasture (OR = 2.9) showed a significantly increased likelihood of *L. monocytogenes* isolation compared with samples collected far from water and pasture. These findings validated a subset of previously developed rules that predict *L. monocytogenes* prevalence for produce production environments. To address *Aim II*, two spinach fields, with a high and a low predicted risk of *L. monocytogenes* isolation, were sampled 24, 48, 72 and 144–192 h following irrigation and rain events. For this aim a total of 1,492 samples (1,092 soil, 334 leaf, 14 fecal, and 52 water samples) were collected. Based on a generalized linear mixed model (GLMM), the likelihood of *L. monocytogenes* isolation from soil samples was highest during the 24 h immediately following a rain or irrigation event (OR = 25). Additionally, *L. monocytogenes* isolates associated with irrigation events showed significantly lower *sigB* allelic type (AT) diversity (as determined by Shannon-Weiner Index) than isolates associated with precipitation events (*P* = <0.001; T-Hutcheson test), suggesting that irrigation water may be a point source for *L. monocytogenes* contamination. Our findings suggest that small changes in management practices (e.g., not irrigating before harvest, planting high risk crops in low risk areas, such as areas far from water) may reduce the risk of *L. monocytogenes* contamination of fresh produce. Importantly, our research (i) provides specific insights into weather and land-use factors that increase the likelihood of *Listeria* detection in produce fields, and (ii) demonstrates that GIS-based strategies can be used to correctly predict weather and land-use factors that increase the likelihood of pathogen detection in fields. This information can help growers manage food safety risks at the field level and suggests that efforts to develop GIS-based strategies to manage other pathogens (e.g., *Salmonella*) and pathogen sources (e.g., surface water used for irrigation) are warranted.

Background

Fresh produce presents a unique food safety challenge due to the absence of a kill-step between harvest and consumption. Prevention of produce contamination in production environments is therefore a concern for growers, the produce industry and public health professionals. To develop effective prevention strategies, it is important to understand the ecological processes and environmental factors that affect foodborne pathogen prevalence in produce production environments. Numerous studies have examined the relationship between management practices, environmental factors and the prevalence of *L. monocytogenes* (*4*, *5*, *16*, *20*) in produce production environments. Many of these studies (*5*, *16*, *20*) determined that water-related factors (e.g., irrigation, rain, proximity to water) were significantly associated with the isolation of *L. monocytogenes* from environmental samples. Studies conducted in non-agricultural environments also found similar results (*5*, *9*, *10*). For example, Ivanek et al. (*9*) found that the isolation of *Listeria* spp. from samples collected in forested environments was positively associated with rainfall. Irrigation has also been repeatedly associated with an increased risk of pre-harvest produce contamination by *L. monocytogenes* (*8*, *16*, *20*). In fact, two studies (*16*, *20*) have found that irrigation was one of the most important risk factors associated with *L. monocytogenes* isolation from samples collected in pre-harvest environments. Despite the repeated identification of irrigation and rain as risk factors for pre-harvest produce contamination, no study, to the authors' knowledge, has reported, quantitatively, the impact of irrigation or rain over time (i.e., over subsequent 24-h periods following an irrigation event) on the risk of produce contamination in production environments.

Past studies have also examined how spatial variation in environmental risk factors can affect the likelihood of isolating *L. monocytogenes* from produce production environments. For example, Chapin et al. (*5*) used GIS to organize and extract remotely sensed data to show that different species of *Listeria* occupy distinct ecological niches in agricultural and natural environments. Despite a number of studies that have used GIS to extract or visualize remotely sensed data (*5*, *9*, *16*), only one study (*16*) has used GIS to predict the distribution and prevalence of a specific foodborne pathogen in produce production environments. This study by Strawn et al. (*16*) used classification tree analysis (CART) to develop a geospatial model that predicts the prevalence of *L. monocytogenes* in New York State (NYS) produce fields. This model consisted of a set of hierarchical rules based on, in order, proximity of fields to surface water, temperature, proximity of fields to impervious cover, available water storage (AWS) and proximity of fields to pasture (*16*). Studies in other disease systems have developed and validated (*2*, *12*, *14*, *15*) geospatial, predictive risk models. These validation studies (*2*, *12*, *14*) demonstrate the utility of geospatial risk models, like the model developed by Strawn et al. (*16*), for accurately and prospectively predicting pathogen prevalence. Additionally, these studies (*2*, *12*, *14*) used the output of their models to prioritize and identify risk management strategies, suggesting that geospatial models can also be integrated with on-farm food safety plans to develop targeted approaches to disease prevention.

Thus, the aims of this research were (*I*) to validate the ability of the model developed by Strawn et al. (*16*) to predict on-farm areas with a significantly higher or lower prevalence of *L. monocytogenes* (Objectives 1 and 2), and (*II*) to quantify the effect of time after precipitation and amount of precipitation on the frequency of *L. monocytogenes* detection (Objective 3). Through these aims, this project improved our understanding of foodborne pathogen ecology, facilitating development of targeted mitigation strategies for risk management in produce production environments (e.g., tailored on-farm food safety approaches).

Research Methods and Results

AIM I: In *Aim I* we addressed **Objective 1** and **Objective 2**. For a detailed description of the methods and results from *Aim I*, please refer to the publication by Weller et al. (*18)* describing the results from this aim; a brief summary of the methods is included below.

AIM I Methods

Study design. A cross-sectional study was conducted over a six-week period in July and August of 2014 on four produce farms in NYS. Farms were not selected based on geographic location or management practices; farms were enrolled based on the willingness of the grower to participate.

All fields within a farm were classified into four high risk categories and one low risk category (see Figure 1) based on a set of hierarchical rules that were adapted from Strawn et al. (*16*) using classification tree analysis. Briefly, we adapted that model by removing the meteorological factors so the model only included spatial factors (i.e., proximity to water, proximity to impervious cover, AWS, and proximity to pastures). This adapted model will be referred to as the CART model throughout this report. The CART model had four splits/rules, which, in order, will be referred to as the Water Rule, the Impervious Cover Rule, the AWS Rule, and the Pasture Rule (a given rule specifies which factors increases the likelihood of *L. monocytogenes* isolation; for example the water rule indicates that proximity to surface water increases the likelihood of *L. monocytogenes* isolation). All field areas classified into a given category were then divided into 5 x 5 m plots, and a subset of plots were randomly selected from each category for sampling. One area drag swab was collected per plot.

Sample collection, preparation and processing. Samples were collected, prepared and processed as previously described by Strawn et al. (*16*). Briefly, for each plot, a pre-moistened drag swab was dragged around the perimeter and diagonals of the plot for 3–5 minutes. All samples were transported on ice, stored at 4°C and processed within 24 h of collection. In the lab each sample was diluted 1:10 with buffered *Listeria* enrichment broth (Becton Dickinson), followed by incubation at 30°C. After 4 h, *Listeria* selective enrichment supplement (Oxoid, Cambridge, UK) was added to each enrichment. After incubating for 24 and 48 h, 50 µl of each enrichment was streaked onto *Listeria monocytogenes* plating medium (LMPM; Biosynth International, Itasca, IL) and Modified Oxford agar (MOX; Becton Dickinson); the plates were then incubated for 48 h at 35 and 30°C, respectively. After incubation for 48 h at 35°C (LMPM) and 30°C (MOX), up to four presumptive *Listeria* colonies were sub-streaked from LMPM and MOX onto brain-heart infusion agar plates (BHI; Becton Dickinson). The BHI plates were then incubated at 37°C for 24 h. Following incubation, the species and *sigB* AT of one presumptive *L. monocytogenes* colony per sample was determined by PCR amplification and sequencing of the partial *sigB* gene as previously described (*3*, *6*, *11*).

Positive and negative controls were processed in parallel with field samples. *L. monocytogenes* FSL R3-0001 (*13*) and uninoculated enrichment media were used as the positive and negative controls, respectively. All isolates were preserved at -80°C and isolate information can be found at www.FoodMicrobeTracker.com.

Statistical analysis. All statistical analyses were performed in R (version 3.1, R Core Team, Vienna, Austria). The frequency and prevalence of *L. monocytogenes* was calculated for each predicted risk area for each rule. Although the outcome of the CART model was a predicted prevalence for *L. monocytogenes*, all statistical analyses were performed for both (i) *L. monocytogenes* and (ii) *Listeria* spp. (including *L. monocytogenes*) since *Listeria* spp. is more common than *L. monocytogenes* in NYS produce production environments and is often used as an index organism for *L. monocytogenes*.

To test the ability of each rule to accurately predict the prevalence of *Listeria* spp. and *L. monocytogenes* in produce fields, and to validate the CART model, multivariable logistic regression analyses were performed using the lme4 package (*1*). The multivariable model originally contained all four rules, but was reduced using backwards selection. The outcome for the multivariable model was the presence of *Listeria* spp. or *L. monocytogenes*. Farm was included as a random effect.

AIM I Results

The overall prevalence of *L. monocytogenes* for field drag swabs collected from NYS produce farms was 20% and 12%, respectively. Overall, *Listeria* spp. (including *L. monocytogenes*) was isolated from 20% (208/1056) of samples. *L. monocytogenes* was isolated from 12% (128/1056) of samples, *L. innocua* was isolated from 4.0% (42/1056) of samples, *L. seeligeri* was isolated from 2.0% (21/1056) of samples, and *L. welshimeri* was isolated from 1.6% (17/1056) of samples. Overall, the prevalence of *Listeria* spp. was greater for all field areas with a high predicted prevalence of *L. monocytogenes* isolation compared to field areas with a low predicted prevalence (Table 1).

The prevalence of *L. monocytogenes* was greater for all field areas with a high predicted prevalence of *L. monocytogenes* isolation compared to the field areas with a low predicted prevalence according to the Water, Pasture and AWS Rules (Table 1).

Rules based on surface water and pasture proximity accurately predict *L. monocytogenes* prevalence in environmental samples collected from NYS produce production environments. Logistic regression was performed to test the ability of each rule to accurately predict *L. monocytogenes* prevalence in NYS produce production environments. Logistic regression analysis showed that only the Water and Pasture Rules accurately predicted the prevalence of *L. monocytogenes* in NYS produce production environments (Table 2). Samples collected from field areas that had a high predicted prevalence of *L. monocytogenes* isolation by the Water Rule had an increased odds of *L. monocytogenes* isolation (OR = 3.0; 95% CI = 2.0, 4.6), compared with samples collected from field areas that had a low predicted prevalence. Samples collected from field areas that had a high predicted prevalence for *L. monocytogenes* by the Pasture Rule had an increased odds of *L. monocytogenes* isolation (OR = 2.9; 95% CI = 1.4, 6.0), compared with samples collected from field areas that had a low predicted prevalence.

While the outcome of the CART model was *L. monocytogenes* prevalence, the ability of the model to predict *Listeria* spp. prevalence was also validated because *Listeria* spp. are more common than *L. monocytogenes* and, as a result, the findings based on *Listeria* spp. are more robust. Multivariable logistic regression showed that only the Water Rule was found to accurately predict the prevalence of *Listeria* spp. in NYS produce production environments (Table 2). Samples collected from field areas that had a high predicted *L. monocytogenes* prevalence by the Water Rule had an increased odds of *Listeria* spp. isolation (OR = 1.6; 95% CI = 1.1, 2.4) compared with samples collected from field areas that had a low predicted prevalence.

AIM II: In *Aim II* we addressed **Objective 3**. For a detailed description of the methods used in and results of *Aim II* see Weller et al. (*19*). A brief summary is included below.

AIM II Methods

Study design. A longitudinal study was conducted in two spinach fields at the Homer C. Thompson Vegetable Research Farm over a seven-week period in May, June, and July 2014. Two 0.2-ha fields (Figure 2) were selected based on their respective predicted prevalence of *L. monocytogenes* (i.e., one high and one low risk field), which was a function of the fields' proximity to water and roads. Briefly, a field was considered at high risk for *L. monocytogenes* if it was ≤37.5 m from water and ≤9.5 m from a road (*16*). A field was considered at low risk for *L. monocytogenes* if it was >37.5 m from water and >9.5 m from a road.

Each field was divided into 21 13x13 m plots. Soil sample sites were randomly selected from within each plot for each sampling trip (i.e., the same location within each plot was only sampled once during the course of the study). Soil samples were collected 24, 48, 72, and 144–

192 h after an "irrigation" or "rain" event. An irrigation event was defined as any time irrigation water was applied to the field. A rain event was defined as >6 mm of rain over a 24-h period (i.e., 9 am to 9 am). If a rain or irrigation event did not occur between 144–192 h after a rain event, a "dry" event sampling was performed. Each set of samples (i.e., 24, 48, 72 h and 144–192 h, if collected,) was defined as representing either an irrigation or rain event depending on which "event type" initiated sample collection. Overall, seven sets of samples were collected: (i) five sets that represented rain events, including three sets of samples collected 144–192 h after the event, and (ii) two sets that represented irrigation events, including one set of samples collected 144–192 h after the event. Overall, each plot was sampled 26 times.

Water, leaf and fecal samples were also collected. Water samples were collected from Fall Creek (Figure 2), the water source used for irrigation. Fecal samples were collected when observed within 5 m of the sampled fields or Fall Creek. Composite leaf samples were collected for each plot once the spinach plants were large enough to survive harvesting (i.e., 36 d after planting). Composite leaf samples were hand collected by gathering leaves from 6–12 spinach plants growing along the perimeter and diagonals of each plot. Global positioning system (GPS) coordinates were recorded for each soil and water sample. In total, 1,092 soil, 52 water, 334 leaf, and 14 fecal samples were collected (n=1,492 total).

Sample collection and preparation. Samples were collected and tested as previously described by Strawn et al. (*16*). Briefly, soil samples were collected from approximately 4 in (10.16 cm) below the soil surface using 5-mL sterile scoops and then placed in a sterile Whirl-Pak bag. Twenty-five g of soil were then weighed into a separate sterile filter Whirl-Pak bag. Water samples were collected directly into sterile jars using a sampling pole (Nasco) and processed according to the Environmental Protection Agency (EPA) standard methods (*17*). Additionally, 10 g of each fecal sample and 25 g of each composite leaf sample were weighed out and aseptically transferred to separate sterile filter Whirl-Pak bags. All samples were transported on ice and processed within 3 h of collection.

Bacterial enrichment and isolation. Detection and isolation of *Listeria* spp. and *L. monocytogenes* was performed as described above in *Aim I.*

Meteorological data. Meteorological data were obtained from the Cornell University weather station located at the Homer C. Thompson Vegetable Research Farm (Rainwise Inc., Trenton, NJ). Data on leaf wetness were obtained from the Cornell University Network for the Environment and Weather Applications. Data were downloaded for each sample collection date and the three preceding 24-h periods (i.e., 9 am to 9 am). Average values for each factor for 0 to 1, 0 to 2, and 0 to 3 d before sample collection were also calculated.

Statistical analysis. All statistical analyses were performed in R (version 3.1). Prevalence was calculated for each field (high or low risk), time period (24, 48, 72 and 144–192 h), event type (rain versus irrigation event) and sample type (leaf, soil and water). The total number of ATs for *Listeria* spp. and *L. monocytogenes* was determined and the Shannon-Wiener index was calculated. A T-Hutcheson test (*7*) was performed to compare the Shannon-Wiener indices for the high risk and low risk fields, and for irrigation and rain events.

Univariable analyses were performed to determine the effect of spatial and meteorological factors, time since event, predicted field risk, and event type on the odds of *Listeria* spp. and *L. monocytogenes* isolation. Correlation between significant factors (at $P \leq 0.20$) was assessed. Principal component analysis (PCA) was performed on each set of meteorological factors (e.g., all humidity factors), with the exception of rainfall, if (i) the factors were significant by univariable analysis and correlated, and (ii) if combination was biologically plausible. The first eigenvector from each PCA was added to the dataset as a potential covariate for inclusion in the final model. Factors that were identified as significant by univariable analysis but not included in a PCA were also included as potential covariates in the final model.

GLMM (*1*) were built using a backwards selection method. The outcome was the presence or absence of *Listeria* spp. or *L. monocytogenes*. Event type, hours and predicted field risk were included as fixed effects. Set and plot were included as random effects.

AIM II Results

Prevalence and diversity of *L. monocytogenes,* and *Listeria* spp. in produce production environments. The overall prevalence of *L. monocytogenes* was 9% (130/1,492). The prevalence of *L. monocytogenes* was higher in fecal samples, 64% (9/14) and water samples, 63% (33/52), compared with soil samples, 8% (86/1092) and leaf samples, 0.6% (2/334; Table 3). The prevalence of *L. monocytogenes* was higher in soil samples collected 24 h after irrigation and rain events, 18% (52/294), compared with soil samples collected 48 h, 6% (18/294), 72 h, 4% (11/294) and 144–192 h, 1% (2/168), after irrigation and rain events (Table 4). Lastly, the prevalence of *L. monocytogenes* was higher in soil samples collected after irrigation events, 12% (34/294), compared with after rain events, 6% (49/756; Table 4; Figure 3).

Nine different *L. monocytogenes* ATs were isolated from *L. monocytogenes* positive soil samples. The diversity of ATs in soil samples collected from the low risk field compared with the high risk field was not significantly different according to T-Hutcheson's test (*P* = 0.39; Table 5; Figure 4). The diversity of *L. monocytogenes* ATs isolated from soil samples following rain events was significantly greater (*P* < 0.001), than the diversity of *L. monocytogenes* ATs isolated from soil samples following irrigation events (Table 5).

The overall prevalence of *Listeria* spp. was 14% (204/1492). Twenty-seven different *Listeria* spp. ATs were isolated from the *Listeria* spp. positive soil samples collected in this study. There was no significant difference in the diversity of ATs in soil samples collected from the low risk field compared with the high risk field according to T-Hutcheson's test (*P* = 0.08; Table 5; Fig 7). The diversity of *Listeria* spp. AT types isolated from soil samples following rain events was significantly greater (*P* < 0.001) than the diversity of ATs isolated from soil samples following irrigation events (Table 5).

Risk factors associated with *L. monocytogenes* isolation from soil samples. Of the 107 factors that were evaluated, 46 were significantly associated with *L. monocytogenes*-positive soil samples by univariable analysis, including two study parameters, two spatial factors, five dew point factors, ten humidity factors, three irrigation factors, two leaf wetness factors, 20 temperature factors, one precipitation factor, and one wind direction factor. PCA was performed for the dew point factors as a group, the humidity factors as a group, the leaf wetness factors as a group, and the temperature factors as a group.

In the multivariable analysis, three factors (hours since event occurred, amount of irrigation water applied to the fields two days before sampling, and predicted field risk) were retained (Table 6). Although event type was not found to be significant by multivariable analysis, it was retained in the final model so the effect of irrigation events compared to rain events could be quantified as this was of interest to the study. No significant interactions between any factors were identified. No significant interactions between any factors were identified.

Risk factors associated with *Listeria* spp. isolation from soil samples. Of the 107 factors that were evaluated, 39 factors were significantly associated with *Listeria* spp.-positive soil samples by univariable analysis, including two study parameters, two spatial factors, one dew point factors, six humidity factors, three irrigation factors, three leaf wetness factors, 15 temperature factors, three precipitation factors, and four wind speed factors. PCA was performed for the leaf wetness factors as a group, the temperature factors as a group, and the wind speed factors as a group.

In the multivariable analysis, four factors (hours since event occurred, amount of irrigation water applied to the fields two days before sampling, amount of rain water that precipitated two days before sampling, predicted field risk) were retained (Table 6). Although event type was not significant, it was retained in the final model.

Outcomes and Accomplishments (covering both Aims I and II)

Overall this project provided new insights into the ecology of *L. monocytogenes* in produce pre-harvest environments. This study yielded quantitative data that showed that *L. monocytogenes* contamination on produce farms is (i) dependent on the specific ecological context of a produce field, and (ii) that geospatial models can be used to prospectively predict *L. monocytogenes* prevalence for NYS produce production environments. Specific key scientific accomplishments include (i) development of predictive risk maps to identify potential on-farm reservoirs of *L. monocytogenes*, (ii) validation of the geospatial model developed by Strawn et al. (*16*), and (iii) quantification of the impact of irrigation and rain events on the prevalence of *L. monocytogenes*. The implementation of geospatial predictive models by the produce industry will allow for the development of individualized preventive measures on produce farms, by enabling growers to proactively assess and address environmental factors that may increase the risk of contamination events on their specific farms. For example, predictive risk maps can identify areas of high predicted pathogen prevalence within farms, and enable growers to make more informed decisions about the management of crops in these areas, including targeted pathogen surveillance programs and altered management practices. Our findings also suggest that small changes in management practices may have a significant effect on the risk of *L. monocytogenes* contamination in produce production environments. For example, growers may reduce *L. monocytogenes* contamination risk by waiting 24 h to harvest crops following rain events, or by not irrigating within 24 h of harvest. Additionally, interventions at the irrigation-level, such as treatment of irrigation water (e.g., by chlorine tabs), may reduce the risk of pre-harvest contamination. Other potential intervention strategies may include constructing buffer zones or conserving wetlands around fields near water, altering cropping schemes (e.g., planting high risk crops in low risk fields), and monitoring pathogen levels in irrigation water.

In addition to the scientific outcomes, this project also continued the training of future food safety researchers and professionals with expertise in produce food safety. For example, Dr. Laura Strawn, who contributed to the preparation of the original proposal when she was still at Cornell, has moved to Virginia Polytechnic University as an Assistant Professor, where she continues to perform produce safety research.

Summary of Findings and Recommendations

<u>Aim I</u>

Key findings

- Proximity to surface water and pasture were significantly associated with *L. monocytogenes* isolation from produce production environments.
- By validating two of the risk factors identified in previous models (proximity to water and proximity to pastures) that can be used to predict field areas with increased risk of *L. monocytogenes* detection, our study demonstrates that geospatial models can be used to accurately and prospectively predict fields and areas in produce fields with an increased risk of pathogen detection.

Recommendations

- Growers for whom *L. monocytogenes* is a pathogen may want to carefully manage growing areas in proximity to surface water and pastures. Similarly, processors or growers that conduct trace-back investigations (e.g., based on a finished product positive) may want to more heavily focus sampling on field sites in proximity to surface water and pastures when trying to identify pre-harvest *L. monocytogenes* sources.
- To facilitate identification of additional risk factors and additional control strategies, future models should account for temporal (e.g., changes in management practices or meteorological factors over time) and farm size.

Aim II

Key findings

- Proximity to surface water and roads (i.e., two rules that were the basis for predicted risk in *Aim II*) were associated with an increased likelihood of isolating *L. monocytogenes* from soil samples collected in produce fields.
- The likelihood of isolating *Listeria* spp. and *L. monocytogenes* was greatest during the 24 h immediately following rain or irrigation events.
- The diversity of *Listeria* spp. and *L. monocytogenes* subtypes (ATs) was lower after irrigation events than after rain events.

Recommendations

- Interventions to reduce the risk of pathogen contamination in fields may need to take into account the water source (i.e., surface water versus rain).
- Waiting 24 h after irrigation and rain events to harvest crops may significantly reduce the risk of *L. monocytogenes* contamination.
- The use of land-use factors to predict risk and to tailor cropping schemes to reduce risk (e.g., planting high risk crops in low risk areas) may be useful for developing targeted on-farm food safety risk management plans.

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APPENDICES

Publications and Presentations

Publications

Weller, D., M. Wiedmann and L. K. Strawn. 2015. Spatial and temporal factors associated with *Listeria monocytogenes* contamination of spinach fields in New York State. *Applied and Environmental Microbiology.* 81 (17): 6059-6069.

Weller, D., S. Swakoti, P. Bergholz, Y. Grohn, M. Wiedmann and L. K. Strawn. 2015. Validation of a previously developed geospatial model that predicts *Listeria monocytogenes* prevalence for New York State produce fields. *Applied and Environmental Microbiology.* 82 (3): 797-807.

Presentations

Weller, D., S. Swakoti, P. Bergholz, Y. Grohn, M. Wiedmann and L. K. Strawn. 2015. *Validation of a previously developed geospatial model that predicts Listeria monocytogenes prevalence for New York State produce fields*. Presented at the Cornell Geospatial Forum, October 13, 2015, Ithaca, NY.

Weller, D., L. K. Strawn and M. Wiedmann. 2015. *Microbe detectives: using geographic information systems (GIS) to track and find foodborne pathogens in produce productions systems*. Poster at the Cornell Institute for Food Systems Summit, October 12, 2015, Ithaca, NY.

Weller, D., S. Swakoti, P. Bergholz, Y. Grohn, M. Wiedmann and L. K. Strawn. 2015. *The use of geographic information systems to predict the risk of Listeria monocytogenes contamination in produce fields*. Presented by Laura Strawn at the International Association for Food Protection Conference, July 16, 2015, Portland, OR.

Weller, D., M. Wiedmann and L. K. Strawn. 2015. *Time since irrigation and rain events is significantly associated with an increased prevalence of Listeria monocytogenes in spinach fields in New York State*. Presented at the International Association for Food Protection Conference, July 16, 2015, Portland, OR.

Weller, D. 2015. *Microbe Hunters: Using geographic information systems to identify risk factors for Listeria monocytogenes contamination in produce production environments*. Presented at the Food Science and Technology Spring Seminar Series, Cornell University, April 21, 2015, Ithaca, NY.

Weller, D., L. K. Strawn and M. Wiedmann. 2014. *Microbe detectives: using geographic information systems (GIS) to track and find foodborne pathogens in produce productions systems*. Poster at the Cornell Institute for Food Systems Summit, December 8, 2014, Ithaca, NY.

Weller, D. 2014. *Integrating geographic information systems and produce safety to develop science-based recommendations for disease prevention*. Presented at the Cornell Geospatial Forum, October 14, 2014, Ithaca, NY.

Budget Summary

Funds utilized as of: 2/29/16

Cornell University

Salaries	$ 118,317
Fringe benefits	$ 61,691
Travel	$ 3,819
Supplies	$ 72,394
Publication	$ 1,952
Services	$ 9,736
Communications	$ 219
Indirect Costs	$ 9,001

North Dakota State University

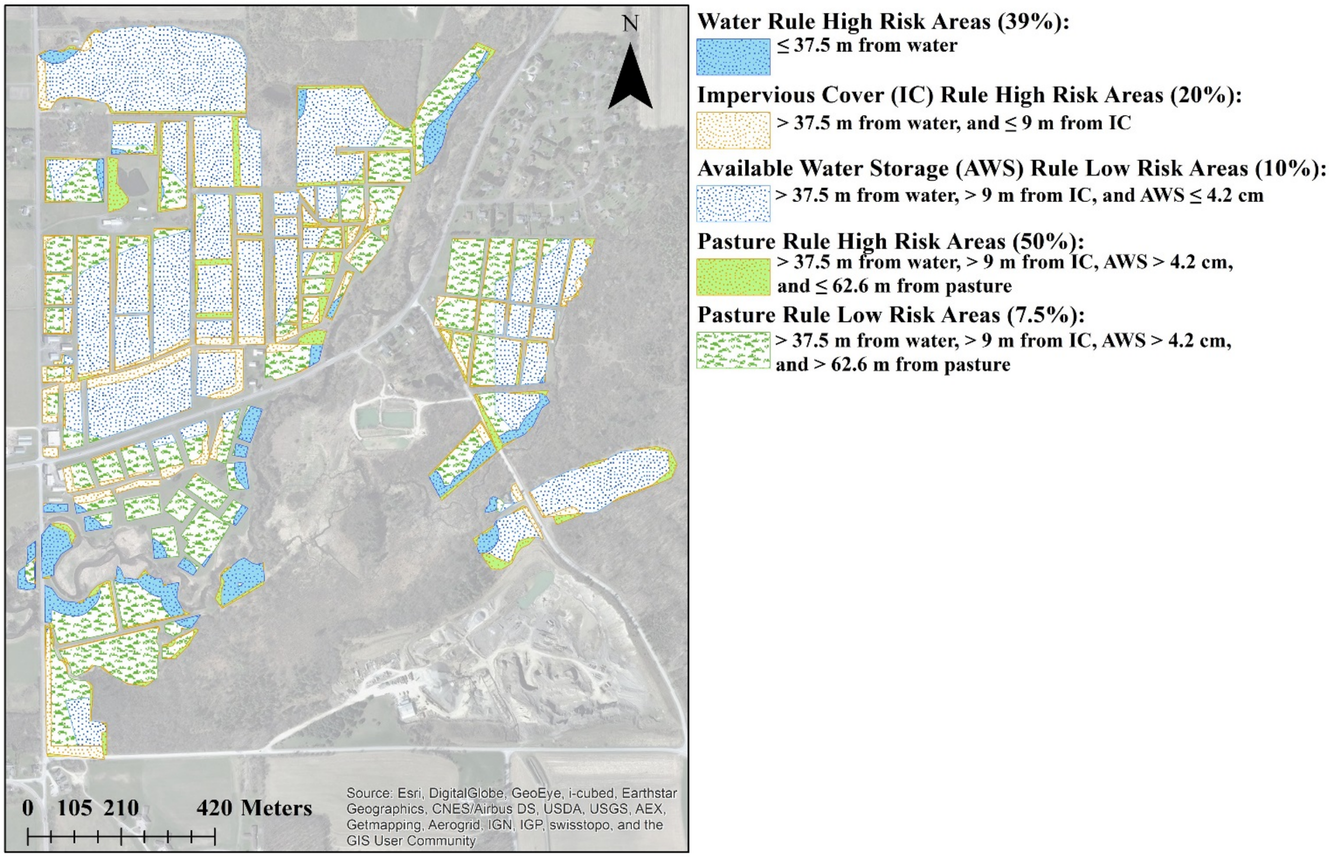
Sub-award	$ 11,859

Total Expenses	$ 288,988

We expect to use all funds awarded to our project. While the award is not yet fully spent, expenses for travel to the June 2016 CPS meeting will fully expend the funds.

Tables and Figures

Figure 1. Map of predicted risk for *L. monocytogenes* prevalence for the Homer C. Thompson Vegetable Research Farm at Cornell University; the expected prevalence of *L. monocytogenes* is listed in parentheses in the legend. Note this map is not based on any of the farms included in this study (for confidentiality reasons).



Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2. Locations of the low and high risk fields, and the surface water sampling sites included in this study.

Figure 3. Distribution of *Listeria* spp. (including *L. monocytogenes*) positive (red) and negative (yellow) samples in the low risk (A) and high risk (B) fields, and of L. monocytogenes positive (red) and negative (yellow) samples in the low risk (C) and high risk (D) fields. Fall Creek, the source of irrigation water in this study, is visible in the bottom right-hand corner of B and D.

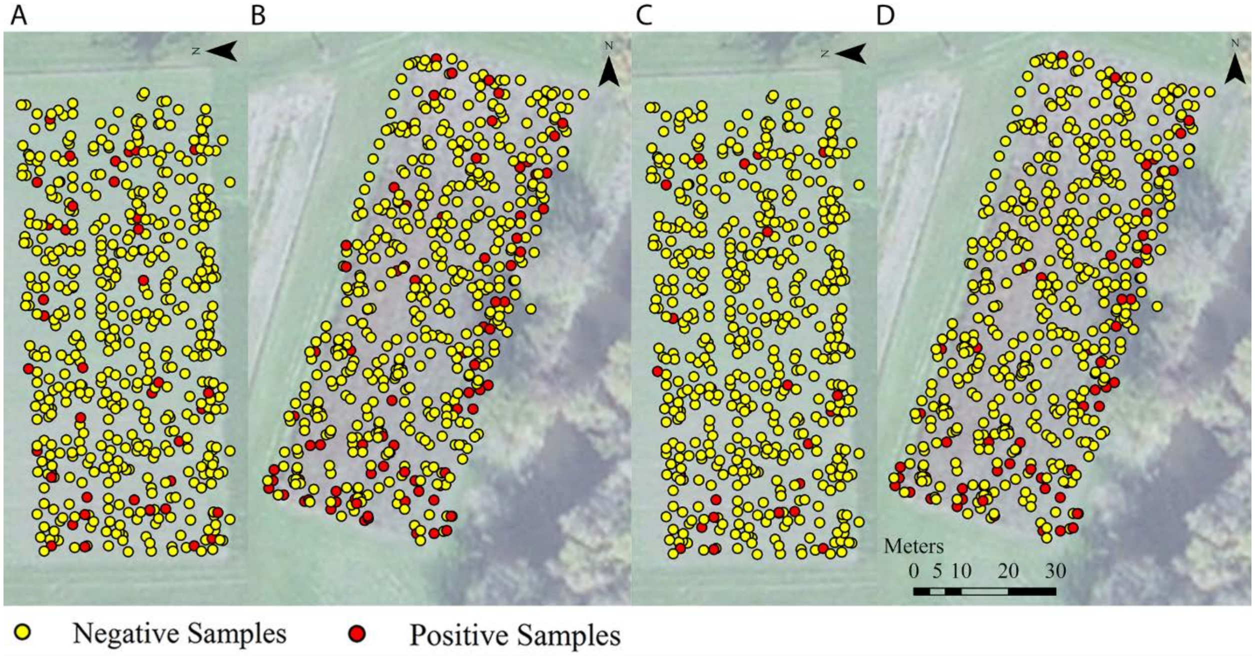


Figure 4. Distribution of *L. monocytogenes* allelic types (AT 57 = red circle; AT 58 = orange circle; AT 59 = yellow circle; AT 61 = light blue circle; AT 64= dark blue circle; AT 67= pink circle; AT 78= light purple circle, AT 159 = purple circle; AT 160=brown circle) in the low risk (A) and high risk (B) fields. Fall Creek, the source of irrigation water in this study, is visible in the bottom right-hand corner of B.

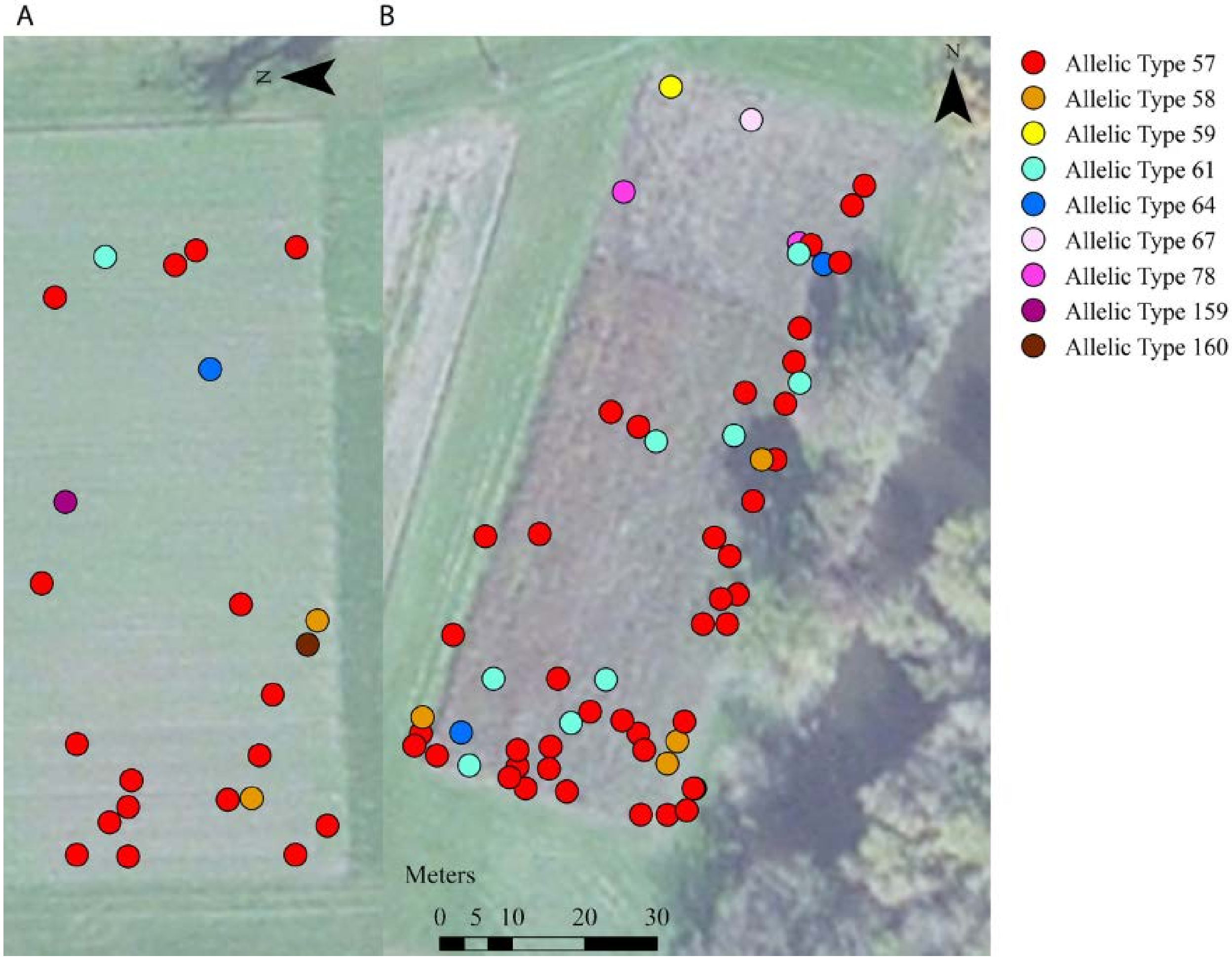


Table 1. Frequency and prevalence of positive *Listeria* spp. and *L. monocytogenes* samples for farm fields that had either a high or a low predicted risk of *L. monocytogenes* isolation based on land cover factors.

Rules	Description of		No. of samples positive for (prevalence of)			
	High Predicted Prevalence (No. of Samples)	Low Predicted Prevalence (No. of Samples)	*Listeria* spp.[a]		*L. monocytogenes*	
			High Predicted Risk	Low Predicted Risk	High Predicted Risk	Low Predicted Risk
Water	≤ 37.5 m from water (195)	> 37.5 m from water (861)	51 (26%)	157 (18%)	43 (22%)	85 (10%)
Road	≤ 9 m from roads (168)	> 9 m from roads (693)	36 (21%)	121 (17%)	11 (7%)	74 (11%)
AWS[b]	> 4.2 cm^3 AWS (106)	≤ 4.2 cm^3 AWS (587)	23 (22%)	98 (17%)	20 (19%)	54 (9%)
Pasture	≤ 62.5 m from pasture (49)	> 62.5 m from pasture (57)	12 (24%)	11 (19%)	11 (22%)	9 (15%)
Total (1056)			208 (20%)		128 (12%)	

[a] *Listeria* spp. includes *L. monocytogenes.*

[b] Available water storage (AWS).

Table 2. Results of multivariable analyses built using backwards regression (i.e., only factors with $P \leq 0.05$ were retained) that tested previously identified rules to accurately predict the effect of different binary land cover factors (e.g., either far away from or close to water) on the likelihood of *Listeria* spp. and *L. monocytogenes* isolation.

Rules	Odds Ratio for *Listeria* spp. or *L. monocytogenes detection*	95 % CI [a]	*P*-value
Listeria spp.[b]			
Water[c]	1.6	1.1, 2.4	0.008
L. monocytogenes			
Pasture[d]	2.9	1.4, 6.0	0.005
Water[c]	3.0	2.0, 4.6	< 0.001

[a] Confidence Interval.

[b] *Listeria* spp. includes *L. monocytogenes.*

[c] The Water Rule predicts a high prevalence of *L. monocytogenes* for areas within 37.5 m of surface water, and a low prevalence for areas farther than 37.5 m from surface water.

[d] The Pasture Rule predicts a high prevalence of *L. monocytogenes* for areas within 62.5 m of pasture, and a low prevalence for areas farther than 62.5 m from surface water.

Table 3. Effect of sample type on frequency and prevalence of *Listeria* spp. and *L. monocytogenes* isolated from soil samples collected from spinach fields previously identified as high or low risk for *L. monocytogenes* isolation.

Sample Type (No. of Samples)	No. of samples positive for (prevalence in %)	
	Listeria spp.[a]	*L. monocytogenes*
High Risk Field (n = 726)	109 (15 %)	73 (10 %)
Fecal (n = 13)	11 (85 %)	9 (69 %)
Leaf (n = 167)	14 (8 %)	2 (1 %)
Soil (n = 546)	84 (15 %)	62 (11 %)
Low Risk Field (n = 714)	48 (7 %)	24 (3 %)
Fecal (n = 1)	0 (0 %)	0 (0 %)
Leaf (n = 167)	5 (3 %)	0 (0 %)
Soil (n = 546)	43 (8 %)	24 (4 %)
Surface Water[b] (n = 52)	47 (90 %)	33 (63 %)

[a] *Listeria* spp. includes *L. monocytogenes.*

[b] Surface water used for irrigation.

Table 4. Frequency and prevalence of *Listeria* spp. and *L monocytogenes* in soil samples collected 24, 48, 72 and 144-192 h after irrigation and rain events from two spinach fields previously identified as high or low risk for *L. monocytogenes* isolation.

Event Type[a]	Time in h[b] (No. of Samples)	No. of samples positive for (prevalence in %)	
		Listeria spp.[c]	*L. monocytogenes*
Low Risk Field			
Pre-sample	NA (21)	1 (5 %)	1 (5 %)
Irrigation	24 (42)	8 (19 %)	7 (17 %)
	48 (42)	3 (7 %)	2 (5 %)
	72 (42)	2 (5 %)	2 (5 %)
	144-192 (21)	0 (0 %)	0 (0 %)
Rain	24 (105)	16 (15 %)	7 (7 %)
	48 (105)	7 (7 %)	2 (3 %)
	72 (105)	4 (4 %)	2 (3 %)
	144-192 (63)	2 (3 %)	1 (1 %)
High Risk Field			
Pre-sample	NA (21)	4 (19 %)	2 (10 %)
Irrigation	24 (42)	11 (26 %)	10 (24 %)
	48 (42)	11 (26 %)	11 (26 %)
	72 (42)	5 (12 %)	2 (5 %)
	144-192 (21)	0 (0 %)	0 (0 %)
Rain	24 (105)	33 (31 %)	28 (27 %)
	48 (105)	7 (7 %)	3 (3 %)
	72 (105)	3 (3 %)	5 (12 %)
	144-192 (63)	3 (5 %)	1 (16 %)

[a] Event type (i.e., irrigation or rain event) that initiated sample collection.

[b] Time in hours (i.e., 24, 48, 72 or 144-192) since event; NA (not applicable) is used to indicate samples collected before study initiation.

[c] *Listeria* spp. includes *L. monocytogenes.*

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Table 5. Diversity of *Listeria* spp. and *L. monocytogenes* allelic types isolated from soiland water samples collected from spinach fields previously identified as being at high or low risk for *L. monocytogenes* isolation.

Predicted Field Risk	Event Type [a]	*Listeria* spp.[b]		*L. monocytogenes*	
		No. of Allelic Types	Shannon-Weiner Index	No. of Allelic Types	Shannon-Weiner Index
Low	---[c]	18	2.4	7	1.2
	Irrigation	4	0.84	2	0.33
	Rain	16	2.5	6	1.4
High	--- [c]	21	2.0	7	1.1
	Irrigation	8	1.1	4	0.53
	Rain	18	2.2	6	1.2
Surface Water	--- [c]	14	0.85	6	0.99
	Irrigation	4	0.67	3	0.39
	Rain	12	1.1	5	0.60

[a] Event type (i.e., irrigation or rain event) that initiated sample collection.

[b] *Listeria* spp. includes *L. monocytogenes.*

[c] This line includes information for all samples collected from the high risk field, low risk field, or surface water regardless of the event type that initiated collection. Number of allelic types for this line is not a simple summation of the numbers of AT found following irrigation and rain events, as some ATs may have been found following both event types.

Table 6. Final multivariable model for the likelihood of isolating *Listeria* spp. and *L. monocytogenes* from spinach fields based on testing of soil samples and given a cutoff *P*-value of 0.05.

Factor	OR[a]	95% CI[b]	*P-value*
Factors significant for *Listeria* spp.[c]			
Amt of irrigation water (mm) applied to the fields 2 days before sample collection	1.1	1.0, 1.2	0.04
Event type that initiated sample collection			
Irrigation	0.71	0.40, 1.2	0.22
Rain	1.0	-	-
Hours since event occurred			
24 h	7.7	2.9, 20	< 0.01
48 h	2.1	0.74, 6.2	0.16
72 h	2.5	0.94, 6.9	0.07
144-192 h	1.0	-	-
Predicted field risk			
Low	1.0	-	-
High	2.3	1.5, 3.5	< 0.01
Total amt of rain (mm) on day 2 before sample collection	1.4	1.1, 1.8	< 0.01
Factors significant for *L. monocytogenes*			
Amt of irrigation water applied to the fields 2 days before sample collection	1.2	1.1, 1.3	< 0.01
Event type that initiated sample collection			
Irrigation	0.74	0.41, 1.3	0.33
Rain	1.0	-	-
Hours since event occurred			
24 h	25	5.7, 99	< 0.01
48 h	2.5	0.49, 12	0.27
72 h	3.4	0.74, 15	0.11
144-192 h	1.0	-	-
Predicted field risk			
Low	1.0	-	-
High	3.5	2.0, 6.0	< 0.01

[a] For continuous factors, OR refers to the change in the odds of isolating *Listeria* spp. or *L. monocytogenes* associated with a one unit increase in the factor (e.g., a one mm increase in the amount of irrigation water applied).

[b] Confidence interval for odds ratio.

[c] *Listeria* spp. includes *L. monocytogenes.*