PREVALENCE OF *LISTERIA MONOCYTOGENES* IN THE PRE-HARVEST ENVIRONMENT; A LANDSCAPE EPIDEMIOLOGY APPROACH

A Paper
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Suvash Shiwakoti

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Veterinary and Microbiological Science

December 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

PREVALENCE OF *LISTERIA MONOCYTOGENES* IN THE PREHARVEST ENVIRONMENT; A LANDSCAPE EPIDEMOLOGY APPROACH

By

Suvash Shiwakoti

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. John McEvoy
Chair

Dr. Jane Schuh

Dr. Sheela Ramamoorthy

Dr. Mark Clark

Approved:

12/28/2015
Date

Dr. Neil Dyer
Department Chair

ABSTRACT

Listeria monocytogenes is the causative agent of the foodborne disease listeriosis. Although the incidence of listeriosis is lower than that of other foodborne diseases, it's much higher mortality rate makes it a cause for serious concern. *Listeria monocytogenes* is a saprophyte in the environment but it can become pathogenic for humans and animals. It is well adapted for survival in soil, water, and livestock manure from where it can contaminate fruits and vegetables. Produce-associated listeriosis outbreaks are frequently caused by contamination occurring in the pre-harvest environment, so there is a need for more effective control measures targeted at produce fields. The combination of epidemiological data and advanced computational tools, such as GIS and machine learning, have made it possible to develop models that predict *L. monocytogenes* prevalence across different landscapes. The predictive model can assist fresh-produce farmers in selecting the most effective controls to reduce contamination in the pre-harvest environment.

ACKNOWLEDGEMENTS

At first I would like to express my indebtedness to the North Dakota State University, Department of Veterinary and Microbiological Science for the successful planning of the program and providing me this opportunity.

I am immensely grateful to my academic advisor, Dr. John McEvoy, Associate Professor, North Dakota State University, for his valuable guidance, critical comments of this report, praiseworthy suggestions, encouragement, inspiration, and cooperation throughout the period.

I would also like to thank my committee members Dr. Jane Schuh, Dr. Sheela Ramamoorthy and Dr. Mark Clark for providing useful feedback throughout this process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CNS……………………………... Central nervous system

DNA…………………………….. Deoxyribonucleic acid

GAP……………………………... Good agriculture practices

GIS……………………………… Geographic information system

PCR……………………………... Polymerase chain reaction

RAPD…………………………… Randomly amplified polymorphic DNA

RTE……………………………... Ready to eat

INTRODUCTION

Diseases caused by foodborne pathogens have major economic and public health impact worldwide. *Listeria monocytogenes* is a ubiquitous environmental saprophyte (53, 71) that can cause foodborne disease (51, 53). Listeriosis, the disease caused by *L. monocytogenes*, can present as a mild flu-like illness, but systemic involvement in susceptible populations can have severe consequences, particularly for a pregnant woman and her developing fetuses (26). The high mortality (more than 25%) associated with listeriosis is what sets it apart from other foodborne pathogens (74). Outbreaks of foodborne listeriosis and frequent food recalls (9, 12, 13) cause consumers to avoid consumption of affected foods. Recent outbreaks associated with fresh produce (9, 12, 13, 71, 72) are of particular concern because produce consumption is necessary for a healthy diet. *Listeria monocytogenes* persistence in food processing environments has been well documented, but comparatively little is known about persistence in pre-harvest environments, which are the major source of fresh produce contamination. Food producers and regulators in the US have taken a number of steps to eliminate *Listeria* from ready-to-eat (RTE) foods, but more is required to prevent contamination in the pre-harvest environment. To develop effective controls, it is necessary to understand the areas where *L. monocytogenes* is most likely to be present in the pre-harvest environment (34, 71, 79). Recently, a computational approach, using GIS and machine learning, has been used to model *L. monocytogenes* persistence in farm environments (34, 71, 78, 80). This review paper discusses the determinants of *L. monocytogenes* persistence in pre-harvest environments that serve as inputs for predictive modeling, and it proposes an enhanced model to predict *L. monocytogenes* prevalence over larger areas.

EPIDEMIOLOGY AND PUBLIC HEALTH OF *LISTERIA MONOCYTOGENES*

Listeriosis is the second leading cause of death due to foodborne illness in the USA, after nontyphoidal salmonellosis (66). It has an incubation period of up to 10 weeks (52), which complicates epidemiological investigations and limits the effectiveness of food recalls.

Listeria monocytogenes can cause mild symptoms such as febrile gastroenteritis (6, 51), or more serious systemic illness with involvement of the gravid uterus and central nervous system (CNS) (42, 51). Establishment of *L. monocytogenes* in the CNS and uterus leads to the severe symptoms of meningitis and abortion/still birth, respectively (42, 51). A schematic diagram of the movement of *L. monocytogenes* in human body is presented in Figure 1.

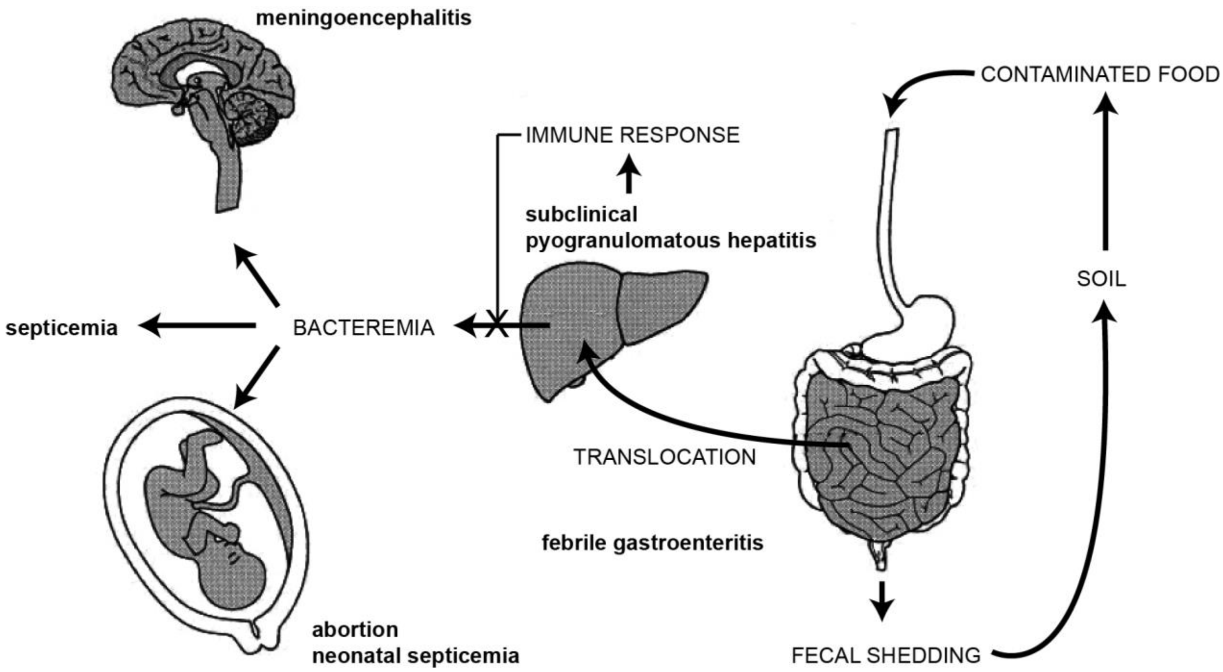

Figure 1. Schematic diagram showing the pathophysiology of listeriosis in humans (76)

Those with impaired T-cell production are most at risk of developing invasive listeriosis (76). Immunocompetent individuals produce memory T-cells that direct the cell mediated immune response against *L. monocytogenes* (76). Pregnant women are susceptible to invasive listeriosis due to reduced cell mediated immunity and the tropism of *L. monocytogenes* for the gravid uterus (51). In the US most cases of listeriosis have been reported in neonates and elderly people (11). The greater number of listeriosis cases in those aged 60 years and older can be explained by decreased immune function, reduced physical activity, and chronic diseases related to aging (38).

Compiling listeriosis cases from 2009 to 2013 (12) and categorizing them by month shows that the number of cases increases in June and reaches a peak in August (Figure 2). This summer peak, which is consistent across different years, could be due to a number of factors, including increased environmental temperature, increased outdoor activities, and increased production and consumption of fresh produce.

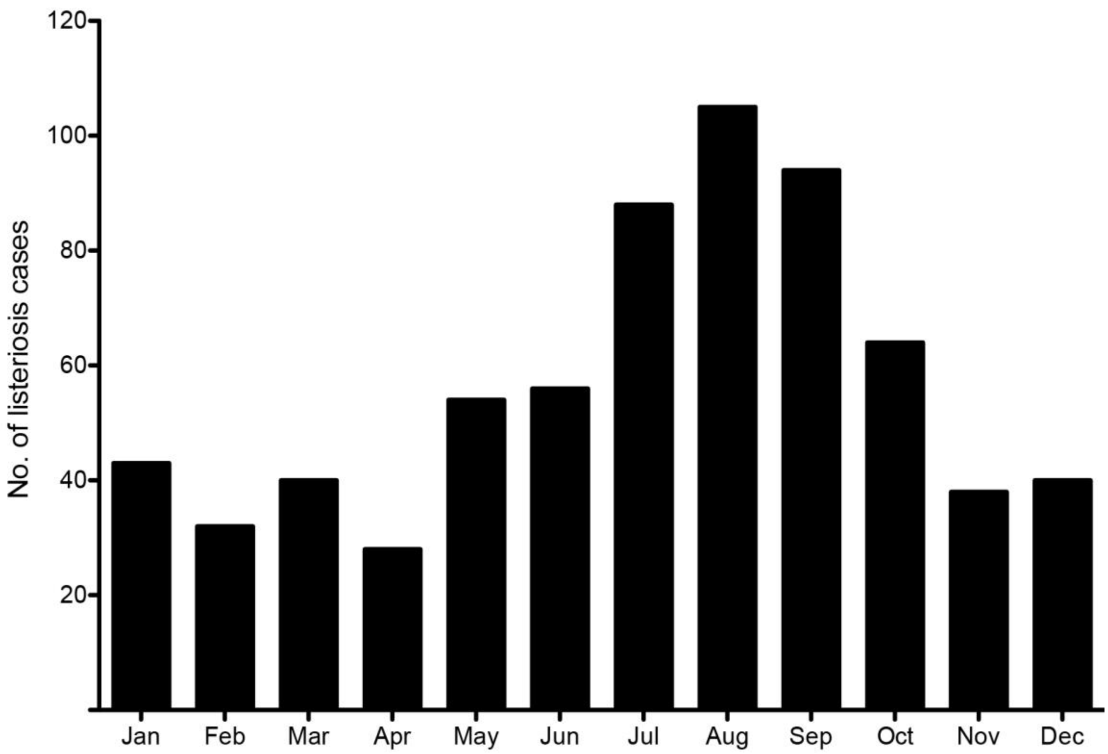

Figure 2. Seasonal variation in reported listeriosis cases from 2009 to 2013. The highest number of listeriosis cases is reported in August (12)

OUTBREAKS OF LISTERIOSIS IN THE US

Clinical cases of listeriosis have been reported worldwide (10, 50, 64). The number of reported cases is higher in developed than developing countries, which may be explained by better surveillance and healthcare (15, 55). Listeriosis outbreaks are frequently associated with the consumption of fresh produce, unpasteurized milk and its products, and ready-to-eat meats (52).

The first recognized outbreak of listeriosis in the US was in 1983 with 49 clinical cases and 14 deaths (8, 24). Pasteurized milk was identified as the cause of the outbreak, which was surprising because standard pasteurization has been shown to kill *L. monocytogenes* (7, 20). The fact that there have been no subsequent outbreaks associated with pasteurized milk suggests that pasteurization is indeed an effective method to inactivate *L. monocytogenes* in milk. The Centers for Disease Control and prevention has reported 24 confirmed major outbreaks of listeriosis between 1998 and 2008 (8). A major outbreak in 2011, associated with cantaloupe produced in Colorado, had 147 registered clinical cases and a fatality rate of 22 % (52, 9). Eighty six percent of cases were in people greater than 60 years old (41).

The association of *L. monocytogenes* with fresh produce has motivated researchers to identify and mitigate sources of contamination in the pre-harvest produce production environment. The remainder of this paper will focus on *L. monocytogenes* in the pre-harvest environment and discuss novel computational approaches to model the likelihood that *L. monocytogenes* will be prevalent in different produce production fields.

PERSISTENCE OF *LISTERIA MONOCYTOGENES* IN SOIL

The reported prevalence of *L. monocytogenes* in soil varies from 0.4 to 91.6% (Table 1). When prevalence data are categorized by land use pattern, the prevalence in soil from livestock farms (mean of 29.7% and a range of 3.0 to 50.0%) is greater than that in all other soil types. This can be explained by the relatively high prevalence of *L. monocytogenes* in livestock and the application of fecal waste to land either directly from the animal or as fertilizer. In one study, Jiang et al. reported that 35.4% of cattle in a herd were shedding *L. monocytogenes* in feces (36), while other studies have reported 20.0 – 29.5% prevalence in cattle (57, 75). The prevalence in pigs (16.0 – 46.6%) is similar to that in cattle, and pig slurry is frequently spread onto pastures (5, 75). The reported prevalence in sheep is in the range 10.7 – 50.0% (57, 82). The presence of *L. monocytogenes* in the environment of livestock facilities can result in contamination of adjacent areas that are not used for livestock production (72). Surface water, in particular, can rapidly transport *L. monocytogenes* across the landscape and, via irrigation, to crops and produce (79).

The reported prevalence of *L. monocytogenes* in soil from produce fields ranges from 7.1 to 91.6 % (Table 1). The high end of this range, which was reported in a study conducted in the early 1970s (35), should be interpreted with caution because the study was conducted while the taxonomic classification of *L. monocytogenes* was still in flux and therefore non-*monocytogenes* species could have been included. Excluding this outlier, the mean prevalence of *L. monocytogenes* in produce fields is 9.7%, and the range is 7.1 – 17.2%.

Table 1. Prevalence of *Listeria monocytogenes* in different soils

Country	Prevalence (%)				Reference
	Produce field	Livestock farm	Forest	Urban	
USA	91.6	-	-	85.7	(35)*
Germany	17.2	-	23.1	-	(77)
USA	-	-	-	0.7	(49)
Spain	-	8.3	-	-	(27)
Canada	8.3	30.8	-	-	(19)
USA	-	30.0	-	-	(57)
Ireland	-	3.0	-	-	(25)
USA	7.1	-	0.4	10.7	(65)
France	-	32.5	-	-	(44)
USA	9.7	50.0	-	20.0	(71)
USA	16.0	-	-	-	(79)
USA	7.9	-	-	-	(80)

*Data reported in this reference should be used cautiously, because this study was conducted before current taxonomic system of *Listeria species*.

Although soil can be a natural reservoir of *L. monocytogenes*, the application of livestock manure to produce fields can increase its prevalence. Manure must be treated appropriately to kill *L. monocytogenes* and other potential pathogens before it is used as fertilizer in produce fields. *Listeria monocytogenes* survived for 43 days in manure-amended soil under laboratory conditions (36), and 128 days in fecal waste applied to land (33). In contrast, *L. monocytogenes* survived for just 21 days in composted manure (75). A 4 log reduction was obtained when manure was composted and dried for 6 days and treated with ammonia (32); however, these data must be interpreted with caution because bacteria can remain viable but non-culturable in composted manure (21, 75). Also, farmers do not always observe good agriculture practice (GAP) for manure storage, instead applying fresh or improperly composted manure to the produce fields (29, 54, 67-69). When surveyed, California cattle farmers reported that liquid manure from cattle was used without treatment in periodic and seasonal fertilization of pasture land and produce fields to supply adequate nitrogen (54). The vast majority of farms (95.9%) used flushing and collection in tanks or ponds, and only 4.1% of the dairy farms identified composting as a management technique for solid manure (54). *Listeria monocytogenes* survived for 90 days in stored slurry, and it survived for more than 32 days when the slurry was applied to land (56).

Temperature, pH, moisture, and competing microbes can affect *L. monocytogenes* persistence in soil (45, 53). *Listeria monocytogenes* can grow at temperatures ranging from 1.1 to 45°C (37, 53). In a study conducted in nutrient-rich growth media, *L. monocytogenes* grew best at 30°C followed by 25°C and 8°C (53). By contrast, numbers declined slowly at each of these temperatures in soil (53), although persistence was greater at 8°C (14 days) than 25°C (7 days) or 30°C (7 days) (53). This is consistent with studies showing that *L. monocytogenes* is more likely to be isolated from soil when the temperature is few degrees Celsius below the average for that time and location (34, 71), and that prevalence is higher in winter-spring than summer (34, 71). Overall, production of fresh produce in the US decreases during the winter months, but California, Colorado, and Florida also produce winter vegetables (31).

Although a neutral pH is most favorable for *L. monocytogenes* survival in soil, it can survive for more than 84 days at a pH between 5.5 and 8.0 (45). When the pH is less than 5.5, survival decreases significantly compared to higher pH soils (45, 53, 77). Enhanced persistence of *L. monocytogenes* at a neutral or close to neutral pH has consequences for produce production because the pH of agricultural soils is generally maintained within this range to decrease the toxicity and enhance the absorption of micronutrients by plants (43).

High moisture also favors the persistence of *L. monocytogenes* in soil (53, 71, 81). *Listeria monocytogenes* survived almost 180 days in clay type soil and 300 days in fertile soil with the moisture level maintained at 7% and 17%, respectively, but survival was less than 60 days when moisture was allowed to evaporate from these soils (81). Other studies have found detection of *L. monocytogenes* in soil to be positively correlated with a rainfall event 2 to 3 days before sample collection (34, 79). Rainfall and irrigation of the produce fields increases moisture levels, which could favor *L. monocytogenes* survival.

Survival of *L. monocytogenes* is greater in sterile than non-sterile soil, suggesting that it doesn't compete well with other soil microflora (45, 53). However, competing microflora appears to have less effect on *L. monocytogenes* survival at a pH <5.5 (45).

Only two studies have reported on the prevalence of *L. monocytogenes* in forest soil (Table 1). While the sources of *L. monocytogenes* in forest soil have not been studied, wildlife are likely to be a more significant source than livestock. Few studies have examined the prevalence of *L. monocytogenes* in wildlife. Weis and Seeliger detected *L. monocytogenes* in 15.7% of fecal samples from deer (77). A study on *L. monocytogenes* prevalence in trapped and hunted wild animals (deer, otter, raccoon and moose) reported a prevalence of 8.3% (47). Fenlon reported a prevalence of 7.8 % in the feces of rooks and gulls from Scotland (23). While these studies clearly show that wildlife shed *L. monocytogenes* in their feces, there is insufficient information to support the conclusion that wild animals are a major source of contamination in produce fields.

LISTERIA MONOCYTOGENES IN WATER

Listeria monocytogenes has been isolated in ground water and surface water (Table 2), with an average prevalence of 24.8% (range: 4.0 – 63.0%) which is similar to the prevalence in soil ($P > 0.05$). The prevalence appears to be significantly higher ($P < 0.05$) in water from North America (38.7%) than Europe (11.7%).

Runoff from livestock fields is the major source of microbial contamination of surface water (70, 83). In one study, 62% of freshwater tributaries impacted by livestock were positive for *L. monocytogenes* (16). By contrast, in rivers not impacted by livestock, the prevalence of *L. monocytogenes* was only 5.9 % (2). Industrial and urban wastewater and feces of wild animals are the other sources of contamination of water with *L. monocytogenes*. About 60% of treated water samples from urban wastewater treatment plant in France were positive for *L. monocytogenes* (1, 59). Therefore, pumping treated wastewater into rivers can result in *L. monocytogenes* contamination, and this contamination can be transmitted to produce fields during irrigation. In one study, the prevalence of *L. monocytogenes* in a produce field was greater after irrigation (12%) than after rainfall (6%) (80).

Few data are available on the prevalence of *L. monocytogenes* in ground water and marine water environments. In Belgium, a 5.0% prevalence was reported in ground water (75) and in Finland, it appeared to be 3.3% (40). Marine water has been found to have a prevalence ranging from 7 to 33% (16, 73). *Listeria monocytogenes* has been reported in marine animals such as fish, shrimp, and oysters, indicating that marine water is also contaminated.

The relative contribution of persistence and recontamination on *L. monocytogenes* prevalence in water is not known. Water used for irrigation must be considered as a source of *L. monocytogenes* in the produce field.

Table 2. Prevalence of *Listeria monocytogenes* in water

Type of water	Country	Identification method	Prevalence (%)	Reference
Surface	Netherlands	Enrichment & culture	21	(18)
Surface	Italy	Enrichment & culture	5	(46)
Fresh and low salinity	USA, CA	Enrichment, culture, & biochemical confirmation	62	(16)
Ground	Belgium	Enrichment & culture	5	(75)
Surface	Italy	Enrichment & culture	43	(4)
Ground	Finland	Enrichment, culture, & biochemical confirmation	3.3	(40)
Marine	Australia	Enrichment & culture	6.6	(73)
Surface (near sheep farm)	Spain	Enrichment, culture, & DNA hybridization	7.8	(27)
Surface	Greece	Enrichment & culture	5.9	(2)
Surface (fish farm)	Denmark	Enrichment, culture, & RAPD typing	16	(30)
Surface	Canada	Enrichment, culture, biochemical confirmation & PCR	10	(48)
Surface	Canada	Enrichment, culture, biochemical confirmation & PCR	19	(83)
Surface	USA, NY	Enrichment & culture	27.5	(71)
Surface (used for irrigation)	USA, NY	Enrichment, culture, PCR & sequencing	63.5	(80)
Surface (used for irrigation)	USA, NY	Enrichment, culture, PCR & sequencing	11.1	(79)
Surface (used for irrigation)	USA, NY	Enrichment, culture, PCR & sequencing	53.1	(79)

GIS ASSISTED LANDSCAPE EPIDEMIOLOGY FOR THE PREVALENCE PREDICTION OF *LISTERIA MONOCYTOGENES*

Landscape epidemiology is an emerging discipline that seeks to predict pathogen prevalence in the environment by understanding how pathogen dynamics is affected by host and vector availability and by edaphic (soil moisture, available water in soil, soil pH and type of soil), topographic, and meteorological (temperature and precipitation) variables (14, 22, 39, 58, 61, 71). GIS is a major tool in landscape epidemiology, as has been demonstrated in studies on viruses (17, 60, 84) and foodborne pathogens (34, 65, 71, 80).

Strawn et al. (71) used GIS to study the landscape epidemiology of foodborne pathogens, including *L. monocytogenes*, on produce farms in New York state. Using a rule-based prevalence prediction model, they categorized a single produce field into areas with high or low predicted *L. monocytogenes* prevalence. This model was recently validated for water and pasture rule with the likelihood of isolating the *L. monocytogenes* were significantly higher in high predicted prevalence area than low predicted prevalence area (78).

Building on the success of this farm-scale prevalence prediction model, I propose that it can be expanded to cover a larger area, such as a county within a state, and, eventually, an entire state or region. Increasing the scale would require modifications to model parameters and the use of the extrapolation function in ArcGIS to predict prevalence at unobserved points or areas using observed or known data.

In landscape epidemiology, machine learning, which uses a basic decision tree method, has replaced statistical techniques such as linear regression to better model complex relationships between a pathogen and multiple environmental variables (3, 14, 84). Machine learning is a branch of computer science that uses artificial intelligence and pattern recognition to build a knowledge-based system by inductive inference from examples (63).

The proposed model would use seven variables to predict *L. monocytogenes* prevalence in a county: 1) livestock density, 2) average annual precipitation, 3) source of irrigation water, 4) air temperature 5), soil moisture, 6) soil pH, and 7) incidence of *L. monocytogenes* in soil. Variables 1-7of the model will be constant for each county. Variables 4–7 of the model will vary among samples collected. Guided by these variables and data on the presence/absence of *L. monocytogenes* in soil samples, machine learning will generate a decision tree that predicts the prevalence of *L. monocytogenes* in an area. An outline of the predictive model for *L. monocytogenes* is given in Figure 3.

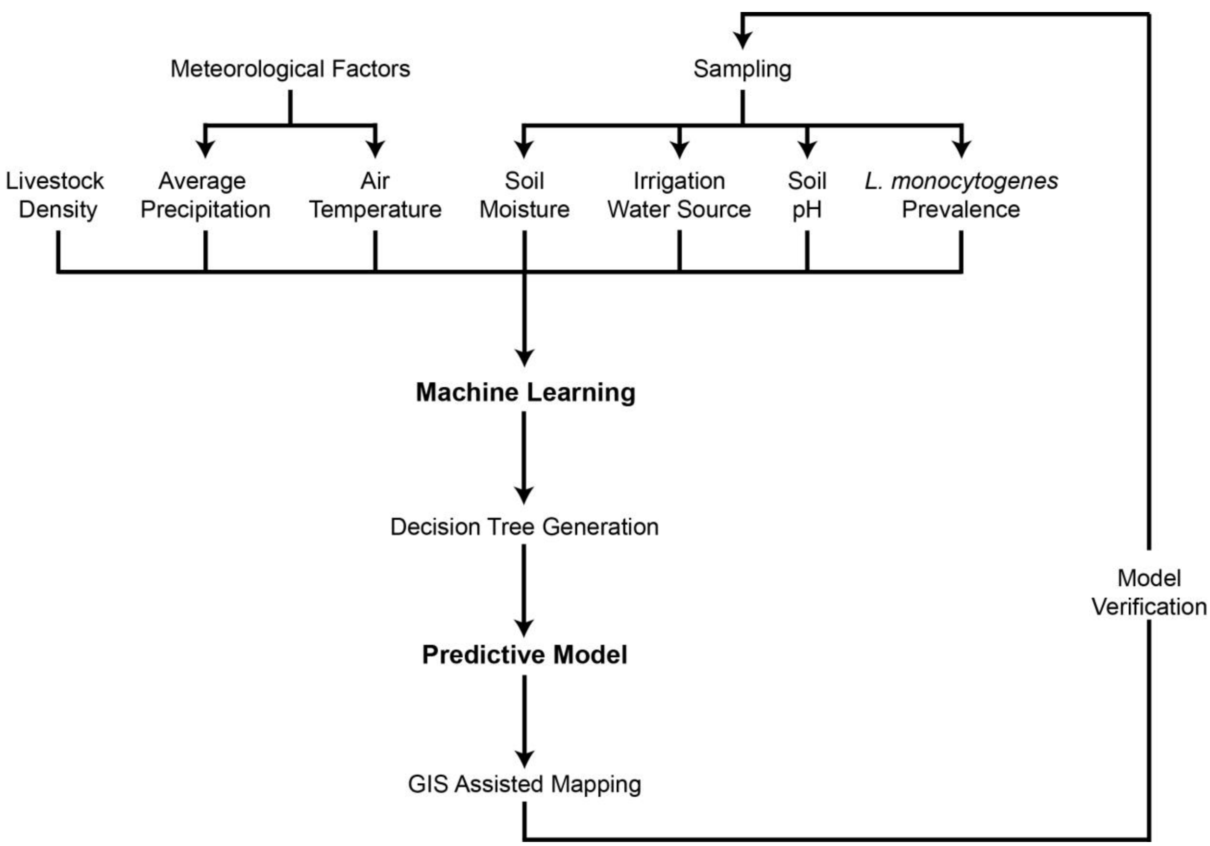

Figure 3. Simplified flow chart to model the prevalence prediction of *Listeria monocytogenes* in the produce field.

Variables of the model

Livestock can contribute to *L. monocytogenes* prevalence in the produce field in three ways: application of livestock manure as fertilizer, contamination of irrigation water through runoff from livestock facilities, and contamination of produce fields by direct runoff from livestock from the adjacent facilities. The model assumes that *L. monocytogenes* prevalence in produce fields will be correlated with livestock density. Rather than calculating the impact of livestock density for each sample location, the initial model will use the average livestock density for the county. More refined spatial density data can be incorporated into the model to increase its sophistication.

Precipitation increases the moisture level in the soil, which enhances *L. monocytogenes* persistence. Heavy precipitation also can increase overland transport of bacteria from adjacent livestock operations to produce fields. All else being equal, soil from counties with higher average annual precipitation are expected to have higher prevalence of *L. monocytogenes*.

Areas with lower temperature are expected to have higher prevalence of *L. monocytogenes* than areas with higher temperature (see section on persistence of *Listeria monocytogenes* in soil). To mitigate the seasonal differences in the temperature even within the same county, the model will use the average annual temperature, in degrees Fahrenheit, of the county. This information would be obtained from the nearest climatological center.

The greater prevalence of *L. monocytogenes* in surface water than ground water results in a greater likelihood of *L. monocytogenes* contamination in produce fields irrigated with surface water (see section on *Listeria monocytogenes* in water). However, data on *L. monocytogenes* prevalence in ground water are limited and more studies are needed to improve the predictive value of irrigation water source in the model.

Listeria monocytogenes prevalence increases with the increasing moisture level in the soil (see section on persistence of *Listeria monocytogenes* in soil). Collected soil samples will be stored in a sealed container upon collection to prevent the evaporation of the moisture.

Listeria monocytogenes prevalence is expected to be higher in soil with at slightly acidic pH than in soil with a neutral or basic pH (see section on persistence of *Listeria monocytogenes* in soil).

Incidence of *L. monocytogenes* in the soil samples is another variable and its presence or absence is dependent upon all other six variables.

Wildlife is also likely to contribute to *L. monocytogenes* prevalence in produce fields, but current data are insufficient for wildlife to be included as a variable in this model.

Sample collection

Model generation using machine learning requires between 400 and 2000 data sets (14, 71, 84). The proposed model will use more than 2000 data sets to train the machine and generate the model. To increase the variation in the variables and reliability of the model, counties from a state will be selected such that there will be wide variations in the values of the independent variables. For example, in North Dakota, counties can be selected from western, central, and eastern parts of the state because of differences in the average annual precipitation (28, 62). At least 200 soil samples will be collected from the produce fields of each selected counties. Samples collected will be evaluated for the presence or absence of *L. monocytogenes* using standard assays (71). Data previously obtained will be used to generate the model.

Model generation and validation

Data sets from the determinants listed above in the collected samples will be aggregated in a table. Presence or absence of *L. monocytogenes* will be the dependent variable and other environmental factors will be independent variables. Training data will be used to develop the algorithm, train the machine, and generate a decision tree. Once the decision tree is generated, ArcGIS will be used to map the prediction of *L. monocytogenes* prevalence in several areas in the test counties based on the decision tree output. Predicted values in sampled (observed) areas of the county can be extrapolated to the remaining (unobserved) areas using the extrapolation function in ArcGIS. In this way, the extrapolation function in ArcGIS allows us to predict the prevalence of *L. monocytogenes* in the entire county. Counties can be selected to validate the model but care should be taken to exclude the counties used to generate the model. Soil samples from produce fields of the test counties will be collected using standard technique (71). Mean error values associated with predicted values will be used to indicate the accuracy of the model. Mean error is the mean absolute difference between the actual values and predicted values. Smaller mean error values would indicate less error and therefore a stronger model. Correlation coefficient can be calculated between *L. monocytogenes* prevalence and values for each independent variable. Increasing the weighting on the most highly correlated variable can be used to refine the model.

CONCLUSION

The proposed county-scale model to predict *L. monocytogenes* prevalence in produce fields is expected to inform the development of more targeted and effective controls. For example, if a producer knows that they are likely to have a higher prevalence of *L. monocytogenes*, they can modify irrigation practices. Because the scale of this model is considerably greater than previously reported, it requires the novel use of the extrapolation function in ArcGIS. Also, to make the model feasible in the short term, some model variables (livestock density, air temperature, and precipitation) are averaged for each county. In the longer term, the model can be refined to improve accuracy and resolution by incorporating more detailed geospatial data. For example, more refined model iterations could use more localized livestock data, including proximity to produce fields. The model can also be progressively expanded to eventually cover an entire state or multiple states.

REFERENCES

1. **Al-Ghazali, M. R., and S. K. Al-Azawi**. 1988. Effects of sewage treatment on the removal of *Listeria monocytogenes*. J. Appl. Bacteriol. **65**:203-208.

2. **Arvanitidou, M., A. Papa, T. C. Constantinidis, V. Danielides, and V. Katsouyannopoulos**. 1997. The occurrence of *Listeria* spp. and *Salmonella* spp. in surface waters. Microbiol. Res. **152**:395-397.

3. **Bennett, C. C., and K. Hauser**. 2013. Artificial intelligence framework for simulating clinical decision-making: A Markov decision process approach. Artif. Intell. Med. **57**:9-19.

4. **Bernagozzi, M., F. Bianucci, R. Sacchetti, and P. Bisbini**. 1994. Study of the prevalence of *Listeria* spp. in surface water. Zentralbl. Hyg. Umweltmed. **196**:237-244.

5. **Boscher, E., E. Houard, and M. Denis**. 2012. Prevalence and distribution of *Listeria monocytogenes* serotypes and pulsotypes in sows and fattening pigs in farrow-to-finish farms (France, 2008). J. Food Prot. **75**:889-895.

6. **Bou Ghanem, E. N., G. S. Jones, T. Myers-Morales, P. D. Patil, A. N. Hidayatullah, and S. E. D'Orazio**. 2012. InlA promotes dissemination of *Listeria monocytogenes* to the mesenteric lymph nodes during food borne infection of mice. PLoS Pathog **8**:e1003015.

7. **Bradshaw, J. G., J. T. Peeler, J. J. Corwin, J. M. Hunt, J. T. Tierney, E. P. Larkin, and R. M. Twedt**. 1985. Thermal resistance of *Listeria monocytogenes* in milk. J. Food Prot. **48**:743-745.

8. **Cartwright, E. J., K. A. Jackson, S. D. Johnson, L. M. Graves, B. J. Silk, and B. E. Mahon**. 2013. Listeriosis outbreaks and associated food vehicles, United States, 1998-2008. Emerg. Infect. Dis. **19**:1-9; quiz 184.

9. **CDC**. 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe--United States, August-September 2011. MMWR Morb. Mortal. Wkly. Rep. **60**:1357-1358.

10. **CDC**. 2013. National Listeria Surveillance Annual Summary, 2011, p. 7. vol. 345. Centers for Disease Control and Prevention, Atlanta, Georgia.

11. **CDC**. 2013. National *Listeria* surveillance annual summary, 2011. Centers for Disease Control and Prevention, Atlanta, Georgia.

12. **CDC**. 2015. *Listeria* (Listeriosis). Centers for Disease Control and Prevention. http://www.cdc.gov/listeria/statistics.html. Accessed 08/29/2015 2045.

13. **CDC**. 2015. Multistate outbreak of listeriosis linked to commercially produced, prepackaged caramel apples made from Bidart Bros. apples. Centers for Disease Control and Prevention, Atlanta, Georgia.

14. **Cesare, F., M. Neteler, S. Merler, S. Menegon, S. Fontanari, A. Donini, A. Rizzoli, and C. Chemini**. 2003. GIS and the random forest predictor: Integration in R for tick-

borne disease risk assessment. In K. Hornik, F. Leisch, and A. Zeileis (eds.), 3rd International Workshop on Distributed Statistical Computing, Technische Universität Wien, Vienna, Austria.

15. **Chen, J., X. Zhang, L. Mei, L. Jiang, and W. Fang**. 2009. Prevalence of *Listeria* in Chinese food products from 13 provinces between 2000 and 2007 and virulence characterization of *Listeria monocytogenes* isolates. Foodborne Pathog. Dis. **6**:7-14.

16. **Colburn, K. G., C. A. Kaysner, C. Abeyta, Jr., and M. M. Wekell**. 1990. *Listeria* species in a California coast estuarine environment. Appl. Environ. Microbiol. **56**:2007-2011.

17. **Dhingra, M. S., R. Dissanayake, A. B. Negi, M. Oberoi, D. Castellan, M. Thrusfield, C. Linard, and M. Gilbert**. 2014. Spatio-temporal epidemiology of highly pathogenic avian influenza (subtype H5N1) in poultry in eastern India. Spat Spatiotemporal Epidemiol **11**:45-57.

18. **Dijkstra, R. G.** 1982. The occurrence of *Listeria monocytogenes* in surface water of canals and lakes, in ditches of one big polder and in the effluents and canals of a sewage treatment plant. Zentralbl Bakteriol Mikrobiol Hyg B **176**:202-205.

19. **Dowe, M. J., E. D. Jackson, J. G. Mori, and C. R. Bell**. 1997. *Listeria monocytogenes* survival in soil and incidence in agricultural soils. J. Food Prot. **60**:1201-1207.

20. **Doyle, M. P., K. A. Glass, J. T. Beery, G. A. Garcia, D. J. Pollard, and R. D. Schultz**. 1987. Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization. Appl. Environ. Microbiol. **53**:1433-1438.

21. **Droffner, M. L., and W. F. Brinton**. 1996. Occurrence and detection of viable *Listeria* in food scrap compost. Zentralbl. Hyg. Umweltmed. **199**:51-59.

22. **Emmanuel, N., N. Loha, M. Okolo, and K. Ikennax**. 2011 Landscape epidemiology: An emerging perspective in the mapping and modelling of disease and disease risk factors. Asian Pacific Journal of Tropical Disease **1**:247-250.

23. **Fenlon, D. R.** 1985. Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. J. Appl. Bacteriol. **59**:537-543.

24. **Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold**. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404-407.

25. **Fox, E., T. O'Mahony, M. Clancy, R. Dempsey, M. O'Brien, and K. Jordan**. 2009. *Listeria monocytogenes* in the Irish dairy farm environment. J. Food Prot. **72**:1450-1456.

26. **Freitag, N. E., G. C. Port, and M. D. Miner**. 2009. *Listeria monocytogenes* - from saprophyte to intracellular pathogen. Nat. Rev. Microbiol. **7**:623-628.

27. **Garcia, E., M. DePaz, J. L. Rodriguez, P. Gaya, M. Medina, and M. Nunez**. 1996. Exogenous sources of *Listeria* contamination in raw ewe's milk. J. Food Prot. **59**:950-954.

28. **George, T. L., A. C. Fowler, R. L. Knight, and L. C. McEwen**. 1992. Impacts of a severe drought on grassland birds in Western North Dakota. Ecol. Appl. **2**:275.

29. **Grewal, S. K., S. Rajeev, S. Sreevatsan, and F. C. Michel, Jr.** 2006. Persistence of *Mycobacterium avium* subsp. paratuberculosis and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. Appl. Environ. Microbiol. **72**:565-574.

30. **Hansen, C. H., B. F. Vogel, and L. Gram**. 2006. Prevalence and survival of *Listeria monocytogenes* in Danish aquatic and fish-processing environments. J. Food Prot. **69**:2113-2122.

31. **Hansen, J. W., J. W. Jones, C. F. Kiker, and A. W. Hodges**. 1999. El Niño–Southern Oscillation Impacts on Winter Vegetable Production in Florida*. J. Clim. **12**:92-102.

32. **Himathongkham, S., and H. Riemann**. 1999. Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. FEMS Microbiol. Lett. **171**:179-182.

33. **Hutchison, M. L., L. D. Walters, T. Moore, D. J. I. Thomas, and S. M. Avery**. 2005. Fate of pathogens present in livestock wastes spread onto fescue plots. Appl. Environ. Microbiol. **71**:691-696.

34. **Ivanek, R., Y. T. Grohn, M. T. Wells, A. J. Lembo, Jr., B. D. Sauders, and M. Wiedmann**. 2009. Modeling of spatially referenced environmental and meteorological factors influencing the probability of *Listeria* species isolation from natural environments. Appl. Environ. Microbiol. **75**:5893-5909.

35. **J., W. H., and J. Donkervo**. 1971. *Listeria monocytogenes* in nature. Appl. Microbiol. **21**:516-&.

36. **Jiang, X. P., M. Islam, J. Morgan, and M. P. Doyle**. 2004. Fate of *Listeria monocytogenes* in bovine manure-amended soil. J. Food Prot. **67**:1676-1681.

37. **Junttila, J. R., S. I. Niemelä, and J. Hirn**. 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic *Listeria*. J. Appl. Bacteriol. **65**:321-327.

38. **Kendall, P. A., V. V. Hillers, and L. C. Medeiros**. 2006. Food safety guidance for older adults. Clin. Infect. Dis. **42**:1298-1304.

39. **Kitron, U.** 1998. Landscape ecology and epidemiology of vector-borne diseases: Tools for spatial analysis. J. Med. Entomol. **35**:435-445.

40. **Korhonen, L. K., M. Niskanen, H. HeinonenTanski, P. J. Martikainen, L. Salonen, and I. Taipalinen**. 1996. Groundwater quality in wells in central rural Finland: A microbiological and radiochemical survey. Ambio **25**:343-349.

41. **Laksanalamai, P., L. A. Joseph, B. J. Silk, L. S. Burall, L. T. C, P. Gerner-Smidt, and A. R. Datta**. 2012. Genomic characterization of *Listeria monocytogenes* strains

involved in a multistate listeriosis outbreak associated with cantaloupe in US. PLoS One **7**:e42448.

42. **Limmahakhun, S., and M. Chayakulkeeree**. 2013. *Listeria monocytogenes* brain abscess: two cases and review of the literature. Southeast Asian J. Trop. Med. Public Health **44**:468-478.

43. **Liu, G. Y., and E. Hanlon**. 2015. Soil pH Range for Optimum Commercial Vegetable Production. http://www.agiweb.org/education/aapg/invest/PreferencesforpH.pdf. Accessed 11/15/15.

44. **Locatelli, A., G. Depret, C. Jolivet, S. Henry, S. Dequiedt, P. Piveteau, and A. Hartmann**. 2013. Nationwide study of the occurrence of *Listeria monocytogenes* in French soils using culture-based and molecular detection methods. J. Microbiol. Methods **93**:242-250.

45. **Locatelli, A., A. Spor, C. Jolivet, P. Piveteau, and A. Hartmann**. 2013. Biotic and abiotic soil properties influence survival of *Listeria monocytogenes* in soil. PLoS One **8**:e75969.

46. **Luppi, A., G. Bucci, P. Maini, and J. Rocourt**. 1988. Ecological survey of *Listeria* in the Ferrara area (northern Italy). Zentralbl. Bakteriol. Mikrobiol. Hyg. A **269**:266-275.

47. **Lyautey, E., A. Hartmann, F. Pagotto, K. M. Tyler, D. R. Lapen, G. Wilkes, P. Piveteau, A. Rieu, W. J. Robertson, D. T. Medeiros, T. A. Edge, V. Gannon, and E.**

Topp. 2007. Characteristics and frequency of detection of fecal *Listeria monocytogenes* shed by livestock, wildlife, and humans. Can. J. Microbiol. **53**:1158-1167.

48. **Lyautey, E., D. R. Lapen, G. Wilkes, K. McCleary, F. Pagotto, K. Tyler, A. Hartmann, P. Piveteau, A. Rieu, W. J. Robertson, D. T. Medeiros, T. A. Edge, V. Gannon, and E. Topp**. 2007. Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. Appl. Environ. Microbiol. **73**:5401-5410.

49. **MacGowan, A. P., K. Bowker, J. McLauchlin, P. M. Bennett, and D. S. Reeves**. 1994. The occurrence and seasonal changes in the isolation of *Listeria spp*. in shop bought food stuffs, human faeces, sewage and soil from urban sources. Int. J. Food Microbiol. **21**:325-334.

50. **Makino, S. I., K. Kawamoto, K. Takeshi, Y. Okada, A. Yamasaki, S. Yamamoto, and S. Igimi**. 2005. An outbreak of food-borne listeriosis due to cheese in Japan, during 2001. Int. J. Food Microbiol. **104**:189-196.

51. **Mateus, T., J. Silva, R. L. Maia, and P. Teixeira**. 2013. Listeriosis during pregnancy: A public health concern. ISRN Obstet. Gynecol. **2013**:851712.

52. **McCollum, J. T., A. B. Cronquist, B. J. Silk, K. A. Jackson, K. A. O'Connor, S. Cosgrove, J. P. Gossack, S. S. Parachini, N. S. Jain, P. Ettestad, M. Ibraheem, V. Cantu, M. Joshi, T. DuVernoy, N. W. Fogg, Jr., J. R. Gorny, K. M. Mogen, C. Spires, P. Teitell, L. A. Joseph, C. L. Tarr, M. Imanishi, K. P. Neil, R. V. Tauxe,**

and B. E. Mahon. 2013. Multistate outbreak of listeriosis associated with cantaloupe. N. Engl. J. Med. **369**:944-953.

53. **McLaughlin, H. P., P. G. Casey, J. Cotter, C. G. M. Gahan, and C. Hill**. 2011. Factors affecting survival of *Listeria monocytogenes* and *Listeria innocua* in soil samples. Arch. Microbiol. **193**:775-785.

54. **Morse Meyer, D., I. Garnett, and J. C. Guthrie**. 1997. A survey of dairy manure management practices in California. J. Dairy Sci. **80**:1841-1845.

55. **Nero, L. A., M. R. de Mattos, A. Barros Mde, M. B. Ortolani, V. Beloti, and B. D. Franco**. 2008. *Listeria monocytogenes* and *Salmonella* spp. in raw milk produced in Brazil: occurrence and interference of indigenous microbiota in their isolation and development. Zoonoses and public health **55**:299-305.

56. **Nicholson, F. A., S. J. Groves, and B. J. Chambers**. 2005. Pathogen survival during livestock manure storage and following land application. Bioresour. Technol. **96**:135-143.

57. **Nightingale, K. K., Y. H. Schukken, C. R. Nightingale, E. D. Fortes, A. J. Ho, Z. Her, Y. T. Grohn, P. L. McDonough, and M. Wiedmann**. 2004. Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. Appl. Environ. Microbiol. **70**:4458-4467.

58. **Nikanorovich, P. E.** 1966. Natural nidality of transmissible diseases: with special reference to the landscape epidemiology of zooanthroponoses. University of Illinois Press, Urbana, IL.

59. **Paillard, D., W. Dubois, R. Thiebaut, F. Nathier, E. Hoogland, P. Caumette, and C. Quentin**. 2005. Occurrence of *Listeria* spp. in effluents of French urban wastewater treatment plants. Appl. Environ. Microbiol. **71**:7562-7566.

60. **Racloz, V., C. Griot, and K. D. C. Stark**. 2006. Sentinel surveillance systems with special focus on vector-borne diseases. Anim. Health Res. Rev. **7**:71-79.

61. **Reisen, W. K.** 2010. Landscape epidemiology of vector-borne diseases. Annu. Rev. Entomol. **55**:461-483.

62. **Current Results** 2015. Average annual precipitation for North Dakota http://www.currentresults.com/Weather/North-Dakota/average-yearly-precipitation.php. Accessed 12/03/2015 2015.

63. **Ross, Q. J.** 1986. Induction of decision trees. Machine learning **1**:81-106.

64. **Ross, T., S. Rasmussen, A. Fazil, G. Paoli, and J. Sumner**. 2009. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. Int. J. Food Microbiol. **131**:128-137.

65. **Sauders, B. D., J. Overdevest, E. Fortes, K. Windham, Y. Schukken, A. Lembo, and M. Wiedmann**. 2012. Diversity of *Listeria* species in urban and natural environments. Appl. Environ. Microbiol. **78**:4420-4433.

66. **Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin**. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. **17**:7-15.

67. **Smith, K. A., A. J. Brewer, J. Crabb, and A. Dauven**. 2001. A survey of the production and use of animal manures in England and Wales. II. Poultry manure. Soil Use and Management **17**:48-56.

68. **Smith, K. A., A. J. Brewer, J. Crabb, and A. Dauven**. 2001. A survey of the production and use of animal manures in England and Wales. III. Cattle manures. Soil Use and Management **17**:77-87.

69. **Smith, K. A., A. J. Brewer, A. Dauven, and D. W. Wilson**. 2000. A survey of the production and use of animal manures in England and Wales. I. Pig manure. Soil Use and Management **16**:124-132.

70. **Soupir, M. L., S. Mostaghimi, E. R. Yagow, C. Hagedorn, and D. H. Vaughan**. 2006. Transport of fecal bacteria from poultry litter and cattle manures applied to pastureland. Water Air and Soil Pollution **169**:125-136.

71. **Strawn, L. K., E. D. Fortes, E. A. Bihn, K. K. Nightingale, Y. T. Grohn, R. W. Worobo, M. Wiedmann, and P. W. Bergholz**. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Appl. Environ. Microbiol. **79**:588-600.

72. **Strawn, L. K., Y. T. Grohn, S. Warchocki, R. W. Worobo, E. A. Bihn, and M. Wiedmann**. 2013. Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. Appl. Environ. Microbiol. **79**:7618-7627.

73. **Tienungoon, S., and C. Garland**. 1995. The occurrence of *Listeria* in temperate aquatic habitats, XII International Symposium on Problems of Listeriosis, Perth, Western Australia.

74. **Van Boeijen, I. K., P. G. Casey, C. Hill, R. Moezelaar, M. H. Zwietering, C. G. Gahan, and T. Abee**. 2013. Virulence aspects of *Listeria monocytogenes* LO28 high pressure-resistant variants. Microb. Pathog. **59-60**:48-51.

75. **Vanrenterghem, B., F. Huysman, R. Rygole, and W. Verstraete**. 1991. Detection and prevalence of *Listeria monocytogenes* in the agricultural ecosystem. J. Appl. Bacteriol. **71**:211-217.

76. **Vazquez-Boland, J. A., M. Kuhn, P. Berche, T. Chakraborty, G. Dominguez-Bernal, W. Goebel, B. Gonzalez-Zorn, J. Wehland, and J. Kreft**. 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. **14**:584-640.

77. **Weis, J., and H. P. Seeliger**. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. **30**:29-32.

78. **Weller, D., S. Shiwakoti, P. Bergholz, Y. Grohn, M. Wiedmann, and L. K. Strawn**. 2015. Validation of a previously developed geospatial model that predicts *Listeria*

monocytogenes prevalence for New York State produce fields. Appl. Environ. Microbiol.:epub ahead of print.

79. **Weller, D., M. Wiedmann, and L. K. Strawn**. 2015. Irrigation Is significantly associated with an increased prevalence of *Listeria monocytogenes* in produce production environments in New York State. J. Food Prot. **78**:1132-1141.

80. **Weller, D., M. Wiedmann, and L. K. Strawn**. 2015. Spatial and temporal factors associated with an increased prevalence of *Listeria monocytogenes* in spinach fields in New York State. Appl. Environ. Microbiol. **81**:6059-6069.

81. **Welshimer, H. J.** 1960. Survival of *Listeria monocytogenes* in soil. J. Bacteriol. **80**:316-320.

82. **Wesley, I.** 2007. Listeriosis in animals, p. 55-85. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, listeriosis and food safety, 3rd ed ed. Marcel Decker, Inc.,, New York, N.Y.

83. **Wilkes, G., T. A. Edge, V. P. J. Gannon, C. Jokinen, E. Lyautey, N. F. Neumann, N. Ruecker, A. Scott, M. Sunohara, E. Topp, and D. R. Lapen**. 2011. Associations among pathogenic bacteria, parasites, and environmental and land use factors in multiple mixed-use watersheds. Water Res. **45**:5807-5825.

84. **Young, S. G., J. A. Tullis, and J. Cothren**. 2013. A remote sensing and GIS-assisted landscape epidemiology approach to West Nile virus. Applied Geography **45**:241-249.