Risk Factors Associated with \textit{Salmonella} and \textit{Listeria monocytogenes} Contamination of Produce Fields

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Abstract

Identification of management practices associated with preharvest pathogen contamination of produce fields is crucial to the development of effective Good Agricultural Practices (GAPs). A cross-sectional study was conducted to (i) determine management practices associated with a *Salmonella* or *Listeria monocytogenes* positive field and (ii) quantify the frequency of these pathogens in irrigation and non-irrigation water sources. Over five weeks, 21 produce farms in New York State were visited. Field-level management practices were recorded for 263 fields, and 600 environmental samples (soil, drag swab, and water) were collected and analyzed for *Salmonella* and *L. monocytogenes*. Management practices were evaluated for their association with the presence of a pathogen-positive field. *Salmonella* and *L. monocytogenes* were detected in 6.1% and 17.5% of fields (n=263), and 11% and 30% of water samples (n=74), respectively. The majority of pathogen-positive water samples were from non-irrigation surface water sources. Multivariate analysis showed that manure application within a year increased the odds of a *Salmonella*-positive field (odds ratio [OR] 16.7), while presence of a buffer zone had a protective effect (OR 0.1). Irrigation (within 3 days of sample collection, OR 6.0), reported wildlife observation (within 3 days of sample collection, OR 6.1), and soil cultivation (within 7 days of sample collection, OR 2.9) all increased the likelihood of an *L. monocytogenes*-positive field. Our findings provide new data that will assist growers with science-based evaluation of their current GAPs and implementation of preventive controls that reduce the risk of preharvest contamination.
Introduction

Produce commodities have been estimated to account for an estimated 46%, 38% and 23% of foodborne illnesses, hospitalizations and deaths in the United States (US), respectively (1). The fact that produce commodities are often consumed raw or with minimal processing likely contributes to the risk of foodborne disease associated with produce. *Salmonella* and *Listeria monocytogenes* are two bacterial foodborne pathogens that represent a substantial burden to the produce industry. Produce-borne *Salmonella* outbreaks have been responsible for a considerable number of foodborne illness cases (2-6). For example, a *Salmonella* outbreak in 2005, associated with tomatoes, resulted in 459 illnesses across 21 US states (7). In 2008, an outbreak of *Salmonella*, linked to jalapeno peppers, sickened approximately 1500 individuals from 43 states, the “District of Colombia” and Canada; this became the largest known outbreak of foodborne illness in the US within the past decade (8). *L. monocytogenes* was responsible for a 2011 produce-borne outbreak, in the US, with 147 illnesses, 33 deaths and 1 miscarriage, due to consumption of cantaloupe (9). In addition, a considerable number of produce recalls have occurred in the past three years as a result of *L. monocytogenes* contamination (e.g., spinach, lettuce (10)). Both *Salmonella* and *L. monocytogenes* can contaminate, persist, and amplify at any point along the farm-to-fork continuum from production to consumption; therefore, minimizing the risk of contamination by these pathogens throughout the supply chain is essential to reducing foodborne illness risks (11-13).

The risk of produce contamination can be reduced by controlling for conditions that favor pathogen introduction and growth in the preharvest environment. Preharvest produce safety is complicated by the fact that each farm has a distinct combination of environmental risk factors (e.g., topography, land-use interactions, and climate). Combinations of these environmental
factors influence the frequency and transmission of foodborne pathogens, and subsequently impact the risk of produce contamination (14). Mitigating contamination risks from environmental factors may be complex and challenging (e.g., as it is difficult to modify farm landscapes); however, modifying management practices to minimize contamination risks may be a more achievable approach. Eighty-nine percent of growers in the US have already reported implementing at least one on-farm food safety measure due to pressure from auditors, inspectors, buyers, and other food safety professionals (15). Examples of food safety measures that were implemented include removing riparian areas, treating irrigation water, installing fences, and using poison bait to control rodents. While these practices were initially used to limit food safety risks in high risk crops (e.g., leafy greens, tomatoes), a follow-up study determined these practices were also being applied to low risk crops (e.g., potatoes, squash), thus increasing the cost of production (16). In addition, some of these practices may also have negative effects on landscape health (17). Average per acre cost to growers to implement food safety modifications to meet the “Leafy Green Marketing Agreement” (LGMA) was $13.60 based on a survey conducted in 2008 and 2009 (18).

Sources of preharvest contamination with foodborne pathogens can occur from a variety of sources (e.g., irrigation and run-off water, soil amendments, such as manure, fecal deposition from intruding domesticated and wild animals). In addition, management practices (e.g., worker hygiene, buffer zones) and geospatial factors (e.g., soil characteristics) can significantly modulate the risk of contamination from different sources (2, 3, 12, 19-21). A number of studies have shown that water can act as both a source of pathogens and vehicle of pathogen introduction to preharvest environments and produce (20, 22-25). For example, surface water has been reported to have a wide range of *Salmonella* (6% to 80%) and *L. monocytogenes* (6.4% to 93% and so on...
62%) prevalence (24, 26-29). In particular, *Salmonella* prevalence of 6-9% has been reported for water samples obtained from produce growing regions in California (CA) and New York State (NYS) (14, 23). Manas et al. (30) determined that lettuce plants irrigated with non-potable water had significantly higher rates of total coliforms and *Salmonella* contamination than lettuce irrigated with drinking water. A number of studies also have linked sporadic or repeated contamination events in produce fields to wildlife fecal deposits (21) with a variety of bacterial foodborne pathogens, including *Salmonella* and *L. monocytogenes* regularly isolated from fecal samples collected from wildlife and domesticated animals (3, 31-36). *Salmonella* can also survive in the soil for long periods of time (e.g., up to 230 days in one study (37)) when introduced by contaminated poultry or cow manure. A study of farm management practices in Minnesota and Wisconsin found that the use of manure significantly increased the risk of *E. coli* contamination in organic (OR 13.2) and semi-organic (OR 12.9) produce (38). Another study demonstrated that worker hygiene (e.g., portable toilets, hand-washing stations) and trainings were important in reducing the likelihood of generic *E. coli* contamination at the preharvest level (39).

While a number of studies (3, 12, 23, 38-41) have suggested that specific farm management practices may impact pathogen contamination in the preharvest environment, we are not aware of any studies that used statistical methods to quantitatively assess the risk of pathogen contamination associated with specific field-level management practices. These types of data are essential to allow for identification of practices that can significantly increase or decrease the likelihood of field-level contamination, in order to facilitate implementation of science-based preventive controls. Thus, the purpose of this study was to (i) evaluate the prevalence of *Salmonella* and *L. monocytogenes* isolated from environmental samples (soil, drag...
swab, and water) and (ii) identify field-level management practices associated with presence of *Salmonella* or *L. monocytogenes*.

**Materials and Methods**

**Study Design.** Twenty-one produce farms in NYS were enrolled in a cross-sectional study. Enrollment was based on the willingness of the grower to participate in the study. Participation entailed permission to collect environmental samples from produce fields on the farm and agreement to fill out a questionnaire regarding field-level management practices associated with each field that was sampled. Farms were located in three regions of NYS with five in western New York, 12 in central New York and four in eastern New York. Farm visits were performed over a five-week period in June and July 2012. At least ten fields were selected per farm. A single composite soil sample (consisting of five subsamples of soil from five locations in the field) and an area drag swab sample were collected for each field (using a sampling area of approximately 0.2 ha). Additionally, samples were collected from water sources that were (i) used for field irrigation (n=23) or (ii) not used for field irrigation, but were within 50 m from a sampled field (n=51). Six hundred samples were collected for the study (263 composite soil samples, 263 area drag swab samples and 74 water samples).

**Questionnaire Design.** A questionnaire was developed to obtain data on field-level practices identified in literature as possible factors (e.g., manure application, irrigation water) that influence the risk of preharvest contamination (see supplemental material, S1). The interview form included questions to (i) obtain general farm characteristics (15 questions) and (ii) information on sampled fields (11 field questions). Seven of the 11 field-specific questions were time-dependent. For instance, growers were asked the last time a sampled field was irrigated,
with answer options: within 3 d, 4-7 d, 8-14 d, and over 14 d/never. One of the time-dependent questions (frequency of irrigation) had two follow-up questions. The two follow-up questions were (i) source of irrigation water (e.g., pond) and (ii) type of irrigation system used (e.g., drip). The remaining four specific field questions were not time-dependent. For example, growers were asked if the field had a buffer zone (i.e., defined as at least a 5 m strip where no produce was grown). Questionnaires were administered by a single interviewer (LS) and completed at the time of sample collection in a face-to-face interview, which lasted approximately 1 h. Data were coded from the questionnaires, entered into Excel (Microsoft, Redmond, WA) and imported into SAS 9.3 (SAS Institute Inc., Cary, NC).

Sample Collection. Samples were collected as previously detailed by Strawn et al. (14). Briefly, latex gloves and disposable plastic boot covers (Nasco, Fort Atkinson, WI) disinfected with 70% ethanol were worn and changed for sample collection at each field. Five soil samples per field were taken using sterile scoops (Fisher Scientific, Hampton, NH) at least six inches (15.2 cm) below the surface (sub-surface soil) and deposited in separate sterile Whirl-Pak bags (Nasco). A pre-moistened drag swab (30 mL of buffered peptone water (BPW; Becton Dickinson, Franklin Lakes, NJ) in a sterile Whirl-Pak bag), as previously described by Uesugi et al. (42), was dragged through the field (side to side in 10-m increments, around perimeter of field) for 10 min. Water samples (n=74) were collected directly into sterile 250 mL jars; a sampling pole (Nasco) was used if necessary (i.e., for creeks, ponds). Surface water samples were taken a minimum of 2 m from the water's edge and 0.3 m below the surface. All samples were transported on ice, stored at 4±2°C and processed within 24 h of collection.

Sample Preparation. Samples were prepared for two enrichment schemes to allow for separate isolation and detection of Salmonella and L. monocytogenes. Composite soil samples were
prepared by combining 5 g portions of each of the five subsamples of soil collected in a field in duplicate. Both 25 g composite soil samples were deposited into sterile filter Whirl-Pak bags. Individual drag swab samples were combined with BPW and hand massaged for 2 min, squeezed, and 10 mL of the liquid contents aseptically transferred to each of two sterile filter Whirl-Pak bags. Water samples were tested according to Environmental Protection Agency (EPA) standard methods (43, 44). Briefly, each water sample collected (250 mL) was passed through a 0.45 \( \mu \)m filter unit (Nalgene, Rochester, NY). The subsequent filter was then aseptically removed and cut in half, and each portion transferred to a separate sterile filter Whirl-Pak bag.

**Salmonella and L. monocytogenes Detection and Isolation.** *Salmonella* (45) and *L. monocytogenes* (46) detection and isolation was performed using modified versions of the procedures outlined in the US Food and Drug Administration’s Bacteriological Analytical Manual (FDA BAM). No quantification of *Salmonella* or *L. monocytogenes* was performed. Briefly, for *Salmonella* detection and isolation, samples were diluted 1:10 with tryptic soy broth (TSB; Becton Dickinson) and allowed to stand for 2 h at room temperature (23±2°C). After incubation at 35±2°C for an additional 24 h, two aliquots (1.0 and 0.1 mL) were transferred to 9 and 9.9 mL of tetrathionate (TT; Oxoid; Cambridge, UK) and Rappaport Vassiliadis (RV; Oxoid) broths, respectively. Both selective enrichment broths were incubated at 42°C in a shaking water bath for 24 h. A 50 \( \mu \)l aliquot of TT and RV broths were plated onto xylose lysine deoxycholate agar (XLD; Neogen, Lansing, MI) and CHROMagar *Salmonella* (CHROMagar Company; Paris, France), and incubated at 35 and 37±2°C for 24 and 48 h, respectively. Up to four presumptive *Salmonella* colonies per selective enrichment and plating medium combination (e.g., TT-XLD, RV-XLD) were sub-streaked to brain heart infusion agar (BHI; Becton
Dickinson) and incubated at 37±2°C for 24 h. Presumptive *Salmonella* colonies were confirmed by a polymerase chain reaction (PCR) assay that detects the gene, *invA* (47). For *L. monocytogenes*, all samples were diluted 1:10 with buffered *Listeria* enrichment broth (BLEB; Becton Dickinson) and incubated at 30±2°C for 24 h. *Listeria Selective Enrichment Supplement* (Oxoid) was added to enrichments at 4 h. At 24 and 48 h, 50 μl of enrichment was streaked onto modified Oxford agar (MOX, Becton Dickinson) 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. Up to four *L. monocytogenes* presumptive colonies per plating medium and time combination (e.g., MOX 24 h or LMPM 48 h) were sub-streaked onto BHI. BHI agar plates were incubated for 37±2°C for 24 h. Presumptive *L. monocytogenes* colonies were confirmed by PCR amplification and sequencing of the partial *sigB* gene (48-50). Controls were processed in parallel with each pathogen detection and isolation scheme. *Salmonella* ATCC 700408 (FSL F6-826; (51) and *L. monocytogenes* FSL R3-001 (52), were used as positive controls. Sterile enrichment media were used as negative controls. **Classification of Isolates.** There were four isolation schemes for each *Salmonella* (TT-XLD, RV-XLD, TT-CHROME, and RV-CHROME) and *L. monocytogenes* (LMPM 24 h, MOX 24 h, LMPM 48 h, and MOX 48 h); one isolate from each “isolation scheme” was used for subtyping (as detailed below), yielding up to four representative isolates per pathogen-positive. Representative isolates were streaked from frozen culture onto BHI and incubated at 37°C for 18 h, and a well-isolated colony selected. *Salmonella* serotyping using the White-Kauffman-Le Minor scheme (53) was conducted by the Wadsworth Center, New York State Department of Health (Albany, NY). Nucleotide sequences of *sigB* from *L. monocytogenes* isolates were obtained by Sanger sequencing performed by the Cornell University Life Sciences Core.
Laboratories Center. Allelic types (AT), as defined by a unique combination of polymorphisms (54, 55), were assigned by comparison of sigB sequences to an internal reference database.

Statistical Analysis. Separate statistical analyses for *Salmonella* and *L. monocytogenes* were performed in SAS 9.3. An initial descriptive analysis was performed to calculate *Salmonella* and *L. monocytogenes* prevalence for all samples (n=600) and each sample type collected: soil (n=263), drag swab (n=263) and water (n=74). Univariate associations between pathogen-positive terrestrial samples and region and week sampled were determined using a chi-square test or Fisher’s exact test (if the expected frequency in any cell was less than 5). Confidence intervals (95%) were calculated assuming a binominal distribution. Individual *p* values are reported for each test.

A field was used as the unit of analysis for model development to identify field-level risk factors associated with *Salmonella* and *L. monocytogenes* contamination in produce fields. A field was considered positive if either a soil or drag swab sample collected from that field was confirmed culture positive for the respective pathogen. Chi-square or Fisher’s exact tests were computed for each of the 11 specific field questions (i.e., factors). Factors determined to be significant (*P* ≤ 0.05) were retained as candidates for subsequent multivariate analysis. General linear mixed models (GLIMMIX) procedure was used to model the association between each candidate factor (univariate analysis) or factors (multivariate analysis) and the outcome (*Salmonella* or *L. monocytogenes*-positive/negative field). Fields within farm were not independent; therefore, farm was included in the model (as a random effect). Effect estimates (β), standard errors (SE), odds ratios (OR), 95% confidence intervals (CI), and *P* values were determined for each candidate factor. Potential collinearity among the candidate factors was evaluated by Spearman’s rank correlation coefficient test. Multivariable models were built using...
a stepwise selection method and accessed by fit statistics, such as Akaike’s information criteria and Schwarz’ Bayesian criterion. The final model retained only variables that significantly improved the fit of the model ($P \leq 0.05$). Interaction terms were also tested, but none were significant.

**Isolate Storage and Data Access.** All isolates were preserved at -80°C in 15% glycerol. Isolate information and subtyping data from this study are archived and available through the Food Microbe Tracker database (http://www.foodmicrobetracker.com).

**Results**

**Salmonella and L. monocytogenes Prevalence in Terrestrial Samples.** The prevalence of *Salmonella* in terrestrial samples (n=263 soil and n=263 drag swab) was 3.4% (18/526). *Salmonella* prevalence was higher among soil samples (13/263), compared to drag swab samples (5/263). *Salmonella* was detected in 6.1% of fields sampled (16/263). For two fields, both soil and drag swab samples were positive for *Salmonella*. No significant difference was observed in the *Salmonella* prevalence in soil and drag swab samples by region ($P = 0.4$ and $0.9$, respectively) and week sampled ($P = 0.9$ and 0.6, respectively). Furthermore, no significant difference was observed for the field-level prevalence of *Salmonella* by region ($P = 0.8$) and week sampled ($P = 0.9$).

The prevalence of *L. monocytogenes* in terrestrial samples (n=263 soil and n=263 drag swab) was 9.7% (51/526). *L. monocytogenes* prevalence in soil and drag swab samples was 11% (30/263) and 8% (21/263), respectively. *L. monocytogenes* was detected in 46 of the 263 fields sampled (17.5%). Five fields had both soil and drag swab samples that were positive for *L. monocytogenes*. No significant difference was found in the *L. monocytogenes* prevalence in soil...
and drag swab samples by region ($P = 0.2$ and 0.3, respectively) and week sampled ($P = 0.7$ and 0.2, respectively). In addition, no significant difference was found for the field-level prevalence of *L. monocytogenes* by region ($P = 0.9$) and week sampled ($P = 0.1$).

*Salmonella* and *L. monocytogenes* Prevalence in Water Samples. The prevalence of *Salmonella* and *L. monocytogenes* in water samples was 11% (8/74) and 30% (22/74), respectively. Samples were collected from irrigation (n = 23) and non-irrigation water sources (within 50 m of a sampled field; n=51) (Table 1).

The prevalence of *Salmonella* and *L. monocytogenes* in water samples used for irrigation was 4% (1/23) and 9% (2/23), respectively. Fourteen of the samples collected from irrigation sources were obtained from engineered water sources (e.g., well, municipal), which were of potable water quality; all of the samples were negative for *Salmonella* and *L. monocytogenes*. The remaining nine water samples were from surface water sources (1 creek and 8 pond samples); three samples from ponds used for field irrigation tested positive for *Salmonella* (1 sample) and *L. monocytogenes* (two samples). All fields sampled using these irrigation water sources were negative for the presence of *Salmonella* and *L. monocytogenes* (Table 1).

*Salmonella* and *L. monocytogenes* were detected in 14% (7/51) and 39% (20/51), respectively, of water samples obtained from non-irrigation sources and within 50 m of a sampled field. Water samples were collected from three source types: ponds (n=17), roadside or field buffer ditches (n=13), and flowing surface water (e.g., rivers, creeks, or streams (n=21)).

The prevalence of *Salmonella* was higher in roadside or field buffer ditch samples (23%, 3/13), compared to pond (12%, 2/17) and flowing surface water (10%, 2/21) samples. The prevalence of *L. monocytogenes* was highest in pond samples (59%, 10/17), compared to roadside or field buffer ditch (39%, 5/13) and flowing surface water (24%, 5/21) samples.
Characterization of *Salmonella* and *L. monocytogenes* Isolated from Terrestrial and Water Samples. Serotyping was performed on one representative *Salmonella* isolate per isolation scheme, which yielded 35 *Salmonella* isolates from the 26 positive samples. Three of the 26 samples yielded isolates with more than one serotype. *Salmonella* Give and Typhimurium were isolated from a single water sample (isolation schemes TT-Chrome and TT-XLD, respectively), *Salmonella* Agona and Tennessee were isolated from a drag swab sample (isolation schemes RV-Chrome and RV-XLD) and *Salmonella* Senftenburg and Newport were isolated from a soil sample (isolation schemes RV-Chrome and RV-XLD, respectively). The remaining 23 *Salmonella*-positive samples represented one serotype. These isolates were identified as *Salmonella* serotypes Newport (8 samples), Cerro (5 samples), Thompson (5 samples), Agona (2 samples), IV 40:z4,z32:- (2 samples), and Give (1 sample). For the two fields where *Salmonella* was isolated in both soil and drag swab samples, the same serotype (Cerro) was isolated in both sample types from one field, whereas different serotypes (Thompson and Cerro) were isolated in the soil and drag swab samples from the other field.

Two-hundred and sixteen *L. monocytogenes* isolates (one isolate per isolation scheme) were subtyped based on alignment of *sigB* nucleotide sequences. None of the four isolation schemes yielded different subtypes for any sample. The 73 representative *L. monocytogenes* isolates (from the 73 *L. monocytogenes*-positive samples) yielded nine different allelic types that represented *L. monocytogenes* lineage I (29 isolates, 5 ATs), II (41 isolates, 3 ATs), and IIIa (3 isolates, 1 AT). *L. monocytogenes* was detected in both soil and drag swab samples for five fields. The same subtype was identified in soil and drag swab samples in two fields (AT 57 and AT 59), whereas different subtypes (ATs 57 and 61, ATs 78 and 137, and ATs 57 and 58) were isolated in the soil and drag swab samples from three fields.
Risk Factors Associated with *Salmonella* Contamination of Produce Fields. Three of the 11 field management practices evaluated were significantly associated with a *Salmonella*-positive field by univariate analysis (manure application, soil cultivation, and buffer zone; Table 2). Fields where manure was applied within a year prior to sample collection had higher odds of *Salmonella* isolation (OR = 19.0, 95% CI = 4.9, 77.0), compared with fields where manure had not been applied. Fields where soil was cultivated within 7 d prior to sample collection were approximately 6 times more likely (OR = 6.3, 95% CI = 1.6, 23.0) to be *Salmonella*-positive, compared with fields where soil was not cultivated for at least 30 d. The presence of a buffer zone was shown to have a protective effect and reduced the likelihood of a *Salmonella*-positive field by 5 times (OR = 0.2, 95% CI = 0.1, 0.5; Table 2).

Examination of Spearman’s rank correlation coefficients for the three retained candidate factors from the univariate analysis showed a correlation between application of manure and soil cultivation of a field. Therefore, three multivariable models were evaluated: model 1 = manure application, soil cultivation, and buffer zone; model 2 = manure application and buffer zone; and model 3 = soil cultivation and buffer zone. In the multivariate model with the best fit (i.e., model 2; Table 3), application of manure to a field within a year prior to sample collection was associated with a higher likelihood of *Salmonella* being detected in a field (OR = 16.7, 95% CI = 3.0, 94.4) as compared with fields where manure had not been applied. Presence of a buffer zone was associated with a lower likelihood of *Salmonella* being detected in a field (OR = 0.1, 95% CI = 0.03, 0.6), as compared to absence of a buffer zone (Table 3).

Risk Factors Associated with *L. monocytogenes* Contamination in Produce Fields. Six of the 11 field management practices were significantly associated with a *L. monocytogenes*-positive field by univariate analysis (manure application, reporting of wildlife, worker activity, irrigation,
soil cultivation and reporting of a buffer zone; Table 4); five of these six factors were time-
dependent. Fields where manure was applied within a year prior to sample collection had 7 times
higher odds of *L. monocytogenes* isolation (OR = 7.0, 95% CI = 3.1, 15.4), compared with fields
where manure had not been applied. Fields where growers reported observation of wildlife
within 3 d prior to sample collection had higher odds of *L. monocytogenes* isolation (OR = 4.4,
95% CI = 1.2, 15.6), compared with fields where growers did not report observation of wildlife
for at least 7 d. Fields where soil was cultivated within 7 d prior to sample collection were
approximately 8 times more likely (OR = 8.1, 95% CI = 3.3, 19.6) to be *L. monocytogenes-
positive, compared with fields where soil was not cultivated for at least 30 d. Fields with recent
worker activity (within 3 d prior to sample collection) had 10.5 times higher odds of *L.
monocytogenes* isolation (OR = 10.5, 95% CI = 2.3, 47.5), compared with fields where workers
had been absent for longer than 30 d. A number of other worker related factors did not show
significant associations with *L. monocytogenes* contamination, including delivery of food safety
training (in the native language), presence of portable toilets and handwashing stations (within a
quarter mile of fields), cleaning frequency of toilets, and posting of signs advocating food safety
and or sanitation best practices in changing areas; for most of these factors a high level of
compliance with “best practices” was reported (e.g., all farms reported cleaning toilets at least
once a week). Fields irrigated within 3 d prior to sample collection had nearly 5.5 times higher
odds of *L. monocytogenes* isolation (OR = 5.3, 95% CI = 2.4, 12.0), compared with fields
irrigated at least 14 d ago. Furthermore, no significant difference was observed in *L.
monocytogenes*-positive fields for irrigation type (overhead versus drip). Lastly, presence of a
buffer zone was shown to have a protective effect and reduced the likelihood of a *L.
monocytogenes*-positive field (OR = 0.5, 95% CI = 0.2, 0.9; Table 4).
Correlation was evaluated between the six factors retained by univariate analysis using Spearman’s rank correlation coefficients. Similar to the findings for *Salmonella*, a correlation was observed between manure application and soil cultivation of a field. The three multivariable models evaluated were these (i) model 1 = manure application, reported observation of wildlife, worker activity, irrigation, soil cultivation, and buffer zone; (ii) model 2 = manure application, reported observation of wildlife, worker activity, irrigation, and buffer zone; and (iii) model 3 = reported observation of wildlife, worker activity, irrigation, soil cultivation, and buffer zone. The multivariate model with the best fit was model 3 (Table 5). In this model, reported observation of wildlife in a field (OR = 6.1, 95% CI = 1.3, 28.4) and irrigation of a field (OR = 6.0, 95% CI = 2.0, 18.1) within 3 d prior to sample collection were associated with higher odds of *L. monocytogenes* isolation. Fields where soil was cultivated within 7 d prior to sample collection were nearly 3 times more likely to be *L. monocytogenes*-positive, compared with fields where soil was cultivated at least 30 d ago (OR = 2.9, 95% CI = 1.1, 8.6; Table 5).

**Discussion**

Our study reported here is one of the first to quantitatively identify management practices that are associated with an increased or decreased likelihood of *Salmonella* and *L. monocytogenes* isolation in produce fields. In a univariate analysis, six factors (manure application, reported observation of wildlife, worker activity, irrigation, soil cultivation, and buffer zone presence) were identified as significant risk factors for *Salmonella* or *L. monocytogenes* contamination. Five of the six risk factors were influenced by time of application to fields, suggesting that adjustments to current practices may reduce the potential for produce contamination with minimal costs to growers.
Some Risk Factors Influence the Likelihood of both *Salmonella* and *L. monocytogenes* Isolation in Fields. Based on the separate univariate analysis of *Salmonella* and *L. monocytogenes* data, we identified three risk factors that significantly affected the likelihood of both *Salmonella* and *L. monocytogenes* detection. As adjustments of management factors related to these risk factors have the potential to reduce contamination with both of these key pathogens, these three factors are discussed below.

Our data specifically showed that recent cultivation of fields (i.e., within 7 days of sample collection) was significantly associated with an increased likelihood of both *Salmonella* and *L. monocytogenes* isolation from fields. Soil cultivation was also found to be a significant risk factor in the final multivariate model for *L. monocytogenes* isolation. A likely explanation for these findings is that pathogens present in the sub-surface soil are exposed to the surface when soil is cultivated, making them more likely to be detected, and possibly also more likely to contaminate produce. Furthermore, the likelihood of pathogen isolation will decrease over time after cultivation, due to exposure to environmental conditions (e.g., UV light) that reduce pathogen loads. This model is supported by previous studies (14, 23, 42) that have shown the presence and persistence of *Salmonella* and *L. monocytogenes* in sub-surface soil. For example, *Salmonella* was detected in 2.6% and 2.0% of soil samples collected from produce growing regions in CA and NYS, respectively; while *L. monocytogenes* prevalence in soil was 9% in NYS preharvest environments (14, 23). Interestingly, Park et al. (39) observed that spinach contamination with generic *E. coli* was less likely when a field was cultivated prior to the growing season; this may reflect that cultivation at time points considerably before sampling (e.g., 7 d before) will reduce overall pathogen loads by exposing pathogens present in the sub-surface soil to UV and other inactivating conditions (e.g., desiccation). This hypothesis is
supported by the observation that *Salmonella* and *Listeria* numbers in inoculated livestock waste declined more rapidly when this material was spread on the surface of soil as compared with incorporation into the soil where it would be protected from exposure to environmental conditions (e.g., UV light, harsh temperatures) (56).

Application of manure was also identified as a significant factor that increased the odds of both *Salmonella* and *L. monocytogenes* isolation in fields. Numerous studies (37, 39, 41, 57-64) have demonstrated that the application of manure to soils can introduce pathogens and may facilitate long term persistence of pathogens in soil. One study observed *Salmonella* to persist in manure, manure-amended non-sterilized soil, and manure-amended sterilized soil for 184, 332, and 405 days, respectively (60). However, the association between pathogen contamination of fields and manure application has not been previously described using commercial produce farms. Some studies (38, 39) have investigated the association between generic *E. coli* contamination of preharvest produce samples and application of manure to fields. One study observed that generic *E. coli* contamination was lower in spinach samples collected over a two year period if the application of manure occurred greater than 200 d prior to sample collection (39), while another study observed that *E. coli* prevalence in preharvest produce samples collected was not affected by the application of manure between 90 to 120 d prior to sampling (38). Our results suggest that application of manure to fields can significantly influence the risk of both *Salmonella* and *L. monocytogenes* contamination; therefore, management of manure before application is essential. Manure management practices, such as aging, treating, and handling of manure before application have been shown to affect the survival of foodborne pathogens in manure (38, 56, 65). For example, one study (65) showed that composting cow manure before application was effective at killing *Salmonella*, supporting that management of
manure before application to fields may limit or reduce the risks associated with manure use in
produce preharvest environments.

In addition, the likelihood of *Salmonella* and *L. monocytogenes* isolation in fields was
significantly decreased if growers reported presence of a buffer zone, defined as a zone of at
least 5 m separating the edge of produce fields from potential environmental pathogen reservoirs
(e.g., forests, roads, waterways, livestock operations). These data suggest that even buffer zones
narrower than the 10 m (30 ft) recommended in the 2012 version of the LGMA (table 6 (66)) are
associated with reduced pathogen prevalence. Surprisingly, there is little science-based research
to support the hypothesis that presence of a buffer zone is associated with decreased pathogen
prevalence in preharvest environments. Therefore, in our study we formally tested the hypothesis
that presence of a buffer zone is associated with decreased pathogen prevalence (i.e., *Salmonella*
and *L. monocytogenes*) in produce fields. Some previous studies (67-69) suggest that vegetative
buffer zones may be effective in reducing bacterial pathogen loads in sewage runoff and
wastewater from animal facilities. Vegetative buffer zones and non-agricultural lands adjacent to
produce fields (e.g., riparian, wetlands, grasslands) also offer a variety of ecological benefits (16,
17, 69, 70). Combined these data suggest that the effects, on pathogen prevalence, of buffer
zones and non-agricultural lands adjacent to produce fields may be driven by complex ecological
interactions that will require further field studies that include mathematical modeling efforts.

These research efforts will also need to define the effects of different types of buffer zones (i.e.,
bare strips, specific vegetation) and the quantitative relationship between buffer zone width and
type, and pathogen reduction.

**Some Risk Factors Specifically Increase the Likelihood of *L. monocytogenes* Isolation in
Fields.** While some risk factors increased or reduced the likelihood of both *Salmonella* and *L.*
monocytogenes, others (worker activity, reported wildlife observation, and irrigation) were solely identified to increase the likelihood of L. monocytogenes detection in fields. Worker activity was significantly associated with an increased likelihood of L. monocytogenes isolation in fields by univariate analysis, but was not significant in the multivariate analysis. However, reported observation of wildlife and irrigation of fields were significantly associated with higher odds of L. monocytogenes isolation by multivariate analysis and are discussed below.

Reported observation of wildlife was based on visual confirmation (i.e., sighting of wildlife in a field) by the grower or his/her staff (e.g., field supervisor). We acknowledge that growers who have their farms and food safety programs (e.g., GAPs) frequently audited may be less inclined to report presence of wildlife because they are aware of the risks associated with wildlife in fields. While growers who have their farms and food safety programs infrequently audited may be more forthcoming to report presence of wildlife. Future studies may choose to measure the impact of wildlife and potential pathogen contamination by objective measures (e.g., the use of infrared cameras to detect wildlife in fields). Our study does provide quantitative data to support previous studies (2, 12, 19, 25, 32) that suggested that wildlife may be a source of pathogen contamination in fields. Furthermore, wildlife has also been suspected as the source of pathogen contamination in a number of produce-associated outbreaks (13, 70, 71). While reported observation of wildlife was shown to be a risk factor increasing the likelihood of L. monocytogenes isolation in fields, this finding may be site specific to NYS, or parts of NYS; Langholz and Jay-Russell have discussed that pathogen prevalence in wildlife may be dependent on geographic location and local landscape characteristics (70).

Recent irrigation was also shown to significantly increase the odds of L. monocytogenes isolation in fields. Water has been identified as a major reservoir for pathogens and irrigation a
vehicle for transmission of pathogens to fields and produce (12, 30, 41, 72-75). *L. monocytogenes* is often found in various water sources with prevalence reported from <1% to 29% (14, 76, 77). We also observed, here, a high prevalence of *L. monocytogenes* in water, particularly surface water sources (e.g., ponds). Steele and Odumeru (72) observed surface water had the most variable microbial quality, and if contaminated, could lead to widespread contamination of crops. Our findings suggest that detection of *L. monocytogenes* in fields was only more likely if irrigation occurred within a couple of days prior to sample collection. Two studies have also shown an association between pathogen detection and time of irrigation or water application. One study observed that *Salmonella* when sprayed on tomatoes was not able to be recovered from the tomatoes after two days (78). The second study observed that the risk of *E. coli* contamination in spinach samples decreased when irrigation in a field occurred >5 d prior to sample collection. In addition to *L. monocytogenes* introduction with irrigation water, the association of irrigation with an increased frequency of *L. monocytogenes* detection may also reflect the fact that moist soils may facilitate *L. monocytogenes* growth or detection, consistent with previous studies that reported a higher *L. monocytogenes* prevalence in moist soils (14, 79). Overall, our data suggest that avoiding irrigation at least 3 d before harvest (if possible and feasible) may reduce potential *L. monocytogenes* contamination to produce and possibly the transfer into packinghouses, from soil in the fields.

**Conclusions.** This study provides quantitative data on management practices that represent potential risk factors for produce field contamination. A majority of research previously conducted to investigate these risk factors has been pathogen inoculation-based or targeted presence of indicator organisms (i.e., generic *E. coli*). Such studies are commonly employed because the prevalence of foodborne pathogens (*Salmonella*, Shiga toxin producing *E. coli*) in
produce production environments is low. Statistically robust analyses are difficult to conduct unless a sufficient number of pathogen-positive samples are obtained, and this generally requires an extremely large sample size. Large sample sizes in environmental field studies are often difficult to achieve due to considerable labor and financial costs, and difficulties gaining access to commercial operations. We focused on only eleven key management practices previously discussed as risk factors for preharvest contamination, limited the number of levels within each factor, and opted for a statistical procedure to deal with farm as a confounder; in order, to prevent bias and misinterpretation of results (e.g., spurious relationships). This study was conducted in NYS, thus risk factors identified may not always be appropriate in other produce growing regions in the US or elsewhere. Additionally, fields were sampled over a five-week period in June and July, as a result risk factors identified may not be applicable to other time periods (e.g., late in the growing season). Despite some limitations, this study is one of the first to use field collected data to provide quantitative data on management practices associated with detection of *Salmonella* and *L. monocytogenes* (two foodborne pathogens of concern to the produce industry). These findings will assist growers in (i) evaluating their current on-farm food safety plans (e.g., GAPs), (ii) implementing preventive controls that reduce the risk of preharvest contamination, and (iii) making more informed decisions related to field practices prior to harvest.

**Acknowledgements.**

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References.


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TABLE 1 *Salmonella* and *L. monocytogenes* prevalence in water samples collected from irrigation and non-irrigation water sources

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
<th>Prevalence (Frequency) of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
</tr>
<tr>
<td>Not used for irrigation&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51</td>
<td>14 (7)</td>
</tr>
<tr>
<td>Pond</td>
<td>17</td>
<td>12 (2)</td>
</tr>
<tr>
<td>Ditch&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13</td>
<td>23 (3)</td>
</tr>
<tr>
<td>River/creek/stream</td>
<td>21</td>
<td>10 (2)</td>
</tr>
<tr>
<td>Used for irrigation</td>
<td>23</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Engineered&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pond</td>
<td>8</td>
<td>13 (1)</td>
</tr>
<tr>
<td>River/creek/stream</td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>11 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Water samples not used for irrigation were collected within 50 m from a sampled field.

<sup>b</sup> Ditch was defined as either a roadside ditch (located between road and field) or a runoff ditch (located between landscape feature (e.g., a pasture) and field; often part of a buffer zone).

<sup>c</sup> Engineered water was defined as water from a well or municipal source (i.e., a potable water source).
**TABLE 2** Univariate analyses of management practices that influence the likelihood of *Salmonella* being detected in a produce field (based on testing of soil and drag swab samples)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
<th>β-coefficient</th>
<th>SE(^a)</th>
<th>OR(^b)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure</td>
<td>Last time manure was applied to field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = within 365 d</td>
<td>3.0</td>
<td>0.7</td>
<td>19</td>
<td>4.9, 77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2 = over 365 d</td>
<td>0.4</td>
<td>0.9</td>
<td>1.5</td>
<td>0.2, 9.4</td>
<td>0.681</td>
</tr>
<tr>
<td></td>
<td>3 = not been applied</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Soil Cultivation</td>
<td>Last time soil in field was cultivated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = within 7 d</td>
<td>1.8</td>
<td>0.7</td>
<td>6.3</td>
<td>1.6, 23</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>2 = 8 to 14 d</td>
<td>0.5</td>
<td>0.9</td>
<td>1.6</td>
<td>0.3, 9.9</td>
<td>0.625</td>
</tr>
<tr>
<td></td>
<td>3 = 15 to 30 d</td>
<td>-0.9</td>
<td>1.2</td>
<td>0.4</td>
<td>0.1, 4.2</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>4 = over 30 d</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Buffer zone(^d)</td>
<td>Does field have a buffer zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = yes</td>
<td>-1.7</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1, 0.5</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>2 = no</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) standard error

\(^b\) odds ratio
...confidence interval

...buffer zone was defined as a strip of land where no produce was grown, approximately 5 m wide
TABLE 3 Multivariate final model\(^a\) of risk factors that influence the likelihood of *Salmonella* being detected in a produce field (based on testing of soil and drag swab samples)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
<th>(\hat{\beta})-coefficient</th>
<th>SE(^b)</th>
<th>OR(^c)</th>
<th>95% CI(^d)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manure</td>
<td>Last time manure was applied to field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = within 365 d</td>
<td>2.8</td>
<td>0.9</td>
<td>16.7</td>
<td>3.0</td>
<td>94.4 0.002</td>
<td></td>
</tr>
<tr>
<td>2 = over 365 d</td>
<td>0.3</td>
<td>1.1</td>
<td>1.3</td>
<td>0.2</td>
<td>11 0.789</td>
<td></td>
</tr>
<tr>
<td>3 = not been applied</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffer zone(^e)</td>
<td>Does field have a buffer zone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = yes</td>
<td>-2.0</td>
<td>0.7</td>
<td>0.1</td>
<td>0.03</td>
<td>0.6 0.008</td>
<td></td>
</tr>
<tr>
<td>2 = no</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm</td>
<td>Random effect</td>
<td>1.6</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) final model developed in PROC GLIMMIX; farm as random effect.

\(^b\) standard error

\(^c\) odds ratio

\(^d\) confidence interval

\(^e\) buffer zone was defined as a strip of land where no produce was grown, approximately 5 m wide
TABLE 4 Univariate analyses of management practices that influence the likelihood of *L. monocytogenes* being detected in a produce field (based on testing of soil and drag swab samples)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
<th>β-coefficient</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manure</strong></td>
<td>Last time manure was applied to field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = within 365 d</td>
<td>1.9</td>
<td>0.4</td>
<td>7.0</td>
<td>3.1</td>
<td>15.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 = over 365 d</td>
<td>-0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
<td>1.7</td>
<td>0.381</td>
</tr>
<tr>
<td>3 = not been applied</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wildlife</strong></td>
<td>Last time wildlife was observed in field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = within 3 d</td>
<td>1.5</td>
<td>0.6</td>
<td>4.4</td>
<td>1.2</td>
<td>15.6</td>
<td>0.022</td>
</tr>
<tr>
<td>2 = 4 to 7 d</td>
<td>-0.2</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
<td>3.1</td>
<td>0.725</td>
</tr>
<tr>
<td>3 = 8 to 30 d</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Worker Activity</strong></td>
<td>Last time workers were in the field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = within 3 d</td>
<td>2.4</td>
<td>0.8</td>
<td>10.5</td>
<td>2.3</td>
<td>47.5</td>
<td>0.003</td>
</tr>
<tr>
<td>2 = 4 to 7 d</td>
<td>0.7</td>
<td>0.8</td>
<td>1.9</td>
<td>0.4</td>
<td>9.9</td>
<td>0.439</td>
</tr>
<tr>
<td>3 = 8 to 30 d</td>
<td>1.0</td>
<td>0.9</td>
<td>2.6</td>
<td>0.5</td>
<td>14.7</td>
<td>0.281</td>
</tr>
<tr>
<td>4 = over 30 d</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irrigation</td>
<td>Last time field was irrigated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 = within 3 d</td>
<td>1.7</td>
<td>0.4</td>
<td>5.3</td>
<td>2.4, 12.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>2 = 4 to 7 d</td>
<td>-0.3</td>
<td>0.5</td>
<td>0.8</td>
<td>0.3, 2.2</td>
<td>0.599</td>
<td></td>
</tr>
<tr>
<td>3 = 8 to 14 d</td>
<td>-1.3</td>
<td>0.7</td>
<td>0.3</td>
<td>0.1, 1.1</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>4 = over 14 d/not irrigated</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil Cultivation</th>
<th>Last time soil in field was cultivated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = within 7 d</td>
<td>2.1</td>
</tr>
<tr>
<td>2 = 8 to 14 d</td>
<td>0.6</td>
</tr>
<tr>
<td>3 = 15 to 30 d</td>
<td>-0.4</td>
</tr>
<tr>
<td>4 = over 30 d</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Buffer zone&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Does field have a buffer zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = yes</td>
<td>-0.8</td>
</tr>
<tr>
<td>2 = no</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> standard error  
<sup>b</sup> odds ratio  
<sup>c</sup> confidence interval  
<sup>d</sup> answer option 4 (never) was not selected in the questionnaire; therefore it was excluded from analysis
a worker constituted a man or woman in the field, not in the cab of farm equipment (e.g., tractor)

buffer zone was defined as a strip of land where no produce was grown, approximately 5 m wide
TABLE 5 Multivariate final model of risk factors that influence the likelihood of *L. monocytogenes* being detected in a produce field (based on testing of soil and drag swab samples)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
<th>β-coefficient</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wildlife</em></td>
<td>Last time wildlife was observed in field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = within 3 d</td>
<td>1.8</td>
<td>0.8</td>
<td>6.1</td>
<td>1.3, 28.4</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>2 = 4 to 7 d</td>
<td>-0.02</td>
<td>0.8</td>
<td>1.0</td>
<td>0.2, 4.8</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>3 = 8 to 30 d</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Irrigation</em></td>
<td>Last time field was irrigated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 = within 3 d</td>
<td>1.8</td>
<td>0.6</td>
<td>6.0</td>
<td>2.0, 18.1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>2 = 4 to 7 d</td>
<td>0.2</td>
<td>0.7</td>
<td>1.2</td>
<td>0.3, 4.5</td>
<td>0.793</td>
</tr>
<tr>
<td></td>
<td>3 = 8 to 14 d</td>
<td>-0.8</td>
<td>0.8</td>
<td>0.4</td>
<td>0.1, 2.0</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>4 = over 14 d/not irrigated</td>
<td>0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Soil Cultivation</em></td>
<td>Last time soil in field was cultivated</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1 = within 7 d</td>
<td>1.1</td>
<td>0.6</td>
<td>2.9</td>
<td>1.1, 8.6</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>2 = 8 to 14 d</td>
<td>0.3</td>
<td>0.7</td>
<td>1.4</td>
<td>0.4, 5.1</td>
<td>0.660</td>
</tr>
<tr>
<td></td>
<td>3 = 15 to 30 d</td>
<td>-0.9</td>
<td>0.7</td>
<td>0.4</td>
<td>0.1, 1.7</td>
<td>0.224</td>
</tr>
<tr>
<td>Farm</td>
<td>Random effect</td>
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<tr>
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<td>--------------</td>
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</tr>
<tr>
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<td>0.1</td>
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</tr>
<tr>
<td>1.0</td>
<td>3.3</td>
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</tr>
</tbody>
</table>

*a* final model developed in PROC GLIMMIX; farm as random effect

*b* standard error

*c* odds ratio

*d* confidence interval

*e* answer option 4 (never) was not selected in the questionnaire; therefore it was excluded from analysis