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Geographical and Meteorological Factors Associated with Isolation of *Listeria* Species in New York State Produce Production and Natural Environments

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ABSTRACT

Listeria species have been isolated from diverse environments, often at considerable prevalence, and are known to persist in food processing facilities. The presence of *Listeria* spp. has been suggested to be a marker for *Listeria monocytogenes* contamination. Therefore, a study was conducted to (i) determine the prevalence and diversity of *Listeria* spp. in produce production and natural environments and (ii) identify geographical and/or meteorological factors that affect the isolation of *Listeria* spp. in these environments. These data were also used to evaluate *Listeria* spp. as index organisms for *L. monocytogenes* in produce production environments. Environmental samples collected from produce production ($n = 588$) and natural ($n = 734$) environments in New York State were microbiologically analyzed to detect and isolate *Listeria* spp. The prevalence of *Listeria* spp. was approximately 33 and 34% for samples obtained from natural environments and produce production, respectively. Coisolation of *L. monocytogenes* and at least one other species of *Listeria* in a given sample was recorded for 3 and 9% of samples from natural environments and produce production, respectively. Soil moisture and proximity to water and pastures were highly associated with isolation of *Listeria* spp. in produce production environments, while elevation, study site, and proximity to pastures were highly associated with isolation of *Listeria* spp. in natural environments, as determined by randomForest models. These data show that *Listeria* spp. were prevalent in both agricultural and nonagricultural environments and that geographical and meteorological factors associated with isolation of *Listeria* spp. were considerably different between the two environments.

The genus *Listeria* is composed of 15 species, including *L. monocytogenes, L. ivanovii, L. seeligeri, L. welshimeri, L. innocua, L. marthii, L. grayi, L. rocourtiae, L. weihenstephanensis, L. fleischmannii, L. floridensis, L. aquatica, L. cornellensis, L. riparia,* and *L. grandensis (9).* Of the 15 *Listeria* spp., *L. monocytogenes* is a foodborne pathogen that represents a significant burden to public health and accounts for an estimated 1,591 cases of listeriosis, 1,455 hospitalizations, and 255 deaths annually in the United States *(38).* There is an increased concern about *L. monocytogenes* in produce, as a consequence of *L. monocytogenes* outbreaks *(48, 40)* and recalls *(47).* In 2011, there were 147 illnesses, 33 deaths, and one miscarriage due to a *L. monocytogenes* outbreak in cantaloupe *(48).* The remaining species of *Listeria* are generally not considered a concern to public health. For instance, *L. ivanovii* is primarily associated with listeriosis in animals (e.g., sheep) and rarely causes human disease *(49).* In this study, the term *Listeria* spp. refers to all species of *Listeria* (e.g., *L. monocytogenes, L. innocua*); it is explicitly stated when a given statement refers to *Listeria* spp., excluding *L. monocytogenes* (i.e., *Listeria* spp. other than *L. monocytogenes*).

Listeria spp. have been detected in a wide variety of environments, ranging from wilderness areas to retail food establishments *(1, 13, 19, 26, 30, 32, 36).* The range of *Listeria* spp. prevalence in nonagricultural and agricultural environments has been estimated from 3.7 to 81% *(1, 7, 13, 23, 37)* and 5.7 to 51% *(10, 26, 28, 50),* respectively, based on the region of study. Specific environmental factors (e.g., soil moisture and precipitation) may influence the prevalence of *Listeria* spp. *(7, 16, 20, 51, 53).* For example, Ivanek et al. *(20)* observed that *Listeria* spp. were more prevalent in soil samples when it rained two days prior to sample collection. Additional studies *(43, 44, 51, 52)* have observed *L. monocytogenes* to be more prevalent in soil and vegetation samples when soil is moist. Specifically, in one study *(43),* it was predicted that in produce production environments, soils with an available water storage greater than 4 cm (in 0- to 25-cm depth) had a threefold higher

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prevalence of *L. monocytogenes,* compared with less-moist soils. Moisture has also been shown to influence the presence of *Listeria* spp. in food processing plants. Slade *(42)* showed *Listeria* spp. were found more often in processing plant locations in which moisture levels were high. These findings demonstrate the importance of moisture and precipitation on the occurrence of *Listeria* spp. Yet, there are no quantitative data to elucidate if the same or different factors influence the isolation of various *Listeria* spp. in produce production environments.

Listeria spp. detection has often been used to identify conditions that may indicate the presence of *L. monocytogenes*. There has been some confusion over the use of the terms indicator, index, and surrogate organisms in food safety microbiology. Indicator organisms are commonly defined as markers whose presence relate to the general microbiological condition of the food or environment (i.e., hygienic quality), while index organisms are commonly defined as markers whose presence relates to the possible occurrence of ecologically similar pathogens *(4, 21, 29, 33)*. Surrogate organisms are commonly defined as nonpathogenic organisms that correlate with the behavior (e.g., growth and survival) of specific pathogens *(4, 21, 29, 33)*. According to Kornacki *(21),* the term indicator organism has often been used to refer to (i) index organisms, (ii) indicators of hygiene and sanitation on equipment and surfaces, (iii) process controls of spoilage or potential spoilage, and also (iv) surrogate organisms in the context of critical control point validation. As a result, the terms have been used interchangeably throughout the literature. In this article, the term index organism is used in the ''Discussion,'' where the relationship between the occurrence of nonpathogenic *Listeria* spp. and *L. monocytogenes* (ecologically similar pathogen) is examined. Few studies *(1, 13, 20)* have focused on *Listeria* spp. prevalence and its association to geographical and meteorological factors in natural environments, and to the authors' knowledge, there have been no studies that have focused on the ecology and prevalence of *Listeria* spp. (excluding *L. monocytogenes*) in produce production environments. The purpose of this study was to gain a more complete understanding of the ecology of *Listeria* spp. in produce production and natural environments. Specifically, the objectives of this study were to (i) determine the prevalence and diversity of *Listeria* spp. in produce production and natural environments, (ii) identify geographical and meteorological factors associated with isolation of *Listeria* spp. in produce production and natural environments, and (iii) evaluate the application of *Listeria* spp. as index organisms for *L. monocytogenes* in the produce production environment.

MATERIALS AND METHODS

Description of study data. Data were assembled for this study using two field study data sets: one published *(43)* and one unpublished. Only data on *L. monocytogenes* in produce production environments has been previously reported *(43)*; data on nonpathogenic *Listeria* spp. have not been reported previously. Furthermore, the analyses (e.g., randomForest [RF]) and discussion (e.g., *L. monocytogenes* as a marker for *Listeria* spp.) that relates to *L. monocytogenes* in this current article are distinct from previously reported analyses *(43)*. These two data sets were collected to determine the prevalence of *Salmonella,* Shiga toxin–producing *Escherichia coli,* and *L. monocytogenes* in produce production and natural environments. In addition, these two data sets were also used to identify geographical and/or meteorological factors that influenced pathogen prevalence in the produce production *(43)* or natural environment. In the study reported here, these previously collected samples were used, along with previously retrieved geographical and meteorological data, to examine the (i) prevalence of *Listeria* spp. and (ii) geographical and/or meteorological factors that affect *Listeria* spp. prevalence in produce production and natural environments.

A total of 1,322 samples were collected between the two studies; global positioning system coordinates for each sample location were recorded, providing georeferenced samples. Briefly, from produce production environments, 588 georeferenced samples (178 composite soil, 175 drag swab, 174 water, and 61 wildlife and domesticated animal fecal samples) were collected over a 2-year period (summer, fall, winter, and spring of 2009 to 2011) from five produce farms across New York State (NYS). Samples were obtained from four fields within each of the five farms. From natural environments, 734 georeferenced samples (90 composite soil, 90 drag swab, 90 water, and 449 wildlife fecal samples) were collected over a 2-year period (spring, summer, and fall 2009 to 2010) from five natural sites across NYS. Natural sites were defined as undeveloped locations, with minimal human presence that provided a habitat for wildlife (e.g., national forests, wildlife refuges). Samples were obtained from three distinct sampling zones within each of the five natural sites.

Sample collection and preparation. Samples obtained in both studies were collected using sample collection protocols previously described *(43)*. Briefly, latex gloves (Nasco, Fort Atkinson, WI) were worn and changed between each sample field per zone. Five soil samples (per field per site) were collected approximately 15 cm below the topsoil surface using sterile scoops (Fisher Scientific, Hampton, NH) and deposited into sterile Whirl-Pak bags (Nasco). One drag swab was collected at each field or zone; these swabs were tied to sterile string and dragged across the sample area for at least 10 min. Water samples were collected (250 ml directly into a sterile Whirl-Pak bag or jar) from surface (e.g., creek, pond) and engineered (e.g., well, municipal) water sources, when available, closest to each field and zone. Fecal samples were collected when observed in produce fields, and an effort was made to collect 15 fecal samples at each natural site. All samples were transported on ice, stored at $4 \pm 2^{\circ}C$, and processed within 24 h.

Five grams from each of the five soil samples collected in a given field or zone were pooled in a sterile filter Whirl-Pak bag to form one 25-g composite sample per field per zone; this composite soil sample was analyzed and represented the sample unit for all analyses. Each drag swab was hand massaged and then squeezed to remove the liquid. A 10-ml aliquot of the liquid was then aseptically transferred to a new sterile filter Whirl-Pak bag. Water samples were analyzed using the Environmental Protection Agency standard methods *(11, 12)*. Water samples were passed through a 0.45-μm filter unit (Nalgene, Rochester, NY), and a third of the filter was aseptically transferred to a sterile Whirl-Pak bag for *Listeria* enrichment. Each fecal sample was weighed, and 10 g of each fecal sample was deposited into a sterile Whirl-Pak filter bag for *Listeria* testing as detailed in the following.

***Listeria* spp. detection and confirmation.** *Listeria* spp. detection and isolation was performed using a modified version of the U.S. Food and Drug Administration's *Bacteriological Analytical Manual,* as previously described *(30, 32, 36, 37).* Controls were processed in parallel with test samples. *L. monocytogenes* (FSL R3-001; *(35)*) and uninoculated buffered *Listeria* enrichment broth (BD, Franklin Lakes, NJ) were used as the positive and negative control, respectively. Environmental samples were mixed with buffered *Listeria* enrichment broth (225 ml for composite soil samples and 90 ml for drag swab, water, or fecal samples) to enrich *Listeria* spp. Enrichments were incubated at 30 ± 2°C for 48 h, with the addition of the selective supplement (Oxoid, Cambridge, UK) at 4 h. At 24 and 48 h, 50 μl of each enrichment was streaked onto modified Oxford agar (BD), which was subsequently incubated for 48 h at 30 ± 2°C. Up to four presumptive *Listeria* spp. colonies were substreaked to *L. monocytogenes* plating medium (Biosynth International, Itasca, IL) and incubated for 48 h at 35 ± 2°C. On *L. monocytogenes* plating medium, white colonies (representing presumptive nonpathogenic *Listeria*) and blue colonies (representing presumptive pathogenic *Listeria*) were substreaked to brain heart infusion agar (BD), which was subsequently incubated at 37 ± 2°C for 24 h. Presumptive *Listeria* spp. colonies (maximum four colonies per sample) were confirmed by amplification of the *sigB* gene by PCR. *Listeria* spp. identification was performed by sequencing *sigB* (Sanger sequencing performed by the Cornell University Life Sciences Core Laboratories Center) and comparison of sequences to an internal reference database using BLASTN, as previously described *(5, 8, 31). Listeria sigB* allelic types (AT) were also assigned to each isolate. Isolates were preserved at −80°C in 15% glycerol, and information on each isolate can be found at www.FoodMicrobeTracker.com.

Data on geographical and meteorological factors. Data were previously retrieved for each sample location and sample collection date as described by Strawn et al. *(43).* Data on geographical factors were obtained from the U.S. Geological Survey EarthExplorer (http://earthexplorer.usgs.gov/), U.S. Department of Agriculture and Soil Survey Geographic database (http://soils.usda.gov/survey/geography/ssurgo/), and Cornell University Geospatial Information Repository (http://cugir.mannlib.cornell.edu/) using the Geographical Resources Analysis Support System (http://grass.osgeo.org). Data on meteorological factors were obtained from the National Oceanic and Atmospheric Administration's National Climate Data Center Local Climatology Database (http://gis.ncdc.noaa.gov/map/lcd/). Further details on the geographical and meteorological factors obtained for each environment are provided in supplemental material (S1; http://foodsafety.foodscience.cornell.edu/research-and-publications/supplementary-materials-manuscripts/2014).

Categorical analysis. Univariate associations between *Listeria* spp.–positive samples and farm per site, season, and sample type were performed by a chi-square test. Confidence intervals (95%) were calculated for each variable assuming a binomial distribution. In addition, associations between samples testing positive for *Listeria* spp. (excluding *L. monocytogenes*) and samples testing positive for *L. monocytogenes* were evaluated with a chi-square test (separately for produce and natural environments). *P* values less than 0.05 were considered significant. The diversity of ATs within environment was quantified using Simpson's index of diversity (*D*) *(41).* All categorical analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC).

RF analysis. RF analysis is a nonparametric statistical tool, which was used here to determine which geographical and/or meteorological factors were highly associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* in produce production and natural environments. The outcome for each RF model was binary (yes/no) based on whether the target *Listeria* spp. (e.g., *L. innocua, L. seeligeri*) was isolated from a sample. Predictor variables for each RF were the 14 geographical and/or meteorological factors based on location of sample or collection date of sample. A limitation of this analysis is that RF models do not specify the directionality of association (e.g., RF would not determine whether the likelihood of *L. innocua* isolation increases or decreases with proximity to pastures). RF models were performed using the RF package in R 2.13.1 *(22)* and using the following criteria: 10,000 bootstrap iterations (with replacement) and four randomly selected predictor variables for each split. The misclassification rate was calculated for each RF model using out-of-bag samples. Out-of-bag samples (approximately one-third of data set) are withheld from each RF model and used to test the RF predictions (cross-validation). Variable importance (VI) scores were calculated for each factor in each RF model. Briefly, factor values are randomly permuted for each tree, and the VI score represents the amount of prediction lost *(22).* Forty-nine of the 734 samples collected in natural environments were excluded from RF analysis because of missing data.

Bar graphs were used to illustrate the most important factors associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* species in produce production and natural environments. The average VI score (for all 10,000 trees) for each factor was plotted for *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri.* A large VI score signifies a factor that was highly associated with isolation of the respective species of *Listeria*.

RESULTS

Prevalence of *Listeria* spp. in produce production and natural environments. In produce production environments, the prevalence of *Listeria* spp. was 34% (201 of 588; Table 1). Nearly 15% of samples were positive for *L. monocytogenes,* as previously reported *(43),* while approximately 28% of samples were positive for at least one species of *Listeria* (excluding *L. monocytogenes*; Table 1). Approximately 9% of samples were positive for both *L. monocytogenes* and at least one other species of *Listeria* (51 of 588). Farm, season, and sample type were found to be significantly ($P \leq 0.05$) associated with the isolation of *Listeria* spp. (excluding *L. monocytogenes*), as determined by chi-square tests (Table 2). Farms 1, 3, and 5 each had a significantly higher prevalence (36, 32, and 42%, respectively) of *Listeria* spp. (excluding *L. monocytogenes*), compared with farms 2 and 4 (each 12%; Table 2). The prevalence of *Listeria* spp. (excluding *L. monocytogenes*) was significantly higher in winter (41%), compared with fall and summer (24 and 22%, respectively; Table 2). Both winter sampling visits (2010 and 2011) yielded the highest prevalence of *Listeria* spp. (excluding *L. monocytogenes*), compared with all other sampling visits (2009 to 2011). Water samples had a significantly higher prevalence of *Listeria* spp. (excluding *L. monocytogenes;* 51%) compared with composite soil, drag swab, and fecal samples (17, 21, and 16%, respectively; Table 2). All *Listeria* spp. (excluding *L. monocytogenes*)

TABLE 1. *Frequency of positive* Listeria *spp. (by genus and each species of* Listeria*) samples obtained from produce production (*n = *588) and natural (*n = *734) environments*

	Environment	
	Produce production	Natural
Listeria spp. (samples positive for any species in the genus *Listeria*)	201	245
L. monocytogenes (samples positive for *L. monocytogenes* with or without coisolation of at least one species)	88[a]	59
L. innocua (samples positive for *L. innocua* with or without coisolation of at least one species)	81	13
L. seeligeri (samples positive for *L. seeligeri* with or without coisolation of at least one species)	45	129
L. welshimeri (samples positive for *L. welshimeri* with or without coisolation of at least one species)	60	117
L. marthii (samples positive for *L. marthii* with or without coisolation of at least one species)	0	5

[a] Data on *L. monocytogenes*–positive samples from produce production environments have been previously reported in Strawn et al. *(43)*.

positive water samples (88 of 174) were from surface water (e.g., ponds, creeks, or ditches). Of the 28 samples collected from engineered water (e.g., wells, municipal), none were positive for any species of *Listeria*.

In natural environments, the prevalence of *Listeria* spp. was 33% (245 of 734; Table 1). *L. monocytogenes* was isolated from nearly 8% of samples, as previously reported *(6),* whereas at least one species of *Listeria* (excluding *L. monocytogenes*) was isolated from approximately 28% of samples (Table 1). Approximately 3% of samples were positive for both *L. monocytogenes* and at least one other species of *Listeria* (18 of 734). Study site (e.g., national forests, wildlife refuges) and season were found to be significantly ($P \leq 0.05$) associated with the isolation of *Listeria* spp. (excluding *L. monocytogenes*; Table 2). Study site 1 had a significantly higher *Listeria* spp. (excluding *L. monocytogenes*) prevalence (47%) than study sites 2, 3, 4, and 5 (prevalence of 15, 30, 26, and 20%, respectively; Table 2). The prevalence of *Listeria* spp. (excluding *L. monocytogenes*) was significantly higher in summer (37%), compared with fall and spring (each 23%; Table 2). No sampling visits were conducted in winter for natural environments, as many areas within the study sites are not accessible (due to snow and ice conditions) during the winter months in NYS. No significant difference was observed in the prevalence of *Listeria* spp. (excluding *L. monocytogenes*) among sample type (Table 2).

Diversity of *Listeria* spp. in produce production and natural environments. The 164 *Listeria* spp.–positive

TABLE 2. *Effect of factors (farm/site, season, and sample type) on the frequency of positive* Listeria *spp. (excluding* L. monocytogenes*) samples detected in produce production and natural environments*

	Produce production environment		Natural environment	
Factor (category)	No. samples	Frequency (%)[a]	No. samples	Frequency (%)
Farm/site[b]				
1	166	60 (36) A	152	71 (47) A
2	103	12 (12) B	145	22 (15) C
3	113	36 (32) A	146	44 (30) B
4	100	12 (12) B	148	39 (26) BC
5	106	44 (42) A	143	28 (20) BC
Season				
Fall	136	33 (24) B	245	57 (23) B
Winter	125	51 (41) A	NC[c]	—[d]
Spring	134	37 (28) AB	241	55 (23) B
Summer	193	43 (22) B	248	92 (37) A
Sample type				
Composite soil	178	30 (17) B	90	28 (31)
Drag swab	175	36 (21) B	90	32 (36)
Fecal	61	10 (16) B	449	119 (27)
Water	174	88 (51) A	90	20 (22)
Sediment	NC	—	15	5 (33)
Total	588	164 (28)	734	204 (28)

[a] Different letters represent values that are significantly different ($P < 0.05$). No letters represent values that are not significantly different.
[b] Farm and site codes were arbitrary and do not imply spatial proximity. Sites 1, 2, 3, 4, and 5 represent Connecticut Hill Wildlife Management Area, Finger Lakes National Forest, Montezuma National Wildlife Refuge, Catskill Forest Preserve, and Adirondack Forest Preserve, respectively. Produce farm names are confidential.
[c] NC, samples not collected.
[d] —, frequency (%) not calculated; no samples were collected.

samples from produce production environments yielded 426 *Listeria* isolates (excluding *L. monocytogenes*). All isolates were assigned a *sigB* AT. If isolates from the same sample were classified as the same AT, then only one representative isolate was selected for inclusion in the subsequent analyses. The 426 isolates resulted in 186 representative *Listeria* spp. isolates (excluding *L. monocytogenes*). Of the 186 representative isolates, 81, 45, and 60 isolates were identified as *L. innocua, L. seeligeri,* and *L. welshimeri,* respectively (Table 1). The frequency of *L. innocua* isolation was significantly higher than the frequency of *L. seeligeri* isolation in produce production environments. There was a high diversity of ATs within each *Listeria* spp. ($D = 0.91$, 0.87, and 0.88 for *L. innocua, L. seeligeri,* and *L. welshimeri,* respectively). There were 22, 10, and 18 different ATs identified for *L. innocua* ($n = 81$), *L. seeligeri* ($n = 45$), and *L. welshimeri* ($n = 60$), respectively. *L. innocua* AT 26 ($n = 19$), *L. seeligeri* AT 12 ($n = 11$), and *L. welshimeri* AT 27 ($n = 18$) were the most common ATs.

The 204 *Listeria* spp.–positive samples from natural environments yielded 504 *Listeria* isolates (excluding *L. monocytogenes*). The 504 *Listeria* spp. isolates (excluding *L. monocytogenes*) resulted in 264 representative isolates for inclusion in the subsequent analyses. Of the 264 representative isolates, 13, 5, 129, and 117 were identified as *L. innocua, L. marthii, L. seeligeri,* and *L. welshimeri,* respectively (Table 1). *L. marthii* was not included in analyses because it was isolated from only one natural site in a low frequency (5 of 734; 0.7%). The frequencies of *L. seeligeri* and *L. welshimeri* isolation were significantly higher than the frequency of *L. innocua* isolation in natural environments. There was a high diversity of ATs within each *Listeria* species ($D = 0.87$, 0.88, and 0.84 for *L. innocua, L. seeligeri,* and *L. welshimeri,* respectively). There were 7, 14, and 19 different ATs identified for *L. innocua* ($n = 13$), *L. seeligeri* ($n = 129$), and *L. welshimeri* ($n = 117$), respectively. *L. innocua* AT 23 ($n = 4$), *L. seeligeri* AT 3 ($n = 26$), and *L. welshimeri* AT 27 ($n = 40$) were the most common ATs.

Geographical and meteorological factors associated with isolation of *Listeria* spp. in produce production and natural environments. In produce production environments, three factors (soil moisture, proximity to water, and proximity to pastures) were strongly associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri.* These three factors were each ranked in the top five VI scores for the respective species of *Listeria* among all 14 geographical and meteorological factors included in RF models (Fig. 1, 1A through 1D; grey bars). No other factors were ranked in the top five VI scores in each the *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* RF model; however, some factors were common to two or three species of *Listeria*. Specifically, proximity to forests was associated with isolation of *L. innocua, L. seeligeri,* and *L. welshimeri,* as determined by VI scores (rank 5, 2, and 3, respectively; Fig. 1, 1B through 1D). Proximity to impervious surfaces was associated with isolation of *L. monocytogenes* and *L. seeligeri* (rank 3 and 5, respectively; Fig. 1, 1A and 1C). Three factors (temperature, drainage class, and slope) were each highly associated with isolation of only *L. monocytogenes,* only *L. innocua,* and only *L. welshimeri,* respectively, as determined by VI scores (Fig. 1, 1A, 1B, and 1D).

In natural environments, three factors (proximity to pastures, elevation, and study site) were strongly associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri.* These factors were ranked in the top five VI scores for the respective species of *Listeria* among the 14 geographical and meteorological factors included in RF models (Fig. 1, 2A through 2D; grey bars). No other factors were ranked in the top five VI scores in each the *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* RF model; however, four factors (slope, water table depth, and proximity to water and impervious surfaces) were common to two species of *Listeria.* Slope was associated with isolation of *L. monocytogenes* and *L. seeligeri,* as determined by VI scores (rank 1 and 5, respectively; Fig. 1, 2A and 2C). Water table depth was associated with isolation of *L. innocua* and *L. welshimeri* (both rank 5; Fig. 1, 2B and 2D). Proximity to water was associated with isolation of *L. monocytogenes* and *L. innocua* (rank 5 and 4, respectively; Fig. 1, 2A and 2B). Finally, proximity to impervious surfaces was associated with isolation of *L. seeligeri* and *L. welshimeri* (rank 1 and 4, respectively; Fig. 1, 2C and 2D).

DISCUSSION

The goal of this study was to investigate the prevalence of *Listeria* spp. in two distinct environments in NYS and to evaluate associations between the isolation of different species of *Listeria* and geographical and/or meteorological factors. This study also provides data on the distribution and diversity of *Listeria* subtypes in the produce production environment (where field collected data are rare). The prevalence of *Listeria* spp. was similar in the produce production and natural environments sampled; however, the prevalence of *L. monocytogenes* was considerably higher in produce production environments compared with natural environments. Of 14 factors, only proximity to pastures was highly associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* in both produce production and natural environments, suggesting an important role of pastures as a source of *Listeria* spp. The pasture landscape factor represented livestock pens, active pasturages, and or hay grass fields. The data collected also show that the predominant species of *Listeria* differed between produce production and natural environments and that *Listeria* spp. isolation was influenced by different environmental factors in these two environments. Sampling locations were in NYS; hence, further studies are needed to determine if one can extrapolate the findings reported here to other regions.

***Listeria* spp. are found at a high prevalence in produce production and natural environments, but distribution of species is dependent on ecological niche.**

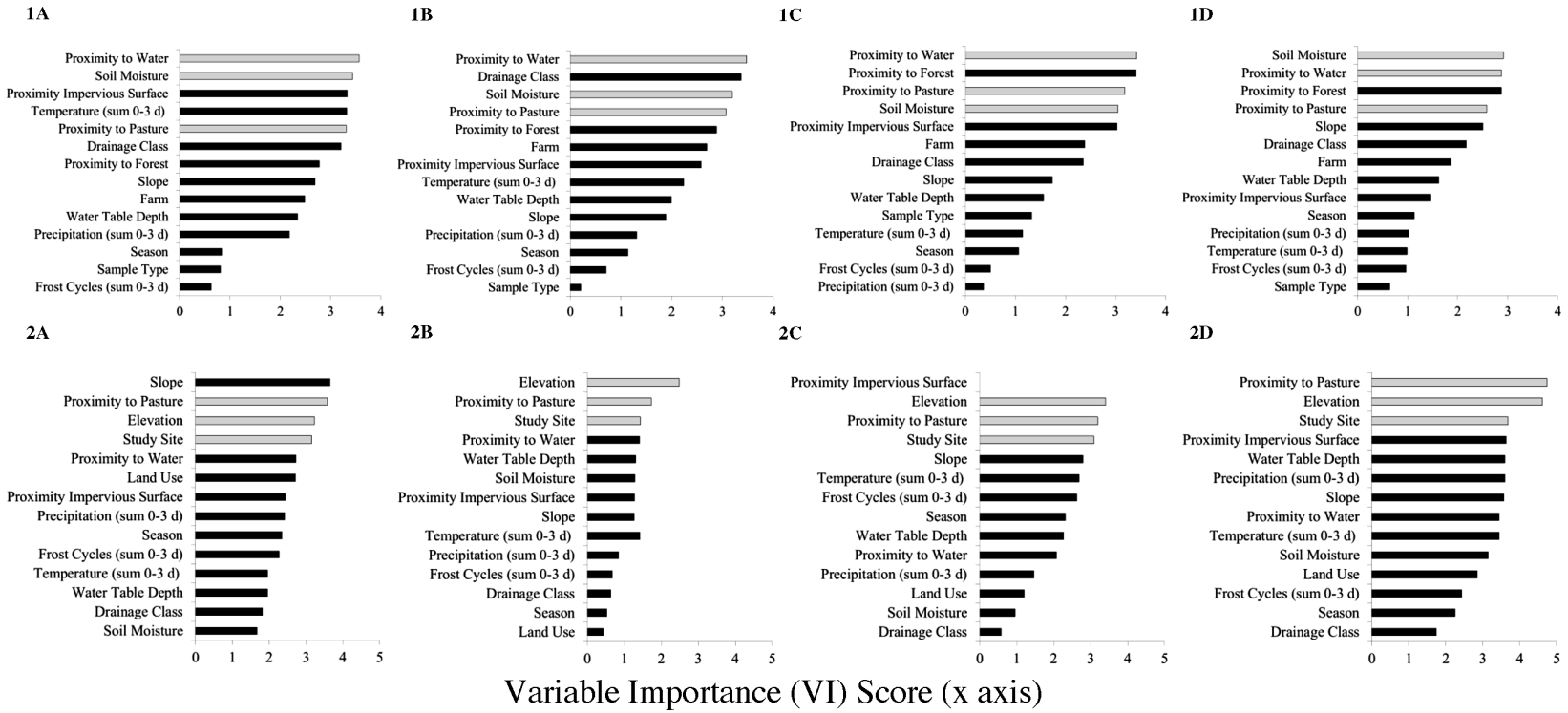

FIGURE 1. *Bar graphs depicting variable importance (VI) scores for each geographical and meteorological factor based on RF analysis of samples obtained from produce production and natural environments. For each bar graph, the* x *axis represents the VI scores, and the* y *axis represents the factors. The top row of bar graphs labeled 1 (A through D) represent the produce production environment, while the bottom row of bar graphs labeled 2 (A through D) represent the natural environment. Letters A through D represent the four species of* Listeria *detected: A,* L. monocytogenes, *B,* L. innocua, *C,* L. seeligeri, *and D,* L. welshimeri. *The VI scores show the loss of predictive power that occurred (over the 10,000 bootstrap iterations) when factor values were randomized for model validation. Geographical and/or meteorological factors with the largest VI score (positioned at the top of each bar graph) are the factors most associated with isolation of the respective species of* Listeria. *Grey bars represent factors of considerable importance (defined as factors ranked in the top five highest VI scores for each species of* Listeria*) for either the produce production or the natural environment.*

Approximately one of every three samples tested for each environment in this study was *Listeria* positive, with most species identified as nonpathogenic *Listeria*. Previous studies *(7, 13, 23, 37, 50)* have shown that the prevalence of *Listeria* spp. can be high (often >20%) in other nonprocessing environments. For example, a 22% prevalence of *Listeria* spp. was observed in samples (e.g., sponge swabs of vending machines, sidewalks, railings) obtained from urban environments *(37)*. In another study, an 81% prevalence of *Listeria* spp. was reported in water samples from an estuarine environment *(7)*. In the two previously mentioned studies, nonpathogenic species of *Listeria* were detected and isolated in greater frequencies than *L. monocytogenes*. The study reported here found the highest prevalence of *Listeria* spp. (excluding *L. monocytogenes*; 51%) in water samples from produce production environments. All of these positive samples were from surface water (e.g., sample from a pond, creek, or ditch) collected near sampled produce fields. Conversely, in natural environments, the study reported here found the highest prevalence of *Listeria* spp. (excluding *L. monocytogenes*) in topsoil (drag swab samples) and the lowest prevalence in water samples. Similar findings were reported in one previous study of NYS forest and wildlife refuges, in this prior study, a higher prevalence of *Listeria* spp. were observed in soil and vegetation samples, compared with water samples *(20)*. Our data reported here suggest surface water in agricultural environments is more likely to be positive for *Listeria* spp. (excluding *L. monocytogenes*) than in nonagricultural environments. Further research is needed to identify potential sources and vehicles (e.g., runoff from livestock operations), that lead to contamination of surface waters in agricultural environments (e.g., produce fields).

Additionally, the species of *Listeria* isolated was highly dependent on the specific environment. Here, *L. monocytogenes* and *L. innocua* were the most prevalent species in produce production environments, whereas *L. seeligeri* and *L. welshimeri* were the most prevalent species in natural environments. Other studies have also shown specific species of *Listeria* to be more common in certain ecological niches *(1, 16, 20, 37)*. For example, *L. marthii* has only been isolated from a distinct area in NYS *(16)*. Interestingly, in the study reported here, *L. marthii* was isolated only in the same area in NYS, where this species was isolated previously *(16)*; *L. marthii* was not isolated from other sampling areas. Consistent with the data reported here for natural environments, one previous study *(37)* found *L. seeligeri* and *L. welshimeri* to be overrepresented among *Listeria* isolates obtained from NYS forest and wildlife refuges. Additionally, in this same study *(38), L. monocytogenes* and *L. innocua* were isolated more frequently from samples collected in urban environments. Similarly, in the study reported here, *L. monocytogenes* and *L. innocua* were also isolated more frequently from samples collected in produce production environments. The *Listeria* spp. diversity isolated in urban and produce production environments thus seems to be more similar to each other as compared with *Listeria* spp. diversity isolated in natural environments, at least for NYS. Overall, these findings are consistent with others, e.g., Gray et al. *(17)* and Sauders et al. *(37),* who also reported distinct populations of *Listeria* in different environments, foods, and/or hosts.

Geographical and meteorological factors associated with isolation of *Listeria* spp. were different between produce production and natural environments. Most factors found to be associated with the isolation of *Listeria* spp. were considerably different between the two sampled environments. Only one factor, proximity to pastures, was identified to be highly associated with the isolation of *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* across both environments. These data suggest that different ecological factors or contamination sources are affecting the isolation of certain species of *Listeria* in produce production and natural environments.

In the produce production environment, soil moisture was shown to be highly associated with the isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri*. Soil properties (soil moisture and loam percentage) have been previously shown to influence the isolation of *Listeria* spp. in soil and vegetation samples from nonagricultural environments *(20, 52)*. Interestingly, in the study reported here, soil moisture was not associated with the isolation of any species of *Listeria* in natural environments. In addition to soil moisture, proximity to water was also highly associated with isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri* in produce production environments. This finding is consistent with other studies *(3, 25, 44, 52)* that have demonstrated water to be an important reservoir for *L. monocytogenes* in both agriculture and nonagriculture environments. Moreover, the data reported here show that water is also an important reservoir for other species of *Listeria* and, subsequently, may contribute to *Listeria* spp. presence in produce production environments.

In the natural environment, elevation and study site were observed to be highly associated with the isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri*. These results are consistent with other previously reported data *(20, 37)*. Ivanek et al. *(20)* identified geographic position of a sampling location (e.g., distance from the equator) as an important factor for the occurrence of a *Listeria* spp.–positive sample from natural environments, as determined by classification tree analysis. Additionally, Sauders et al. *(37)* showed certain species of *Listeria* were highly associated with specific sample sites, such as *L. seeligeri* in the Connecticut Hill Wildlife Management area in NYS. Combined, these data suggest geographic factors specific to location of sample collection are influential predictors of *Listeria* spp. isolation in NYS forests and wildlife refuges.

In both produce production and natural environments, proximity to pastures was found to be highly associated with the isolation of each *L. monocytogenes, L. innocua, L. seeligeri,* and *L. welshimeri*. Several studies *(24, 36, 39)* have found previously that proximity to pastures may be associated with an increased *Listeria* spp. prevalence in nonagricultural environments (e.g., watersheds). Lyautey

et al. *(25)* observed a strong association between proximity of dairy farms and the isolation of *L. monocytogenes* from water samples obtained in a Canadian watershed. The prevalence of *L. monocytogenes* in environmental samples collected from livestock animal operations (e.g., soil, water troughs, bedding, and feedstuff) in two studies *(27, 30),* both conducted in NYS, was estimated at 24 and 46%. The data reported here provide evidence that livestock and/or livestock pasture areas may be reservoirs for other species of *Listeria* as well, and potential sources of contamination in both agriculture and nonagriculture environments.

Detection of *Listeria* spp. in produce production environments may not be an effective strategy to predict *L. monocytogenes* contamination in produce fields. The data set collected here also allowed for assessment of whether *Listeria* spp. could be used as index organisms for *L. monocytogenes* in produce production environments. The use of *Listeria* spp. as index organisms was evaluated in the context of three previously established criteria *(45, 46)*: (i) the index organism should have a higher prevalence than the target pathogen, (ii) the detection of the index organism should have a reasonably strong correlation with the detection of the target pathogen, and (iii) testing for detection of the index organism should be more rapid and/or more cost effective than for the target pathogen.

In this study, *Listeria* spp. were detected in nearly 30% of samples obtained from produce production environments, where half of those samples were confirmed to be positive for *L. monocytogenes* (only 6% of samples were positive solely for *L. monocytogenes*). *Listeria* thus fulfils the first criterion of an acceptable index organism, i.e., that the index organism should have a higher prevalence than the target pathogen. Additionally, previous studies *(1, 23, 26, 28, 37)* have consistently observed a higher prevalence of nonpathogenic species of *Listeria* as compared with *L. monocytogenes*. Interestingly, the prevalence of *L. monocytogenes* in this study (15%) was considerably higher than other pathogenic organisms of concern in produce, such as *Salmonella* or Shiga toxin–producing *Escherichia coli (15, 43, 44, 54)*. The observation of a high *L. monocytogenes* prevalence in NYS may reduce the need for an index organism, as index organisms are commonly used when the prevalence of ecologically similar pathogens is low *(21, 29, 33)*. Studies are needed to address the prevalence of *L. monocytogenes* and nonpathogenic species of *Listeria* in other produce-growing regions to establish the need for index organisms there. For example, in regions where *L. monocytogenes* prevalence is low, *Listeria* spp. may function as a useful index organism.

In this study, 3 of 14 geographical factors (soil moisture, proximity to pastures, and proximity to water) were highly associated with the isolation of pathogenic (*L. monocytogenes*) and nonpathogenic (*L. innocua, L. seeligeri,* and *L. welshimeri*) *Listeria* in produce production environments. This finding suggests that certain environmental conditions increase the likelihood for isolation of both selected nonpathogenic *Listeria* and *L. monocytogenes*. This is further supported by the data reported here that showed *L. monocytogenes* was more likely isolated from a sample positive for nonpathogenic *Listeria* compared with a sample negative for nonpathogenic *Listeria* in produce production environments, while this did not hold true for natural environments. Previous field studies *(20, 37, 53, 54)* on *Listeria* spp. in nonagricultural environments (e.g., natural, urban environments) have not shown a strong association between the detection of nonpathogenic species of *Listeria* and *L. monocytogenes*. Although some other studies *(45, 46)* have suggested that *Listeria* spp. may be reliable as an index organism for *L. monocytogenes* in processing environments, a recent study *(2)* in smoked fish plants suggests limited value of *Listeria* spp. as an index organism for *L. monocytogenes*. Importantly, *Listeria* spp. differ considerably in their metabolic capabilities (e.g., carbohydrate utilization); for example *L. innocua* shows similar carbohydrate utilization patterns to *L. monocytogenes* and may be a better index organism for *L. monocytogenes* than *Listeria* spp. that differ considerably in their carbohydrate utilization patterns from *L. monocytogenes (34)*. Further research is needed to fully address the strength of association between *Listeria* spp. and *L. monocytogenes* in various environments to evaluate whether this second index organism criterion (strong association between index organism and target pathogen) is met in different environments. Association between isolation of *Listeria* spp. and *L. monocytogenes* may also differ depending on which *Listeria* spp. (e.g., *L. innocua* versus more divergent *L. seeligeri*) are present in a given environment *(9, 14)*.

Both traditional and molecular methods can be used to test for *Listeria* in the environment. Traditional methods of *Listeria* detection and isolation require a series of biochemical tests for species differentiation; thus, there is a difference in time and cost to identify *Listeria* spp. versus *L. monocytogenes*. The identification of *Listeria* spp. using traditional methods is more rapid and economical, as it requires fewer biochemical tests to confirm a sample as positive for *Listeria* (genus) versus positive for a specific species of *Listeria (18, 42)*. Most molecular detection methods are targeted to specific organisms, and there is typically no difference in time or cost to identify *Listeria* spp. versus *L. monocytogenes*. Although the third criterion of an acceptable index organism is thus fulfilled when using traditional methods, use of molecular methods may be more suitable for use in the produce industry due to the short shelf life of many produce commodities.

In the context of the three previously established criteria, *Listeria* spp. are generally appropriate index organisms for *L. monocytogenes*; however, application of *Listeria* spp. as index organisms for *L. monocytogenes* in the produce production environment is dependent on location (e.g., NYS, elsewhere) and or detection method (e.g., traditional, molecular). The findings reported here suggest limited value to the application of *Listeria* spp. as index organisms for *L. monocytogenes* in NYS produce production environments. Depending on the regulatory environment, testing directly for *L. monocytogenes* may be more appropriate due to the relatively high prevalence of

this organism, compared with other pathogens (e.g., *Salmonella*), at least in some environments. Further research is needed to evaluate the application of *Listeria* spp. as index organisms of *L. monocytogenes* in produce production environments in other regions of the United States and elsewhere, as *Listeria* spp. (both nonpathogenic species of *Listeria* and *L. monocytogenes*) prevalence differs considerably by region *(1, 7, 10, 13, 23–26, 28, 37, 50).*

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