EPIDEMIOLOGICAL INVESTIGATION OF RISK FACTORS FOR MICROBIAL CONTAMINATION IN PRODUCE AT THE PREHARVEST LEVEL

A Dissertation

by

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ABSTRACT

In the United States, the proportion of outbreaks of microbial foodborne illnesses associated with fresh produce has increased over the past decades. A large proportion of these outbreaks have been caused by enteric pathogens, including *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7. The overall objective of this dissertation was to study the risk factors for preharvest produce contamination with these three pathogens and generic *Escherichia coli*, as an indicator organism of fecal contamination, to improve control of foodborne illnesses associated with fresh produce. This objective was accomplished through three independent studies.

The first study identified and characterized known risk factors for contamination of fruits and vegetables at the preharvest level with *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 by conducting a systematic review. The review identified and evaluated 68 published research articles which indicated soil and irrigation water as the most important routes of produce contamination with pathogens. The review indicated the existence of solid evidence for several additional risk factors, including growing produce on clay-type soil, the use of contaminated or non-pH-stabilized manure fertilizer, and the use of spray irrigation with contaminated water, with a particular risk of contamination on the lower leaf surface. A total of 955 spinach samples were collected from 12 spinach farms in Colorado and Texas for the second and third study. The second study evaluated the effect of farm management and environmental factors on spinach contamination with generic *E. coli* at the preharvest level. The results indicated that spinach contamination

was influenced by the time since last irrigation, the use of pond water for irrigation, workers' personal hygiene, the use of the field prior to planting, and the proximity of a poultry farm. The third study evaluated the role of weather and landscape factors, in addition to the farm management and environmental factors, in occurrence of spinach contamination with generic *E. coli* at the preharvest level. The results indicated that spinach contamination was influenced not only by the amount of rain, but also by workers' personal hygiene, the use of the spinach field prior to planting, and the use of manure fertilizer.

In conclusion, the three studies have identified important risk factors for microbial contamination of produce at the preharvest. The control of several of these risk factors has already been the focus of the currently established Good Management Practices (GMP) in produce production. The novel findings suggest that the GMP may need to account for rainfall and improve workers' personal hygiene in order to further reduce produce contamination with microorganisms.

DEDICATION

To my parents, SUNGJAE PARK and SUNHEE LEE.

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Thank to my parents, my elder brother and his wife, and my younger brother who have been the most supportive with unconditional love. I wanted to become a farmer when I was an elementary school student and changed my dream to a veterinarian when I went to middle school. My family have always believed in my dreams and encouraged me to pursue them, and now I complete PhD dissertation on preharvest produce safety as a veterinarian. Most of all, my parents had done their best to raise and educate me in their lives. Therefore, I ascribe my dissertation to my parents.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Foodborne disease continues to be an important public health threat in the United States (US) and worldwide. In the US alone, each year foodborne disease causes approximately 47.8 million illnesses, resulting in 127,839 hospitalizations and 3,037 deaths *(168, 169)*. This disease also imposes a considerable economic burden to society. The total annual cost of foodborne illness is estimated to $51.0 billion in the US *(170)*. Approximately 63.9% of illnesses, which induce hospitalizations, are caused by bacterial pathogens, and of these illnesses, 64.1% are caused by *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 *(169)*. These zoonotic enteric pathogens are shed into the environment through the feces of infected (albeit usually asymptomatic) human or animal hosts *(68, 110)* and *L. monocytogenes* naturally persist in soil *(110)*. They could contaminate foods, including produce, at any stage of the farm-to-table production chain and cause foodborne illness.

Worldwide, fruits and vegetables consumption increased by 4.5% annually between 1990 and 2004 *(61)*. US per capita consumption of fresh fruits increased by 19.0% in the period from 1976 to 2007 *(189)*, and that of fresh vegetables increased by 64.1% between 1970 and 2011 *(190)*. During the same period, numerous cases of foodborne diseases related to fresh produce have been observed in the US *(78, 169, 177)*. US Food and Drug Administration (FDA) reported that approximately 33.3% outbreaks

were associated with leafy green vegetables, such as lettuce and spinach *(193)*. Since produce is often consumed fresh or minimally processed, produce contamination with pathogens is considered a serious health risk. Moreover, it may be difficult to eliminate the pathogen that occurred before harvest *(135)*. Due to these reasons, it is important to understand the contamination routes and weather and environmental factors affecting pathogens' transmission and survivability on produce at the preharvest level. However, the contamination events of produce with *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 are relatively rare *(84, 120, 148)*. Therefore, a common practice in research of enteric pathogens is to use generic *Escherichia coli*, as an indicator of fecal contamination *(150, 151, 187)* to determine the risk factors for produce contamination with enteric pathogens.

This dissertation explores the role of farm management, environmental, landscape, and weather factors on the preharvest produce contamination with *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, and generic *E. coli*. The risk factors for produce contamination have been studied through application of a systematic review of published literature and a design and conduct of cross-sectional field study. The systematic review was conducted in an effort to summarize the existing knowledge about the risk factors for produce contamination with *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7. Next, the cross-sectional study focused on identification of risk factors for spinach contamination with generic *E. coli*.

1.2 Literature review

1.2.1 Foodborne outbreaks of public health and economic concern

Enteric foodborne pathogens, such as *L. monocytogenes, Salmonella*, and *E. coli* O157:H7, impose a considerable economic burden to the society. *L. monocytogenes* was estimated to cause 1,591 human foodborne disease cases, including 255 deaths annually *(169)*. Likewise, the non-typhoidal *Salmonella* was estimated to cause 1.03 million foodborne disease cases, including 378 deaths, while *E. coli* O157:H7 was estimated to cause 63,153 foodborne illness cases, including 20 deaths *(169)*. The economic burden of illnesses associated with these three pathogens is also considerable. The total health-related annual cost of illness due to foodborne infections from *L. monocytogenes*, non-typhoidal *Salmonella*, *E. coli* O157:H7 was estimated as $2.0 billion, 11.4 billion, and 607 million, respectively *(170)*. In addition to this public health burden, these three pathogens impose huge costs (e.g., the cost of recalled and destroyed products, sampling and testing, and cost to reduce contamination) to the food industry and the government. For example, in the US, annual cost of food safety measures to control *L. monocytogenes* was estimated to be between $0.01 billion and $2.4 billion *(108)*. In the listeriosis outbreak associated with cantaloupes in 2011, 4,800 individual cantaloupe packages were recalled *(35)*. Similarly, in the *E. coli* O157:H7 outbreak associated with spinach, spinach growers suffered from decreased (43%) consumers' demand over a year *(34)*.

1.2.2 Bacterial foodborne pathogens in produce

Global consumption of fruits and vegetables showed an annual increase of 4.5% between 1990 and 2004 *(61)*. Increasing numbers of foodborne illness outbreaks have been traced

to the consumption of fresh produce *(48, 78, 169, 177).* The mean percentage of outbreaks associated with leafy greens has been increased from 6% (1998-1999) to 11% (2006-2008) *(78).* Based on the data on foodborne disease outbreaks reported to the US Centers for Disease Control and Prevention, among almost 68,000 illnesses in outbreaks, the commodities associated with the most outbreak related illnesses were poultry (17%), leafy vegetables (13%), beef (12%), and fruits/nuts (11%) *(78)*. *Salmonella* and *E. coli* O157:H7 have been the main causative agents responsible for foodborne outbreaks associated with leafy green vegetables in the US *(177).* Public health and economic consequences of produce contamination with *L. monocytogenes, Salmonella*, and *E. coli* O157:H7 provide a strong incentive to prevent contamination of fresh produce with these pathogens.

1.2.3 Escherichia coli *as an indicator microorganism*

E. coli is a native inhabitant of the intestines of humans and other warm-blooded animals and is disseminated into environment through their feces. Thus, the presence of *E. coli* is commonly used to indicate fecal contamination and potential contamination with enteric pathogens *(3).* Although this bacteria can survive for a certain periods external to the intestine *(132),* many previous studies have documented the merits of *E. coli* as an indicator organism of fecal contamination and the presence of enteric pathogens *(20, 150, 151, 187).* For example, a study by Natvig et al. *(150)* reported the similarity of *E. coli* and *Salmonella* persistence in manure-fertilized soil and the increased prevalence of *E. coli* on produce grown in such soil. A study by Ogden et al. *(151)* reported the similar die off rate of *E. coli* and *E. coli* O157:H7 on the plots to which slurry was applied. A

study by Baudisova showed the advantage of *E. coli* assessment as an indicator for fecal contamination due to the shorter survival of *E. coli* in river water than of total coliforms or fecal coliforms and better stability of its occurrence in a stream than of total coliforms' *(20)*. Thus, *E. coli* has been generally considered as a good indicator organism of fecal contamination in the food industry *(187)*.

Numerous field studies have been conducted to investigate risk factors for produce contamination with *E. coli* as an indicator of enteric foodborne pathogens *(84, 120, 148)*. These studies reported a significantly increased prevalence of *E. coli* in produce grown on fields fertilized by animal waste, including when manure was not composted long enough or the time since manure spreading was short *(97, 147-149)*. These findings were consistent with the results of previous studies that investigated enteric pathogens, including *Salmonella* and *E. coli* O157:H7 *(102, 105, 150)*.

1.2.4 Epidemiological approaches to identify risk factors for produce contamination

Numerous studies, observational and experimental, have been conducted to investigate risk factors for produce contamination related to farm management factors and local environmental characteristics. However, observational studies were generally focused on farm management risk factors, such as the use of manure fertilizer *(147, 148)* and organic farm practices *(129, 148, 149)*. A few environmental risk factors (e.g., wild animal intrusion *(152)*) were studied using an observational study design, while other environmental (e.g., soil pH, soil type) *(45, 98, 150)* and weather *(27, 37, 38, 40, 51, 52, 74, 131, 150)* (e.g. temperature, rainfall, wind speed) factors were studied using laboratory or field based controlled trials.

When we evaluate the effect of a risk factor on microbial contamination of produce under the controlled conditions, we can miss to consider other factors that may occur under the natural environmental conditions. For example, previous studies evaluated the effect of high temperature on the persistence of microorganisms on the produce *(44, 99)*, but these studies did not consider harsh conditions, such as UV radiation and relatively dry environment and high temperature, which might occur under intense sunlight. Thus, while controlled trials' strength is in identifying causal relationships, generalizability of their findings may be limited. On the other hand, observational studies are limited to identifying associations rather than causal relationship. However, compared to controlled trials, observational studies are generally financially more feasible and their findings may be valid under more diverse natural conditions.

Most observational field studies on microbial contamination of produce examined separately the individual groups of farm management *(129, 148, 149)* and local environmental characteristics *(86)*. Farm management factors and/or local environmental characteristics can have offsetting or synergic effects with each other on microbial contamination of produce. For example, rainfall can splash or transport pathogens onto produce *(37, 38, 131)* and wind can also transport pathogens *(86)*. However, a moderate rainfall on a nearby beef farm may be able to reduce transmission of pathogens in dust by wind. Thus, all risk factors should be evaluated not only separately, but also simultaneously.

Contamination of produce with foodborne pathogens is affected by contamination events and pathogens' survivability. Thus, in order to effectively control produce contamination with foodborne pathogens at the preharvest level, it is important to determine both the contamination routes and weather and environmental factors affecting pathogens' survivability.

1.3. Overall objective of the dissertation

The overall objective of this dissertation was to study the risk factors for microbial contamination of produce at the preharvest level. This objective was accomplished through three independent studies described in this dissertation. The first study involved conduct of a systematic review to identify and characterize known risk factors for produce contamination with *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7 at the preharvest level. The second study determined the effect of farm-management and environmental factors on the probability of spinach contamination with generic *E. coli* at the preharvest level. The third study determined the joint effects of weather, landscape and farm management and environmental factors on generic *E. coli* contamination of spinach at the preharvest level.

CHAPTER II

RISK FACTORS FOR MICROBIAL CONTAMINATION IN FRUITS AND VEGETABLES AT THE PREHARVEST LEVEL: A SYSTEMATIC REVIEW [*]

The objective of this study was to perform a systematic review of risk factors for contamination of fruits and vegetables with *Listeria monocytogenes, Salmonella*, and *Escherichia coli* O157:H7 at the preharvest level. Relevant studies were identified by searching six electronic databases: MEDLINE, EMBASE, CAB Abstracts, AGRIS, AGRICOLA, and FSTA, using the following thesaurus terms: *L. monocytogenes, Salmonella, Escherichia coli* O157 AND *fruit, vegetable*. All search terms were exploded to find all related subheadings. To be eligible, studies had to be prospective controlled trials or observational studies at the preharvest level and had to show clear and sufficient information on the process in which the produce was contaminated. Of the 3,463 citations identified, 68 studies fulfilled the eligibility criteria. Most of these studies were on leafy greens and tomatoes. Six studies assessed produce contamination with respect to animal host-related risk factors, and 20 studies assessed contamination with respect to pathogen characteristics. Sixty-two studies assessed the association between produce contamination and factors related to produce, water, and soil, as well as local ecological conditions of the production location. While evaluations of many risk factors for preharvest-level produce contamination have been reported, the quality assessment of

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the reviewed studies confirmed the existence of solid evidence for only some of them, including growing produce on clay-type soil, the application of contaminated or non-pH-stabilized manure, and use of spray irrigation with contaminated water, with a particular risk of contamination on the lower leaf surface. In conclusion, synthesis of the reviewed studies suggests that reducing microbial contamination of irrigation water and soil are the most effective targets for the prevention and control of produce contamination. Furthermore, this review provides an inventory of the evaluated risk factors, including those requiring more research.

2.1. Introduction

In the United States, between 1976 and 2007 per capita consumption of fresh fruits and vegetables increased approximately 19% *(189)* and 57% *(190)*, respectively. The proportion of outbreaks of foodborne illness attributed to fresh produce also increased from 0.7 % in the 1970s to 6 % in the 1990s *(177)*. Microbial contamination is the main cause of foodborne illness related to fresh produce. Among 1,400 potential food-contaminating microbial species, 58% are zoonotic *(68)*, including *Listeria monocytogenes, Salmonella*, and *Escherichia coli* O157:H7. These three pathogens are among the most important pathogens of concern to produce food safety. Indeed, they have been recovered from a wide variety of produce types *(23, 68)*

Foodborne outbreaks associated with fresh produce contaminated with these three pathogens have been reported from several countries around the world in recent decades. The largest *E. coli* O157:H7 infection outbreak occurred in 1996 in Japan, when

approximately 7,892 school children and 74 school staff and teachers became infected with *E. coli* O157:H7 from white radish sprouts *(140)*. Between 1973 and 1997, *Salmonella* accounted for the largest number (30 of 103 cases) of produce-related outbreaks of foodborne illness in the United States *(177)*. Most recently, in 2011, whole cantaloupes contaminated with *L. monocytogenes* caused 146 illness and 30 deaths in the United States *(35)*.

These three pathogens also constitute a considerable economic burden to the society. In the United States alone, the total economic cost of foodborne illnesses due to *L. monocytogenes*, non-typhoidal *Salmonella*, and *E. coli* O157:H7 have been estimated as $2.0 billion, $4.4 billion, and $607 million, respectively *(170)*. In addition to this public health burden, these three pathogens incur large costs to the food industry and the government. Specifically, the industry costs originate from the cost of recalled and destroyed products (failure costs), sampling and testing (appraisal costs) and cost to reduce contamination (prevention costs) *(108)*. For example, annual cost of *L. monocytogenes* food safety to the meat processing industry in the United States was estimated to be between $0.01 billion and $2.4 billion *(108)*. In the midst of the listeriosis outbreak linked to cantaloupes in 2011, the farm implicated in the outbreak recalled 4,800 individual cantaloupe packages *(35)*. Furthermore, the cantaloupe industry as a whole suffered from lost consumers' demand for the product. Similarly, the consumers' demand for bagged spinach decreased 43% over the year after the spinach-associated *E. coli* O157:H7 outbreak in 2007 *(34)*.

The public health and economic consequences of produce contamination with *L. monocytogenes, Salmonella*, and *E. coli* O157:H7 provide a strong incentive to prevent contamination of fresh produce with these pathogens. It is known that these pathogens are shed into the environment through the feces of colonized or infected animal hosts *(68, 110)*, and *L. monocytogenes* is naturally found in soil *(110)*. Once on the field, under favorable conditions, these pathogens survive and multiply, increasing the risk of produce contamination. From the point of view of prevention, it is important to identify how pathogens are introduced to produce fields and why they persist there. These questions have been the subjects of independent research projects over the past decades. Thus, it is important to summarize the existing knowledge so as to provide comprehensive guidelines for decision making and future research. Therefore, many review studies have been conducted in an effort to summarize the microbial contamination routes and persistence in produce fields. However, of the review studies *(23, 24, 46, 47, 50, 68, 96, 181)* including a scoping study *(96)*, only a few may have taken into account study design and quality in determining causality and association. For example, many of the reviewed studies were crosssectional or in general provided weak evidence. It is also possible that systematic bias occurred in the selection of studies included in these reviews, except in the scoping study *(96)*. Unlike narrative reviews, systematic reviews use explicit methods in data synthesis and assess the quality of methods used in the primary studies for drawing conclusions from the evidence *(36)*. The objective of this study was therefore to conduct a systematic review to identify and

characterize previously considered risk factors for contamination of fruits and vegetables with *L. monocytogenes, Salmonella*, and *E. coli* O157:H7 at the preharvest level.

2.2 Materials and methods

2.2.1 Literature search and study selection

Our systematic review was conducted according to the guidelines of the Centre for Reviews and Dissemination *(36)* and Sargeant et al. *(166, 167)*. Relevant studies were identified by searching six electronic databases: Ovid MEDLINE (beginning in 1950), Ovid EMBASE (beginning in 1980), Ovid CAB Abstracts (beginning in 1910), Ovid AGRIS (beginning in 1991), EBSCO AGRICOLA (beginning in 1978), and Ovid Food Science and Technology Abstracts (FSTA, beginning in 1969), through January 2011. Searches were conducted using the following Medical Subject Heading (MeSH) terms: *Listeria monocytogenes, Salmonella*, and *Escherichia coli* O157. These were combined with the fresh produce - associated MeSH terms of *fruit* and *vegetable*. All search terms were properly modified for each database thesaurus (e.g., *Escherichia* instead of *Escherichia coli* O157 in the FSTA database) and were exploded to find all related subheadings. No language restriction was applied for identifying relevant studies. To identify additional studies, we reviewed the references of published reviews, reports, and original research articles.

To be eligible for inclusion in this review, studies had to be prospective. The target of each study had to be soil grown produce becausethere are inconsistencies among produce grown in soil, in a hydroponic greenhouse, and *in vitro (124)*. However, we

included studies based on hydroponic greenhouses to evaluate the effect of environmental factors on the microbial contamination of plants under field conditions. Studies also had to show clear and sufficient information on the process in which the produce was contaminated by the microorganisms. If only a part of a study agreed with our inclusion criteria only the corresponding results were reviewed. We included reports and conference abstracts, if duplicated data were not reported in a full article, but excluded literature reviews, editorials, letters, and cross-sectional studies.

As part of the systematic review, all citations were first identified and checked for whether duplicates existed by one author (SP). Two of the authors (SP and BS) independently selected relevant papers. Discrepancies between the two reviewers were resolved by discussion and consensus with another reviewer (RI). Review of the articles' full text was performed to determine fulfillment of inclusion criteria. Other languages, including Spanish (n = 2), Russian (n = 1), Norwegian (n = 1), and French (n = 2), were translated using the freely available Web-based Google Translator (Google Inc., Mountain View, CA) before reviewing full papers. The agreement between the reviewers was good, based on Cohen's kappa coefficient (k = 0.76).

2.2.2 Data abstraction

For the studies included, information regarding study type, pathogen, fresh produce type studied, risk factors influencing the contamination of produce, information on inoculation, setting (indoor/outdoor), and country was extracted and entered into standardized electronic forms developed in Microsoft Excel (Microsoft Corp, Redmond, WA). When studies did not show the statistical results or did not make conclusions about

the evaluated risk factor, we used overall test results for extracting data. For example, we confirmed the non-alkaline-pH-stabilized manure as a risk factor when higher numbers of pathogens were found for the entire study duration in produce grown in nonstabilized manure than that in stabilized manure *(101, 102, 104, 105)*. Two authors (SP and RI) developed a categorization of themes based on the study results, and assigned those study results to the defined risk categories as related to the three corners of the epidemiological triad: the animal reservoir, pathogen, and environmental factors that also included relevant characteristics of the produce as the vehicle for human exposure to these pathogens.

2.2.3 Quality assessment and data synthesis

Two reviewers (SP and RG) conducted quality assessment and data extraction. Since the individual studies used different methods in sample collection and different protocols for pathogen detection, we did not perform statistical pooling of the outcomes. Instead, we discerned the risk factors of produce contamination based on the conclusion of individual studies. The methodological quality was assessed using a slightly modified Sargeant's checklist *(166)* available as APPENDIX A. A separate checklist was developed to assess cohort studies and controlled trials. Methodological quality was assessed on the basis of study design, sampling frame, study period, and appropriate statistical analysis. Since blinding is rarely done in studies of microbial contamination of produce at the preharvest level, this factor was not included in the methodological quality assessment.

2.3 Results

2.3.1 Selection of eligible studies

The initial search identified a total of 5,802 unique citations (Figure 1). After screening titles and abstracts, 193 potentially relevant articles were reviewed in detail. Seventy nine studies remained for inclusion, but 12 papers among them were finally excluded because they: represented duplicate data *(17, 31, 117)* or lacked critical information (i.e., did not detect pathogens in both the environment and produce when applying noninoculated irrigation water or a fertilizer *(1, 62, 65, 130, 153, 207)*, detected pathogens in produce before irrigating with contaminated water in the observation study *(7)*, detected pathogens both in the control group and the group treated with wastewater irrigation *(63)*, or did not show the clear causality of produce contamination *(157)*). This left 68 studies with sufficient data to be used in the review.

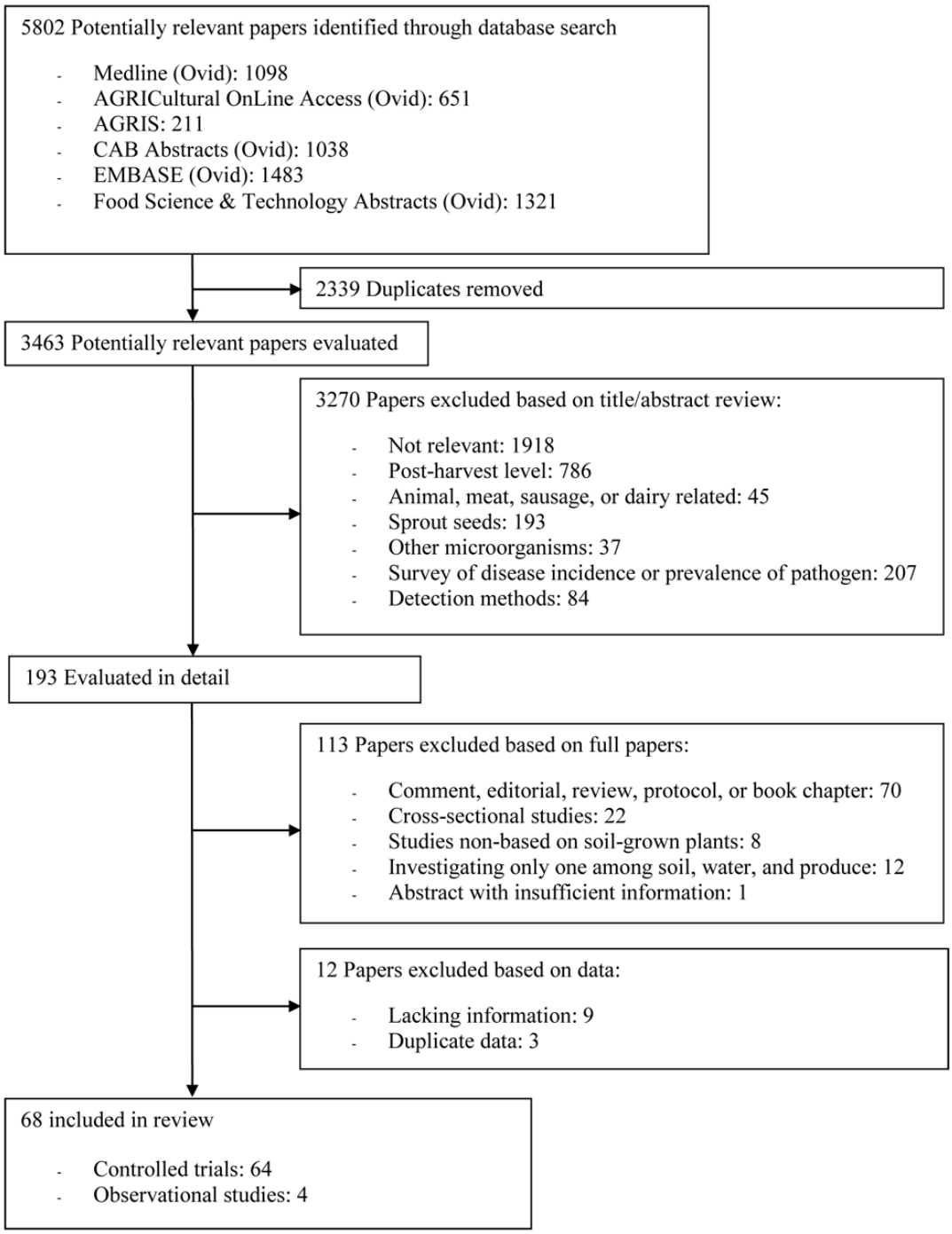


Figure 1. Schematic diagram showing selection of studies throughout the process of the systematic review.

2.3.2 Characteristics of included studies

The risk factors for produce contamination evaluated in the 68 reviewed studies, together with information on the study design, pathogen, produce type, inoculation approach, setting (indoor/outdoor), and results, were summarized (Figure 2). The 68 prospective studies included 64 controlled trials (94%) and 4 observational studies (6%). Only 5 studies focused on *L. monocytogenes*, while the majority focused on *Salmonella* (38 studies) and *E. coli* O157:H7 (34 studies). The main interest in the reviewed studies was in the (i) leafy vegetables, such as lettuce (27 studies) and spinach (12 studies), and (ii) tomatoes (14 studies). Forty-four studies (65%) were conducted under indoor conditions (e.g., growth chamber, climate chamber, laboratory, warehouse, and greenhouse), and 53 studies (78%) applied inoculum suspensions into soil, water, or produce. Forty-six (68%) of the studies were performed in North America, 12 (18%) in Europe, 5 (7%) in Asia, 2 (3%) in South America, 2 (3%) in Africa, and 1 (1%) in Australia (data not shown). These studies are summarized in the next section. Furthermore, Tables 1, 2, and 3 show details about the reviewed studies regarding the animal host factors, pathogen factors, and local environmental factors associated with produce contamination, respectively.

Risk factors associated with produce contamination	Produce								
	Root vegetables			Leafy green vegetables			Fruit vegetables/Fruits		
	S	E	L	S	E	L	S	E	L
Host factors									
Composts from different manure (poultry vs. cow)	104(car↑,ra↑)	102(car↓,on↔)		105(pa↑,le↑)	101(pa↔,le↑)				
Animal host's diet				**69(le↔)**	**69(le↔)**				
Wild animal intrusion							[152](to↑)		
Pathogen factors									
Pathogen species (*Salmonella* vs. *Escherichia coli* O157:H7)				**69(le↔),70(le↓)**	**69(le↔),70(le↓)**		**53(to↑),54(can↑)**, 80(to↑)	**53(to↑),54(can↑)**, 80(to↑)	
Pathogen serotype [a]				69(le↔),70(le↔),**124(le↑)**	**174(sp↑),210(le↔)**		**82(to↑)**,*175(to↑)*		
Pathogen concentration	39(po↑),118(car↔)			**8(sp↑)**,91(sp↔,le↑), 118(sp↔),**164(le↑)**	**57(sp↑)**,58(sp↔),59(sp↑), 91(sp↔,le↑),**158(sp↔)**, **178(le↑)**,**210(le↔)**				
Local environment: produce, water, soil, and local ecological factors									
- **Produce factors**									
Produce type and cultivars [b]	**14(car,ra)**, 104(car,ra)	102(car,on), **103(car,on)**		**14(tu,br,le,rad,en,ci,pa,sp)**, 91(sp,le), 105(pa,le)	**72(al,ry,cl,ha)**, 91(sp,le), 101(pa,le),**142(sp)**, **210(le)**		**14(to)**, 53(to), **54(can)**	**53(to)**,**54(can)**	
Plant age				**21(le↑)**	58(sp↔),59(sp↑),**158(sp↑)**, **145(le↑)**,**210(le↔)**		**85(to↔)**		
Leaf age				**26(le↓)**	**26(le↓)**				
Route of pathogen introduction (soil vs. leaf/stem)					**142(sp↑)**				
Lower surface on the leaf					**57(sp↑,le↔)**,59(le↑),**210(le↑)**				
Physical damage in leaf					**9(le↑)**,**57(sp↓)**				
Physical damage in root				**21(le↑)**	**90(sp↔)**				
- **Water factors**									
Contaminated water without inoculation				32(un↑),133(le↑),[137](le↑,pa↑)			[137](to↑,pi↑)		
Irrigation methods (furrow/spray/drop vs. subsurface drip/surface/mist)				182(le↑)	**57(sp↑)**,**179(le↑)**		182(be↔,can↑)		
Repeated irrigation					**95(le↑)**,**178(le↑)**		**141(to↑)**		
Flood							[152](to↑)		
Other topics [b]				60(cab)	**180(le)**		**113(to)**		
- **Soil factors**									
Contaminated fertilizers without inoculation	39(po↑),118(car↔)	146(ra↔)	128(po↔)	32(un↑),114(co↑),118(sp↑)		4(pa↑,al↑)	45(cu↔)		
Composted or alkaline manure	104(car↓,ra↔)	102(car↓,on↔)		105(pa↓,le↓)	101(pa↔,le↔),120(le↔)				
Soil type ([silty] clay loam vs. [loamy] sand soil)	**150(car↔,ra↑)**			**69(le↔)**,**150(ar↑)**	**69(le↔)**,**92(le↓)**,**93(le↓)**,**95(le↑)**		45(cu↔)		
Soil mixed with contaminated crop debris							**15(to↑)**		
Other topics [b]			**204(car,ra)**	**126(cr)**	**119(le)**,**126(cr)**,**180(le)**				
- **Local ecological factors in cross-contamination and pathogen persistence**									
Insects					**57(le↓)**,**186(sp↑)**				
Nematode					**90(sp↔)**		**25(to↔)**		
Pesticide contamination							80(to↑),[111](sa↔), [112](pe↔)	80(to↑), [112](pe↔)	
Epiphytic bacteria				**43(cr↓)**	**9(le↔)**,**43(cr↓)**,90(sp↔)		**15(to↑)**,*176(to↓)*	**10(to↑)**	
High temperature/humidity	**150(car↔,ra↑)**			**27(ci↑)**,**150(ar↑)**		**51(pa↑)**,**52(pa↑)**			

Figure 2. Risk factors for microbial contamination of produce from the reviewed studies grouped according to the pathogens' epidemiology. Number: controlled trial study, [Number]: observational study. Upward (↑) and downward (↓) arrows indicate the factor was found to be positively and negatively significantly associated, respectively, and horizontal arrows (↔) indicate inconclusive evidence of the association. Bold and italic numbers indicate the studies conducted under indoor (e.g., laboratory, growth chamber, and green house) and unknown conditions, respectively. Underlined numbers indicate the studies with inoculation. [a]Upward arrows indicate the significant differences in the produce contamination among pathogen serotypes. [b]Significance of risk factors was not shown for risk factors. S: *Salmonella*, E: *Escherichia coli* O157:H7, L: *Listeria monocytogenes*.al: alfalfa, ar: argula, be: bell pepper, br: broccoli, cab: cabbage, can: cantaloupe, hcar: carrot, ci: cilantro, cl: clover, co: coriander, cr: cress, cu: cucumber, en: endive, ha: hairy vetch, le: lettuce, on: onion, pa: parsley, pe: persimmon fruit, pi: pimento, po: potato, ra: radish, rad: radicchio, ry: rye, sa: satsuma mandarin, sp: spinach, to: tomato, tu: turnip, u: unknown

2.3.3 Animal host risk factors associated with produce contamination

Animals are considered the primary source of *Salmonella*, *E. coli* O157:H7, and to a lesser extent *L. monocytogenes* (which can persist in the environment) found on contaminated produce. Therefore, risk factors associated with an animal host are of interest in efforts to reduce produce contamination. Four studies *(101, 102, 104, 105)* explored the association between produce contamination and the type of animal waste applied (Table 1). Of these, three studies *(101, 104, 105)* showed that pathogen survival was greater in produce grown in soil amended with manure compost from poultry than in that from cattle, while another study *(102)* showed the opposite result. The other studies *(69, 152)* addressed the influence of the animal host's diet and wild animal intrusion on produce contamination. The appearance of wild animals, such as mice and opossums, contributed to contamination of tomatoes by *Salmonella (152)*. Cattle diet, however, was not found to be associated with the survival of pathogens in plants grown in soil amended with that cattle manure *(69)*.

Table 1. Summary of studies on host factors associated with produce contamination.

Source sorted by host factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Composts from poultry manure					
Islam et al. *(101)*	Split-plot block design with random selection	Lettuce, 11; Parsley, 26	Transplanting plants into soil amended with cattle or poultry manure compost inoculated with *Escherichia. coli* O157:H7	Continuously greater pathogen survival in lettuce grown in soil amended with compost from poultry than from cattle	United States
Islam et al. *(102)*	Split-plot block design with random selection	Carrot, 26; Onion, 13	Transplanting plants into soil amended with cattle or poultry manure compost inoculated with *E. coli* O157:H7	Continuously greater pathogen survival in carrot grown in soil amended with compost from cattle than from poultry	United States
Islam et al. *(104)*	Split-plot block design with random selection	Carrot, 33; radish, 14	Planting seeds into soil amended with cattle or poultry manure compost inoculated with *Salmonella* Typhimurium	Continuously greater pathogen survival in carrots and radishes grown in soil amended with compost from poultry than from cattle	United States
Islam et al. *(105)*	Split-plot block design with random selection	Lettuce, 12; parsley, 35	Transplanting plants into soil amended with cattle or poultry manure compost inoculated with *S.* Typhimurium	Continuously greater pathogen survival in lettuce and parsley grown in soil amended with compost from poultry than from cattle	United States
Animal host's diet					
Franz et al. *(69)*	Completely randomized design (pot placement)	Lettuce, 3	Transplanting lettuce seedlings in soil amended with *E. coli* O157:H7 and *S.* Typhimurium-inoculated manure from dairy cattle fed on straw or grass silage with low or high digestibility plus maize silage	Detection of *E. coli* O157:H7 in only 1 root sample of lettuce grown in soil amended with manure from cattle on highly digestible grass and maize silage diet, but nondetection of *S.* Typhimurium in any plant samples	Nether-lands
Wild animal intrusion					
Orozco et al. *(152)*	(observational study)	Tomato, time unclear	Observations of microbiological quality in a hydroponic tomato farm before, during, and after wild animals (sparrows, mice, and opossums) were entering the greenhouses	Higher level of contamination with *Salmonella* on tomatoes during wild animal invasion, and identical *AvrII* or *XbaI* patterns of *S.* Montevideo among opossums, mice, and tomatoes	United States

2.3.4 Pathogen factors associated with produce contamination

Five studies *(53, 54, 69, 70, 80)* compared the persistence between pathogens on the produce (Table 2). Of these, three studies *(53, 54, 80)* showed longer persistence of *Salmonella* than of *E. coli* O157:H7 on produce, but one study *(70)* reported the opposite. Furthermore, seven studies *(69, 70, 82, 124, 174, 175, 210)* compared produce contamination according to pathogen serotype. All but three of these studies *(69, 70, 210)* showed that produce contamination differed significantly among pathogen serotypes. For example, *Salmonella* serovars Montevideo, Hadar, Newport, and Javiana 6027 were found on 90, 56, 44, and 40% of tomatoes, respectively, when these *Salmonella* serovars were separately inoculated onto the flowers of growing plants *(175)*. Finally, 11 studies *(8, 39, 57-59, 91, 118, 158, 164, 178, 210)* explored the impact of pathogen concentration on produce contamination. Among these, seven studies *(8, 39, 57, 59, 91, 164, 178)* reported that the concentration of the inoculated pathogen and the extent and length of produce contamination were correlated.

Table 2. Summary of studies on pathogen factors associated with produce contamination.

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Pathogen species (*Salmonella* vs. *Escherichia coli* O157:H7)					
Duffy *(53)*	Completely randomized design (treatment)	Tomato, <1	Transplanting tomato plants and spraying leaf surfaces with *E. coli* O157:H7 and *S.* Thompson solutions	Higher levels of *S.* Thompson populations than of *E. coli* O157:H7 populations in the leaves of all tested cultivars	United States
Duffy et al. *(54)*	Not reported	Cantaloupe, 3	Planting cantaloupes and inoculating by watering with suspension containing *E. coli* O157:H7 and *S.* Thompson.	Higher levels of *S.* Thompson populations than *E. coli* O157:H7 populations in rhizosphere of all tested cultivars	United States
Franz et al. *(69)*	Completely randomized design (pot placement)	Lettuce, 3	Transplanting lettuce seedlings into soil mixture to which manure inoculated with *E. coli* O157:H7 and *S.* Typhimurium had been applied	Detection of *E. coli* O157:H7 in only 1 root sample of lettuce, but nondetection of *S.* Typhimurium	Nether-lands
Franz et al. *(70)*	Not reported	Lettuce, 5	Planting lettuce and adding *E. coli* O157:H7 and *S.* Typhimurium to pots later	Significantly higher levels of internalization of *E. coli* O157:H7 than of *S.* Typhimurium	Nether-lands
Guan et al. *(80)*	Row level allocation in two plots	Tomato, 16	Planting tomatoes and spraying fungicide and/or bacteria (*E. coli* O157:H7 and *S.* Enteritidis) combination	Better survival of *S.* Enteritidis than of *E. coli* O157:H7	Canada
Pathogen serotype					
Franz et al. *(69)*	Completely randomized design (pot placement)	Lettuce, 3	Transplanting lettuce seedlings into soil mixture applied to which manure inoculated with *E. coli* O157:H7 and *S.* Typhimurium MAE 119 and 110 had been applied	Nondetection of *S.* Typhimurium in any samples.	Nether-lands
Franz et al. *(70)*	Not reported	Lettuce, 5	Planting lettuce and adding *E. coli* O157:H7 and *S.* Typhimurium MAE 119 and 110 to pots later	No statistical differences between results for *Salmonella* strains.	Nether-lands
Guo et al. *(82)*	Not reported	Tomato, 7	Transplanting tomato plants into potting soil and inoculating mixture of 5 serotypes of *S. enterica* onto the flowers of plant when they started to bloom before fruit set and after fruit set	Persistent by day 49 the greatest for *Salmonella* Montevideo; detection of serotype Poona, Montevideo, Enteritidis, and Michigan in 5, 4, 2, and 2 tomatoes among 11 *Salmonella*-positive tomatoes	United States
Klerks et al. *(124)*	Not reported	Lettuce, 6	Planting lettuce in soil amended with manure inoculated with *S.* Dublin, *S.* Typhimurium, or *S.* Enteritidis	Endophytical colonization of only *S.* Dublin 6 weeks after planting of seeds	Nether-lands

Table 2. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Sharma et al. *(174)*	Not reported	Spinach: 4	Transplanting spinach seedlings in the soil inoculated with *E. coli* O157:H7 suspensions	Detection of pathogens in 78 and 22% in root grown in soil with RM4407/RM5279 and 86-24h11 on day 7, respectively	United States
Shi et al. *(175)*	Not reported	Tomato: 7	Transplanting tomato plants into soil and separately inoculating 10 *S.* serovars onto the flowers of plants	90, 56, 44, and 40% survival of pathogen in tomatoes inoculated with *S.* Montevideo, Hadar, Newport, and Javiana 6027, respectively	Canada
Zhang et al. *(210)*	Not reported	Lettuce: 9	Transplanting lettuce seedlings into soil inoculated soil with 5 *E. coli* O157:H7 strains	Nondetection of any strain of internalized pathogen in lettuce leaves and roots	United States
Pathogen concentration					
Arthurson et al. *(8)*	Not reported	Spinach: 4	Planting spinach seeds in soil amended with cattle slurry inoculated with *S.* Weltevreden at 10^4, 10^5, and 10^6 cells/g	Detection of pathogen only in roots of spinach grown in soil fertilized with manure inoculated with highest dose	Sweden
Chale-Matsau and Snyman *(39)*	Not reported	Potato: 12	Planting potatoes in soil amended with pathogen-rich sludge at rates of 8 and 16 tons/ha	Nondetection of *Salmonella* in the potato core, but detection only in peel of potatoes grown in the soil which had 16 tons of sludge applied per ha	South Africa
Erickson et al. *(57)*	Not reported	Spinach: <1	Transplanting spinach seedlings into pots and applying suspension containing 10^6 or 10^8 CFU of *E. coli* O157:H7 per ml to abaxial or adaxial sides	Detection of internalized pathogen only in spinach leaves treated with inoculum suspension at a higher dose	United States
Erickson et al. *(58)*	Random design (plot)	Spinach: 11	Transplanting spinach into fields and applying *E. coli* O157:H7-contaminated irrigation water (at 0, 2, 4, and 6 log CFU/ml) on 0, 55, and 69 days posttransplantation	No internalization of *E. coli* O157:H7 in spinach roots, except on samples taken 7 days after contamination of plants at 55 days posttransplantation	United States
Erickson et al. *(59)*	Random design (plot) with random selection	Spinach: 11	Transplanting spinach into fields and applying *E. coli* O157:H7-contaminated irrigation water (at 0, 2, 4, and 6 log CFU/ml) at 48 and 69 days posttransplantation	Pathogen detected in 80-95%, 10-30%, and 0% of spinach leaf surfaces immediately after spraying contaminated water with 6, 4, and 2 log CFU of pathogen per ml, respectively	United States
Hutchison et al. *(91)*	Random design (pot placement) with random selection	Spinach, lettuce: 6	Irrigation of lettuce and spinach fields with *E. coli* O157:H7- and *S.* Enteritidis-inoculated water at 2 different concentrations (10^3 and 10^5 CFU/ml)	Greater number of both pathogens in lettuce irrigated with water at a higher concentration (10^5 CFU/ml) of pathogens 1-2 hour after irrigation	United Kingdom

Table 2. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Jimenez et al. *(118)*	Not reported	Spinach: 7, carrot: 12	Planting spinach and carrot seeds in soil treated with Ecological sanitation *(118)* of different quantities of sludge	No clear relationship between the total number of *Salmonella* organisms in spinach stems or leaves and edible portions or leaves of carrots and quantity of sludge used	South Africa
Pu et al. *(158)*	Random design (pot placement, inoculation level, and harvest week)	Spinach: 12	Planting spinach seeds in pots and inoculating 1ml of *E. coli* O157:H7 at 2 concentrations (10^3 and 10^7 CFU/ml) into soil	Nonsignificant differences in contamination prevalence of total and surface of leaves of spinach inoculated pathogen at 2 concentrations	United States
Rodriguez et al. *(164)*	Randomly block design (treatment)	Lettuce: 8	Transplanting lettuce seedlings with and without polyethylene cover into soil amended with compost inoculated with *S.* Typhimurium (1×10^2 and 0.04 microorganisms per g of soil)	Detection of a higher number of pathogens in lettuce grown in soil inoculated with 1×10^2 organisms per g of soil than in those grown in soil inoculated with 0.04 organisms per g of soil	Colombia
Solomon et al. *(178)*	Not reported	Lettuce: 5	Transplanting lettuce plants and spraying irrigation water inoculated with *E. coli* O157:H7 at concentrations of 10^2 or 10^4 CFU/ml	Longer persistence of pathogens on lettuce irrigated with water at higher concentration of *E. coli* O157:H7	United States
Zhang et al. *(210)*	Not reported	Lettuce: 9	Transplanting lettuce seedlings into soil inoculated soil with *E. coli* O157:H7 at 2 concentrations (10^3 and 10^6 CFU/g)	No pathogen internalization detected in lettuce leaves and roots	United States

2.3.5 Local environment: produce factors

Twelve studies *(14, 53, 54, 72, 91, 101-105, 142, 210)* reported impacts of produce type and cultivars on the colonization of pathogens (Table 3). Endive and radicchio had higher incidences of contamination with *Salmonella* than lettuce *(14)*. *E. coli* O157:H7 *(101)* and *Salmonella (105)* were also detected for a longer period of time on parsley than on lettuce. The levels of both *E. coli* O157:H7 and *Salmonella* recovered from lettuce were higher than those recovered from spinach *(91)*. *E. coli* O157:H7 and *Salmonella* persisted longer on carrots than on onions *(102, 103)* and radishes *(104)*, respectively. Brassicaceae family members, such as radishes, turnips, and broccoli, however, had higher incidences of contamination with *Salmonella* than tomatoes, lettuce, and carrots in another study *(14)*. Some of the studies showed that contamination incidences or population numbers of pathogens in produce varied between cultivars within spinach *(142)*, lettuce *(210)*, cantaloupe *(54)*, and tomato *(14, 53)*.

Table 3. Summary of studies on local environmental factors associated with produce contamination.

Source sorted by local environmental factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Produce factors					
Produce type and cultivars					
Barak et al. *(14)*	Not reported	A variety of produce, time unclear	Planting a variety of seeds on soil inoculated with *Salmonella enterica* suspension containing a mixture of 8 strains	Contamination incidence depending on produce type (Brassicaceae>tomato/lettuce/carrot, endive/radicchio >lettuce) and tomato cultivars (Brandywine>Yellow Pear /Nyarous)	United States
Duffy *(53)*	Completely randomized design (treatment)	Tomato, <1	Transplanting 4 tomato cultivars and spraying leaf surfaces with *Escherichia coli* O157:H7 and *S.* Thompson solutions	Higher level of both pathogens on 'Better Boy' and 'Roma' than on 'Supersweet 100' or 'Sweet Million'	United States
Duffy et al. *(54)*	Not reported	Cantaloupe, 3	Planting 4 cantaloupe cultivars and inoculating by watering with suspension containing *E. coli* O157:H7 and *S.* Thompson	The highest population levels of both pathogens in 'Burpee's Ambrosia' and 'Hale's Best' among the cultivars tested	United States
Gagliardi and Karns *(72)*	Random selection	Rye, alfalfa, clover, and hairy vetch, 9	Planting seeds in fallow soil microcosms with or without manure amendment and applying *E. coli* O157:H7-contaminated suspension to the soil	Different persistence of *E. coli* O157:H7 among roots of cover crops (47-96 days on rye roots, 92 days on alfalfa roots, and 25-40 days on clover and hairy vetch roots)	United States
Hutchison et al. *(91)*	Random design (pot placement) with random selection	Spinach and lettuce, 6	Applying irrigation water inoculated with *E. coli* O157:H7 and *S.* Enteritidis to lettuce and spinach fields	Detection of a higher number of both pathogens in lettuce than in spinach 1-2 hour after irrigation	United Kingdom
Islam et al. *(101)*	Split-plot block design with random selection	Lettuce, 11; parsley, 26	Transplanting plants into soil amended with manure compost inoculated with *E. coli* O157:H7 or treated with *E. coli* O157:H7-contaminated irrigation water	Detection of *E. coli* O157:H7 on parsley and lettuce for 177 and 77 days, respectively	United States
Islam et al. *(102)*	Split-plot block design with random selection	Carrot, 26; onion, 13	Transplanting plants into soil amended with manure compost inoculated with *E. coli* O157:H7 or treated with *E. coli* O157:H7-contaminated irrigation water	Detection of *E. coli* O157:H7 on carrots and onions for 168 and 74 days, respectively	United States
Islam et al. *(103)*	Not reported	Carrot, 12; onion, 9	Transplanting plants into soil amended with manure compost inoculated with *E. coli* O157:H7 or treated with *E. coli* O157:H7-contaminated irrigation water	Higher survival rate of pathogen on carrots than on onions	United States
Islam et al. *(104)*	Split-plot block design with random selection	Carrot, 33; radish, 14	Planting seeds in soil amended with manure compost inoculated with *S.* Typhimurium or treated with *S.* Typhimurium-contaminated irrigation water	Detection of *S.* Typhimurium on carrots and radishes for 203 and 84 days, respectively	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Islam et al. *(105)*	Split-plot block design with random selection	Lettuce, 12; parsley, 35	Planting seeds in soil amended with manure compost inoculated with *S.* Typhimurium or treated with *S.* Typhimurium -contaminated irrigation water	Detection of *S.* Typhimurium on parsley and lettuce for 231 and 63 days, respectively	United States
Mitra et al. *(142)*	Not reported	Spinach, 2	Planting seeds and inoculating *E. coli* O157:H7 through leaf drop and soil drench	The highest number of pathogen in cultivar Tyee among three cultivars (Tyee, Space, and Bordeaux) at weeks 2	United States
Zhang et al. *(210)*	Not reported	Lettuce, 9	Transplanting 3 types of lettuce seedlings into soil inoculated with 5 *E. coli* O157:H7 strains	Nondetection of internalized pathogens in the leaves and roots of any lettuce	United States
Plant age					
Bernstein et al. *(21)*	Not reported	Lettuce, 1	Transplanting lettuce plants and inoculating *S.* Newport into soil when the plants were 17 and 33 days old	Detection of pathogens only in lettuce aged 33 days	Israel
Erickson et al. *(58)*	Random design (plot)	Spinach, 11	Transplanting 4- to 8-weeks-old spinach into fields and applying *E. coli* O157:H7-contaminated irrigation water at 0, 55, and 69 days posttransplantation	Nondetection of internalized *E. coli* O157:H7 in spinach roots, except at 7 days after contamination of plants at 55 days posttransplantation	United States
Erickson et al. *(59)*	Random design (plot) with random selection	Spinach, 11	Transplanting spinach into fields and applying *E. coli* O157:H7-contaminated irrigation water (0, 2, 4, and 6 log CFU/ml) at 48 and 69 days posttransplantation	Higher number of pathogens in leaves exposed to the pathogen at 69 days than in those exposed at 48 days when applying relatively high concentration (6 log CFU/ml)	United States
Hintz et al. *(85)*	Random selection	Tomato, 11	Transplanting tomato seedlings and irrigating with water containing *S.* Newport every 7 days	Nonsignificant association between growth stages and presence of pathogen in any of the tissue samples	United States
Mootian et al. *(145)*	Not reported	Lettuce, 5	Transplanting 12- or 30-day-old lettuce into contaminated soil or irrigating with water containing *E. coli* O157:H7	20.5 and 27.1% contamination of 12- and 30-day-old lettuce, respectively, with *E. coli* O157:H7	United States
Pu et al. *(158)*	Random design (pot placement, inoculation level, and harvest week)	Spinach, 12	Planting spinach seeds and inoculating *E. coli* O157:H7 into soil weekly for a total of 5 times	Leaf surface contaminated with pathogen only in spinach aged 3-5 weeks, but not in younger spinach	United States
Zhang et al. *(210)*	Not reported	Lettuce, 8	Transplanting lettuce seedlings and inoculating water and cow manure extract containing *E. coli* O157:H7 on abaxial or adaxial leaf surfaces (around 5 droplets per leaf) 3, 30, and 60 days after transplantation	Differences of number of pathogens in lettuce leaves inoculated with pathogen on abaxial surface among ages	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Leaf age					
Brandl et al. *(26)*	Random design (pot)	Lettuce, <1	Inoculating *E. coli* O157:H7 or *S.* Thompson into potted lettuce plants by immersing aerial part of plant in pathogen suspension when plants were 10th- to 12th-leaf age	Colonization of both pathogens reached approximately 10-fold-higher populations on the surface of young lettuce leaves than of medium-age leaves	United States
Route of pathogen introduction (soil vs. leaf/stem)					
Mitra et al. *(142)*	Not reported	Spinach, 2	Planting seeds and inoculating *E. coli* O157:H7 through leaf drop, soil drench, stab, or pressure inoculation methods	Highest prevalence in the spinach group with soil drench inoculation, and lowest in the group with stab inoculation, except for the pressure inoculation (100% prevalence)	United States
Lower surface on the leaf					
Erickson et al. *(57)*	Not reported	Lettuce and spinach, <1	Transplanting spinach and lettuce seedlings into pots and applying suspension containing *E. coli* O157:H7 into abaxial and adaxial sides	Statistically higher incidences of contaminated abaxial sides of spinach leaves than of adaxial side	United States
Erickson et al. *(59)*	Random design (plot) with random selection	Lettuce, 4	Transplanting germinated lettuce into field and spraying *E. coli* O157:H7 inoculum with a fine mist on abaxial side or spraying with a fine drizzle on adaxial side of leaf surface	Higher number of total surface and internalized pathogens on lettuce leaves sprayed on abaxial surface than on adaxial surface	United States
Zhang et al. *(210)*	Not reported	Lettuce, 9	Transplanting lettuce seedlings and inoculating with water and cow manure extract containing *E. coli* O157:H7 on abaxial or adaxial leaf surfaces	Significantly greater detection of pathogen in leaves inoculated on abaxial side (17.9%) than those on adaxial side (4.7%)	United States
Physical damage in leaf					
Aruscavage et al. *(9)*	Not reported	Lettuce, 2	Transplanting lettuce seedlings and inoculating leaves with *E. coli* O157:H7 suspension by spray method among traumatically damaged, phytopathogen-damaged, and intact plants	Significantly greater populations of pathogens on physically damaged leaves of lettuce than on intact ones over 10-day period	United States
Erickson et al. *(57)*	Not reported	Spinach, 2	Inoculating spinach leaves with *E. coli* O157:H7 suspension by spray and drop-spread inoculation methods followed or not by abrasion damage to leaf surface	Significantly smaller pathogen populations in leaves contaminated by the drop-spread method and then physically damaged than in those not damaged on day 14	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Physical damage in root					
Bernstein et al. *(21)*	Not reported	Lettuce, 1	Transplanting lettuce plants with or without damaged roots and inoculating *S.* Newport into soil when plants were 17 and 33 days old	Increased number of pathogens in lettuce plants aged 33 days with damaged roots (5,130 ± 440 CFU/g) compared with those with intact roots (500 ± 120 CFU/g) at 2 days postinoculation	Israel
Hora et al. *(90)*	Not reported	Spinach, 1	Comparing intact and damaged roots of 5-week-old spinach plants which were irrigated with *E. coli* O157:H7 suspension	Noninternalization of pathogen into aerial leaf in any group, but detection in all surface-sterilized root in both groups	Canada
Water factors					
Contaminated water without inoculation					
Cai et al. *(32)*	Not reported	Unclear cultivar, time unclear	Investigating 3 vegetable farm villages using sewage plant effluent and sludge, as case points, and comparing with 2 farm villages using deep well and night soil, as control points	7.8% and 7.5% detection rates of *Salmonella* in soil samples and 27% and 1.8% detection rates in vegetable samples of case and control points, respectively	China
Manas et al. *(133)*	Not reported	Lettuce, 9	Monitoring 2 lettuce plots irrigated with treated wastewater (30% industrial and 70% of domestic origin) and drinking water for 3 seasons	Significantly high occurrences of lettuce contamination in plants irrigated with wastewater compared with those irrigated with drinking water 40 days after planting	Spain
Melloul et al. *(137)*	(observational study)	Lettuce, parsley, tomato, pimento, time unclear	Investigating wastewater irrigated into fields and crops grown in the fields (parsley and lettuce in winter and tomatoes and pimento in summer)	Contamination both plants grown in winter (parsley and lettuce, 85.7%) and in summer (tomatoes and pimento, 44.4%)	Morocco
Irrigation methods (subsurface drip/spray/drop vs. furrow/surface/mist)					
Erickson et al. *(57)*	Not reported	Spinach, 2	Inoculating spinach leaves with *E. coli* O157:H7 suspension by spray and drop-spread inoculation methods	Higher number of internalized pathogens when small droplets were applied than with mist spraying	United States
Solomon et al. *(179)*	Not reported	Lettuce, 6	Planting lettuce seeds and applying irrigation water inoculated with *E. coli* O157:H7 to plants through surface and spray irrigation	Larger number of positive results of pathogens in plants exposed to pathogen through spray irrigation (90.6%) than surface irrigation (18.8%)	United States
Stine et al. *(182)*	Random selection	Cantaloupe, lettuce, and bell pepper, 2	Pumping reservoir contents into irrigation water, inoculating *Salmonella* into the water, and applying subsurface drip and furrow irrigation to plots	Greater contamination of cantaloupe and iceberg lettuce with 2 to 4 orders of magnitude for furrow irrigation than for subsurface drip irrigation method	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Repeated irrigation					
Ibekwe et al. *(95)*	Completely randomized design	Lettuce, 7	Transplanting lettuce seedlings and irrigating with water contaminated with *E. coli* O157:H7 at transplanting day (day 1) and 15 days later	Increased population of pathogens in the phyllosphere after 2nd contamination at 15 days	United States
Miles et al. *(141)*	Not reported	Tomato, 12	Making 6 tomato plant groups and applying 1-6 times *S.* Montevideo-contaminated water events (7 log CFU/ml; irrigating 350ml)	No detection of pathogens from any stem and leaf samples, but detection in 1 root sample from each of groups 4 and 5 and 3 root samples from group 6	United States
Solomon et al. *(178)*	Not reported	Lettuce, 5	Transplanting lettuce plants and spraying with irrigation water inoculated with *E. coli* O157:H7 on days 1, 7, or 14 or combination of these days	Increased populations of *E. coli* O157:H7 on lettuce after repeated contamination by irrigation water on day 7 or on days 7 and 14 after 1st exposure	United States
Flood					
Orozco et al. *(152)*	(observational study)	Tomato, time unclear	Observations of microbiological quality (*Salmonella*) in a hydroponic tomato farm before, during, and after flood	Detection of *S.* Newport in tomatoes, puddles, and soil during and after flood, despite nondetection before flood	United States
Other topics					
Escaff et al. *(60)*	Not reported	Cabbage, 12	Applying irrigation water containing *Salmonella* to plots with shallow or deep furrow at 2 water flow rates (1 or 8 liters/s)	Detection of pathogens from samples only with high-flow-rate irrigation in plots with shallow (1/19) and deep (1/19 samples) furrows	Chile
Jablasone et al. *(113)*	Not reported	Tomato, 5	Irrigating tomato plants every other day with water contaminated with *S.* Enteritidis	Nondetection of any pathogens from the stem, leaf, and fruit samples for 5 weeks	Canada
Solomon et al. *(180)*	Not reported	Lettuce, <1	Irrigating lettuce with water containing *E. coli* O157:H7	Detection of pathogens in lettuce for up to whole observation period (5 days) after exposures to pathogens	United States
Soil factors					
Contaminated fertilizers without inoculation					
al-Ghazali and al-Azawi *(4)*	Not reported	Parsley, 5; alfalfa, unclear	Planting parsley and alfalfa on soil mixed with sewage sludge cake which was ready to be sold to farmers	Detection of *Listeria monocytogenes* on parsley and alfalfa samples developed in soil mixed with sewage sludge cake	Iraq

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Cai et al. *(32)*	Not reported	Unclear cultivar, unclear	Investigating 3 vegetable farm villages using sewage plant effluent and sludge, as case points, and comparing with 2 farm villages using deep well and night soil, as control points	7.8 and 7.5% detection rate of *Salmonella* in soil samples and 27% and 1.8% detection rate in vegetable samples of case and control points, respectively	China
Chale-Matsau and Snyman *(39)*	Not reported	Potato, 12	Planting potatoes in soil amended with pathogen-rich sludge at application rates of 8 and 16 tons/ha	Detection of *Salmonella* on outside (peel) of cleaned potatoes grown in soil with sludge application rate of 16 tons/ha	South Africa
Cote and Quessy *(45)*	Randomized complete block design	Cucumber, time unclear	Planting pickling cucumber on soil which had liquid hog manure applied	Detection of *Salmonella* for 54 days in soil, but nondetection in any pickling cucumber samples	Canada
Jaeger et al. *(114)*	Not reported	Coriander, time unclear	Applying broiler litter-treated soil for coriander production	Detection of *S.* Sofia and Typhimurium from coriander	Australia
Jimenez *(118)*	Not reported	Spinach, 7; carrot, 12	Planting spinach and carrot seeds in soil applied Ecological sanitation *(118)* with the different quantities of sludge	Detection of *Salmonella* in spinach stems and leaves, but nondetection in edible portion and leaves of carrots	South Africa
Liao et al. *(128)*	Randomized complete block design	Potato, time unclear	Applying liquid dairy manure to the soil production 2 weeks before planting potato	Despite detection of *L. monocytogenes* in manure, nondetection of pathogens in any potato tuber samples at harvest	United States
Mukherjee et al. *(146)*	Random selection	Radish, 10	Monitoring soil and radishes grown in garden amended with cattle manure predicted to be contaminated with *E. coli* O157:H7	Nondetection of pathogen in 4 composite samples of radishes, despite detection of pathogens in the soil	United States
Composted or alkaline manure					
Islam et al. *(101)*	Split-plot block design with random selection	Lettuce, 11; parsley, 26	Transplanting plants into soil amended with compost or alkaline-stabilized compost inoculated with *E. coli* O157:H7	Nonconsistent better survival of pathogen in lettuce and parsley in the entire experimental periods when dairy manure was applied than when alkaline-stabilized compost was applied	United States
Islam et al. *(102)*	Split-plot block design with random selection	Carrot, 26; onion, 13	Transplanting plants on soil amended with compost or alkaline-stabilized compost inoculated with *E. coli* O157:H7	Consistently better survival of pathogens only in carrots in entire experiment periods when dairy manure was applied than when alkaline-stabilized compost was applied	United States
Islam et al. *(104)*	Split-plot block design with random selection	Carrot, 33; radish, 14	Planting seeds into soil amended with compost or alkaline-stabilized compost inoculated with *S.* Typhimurium	Consistently better survival of pathogens only in carrots in entire experiment periods when dairy manure was applied than when alkaline-stabilized compost was applied	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Islam et al. *(105)*	Split-plot block design with random selection	Lettuce, 12; parsley, 35	Transplanting plants into soil amended with compost or alkaline-stabilized compost inoculated with *S.* Typhimurium	Consistently better survival of pathogens in lettuce and parsley in entire experiment periods when dairy manure was applied than alkaline-stabilized compost was applied	United States
Johannessen et al. *(120)*	Randomized complete block design with random selection	Lettuce, 10	Applying slurry, firm manure, and compost of bovine origin without inoculation into soil for lettuce production	Detection of *E. coli* O157:H7 from slurry, firm manure, and soil 7 days after applying fertilizers, but nondetection of any pathogens from lettuce outer leaves at harvest	Norway
Soil type ((silty) clay loam vs. (loamy) sand soil)					
Cote and Quessy *(45)*	Randomized complete block design	Cucumber, time unclear	Planting pickling cucumber in sandy loam or loamy sand soil with liquid hog manure applied	Detection of *Salmonella* for 54 days in soil, but nondetection in any pickling cucumber samples, regardless of soil type	Canada
Franz et al. *(69)*	Completely randomized design (pot placement)	Lettuce, 3	Transplanting lettuce seedlings into loamy and sandy soil with manure inoculated with *E. coli* O157:H7 and *S.* Typhimurium applied	Detection of *E. coli* O157:H7 in only 1 root sample of lettuce grown on amended loamy soil, but nondetection of *S.* Typhimurium in any plant samples	Nether-lands
Ibekwe et al. *(93)*	Completely randomized design (treatment)	Lettuce, 8	Transplanting lettuce seedlings into clay and sandy soil and irrigating with water contaminated with *E. coli* O157:H7	Better survival of pathogens in rhizosphere and phyllosphere of lettuce grown in sandy soil than in those of lettuce grown in clay soil	United States
Ibekwe et al. *(95)*	Completely randomized design (treatment)	Lettuce, 7	Transplanting lettuce seedlings into clay and sandy soil and irrigating with water contaminated with *E. coli* O157:H7 at transplanting day (day 1) and 15 days later	Detection of 170 and 67 CFU *E. coli* O157:H7 per g from lettuce phyllosphere grown on clay and sandy soil, respectively, 12 days after applying irrigation	United States
Ibekwe et al. *(92)*	Completely randomized design (treatment)	Lettuce, 6	Transplanting lettuce seedlings into clay and sandy soil and irrigating with water contaminated with *E. coli* O157:H7	Detection of pathogen up to 1st 21 days and 7 days in lettuce grown on sandy and clay soil, respectively	United States
Natvig et al. *(150)*	Random design (plot)	Radish, arugula, and carrot, 8	Planting vegetables in silty clay loam and loamy sand soil beds amended with bovine manure inoculated with *S.* Typhimurium	More contamination of arugula and radishes by pathogen in silty clay loam soil than in loamy sand soil	United States

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Contaminated crop debris					
Barak and Liang *(15)*	Not reported	Tomato, time unclear	Applying soil mixed with plant debris grown in soil inoculated with *S. enterica* to production of tomatoes	Detection of contamination of subsequent crop through crop debris, and influence of fallow period on contamination	United States
Other topics					
Johannessen et al. *(119)*	Random selection	Lettuce, 7	Transplanting lettuce seedlings into soil with dairy cattle manure inoculated with *E. coli* O157:H7 applied	Detection of pathogen in soil for 8 weeks after fertilizing, but nondetection from edible parts of lettuce, root, or outer leaves for entire study period	Norway
Kupriyanov et al. *(126)*	Not reported	Cress, time unclear	Sowing cress seed in pots containing cattle excreta mixture with *S.* Typhimurium and *E. coli* O157:H7	Detection of both pathogens in phyllosphere and rhizosphere at all time points with gradual decreases in their population	Russia
Solomon et al. *(180)*	Not reported	Lettuce, <1	Applying manure slurry inoculated with *E. coli* O157:H7	Detection of pathogens lettuce for up to whole observation period (3 days) after exposure to pathogens	United States
Van Renterghem et al. *(204)*	Not reported	Radish and carrot, 8	Sowing radish and carrot seed in soil inoculated with *L. monocytogenes*	Detection of pathogens in only 3 radish samples when collecting 6 samples from each vegetable	Belgium
Local ecological factors in cross-contamination and pathogen persistence					
Insects					
Erickson et al. *(57)*	Not reported	Lettuce, 2	Brief exposing lettuce leaves to thrips, aphids, or cabbage loopers and inoculating *E. coli* O157:H7 suspension	Significantly reduced internalization of pathogens in lettuce leaves exposed to insects comparing to internalization of pathogens in leaves not exposed	United States
Talley et al. *(186)*	Not reported	Spinach, <1	Confining house flies on manure or agar medium inoculated with *E. coli* O157:H7 and placing flies on spinach plants within plastic cages	Detection of pathogens in 100% of spinach leaves after exposure to house flies	United States
Nematode					
Beuchat et al. *(25)*	Randomly located pots	Tomato, 4	Planting tomatoes in soil inoculated with *Salmonella* and/or infested with plant-parasitic nematode	Nondetection of pathogens in either leaf or stem samples	United States
Hora et al. *(90)*	Not reported	Spinach, 7	Transplanting spinach plants into soil inoculated with nematodes and irrigating with water containing *E. coli* O157:H7	Noninternalization of pathogens into aerial leaf tissue in any spinach plants	Canada

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
Pesticide contamination					
Guan et al. *(80)*	Row level allocation in two plots	Tomato, 16	Planting tomatoes and spraying with fungicide and/or bacteria (*E. coli* O157:H7 and *S.* Enteritidis) combination	Detection of pathogens in leaves and fruit skin of tomatoes in fungicide and bacteria combination group for 45 hours	Canada
Izumi et al. *(111)*	(observational study)	Satsuma mandarin, time unclear	Observations of potential sources of microbial contamination in the process of satsuma mandarin production	Detection of *Salmonella* in pesticide solution, but nondetection in any fruit samples	Japan
Izumi et al. *(112)*	(observational study)	Persimmon, time unclear	Observations of potential sources of microbial contamination in the process of persimmon production	Detection of *E. coli* O157:H7 and *Salmonella* in agricultural water and of *Salmonella* in soil and pesticide solution, but nondetection in any fruit samples	Japan
Epiphytic bacteria					
Aruscavage et al. *(9)*	Not reported	Lettuce, 2	Transplanting lettuce seedlings and spray inoculating with *E. coli* O157:H7 suspension on leaves among traumatically damaged, phytopathogen-damaged, and intact plants	Nonsignificant difference in the number of pathogens on lettuce phytopathogen-damaged lettuce and on intact or mechanically damaged plants on day 10	United States
Aruscavage et al. *(10)*	Not reported	Tomato, <1	Spraying with *Xanthomonas campestris* or *Pseudomonas syringae* in separated greenhouse rooms, transferring to separated growth chambers, and spraying *E. coli* O157:H7	Significantly higher populations of *E. coli* O157:H7 on *X. campestris*–infected tomato plants than on *P. syringae*–infected tomato plants and control plants	United States
Barak and Liang *(15)*	Not reported	Tomato, time unclear	Inoculating or not inoculating seeds with *X. campestris* and sowing in soil inoculated with high or low concentration of *S. enterica*	Noninfluence of cocolonization on incidence of *S. enterica* contamination in phyllosphere, but growth of *S. enterica in* phyllosphere of cocolonized plant	United States
Cooley et al. *(43)*	Not reported	Cress, 5	Inoculation of *S.* Newport or *E. coli* O157:H7 into seeds and sowing separately on different types of soil	Negative influence of pathogen persistence on cress from presence of *Enterobacter asburiae*	United States
Hora et al. *(90)*	Not reported	Spinach, 1	Transplanting spinach plants into soil and inoculating with cocktail of *P. syringae* and *E. coli* O157:H7	Noninfluence of cocolonization on pathogen populations of *E. coli* O157:H7 on spinach	Canada
Shi et al. *(176)*	Not reported	Tomato, 7	Introducing *S.* Montevideo or Typhimurium suspension onto flowers of tomato plants at flowering stage	Negative influence of pathogen persistence on tomatoes by presence of *Enterobacter* and *Bacillus*	Canada

Table 3. *Continued*

Source sorted by pathogen factor	Randomization	Type of produce, length of follow-up of produce from inoculation (weeks)	Treatment	Outcome reported	Country
High temperature/humidity					
Brandl and Mandrell *(27)*	Randomized pots within growth chambers	Cilantro, <1	Immersing upper part of each plant in *S.* Thompson suspension and incubation at different temperatures	Higher growth rates of pathogen in cilantro phyllosphere at warm temperatures, such as 30°C, and under high humidity	United States
Dreux et al. *(51)*	Random selection of leaves	Parsley, 2	Inoculating parsley leaves with *L. monocytogenes* suspension under saturated humidity during 7 days and shifting humidity from saturated to low relative humidity	Declines in pathogen populations on aerial surface of parsley under low relative humidity, but tending towards about 10^5 CFU per leaf	France
Dreux et al. *(52)*	Random selection of leaves	Parsley, 4	Inoculating parsley leaves with *L. monocytogenes* suspension under low relative humidity (47-69%) and shifting humidity to saturated	Induction of viable but nonculturable pathogens on parsley leaves by dry condition, and induction of increased culturable counts of pathogens by changes of relative humidity from low to 100% only when residual culturable cells remained	France
Natvig et al. *(150)*	Random location for sampling each bed	Radish, arugula, and carrot, 8 (or 17)	Planting vegetables in soil beds amended by bovine manure inoculated with *S.* Typhimurium on 1 March and 1 June	Rare detection of pathogen in vegetables grown on soil with manure applied on 1 March, but frequent detection in vegetables grown on soil with manure applied on 1 June	United States

The association between microbial contamination and plant age has also been evaluated in several studies *(21, 58, 59, 85, 145, 158, 210)*. Several of the studies showed significantly higher levels of contamination of mature than of young plants for lettuce *(21, 145)* and spinach *(59, 158)*. However, there was no significant association between growth stage and pathogen presence in the other studies *(58, 85)*. Interestingly, one study detected pathogens only in the middle-age lettuce leaves inoculated with pathogens on the adaxial surface *(210)*. Furthermore, one study reported a dependence of pathogen dynamics on leaf age: Brandl and Amunson *(26)* observed *E. coli* O157:H7 and *Salmonella enterica* on the lettuce surface for 3 days after immersing the aerial part of lettuce in the pathogen suspension. Both pathogens colonized the surface of young lettuce leaves at levels approximately 10-fold higher than the levels on medium age leaves.

It is expected that bacterial distribution on plants may differ according to the route of exposure. However, only one study *(142)* explored the effect of the contamination route on pathogen colonization or internalization. In that study, there were significant differences in the levels of prevalence and densities of pathogens depending on the route of *E. coli* O157:H7 inoculation (in decreasing order: pressure, soil drench, leaf drop, and stab inoculations). Three studies evaluated the survival or internalization of pathogens in lettuce *(57, 59, 210)* and spinach *(57)* after inoculation on abaxial or adaxial sides of leaf surfaces. All studies showed higher levels of contamination of the lettuce or spinach leaves sprayed on the abaxial surface than of those sprayed on the adaxial surface.

Four studies *(9, 21, 57, 90)* conducted experiments to ascertain whether the physical damage of produce leaves or roots could influence the fate of the pathogen. However, the studies on the effect of leaf damage showed conflicting results *(9, 57)*. One study *(9)* reported that *E. coli* O157:H7 populations remained significantly higher on physically damaged leaves of lettuce compared to intact ones over a 10 day period. However, another study *(57)* showed the opposite effect in spinach as evaluated 14 days postinoculation. Similarly, two studies *(21, 90)* evaluated the influence of mechanical root damage on pathogen internalization in plants. Root decapitation of romaine lettuce increased the number of *Salmonella* organisms in leaves of plants grown on potting medium inoculated with the pathogen on day 2 postinoculation *(21)*. However, mechanical disruption of root hairs and seminal roots in spinach transplanted in soil microcosms inoculated with *E. coli* O157:H7 did not induce the pathogen internalization into plant leaves in another study *(90)*.

2.3.6 Local environment: water factors

Water on the produce fields, either from an irrigation system or flooding, can have an effect on produce contamination. Three studies, conducted in China *(32)*, Morocco *(137)*, and Spain *(133)*, demonstrated that naturally contaminated irrigation water could influence the contamination of plants. The application of contaminated irrigation water, such as sewage plant effluent *(32)* and wastewater *(133, 137)*, in the produce field significantly increased the occurrence of pathogens in plants. Furthermore, three studies evaluated and reported correlations between repeated irrigation and microbial contamination in lettuce *(95, 178)* and tomatoes *(141)*. Regarding the method of

irrigation, three studies *(57, 179, 182)* evaluated how it affects the transfer of pathogens from water to plants. They reported that irrigation through furrow irrigation *(182)*, spray irrigation *(179)*, and spraying with droplets *(57)* resulted in higher produce contamination than subsurface drip irrigation, surface irrigation, and spraying with mist, respectively. The effect of flooding on the occurrence of *Salmonella* in a hydroponic tomato farm has also been reported in one study *(152)*. *Salmonella* Newport was detected on tomatoes and in many environmental samples, such as puddles, soil, and worker's shoes, during and after but not before the flood. We reviewed three additional studies *(60, 113, 180)* regarding water factors. One study evaluated the effect of irrigation water flow rate on produce contamination *(60)* and reported pathogen detection only when the flow rate was high. Two reviewed studies *(113, 180)* conducted experiments using pathogen inoculated irrigation water. In those studies, one study did *(180)* but the other *(113)* did not recover any pathogens from produce samples after exposure to irrigation water contaminated with pathogens.

2.3.7 Local environment: soil factors

The effects of contaminated soil fertilizers, soil types and crop debris as risk factors for produce contamination were also assessed. Eight studies *(4, 32, 39, 45, 114, 118, 128, 146)* evaluated produce contamination from naturally contaminated fertilizer and soil. The application of contaminated fertilizer, such as sludge *(4, 32, 39, 118)*, raw cattle manure *(146)*, and broiler litter- amended soil *(114)*, induced a high occurrence of pathogens in plants. However, there was no increase in produce contamination after fertilization with liquid hog *(45)* and dairy *(128)* manure. Furthermore, five studies *(101,*

102, 104, 105, 120) conducted experiments to evaluate whether composting *(120)* or alkalization of manure *(101, 102, 104, 105)* could most effectively reduce microbial contamination of produce. Some of these studies *(102, 104, 105)* showed consistently lower rates of pathogen survival in produce grown in soil amended with alkaline-pH-stabilized manure than in produce those grown in soil amended with nonstabilized manure. However, Johannessen et al. *(120)* did not detect any pathogens on the outer leaves of harvested lettuce grown in soil fertilized by contaminated bovine slurry, firm manure, and compost. The effect of soil type on pathogen survival was evaluated in six studies *(45, 69, 92, 93, 95, 150).* Two of these studies *(95, 150)* reported a higher likelihood of produce contamination when produce was grown on the clay (loam) soil than on (loamy) sand soil. In contrast, two studies *(92, 93)* by Ibekwe et al. reported better survival of *E. coli* O157:H7 in lettuce grown on sandy soil than on clay soil. Other studies did not report significant differences *(45, 69).* Finally, one study *(15)* evaluated the effect of contaminated crop debris mixed with soil on contamination of the next batch of produce. In the production of tomatoes, these authors used soil mixed with debris of plants grown on soil inoculated with *Salmonella* and detected contamination of a subsequent crop *(15).* In addition to the studies shown in Table 3 under soil factors, we reviewed four studies *(119, 126, 180, 204)* on the application of pathogen-contaminated fertilizers. These studies reported contamination of produce grown in soil inoculated with *E. coli* O157:H7 *(180), Salmonella (126),* and *L. monocytogenes (204),* except for one study *(119)* in which pathogens were not detected in any part of lettuce grown in contaminated soil during the entire study period.

2.3.8 Local ecological conditions in cross-contamination and pathogen persistence

Two studies *(57, 186)* evaluated the role of insects in the microbial contamination of plants. One study *(186)* detected pathogens from all samples of spinach leaves collected after exposure to contaminated house flies. Interestingly, Ericksonet al. *(57)* reported significantly reduced internalization of *E. coli* O157:H7 in lettuce leaves exposed to thrips, aphids, and cabbage loopers prior to pathogen inoculation of the surface of lettuce leaves as compared to its internalization in lettuce that was not exposed to insects. Two studies *(25, 90)* were conducted to determine whether nematodes facilitate the internalization of *Salmonella* into plants. In one study *(25)*, the pathogen was not detected in tomato plants grown in soil inoculated with *Salmonella* and infested with plant-parasitic nematodes. The other study *(90)* reported that the exposure of spinach plants to nematodes did not enhance internalization of *E. coli* O157:H7. The competition between pathogens and epiphytic bacteria was tested in six studies *(9, 10, 15, 43, 90, 176)*. Two of these studies *(10, 15)* reported that epiphytic bacteria contributed to the better survival of pathogens in produce. In contrast, two studies *(43, 176)* reported that the persistence of pathogens on produce was negatively associated with the presence of epiphytic bacteria. The other studies *(9, 90)* reported that the persistence of pathogens was not affected by coinoculation of pathogens with a different epiphytic bacteria. Here we also report studies *(80, 111, 112)* that evaluated whether diluted pesticide solutions may serve as a potential source of microbial contamination on the farm. In two of these observational studies, Izumi et al. recovered *Salmonella (111)* and *E. coli* O157:H7 *(112)* in pesticide solution prepared with agricultural water but did not detect these pathogens

on fruits. However, in the experimental studies, where pathogen-contaminated fungicide was used, pathogens were detected on tomato plant leaves and fruit *(80)*.

Finally, four studies *(27, 51, 52, 150)* evaluated pathogen growth under high-temperature and high-humidity conditions. All of these studies showed that warm temperatures and high humidity facilitate the survival or growth of pathogens on produce. One of these studies *(150)* additionally reported that freeze-thaw cycling substantially reduced the pathogen load on produce.

2.4 Discussion

Our systematic review identified and synthesized existing knowledge on risk factors for produce contamination by three pathogens, *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7, targeted at the preharvest level. In accordance with the requirements of the systematic review approach, the synthesis included only studies with information allowing inferences about the causal effect of risk factors in determining produce contamination. Our results showed that produce contamination is related to a complex interplay between all three corners of the epidemiological triad involving animal reservoirs, pathogens, and the local environment (Figure 2). While all the appraised risk factors were not found to be consistently or conclusively associated with produce contamination, their systematic review provided a comprehensive outline of the current knowledge.

There have been many studies on the effects of animal host species and their diet, pathogen species and serotype, and plant species and cultivar effects on produce

contamination. Prior studies stated that *Salmonella* and *E. coli* O157:H7 populations decrease more rapidly with higher fiber content and higher pH *(69)*, and that *E. coli* O157:H7 survival in soil is shorter when applying solid cattle manure than when applying liquid swine manure *(16)*. However, our review discovered inconsistent results in previous studies focused on animal species and diet. This may not be surprising since the differences in surface morphology and metabolic functions of different parts of fruits and vegetables may provide diverse ecological niches selective for specific species or groups of microorganisms *(28)*. Insufficient concentrations of inoculated pathogens in an animal diet study could be another reason for nondetection of pathogens in produce samples. The reviewed studies also indicate longer persistence of *Salmonella* than of *E. coli* O157:H7 on cantaloupes and tomato plants, but the opposite result on lettuce. These conflicting results between produce types are consistent with the previous *in vitro* studies *(185, 203)*. The serotypes of the two pathogens also significantly impacted the level of produce contamination. These contamination differences, depending on pathogen species or strains may be explained by the biological and physical differences between pathogen strains used in the studies, including biofilm formation, curli and cellulose production, flagella, cell charge, and hydrophobicity *(46)*. The studies examined also revealed that produce species and cultivars play an important role in pathogen persistence. Differences in root exudate quality and quantity *(159)*, nutrient content within the plant *(46)*, stomatal opening and photosynthesis *(125)*, and plant defensive mechanisms (e.g., triggering stomatal closure) *(138)* among produce species and cultivars might be responsible for the observed differences in pathogen persistence

in plants. Thus, the observed inconsistences highlight the importance of considering variability among pathogens, animal hosts and produce types, not only at the species level but also at the serotype and cultivar level when interpreting study results and designing control policies.

Contaminated fomites and vectors, such as fertilizers, irrigation water, wild animal intrusion, insects, nematodes, pesticides/fungicides, crop debris, and flooding may serve as potential sources of microbial contamination of produce. Animal manure, including from feces of animal reservoirs of the considered pathogens *(64, 160, 161),* is extensively used as fertilizer around the world *(68)* and wastewater is commonly used for irrigation in many countries, including in the United States and Canada *(181)*. Thus, manure and irrigation represent the two most important modes of pathogen transmission from human or animal hosts to produce at the preharvest level. Although seemingly rare, wild animals can also act as vehicles for pathogen transmission to produce fields and produce. Indeed, several observational studies determined the presence of the pathogens in wild birds *(89, 206),* boar *(205),* fish *(76),* reptiles *(171)*. House flies are known to play a role in the dissemination of *E. coli* O157:H7 on cattle farms *(5)* and *E. coli* on spinach farms *(186)*. However, interestingly, exposure of produce leaves to insects prior to inoculation of *E. coli* O157:H7 may stimulate plant defenses and decrease the pathogen population on produce leaves *(57)*. Free-living nematodes, such as *Caenorhabditis elegans*, have been hypothesized as vectors for produce contamination at the preharvest level *(6, 33, 121, 122)*. While none of the studies reviewed here focused on the free-living nematodes, two of the studies reviewed failed to show that plant-

parasitic nematodes, such as *Meloidogyne incognita (25)* and *Meloidogyne halpa (90)*, have any effect on the internalization of pathogens into spinach and tomatoes. Following spraying fungicide solution diluted by contaminated water, pathogens were detected on tomatoes and tomato plants *(80)*. This might have happened because this compound was not bactericidal *(81)*. In two observational studies *(111, 112)*, pathogens were found only in pesticide, not in the produce, still suggesting that produce contamination through contaminated pesticide might occur in the field. Barak and Liang *(15)* demonstrated that crop debris grown on the soil contaminated with pathogen can induce contamination of a subsequent crop. These authors also showed that crop debris could introduce other microorganisms (*Xanthomonas campestris* pathovar *vitians) (13)*. Flooding may be able to unexpectedly increase produce contamination by contact with not only river or creek water itself but also such water contaminated with other sources of contamination (e.g., animal feces, sewage, and runoff water).

The condition of soil and fertilizers is another potential factor associated with microbial contamination of produce. For example, lower pathogen survival was observed for produce grown in soil mixed with alkaline-pH-stabilized manure than for produce grown in soil amended with nonstabilized manure. The increased pH caused the release of ammonia and resulted in pathogen reduction *(208)*. Composting has a damaging effect on potential human pathogens *(127)*, but this beneficial effect of composting on manure was not confirmed in the studies reviewed *(120)*. Our review revealed inconsistent results about the impact of soil type and time on produce contamination that to some extent confirms a previous report *(94)* which showed better

short-term survival of pathogens in sandy soil than in clay soil, with the opposite results in the long run *(94)*. While soil type-time interaction may be real, it is also possible that it occurred due to differences in tillage practices and methods of pathogen delivery *(71)*.

Irrigation methods and frequency of irrigation have important roles in produce contamination. The levels of prevalence and densities of pathogens were higher in produce when *E. coli* O157:H7 inoculated water was applied through soil drench than by the leaf drop method *(142)*. This trend may be contributed to by the fact that pathogens on the leaf surface are confronted with harsh conditions, such as insufficient nutrients, relatively dry environment, large fluctuations in temperature, and UV light *(209)*. Some studies *(57, 179, 182)* showed that leaf surface contamination is more likely to occur with the application of irrigation water through the methods of furrow irrigation, spray irrigation, and spraying with droplets than those of subsurface drip irrigation, surface irrigation, and spraying with mist. It is not feasible to cultivate plants without applying irrigation water in most locations where produce farming is abundant. However, produce contamination can be mitigated to an extent by applying irrigation methods that reduce the exposure of leaves to irrigation water, such as furrow irrigation and surface irrigation. This is important because spraying irrigation water on the abaxial leaf surface protects pathogens from the environmental conditions (such as UV light), and so, pathogens from contaminated water can penetrate the plant stomata which is important in gas exchange and water transpiration *(50)* and may play a role in produce contamination. One *in vitro* study showed that *S.* Typhimurium could enter plant tissues through stomata without triggering the immune response of the plant *(125)*.

Interestingly, another *in vitro* study showed that *E. coli* O157:H7 invasion of *Arabidopsis* was restricted by stomatal closure *(138).* Intuitively, repeated application of contaminated irrigation water would increase the risk of produce contamination as is also confirmed from the studies reviewed.

Produce conditions, such as plant age, leaf age, physical damage in leaf and root, and epiphytic bacteria, are also closely associated with produce contamination. Mature produce is more prone to be contaminated with *Salmonella* and *E. coli* O157:H7 than young produce. Intuitively, this might be because of a longer period of exposure. Alternatively, in lettuce for example, a well-developed secondary root system in the mature plant might enhance the likelihood of interaction with pathogens in the soil *(145).* On the other hand, the reported association between young leaves and contamination level *(26)* might be attributed to the high density of nitrogen content in the young leaves, where a relatively high concentration of nitrate in young leaves might have led to the growth of *E. coli* O157:H7. There were conflicting results in the studies exploring the association between physical damage to produce leaves and contamination *(9, 57).* It is possible that attachment and penetration of *E. coli* O157:H7 occurs preferentially to injured or cut leaf surfaces *(9).* However, it is also possible that physical damage of the leaf stimulates plant defense *(57).* Such contradictory results might be attributed to either the different time-point of pathogen inoculation (before or after damage) or the different produce types or *E. coli* O157:H7 serotypes. A significantly higher number of pathogens was detected in the lettuce, which was transplanted to potting medium inoculated with *S.* Newport after damaging roots than in lettuce with

intact roots *(21)*. These studies indicate the importance of preventing leaf and root damage as a means of controlling produce contamination. Two studies *(10, 15)* reported the contribution of epiphytic bacteria to better survival of pathogens. One study suggested that the necrotic lesions formed by epiphytic bacteria enhanced the survival of *E. coli* O157:H7 *(10)*. The study suggested that epiphytic bacteria might overcome the plant immune system, and improve the entrance of both epiphytic bacteria and *S. enterica* into leaf tissue via stomata *(15)*. However, two other studies *(43, 176)* showed the opposite effect of epiphytic bacteria because of the competition between pathogens and epiphytic bacteria. The discrepancy among these results may be related to the different produce types, pathogens, or epiphytic bacteria. Consistent with our conclusions, an *in vitro* study by Cooley et al. *(42)* showed that *Wausteria paucula* and *Enterobacter asburiae* enhanced and decreased, respectively, the survival of *E. coli* O157:H7.

Warm temperature, high humidity, and pathogen concentration also affect produce contamination. In contrast to the pathogens in manure, which declined with increasing temperature between 7 and 33 ˚C *(173)*, warm temperature (e.g., 30˚C) leads to increase of the survival or growth of pathogens in produce *(27, 150)*. *In vitro* studies *(77)* demonstrated that warm culture growth temperatures (e.g., 20 to 30˚C) increases the attachment of *L. monocytogenes*, and it is thus possible that ambient temperature has the same effect on the attachment of these pathogens *in vivo*. High humidity also induced the growth of *S. enterica (51, 52)*, and this finding is consistent with the result of an *in vitro* study *(107)*. The interaction of temperature and relative humidity also significantly

impacted the attachment of *Salmonella* bacteria to tomatoes *(106)*. Higher concentrations of pathogens in contaminated soil or irrigation water increased the population of pathogens in produce or the likelihood of produce contamination. However, some studies *(58, 118, 210)* did not show the correlation between pathogen concentration and produce contamination. We suggest that there might be a threshold in the concentration of pathogens in soil or water necessary for successful produce contamination.

In the assessments of methodological quality of the reviewed papers performed here, sample size was not considered because the methods to collect samples were very heterogeneous (e.g., [periodic] sampling from the same produce and/or the parts of them and sampling from collective produce in the plot). A considerable number of studies had deficiencies in the reporting of random allocation. It is possible that random allocation was properly executed in these studies without being explicitly reported. In the reviewed articles, we considered conducting an assessment of the quality of evidence for the risk factors. However, we could not apply any of the existing assessment systems *(12, 139, 194)* primarily because of the small number of studies for each of the risk factors and produce type considered.

The major limitation of this review originates from the fact that the individual studies reviewed used different methods in sample selection and different protocols for pathogen detection, from which we synthesized the different types of outcomes to assess the risk factors for produce contamination. While grouping of the risk factors by the elements of the epidemiologic triad provided a logical and structured platform for the evaluation and synthesis of risk factors for produce contamination, the grouping may

have also masked other important commonalities and relationships among risk factors. Furthermore, the fact that there were only five studies on *L. monocytogenes* restricted the range of application of the risk factors reviewed. Lastly, all risk factors described in this review had a low external validity mostly because of the deficiencies of observational studies *(156)* and the deficiencies of relevant studies from developing countries *(55, 96)*.

However, our study has important strengths. To our knowledge, this is not only the first systematic review to evaluate the risk factors of produce contamination but also the first systematic review in the field of plant agriculture. The systematic versus narrative review approach has minimized systematic errors and publication bias by nonapplication of language restriction *(144)* and inclusion of grey literature *(88)*, respectively. Thus, our systematic review and recently published scoping study *(96)* may provide cornerstones for the development of the systematically reviewed body of literature in produce food safety.

Finally, the results of this review provided an outline of topics needing future research. Specifically, observational studies should be conducted to assess the risk factors associated with produce contamination in the natural environment. For example, microbial contamination was greater when plant leaves were sprayed on the abaxial surface than when sprayed on the adaxial surface in controlled conditions, but it is unclear whether spraying overhead irrigation water is applicable for minimizing produce contamination in the field. So, future studies should examine the difference between the two spraying methods in produce contamination in the natural environment. Future studies should also attempt to explain the biological basis for the observed variability in

which different pathogen species and/or serotypes contaminate different produce types and/or cultivars as that could lead to the identification of novel intervention and control strategies. More studies evaluating tradeoffs between benefits (e.g., prevention of human illness) and costs (e.g., reduced produce production) need to be conducted to suggest "practical" guidelines for the prevention of produce contamination. Incidentally, one of the main findings of our work is that there is a large body of literature on produce contamination with foodborne pathogens (as evident from almost 3,500 identified unique citations). However, very few (68 studies) of these studies have been conducted and reported in such a way that these studies and their findings about risk factors in determining produce contamination could be considered as evidence of causality (or lack of it). Therefore, our work indicates the need for more stringent adherence to study design and reporting requirements in produce food safety research (as has, for example, been done by the initiative to STrengthen the Reporting of OBservational studies in Epidemiology [STROBE] http://www.strobe-statement.org/). Furthermore, there is a strong need to develop guidelines for conducting systematic reviews in the produce agriculture field, because the existing guidelines in agri-food public health focus only on the production of livestock *(166, 167).*

In conclusion, we reviewed a number of studies investigating risk factors for produce contamination at the preharvest level. Although the number of these studies was not sufficient to yield a review result indicating high-quality evidence for any of the evaluated risk factors, important conclusions did emerge. Specifically, the existing literature suggests that reducing microbial contamination of irrigation water and soil are

the most promising targets for prevention and control of produce contamination. This review also provides an inventory of the evaluated risk factors, including those requiring more research. Based on that, we stress the need for conducting carefully designed prospective studies to clearly confirm the association between risk factors and contamination of produce with *L. monocytogenes, Salmonella*, and *E. coli* O157:H7.

CHAPTER III

GENERIC *ESCHERICHIA COLI* CONTAMINATION OF SPINACH AT THE PREHARVEST LEVEL: THE ROLE OF FARM MANAGEMENT AND ENVIRONMENTAL FACTORS [*]

The objective of this study was to determine the effects of farm management and environmental factors on preharvest spinach contamination with generic *Escherichia coli* as an indicator of fecal contamination. A repeated cross-sectional study was conducted by visiting spinach farms up to four times per growing season over a period of 2 years (2010 to 2011). Spinach samples (n = 955) were collected from 12 spinach farms in Colorado and Texas as representative states of the Western and Southwestern United States, respectively. During each farm visit, farmers were surveyed about farm-related management and environmental factors using a questionnaire. Associations between the prevalence of generic *E. coli* in spinach and farm-related factors were assessed by using a multivariable logistic regression model including random effects for farm and farm visit. Overall, 6.6% of spinach samples were positive for generic *E. coli*. Significant risk factors for spinach contamination with generic *E. coli* were the proximity (within 10 miles) of a poultry farm, the use of pond water for irrigation, a >66 day period since the planting of spinach, farming on fields previously used for grazing, the production of hay before spinach planting, and the farm location in the Southwestern United States.

[*] Reprinted with permission from "Generic *Escherichia coli* contamination of spinach at the preharvest stage: effects of farm management and environmental factors" by Park S, Navratil S, Gregory A, Bauer A, Srinath I, Jun M, Szonyi B, Nightingale K, Anciso J, Ivanek R. 2013. *Applied and Environmental Microbiology*, 79, 4347-58, Copyright 2013 by American Society for Microbiology

Contamination with generic *E. coli* was significantly reduced with an irrigation lapse time of >5 day, as well as by several factors related to field workers including the use of portable toilets, training to use portable toilets, and the use of hand-washing stations. To our knowledge, this is the first report of an association between field workers' personal hygiene and produce contamination with generic *E. coli* at the preharvest level. Collectively, our findings support that practice of good personal hygiene and other good farm-management practices may reduce produce contamination with generic *E. coli* at the preharvest level.

3.1 Introduction

Produce consumption, production, and safety are undergoing rapid changes. Global consumption of fruits and vegetables demonstrated an average annual increase of 4.5% from 1990 to 2004 *(61)*. During the same period, the numbers of foodborne outbreaks and cases linked to produce have also increased *(48)*. Increases in produce-related foodborne disease may have resulted from not only the increase in consumption of produce but also from changes in the farm-management and processing practices *(23)*. Among outbreaks where a pathogen was identified, *Salmonella* (29%) and *Escherichia coli* O157:H7 (13%) were the main pathogens causing foodborne outbreaks in the United States *(177)*. Both of these pathogens are shed through the feces of infected animals and human hosts (including asymptomatic carriers) *(68)*. *Listeria monocytogenes* is another important foodborne pathogen of significant human health concern *(170)*. It is shed through the feces of infected animals and human hosts, but it

can also sustain itself in the environment as a saprophytic microorganism that thrives on decaying plant material *(110)*. Microbial contamination of produce may occur at any point in the farm-to-fork food production chain *(192)*. However, during postharvest stage, it may be difficult to eliminate or counteract contamination that occurred before harvest *(135)*. Produce is often consumed raw or after minimal processing, and therefore pathogen contamination of produce is considered a serious human health risk. Identifying and controlling risk factors for produce contamination at the preharvest level are important steps for reducing this health risk.

The presence of *E. coli* in foods indicates fecal contamination and possibly the presence of pathogens carried in the intestinal tract of animals *(3)*. This bacterium, which is commonly isolated from the intestines of warm-blooded vertebrates, is shed into the environment through feces. *E. coli* contamination of produce fields occurs from various sources such as contaminated soil, fertilizer (manure/compost), wildlife, and irrigation water *(24)*. A previous study *(150)* showed the usefulness of *E. coli* as an indicator organism for evaluating contamination with *Salmonella enterica* serovar Typhimurium originating from manure. Thus, to reduce the incidence of foodborne illnesses attributed to produce, it is of interest to study farm-related risk factors for *E. coli* produce contamination.

Previous research *(155)* has provided a comprehensive systematic review of the current knowledge about the effects of farm management practices, such as planting procedures, manure use, and irrigation application, on the contamination of fruits and vegetables with *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes.* For example,

irrigation methods, such as furrow and surface irrigation, resulted in less produce contamination than spray irrigation. Additionally, the risk of *E. coli* contamination increased with the use of manure aged < 6 months or 12 months as well as the use of cattle manure instead of another type of manure-based fertilizer *(147, 148)*. The use of animal waste as fertilizer increased the risk of *E. coli* contamination of produce in organic and semiorganic farms *(147)*; however, studies of the association between organic farming and *E. coli* contamination have yielded inconsistent results *(148, 149)*. There is a need to reevaluate these and other types of inconsistencies and to assess the currently known risk factors alongside with factors that have not yet been evaluated (e.g., landscape factors and workers' hygiene) in order to determine how they independently and jointly affect produce contamination. Most reported observational studies interested in the role of farm management factors in produce contamination with *E. coli* were conducted only in the Midwestern United States (e.g., Minnesota *(147-149)* and Wisconsin *(97, 147, 149)*), although additional states in the Western and Southwestern United States are important vegetable production areas with region-specific management and landscape factors *(191)*. The objective of this study was to describe the distribution of generic *E. coli* contamination in spinach grown in Colorado and Texas as representative states of the Western and Southwestern United States, and to determine the effects of farm management and environmental factors on the contamination of spinach with generic *E. coli* at the preharvest level.

3.2 Materials and methods

3.2.1 Study design and area

A repeated cross-sectional study over a period of 2 years (2010-2011) was conducted. We recruited 12 spinach farms: 4 in the Western (Colorado) and 8 in the Southwestern (Texas) United States (Figure 3). A total of 955 spinach samples were collected over the duration of the study. Each farm was visited one to four times per growing season, or up to seven times over the 2-year study period, depending on the availability of spinach fields throughout the growing seasons. At each farm visit, we chose one to six fields per farm and collected five spinach samples per field (Table 4). The number of fields sampled per farm depended on the number of available fields with spinach crop at the time of the visit. The spinach-growing season lasts from May to September in Colorado and from November to March in Texas. During the 2010 growing season, the monthly averages of mean daily temperatures around the enrolled farms ranged from 11°C to 21°C in Colorado *(195)* and from 13°C to 22°C in Texas *(196)*. Likewise, the mean monthly precipitations ranged from 10 to 35 mm in Colorado and from 3 to 27 mm in Texas. During the growing season of 2011, the monthly averages of mean daily temperatures ranged from 10 to 22°C in Colorado *(197)* and from 14 to 21°C in Texas *(198-201)*, while the mean monthly rainfalls ranged from 13 to 62 mm in Colorado and from 18 to 90 mm in Texas. In Colorado, most of the sampled spinach was grown on loam soil (70%) followed by clay loam soil (29%). In Texas, on the other hand, there was a greater diversity of soil types, with most of the sampled spinach being grown on silty clay loam soil (63%), followed by clay loam (14%), fine sandy loam soil (7%), and

several other soil types *(202)*. The meteorological and landscape factors, including temperature, precipitation, and soil types, are being investigated in more detail in a separate study.

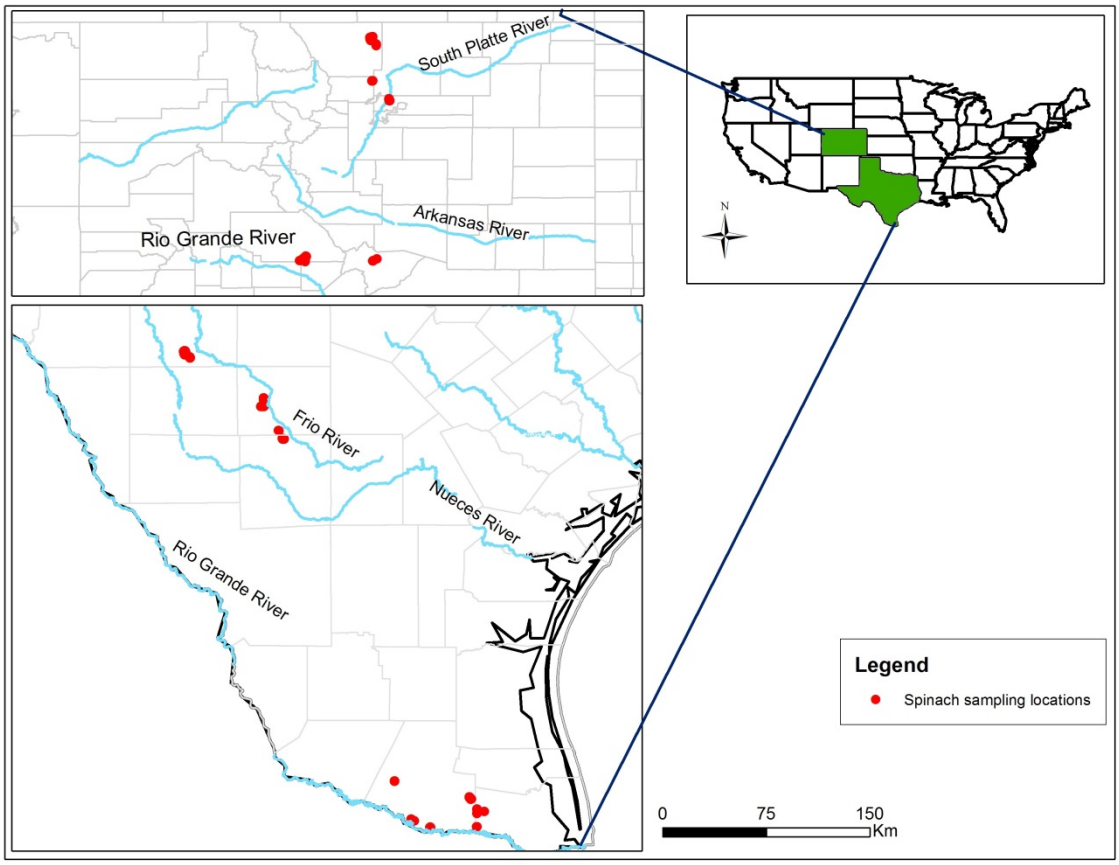


Figure 3. Map of sampling locations in Colorado and Texas.

Table 4. Description of spinach sample collection.

State	Farm	Growing season	Sampling month and year	Number of visits	Number of fields sampled per visit	Number of collected samples	Number of samples testing positive
Colorado	1	1	Jul/Aug/Sep 2010	3	3-4	55	0
		2	May/Jul/Aug 2011	3	4	60	0
	2	1	Jun/Jul/Aug 2010	3	4	60	4
		2	May/Jun/Jul 2011	3	4	60	20
	3	1	Jun/Jul/Aug 2010	3	4	60	2
		2	May/Jun/Jul 2011	3	4	60	0
	4	1	Jun/Aug/Sep 2010	3	4	60	1
		2	Jun/Jul/Aug 2011	3	4	60	0
Texas	1	1	Nov 2010, Jan/Feb 2011	3	4	60	5
		2	Nov/Dec 2011, Jan 2012	3	4	60	6
	2	1	Nov 2010, Jan/Feb 2011	3	4	60	2
		2	Nov/Dec 2011, Jan 2012	3	4	60	2
	3	1	Dec 2010, Jan 2011	2	1	10	1
		2	NS				
	4	1	Dec 2010, Jan/Feb/Mar 2011	4	1-4	45	3
		2	Dec 2011, Jan/Feb 2012	3	2-6	50	4
	5	1	Dec 2010, Jan/Feb 2011	3	1-2	25	7
		2	NS				
	6	1	Jan/Feb/Mar 2011	3	2	30	0
		2	Dec 2011, Jan/Feb 2012	3	2	30	1
	7	1	Dec 2010, Jan 2011	2	1	10	1
		2	NS				
	8	1	NS				
		2	Dec 2011, Jan 2012	2	4	40	4

NS = samples not collected

3.2.2 Description of spinach sample collection

Spinach samples were collected using sterile gloves. Each spinach sample consisted of at least 10 randomly selected individual plant leaves of different maturities, collected in an area within a 5-meter radius. Only random leaves were collected, without harvesting of the whole plants. Samples were placed into sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI). All samples were shipped in coolers with ice packs. In year 1, the

samples were shipped to the Food Safety Laboratory of the Department of Animal Sciences at Colorado State University (Fort Collins, CO). In year 2, samples were shipped to the Department of Animal and Food Sciences at Texas Tech University (Lubbock, TX). The research pesearch protocol and laboratory personnel for microbial detection were identical between the two laboratories. All samples were processed within 48 h after collection.

3.2.3 Microbiological analyses

Each sample was prepared by using 25 g of spinach leaves. The spinach samples were transferred into 75 ml of phosphate-buffered saline (PBS) contained in stomacher bags. The contents of each bag were then mixed by using a laboratory blender (Smasher Lab Blender; AES-Chemunex, France) for 2 min at room temperature. A 1-ml aliquot from the sample bag followed by 1 ml of each of five 1:10 serial dilutions was then plated directly onto Petrifilm *E. coli*/coliform count plates (3 M Microbiology, St. Paul, MN) and then incubated at 37°C ± 2°C. Petrifilm plates were counted, and blue colonies with gas bubbles, which were observed at 48 h, were considered to be *E. coli* colonies according to standard *E. coli* Petrifilm enumeration methods. The limit of detection was 4 CFU/ml of the plated dilution.

3.2.4 Questionnaire

At each farm visit, we administered a comprehensive questionnaire to obtain information on the general farm-related management and environmental factors selected. The farm owner or manager was asked the questions in a face-to-face interview during each farm visit. The farmers referred to their management records to answer questions that required

more detail, such as the date of manure application. The questionnaire can be found in the APPENDIX B. The questionnaire has two parts: parts A and B. Part A inquires about general farm information (such as farm size) that is not expected to change during the growing season. These questions were asked only at the beginning of each growing season. Part B asks about factors that may change during the growing season (e.g., history of farm intrusion by wild animals between two subsequent visits). These questions were asked at each farm visit. The questionnaire responses were coded and entered into an Excel spreadsheet. This spreadsheet was then used to create variables to be considered in the statistical analysis.

3.2.5 Statistical analyses

Data analyses were conducted by using R software (R Project for Statistical Computing, http://www.r-project.org/). Except when stated otherwise, *P* values of <0.05 were considered statistically significant. The outcome of interest was spinach contamination with generic *E. coli* evaluated as a binary variable, i.e., if any generic *E. coli* was detected in a spinach sample, the sample was considered contaminated, otherwise it was considered noncontaminated (meaning that generic *E. coli* were either present below the limit of detection or absent all together). Table 5 lists and describes the 76 explanatory variables considered in the statistical analyses. The causal diagram in Figure 4 shows the hypothesized associations among these variables and the outcome of interest. In the univariate and multivariable analyses, the associations between the explanatory variables (farm management and environmental) and the outcome variable (generic *E. coli* contamination) were evaluated by using a mixed-effect logistic regression model with

farm and farm visit as random effects. Regarding the random effects, there were 12 farms (F1 to F12) each with a total of 1to7 visits (V1 to V7) over the course of the study. The mixed-effect models were fitted by using the "lmer" function of the R package called "lme4" *(19)*. At the univariate analysis level only, the significance of associations was assessed at a liberal cutoff of a *P* value of 0.2 to assure that all potentially important factors and confounders reached the multivariable analysis. The validity of the linearity assumption of the developed mixed-effect logistic regression models was assessed by graphical plotting, lowess smoothing, between the continuous explanatory variables and log odds of the outcome variable *(49)*. Natural log and quadratic transformations of continuous explanatory variables were also considered. However, because the linearity assumption was not confirmed for any of the continuous explanatory variables (including for their transformations), all continuous variables were median-dichotomized before they could be assessed in the univariate and multivariable mixed effect logistic regression models that also controlled for clustering of samples within farms and farm visits. A manual forward stepwise selection procedure was used to select an appropriate multivariable model ($P < 0.05$ based on the Wald Z test). Only those independent variables whose addition significantly reduced residual deviance were included in the expanded model. Significant differences in model deviance between two nested models were evaluated based on the likelihood ratio test ($P < 0.05$). A plot of observed proportions versus mean predicted probabilities was used to determine the goodness-of-fit by the "plot.logistic.fit.fnc" function of the R package, called "languageR" *(11)*. The

proportion of variation explained by clustering levels (farm and farm visit) was calculated for the final model by using a latent variable approach *(49)*.

Table 5. Description of the explanatory variables.

Category and variable name	Description and levels	Unit
Farm management factor		
Human		
workers	Farm uses temporary workers (yes/no)	
workers_#	Number of temporary workers used on the farm (con)	Number
workers_time	Time since the last workers' visit during CGS (con)	Days
foodsafety_training	Food safety training provided to the staff/temporary workers on the farm (yes/no)	
toilets	Portable toilets used in the field (yes/no)	
toilet_training	Training to use portable toilets to staff/temporary workers (yes/no)	
toilet_distances	Portable toilet distances from the work area on the field (con)	Meter
washing_stations	Hand washing stations used in the field (yes/no)	
Farm and field condition		
farm_size	Farm size (con)	Acres
organic	Organic farming practices currently applied on the farm (yes/no)	
organic_duration	Duration of application of organic farming practices on the farm (con)	Years
organic_certified	Organic farming certified by the National Organic Program (yes/no)	
field_grazed	Farming on field previously used for grazing (yes/no)	
before_fallow	Field condition before planting of the spinach during CGS: fallow (yes/no)	
before_rotavated	〃 rotavated (yes/no)	
before_tilled	〃 tilled (yes/no)	
before_cover_crop	〃 cover crop (yes/no)	
before_hay	〃 hay (yes/no)	
before_riped	〃 riped (yes/no)	
tillage	Tilling, rotavating, or aerating soil for CGS (yes/no)	
tillage_time	Time since the last tilling, rotavating, or aerating soil for CGS (con)	Days
Pesticide		
pesticide_application	Pesticide application (yes/no)	
pesticide_time	Time since the last pesticide application during CGS (con)	Days
pesticide_herbicide	Type of pesticide applied to the field for CGS: herbicide (yes/no)	
pesticide_fungicide	〃 fungicide (yes/no)	
pesticide_insecticide	〃 insecticide (yes/no)	
pesticide_method_low	Method for applying pesticide for CGS: low volume spray (yes/no)	
pesticide_method_high	〃 high volume spray (yes/no)	
pesticide_method_foliar	〃 foliar (yes/no)	
pesticide_method_soil	〃 soil (yes/no)	
Chemical fertilizer		
chemical_application	Chemical fertilizer spread on the field for CGS (yes/no)	
chemical_time	Time since the last chemical fertilizer spreading during CGS (con)	Days
chemical_method_fertigation	Method for spreading chemical fertilizer on the field for CGS: fertigation (yes/no)	
chemical_method_spray	〃 foliar spray (yes/no)	
chemical_method_ground	〃 ground application (yes/no)	
Manure fertilizer		
manure_application	Manure spread on the field for CGS (yes/no)	
manure_time	Time since the last manure spreading during CGS (con)	Days
manure_age	Age of the manure spread on the field for CGS (con)	Weeks
manure_source	Source of manure spread on the field for CGS (dairy farm/poultry farm)	
Compost fertilizer		
compost_application	Compost spread on the field for CGS (yes/no)	
compost_time	Time since the last compost spreading during CGS (con)	Days

Table 5. *Continued*

Category and variable name	Description and levels	Unit
Irrigation		
irrigation_time	Time since the last irrigation during CGS (con)	Days
irrigation_source_pond	Source of irrigation water applied during CGS: pond (yes/no)	
irrigation_source_well	〃 well (yes/no)	
irrigation_source_municipal	〃 municipal (yes/no)	
irrigation_source_river	〃 river/stream/creek (yes/no)	
irrigation_source_reservoirs	〃 reservoirs (yes/no)	
irrigation_method_drip	Method of irrigation for CGS: drip (yes/no)	
irrigation_method_overhead	〃 overhead (yes/no)	
irrigation_method_spray	〃 spray (yes/no)	
irrigation_method_flood	〃 flood (yes/no)	
Equipment		
own_equipment	Use of own farm equipment for all operations (yes/no)	
equipment_cleaning	Cleaning of farm equipment (yes/no)	
Microbial_test	Routine microbial test (yes/no)	
Planting_time	Time since planting spinach (con)	Days
Farm environmental factor		
Terrain, buffer zone, and proximity		
terrain	Terrain where the farm is located (flat/sloped)	
buffer	Buffer zone from neighbors, roads etc (yes/no)	
buffer_fence	Type of buffer zone: fence (yes/no)	
buffer_ditch	〃 ditch (yes/no)	
buffer_road	〃 road (yes/no)	
proximity_dairy	Proximity within 10 mile radius: dairy farm (yes/no)	
proximity_beef	〃 beef farm (yes/no)	
proximity_poultry	〃 poultry farm (yes/no)	
proximity_swine	〃 swine farm (yes/no)	
proximity_water	〃 water resources (yes/no)	
proximity_landfill	〃 landfill (yes/no)	
proximity_residential	〃 residential (yes/no)	
proximity_forest	〃 forest (yes/no)	
proximity_roadways	〃 roadways (yes/no)	
Domestic/wild animal		
domestic_animal	Domestic animal intrusion of the field for CGS (yes/no)	
wildlife	Wildlife intrusion of the field for CGS (yes/no)	
wildlife_control	Wildlife control of the farm (yes/no)	
wildlife_control_fences	Wildlife control methods of the farm: fences (yes/no)	
wildlife_control_trap	〃 trap (yes/no)	
wildlife_control_hunting	〃 hunting (yes/no)	
Farm_location	Farm location (Southwestern US/Western US)	

CGS = the current growing season; con = continuous variable; 〃 = indicates that the above text applies

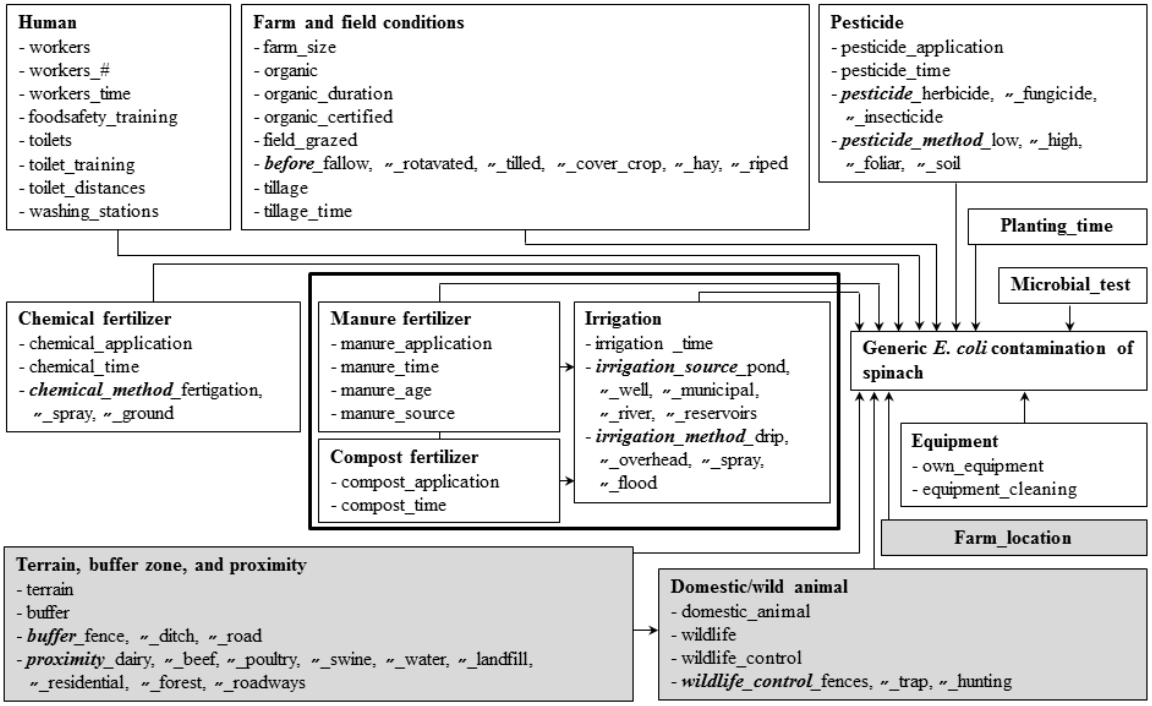


Figure 4. Causal diagram of the hypothesized farm management and environmental risk factors for generic *Escherichia coli* contamination of spinach at the preharvest level.
~ means that the above text in bold italic font applies. Grey shaded boxes indicate environmental factors.

To evaluate the predictive performance of the final multivariable model, we calculated the model sensitivity (Se) and specificity (Sp), with the standard errors (SE) for each of these estimates, along with the model positive predictive value (PPV) and negative predictive value (NPV), based on the explanatory variables appearing in the model. The logistic regression prediction results were recorded on a continuous scale (spanning from 0 to 1) and were dichotomized for comparison with the binary (yes/no) contamination data. To find the optimal cutoff value, the model misclassification costs were estimated over the entire range of possible cutoffs while penalizing false-negative more than false-positive classifications in order to improve the Se of the model. This was

achieved by setting the false-positive cost to 1 and testing the model's predictive performance when the false-negative cost was set to an integer value in the interval [1, 25]. Penalizing false-negatives more than false-positives also compensated for the fact that microbial culture-based tests, such as the one used here to detect spinach contamination, are expected to have better Sp than Se. Consequently, positive microbial test results can be considered true positives, while some negative results might actually be false-negatives.

3.3 Results

Overall, generic *E. coli* was isolated from 63 of 955 (6.6%) of the spinach samples. The median size of enrolled farms was 280 acres (interquartile range [IQR]: 12 to 1,000 cares). In Table 6, we show summary statistics for the remaining continuous explanatory variables to aid in interpretation of the results of univariate (Table 7) and multivariable (Table 8) statistical analyses, where these variables were considered in their median-dichotomized forms. Three of the 12 enrolled farms were organic, and 2 of these were certified organic. All but one of the enrolled farms used hand-washing stations and portable toilets and trained employed workers on how to use them. That farm was also the only one that used their fields for grazing and hay production before spinach planting. Due to the simultaneous occurrence of these farm management factors, we evaluated them by using toilet use as a representative factor for the "hygiene-field status" group of factors. The hygiene-field status group had two levels: "yes" and "no." The yes level indicated the use of portable toilets and hand-washing stations, the

presence of training on the use of portable toilets, and the absence of grazing and hay production in the field before spinach planting.

Table 6. Summary statistics for continuous variables with respect to spinach contamination with generic *Escherichia coli.*

Category and variable name	Total (n = 955)			Positive (n = 63)			Negative (n = 892)		
	Mean	Median	IQR	Mean	Median	IQR	Mean	Median	IQR
Human									
workers_#	99.2	100	8-150	99.4	150	8-150	99.2	100	9-150
workers_time (days)	7.7	3	1-9	3	1.5	1.5-4	8.1	3	1-9
toilet_distances (meter)	186.9	146.3	45.7-402.3	213.6	201.2	201.2-201.2	185.2	91.4	45.7-402.3
Farm and field condition									
organic_duration (years)	13.4	4	3-25	20.2	26	25-26	12.5	4	3-25
tillage_time (days)	42.5	17	15-74	24	15	15-15	45.3	18	15-74
Pesticide/fertilizers/irrigation									
pesticide_time (days)	24.9	10	5-41	24.7	10	5-41	29.2	12	5-50.8
chemical_time (days)	24.1	15	10-32	24.0	15	10-32	24.8	15	10-44.5
manure_time (days)	228.3	200	200-281	202.4	200	200-200	233.9	224	200-281
manure_age (weeks)	12.9	13	9-13	14.6	13	13-13	12.5	13	9-13
compost_time (days)	269.1	275	237-292.5	328	328	328-328	268.6	269	237-291
irrigation_time (days)	12.9	5	2-14	11.4	3.5	3.5-8.5	13	5	2-15
Planting_time (days)	66.8	66	47-82	76.2	77	64-90.5	66.1	65	47-80

IQR = interquartile range

Based on the univariate analyses, the variables that were associated with spinach contamination at the 20% significance level were identified (Table 7). Among the farm management factors, the presence of generic *E. coli* on spinach samples was significantly reduced if they were collected from certified organic farms compared with the noncertified organic farms. Similarly, spinach was less likely to be contaminated if it was collected from fields that used portable toilets, fields that were rotavated before planting of the spinach crop in the season, or fields that used reservoir water for

irrigation. Finally, the prevalence of contamination was lower if the time since the last manure spreading was >200 days or the time since the last irrigation was >5 days. Alternatively, the presence of generic *E. coli* was significantly increased by the use of manure in general, by the use of manure from dairy farms in particular, and when pond water was used for irrigation. Among the farm environmental factors, the presence of generic *E. coli* was significantly reduced by proximity (within 10 miles) of forest or roadways. The presence of generic *E. coli* was significantly increased when the farm was located on a sloped terrain and when domestic animal intrusion on the field was reported. Four additional variables were associated with the outcome at the 20% level albeit with a counterintuitive direction of association, suggesting a possible distorting effect of a confounder *(49)*. Specifically, the probability of generic *E. coli* occurrence was higher when a fenced buffer zone around the farm was present, when the farm applied some means of controlling wildlife, if the farm used their own farm equipment, and when manure applied onto the field was aged longer than 13 weeks. All four variables dropped out during the multivariable analysis, indicating a lack of an association with spinach contamination after controlling for other risk factors.

Table 7. Association between generic *Escherichia coli* contaminated spinach and risk factors assessed in the univariate mixed-effects logistic regression analysis with farm and visit as random effects.

Variable (comparison level)	Frequency [a]	Reference level	OR (95% CI) [b]	*P* value [c]
Farm management factor				
Human				
toilets (yes) [d]	930/955	no	0.08 (0.01, 0.99)	0.049
Farm and field condition				
organic_certified (yes)	175/200	no	0.05 (0.00, 0.65)	0.022
before_rotavated (yes)	490/955	no	0.21 (0.08, 0.54)	0.001
tillage_time (>17 days)	85/185	≤17 days	0.02 (0.00, 0.16)	< 0.001
Fertilizers				
manure_application (yes)	160/955	no	7.9 (1.6, 39.4)	0.011
manure_time (>200 days)	60/140	≤200 days	0.08 (0.01, 0.90)	0.041
manure_age (>13 weeks)	20/150	≤13 weeks	156.6 (0.2, 114,716.7)	0.133
manure_source (poultry farm)	90/150	dairy farm	11.4 (1.1, 123.5)	0.045
compost_application (yes)	140/955	no	0.08 (0.00, 2.02)	0.127
Irrigation				
irrigation_time (>5 days)	365/845	≤5 days	0.17 (0.05, 0.59)	0.005
irrigation_source_pond (yes)	20/955	no	24.4 (2.1, 280.1)	0.010
irrigation_source_well (yes)	635/955	no	0.30 (0.06, 1.51)	0.144
irrigation_source_reservoirs (yes)	160/955	no	0.08 (0.01, 0.40)	0.002
Equipment				
own_equipment (yes)	865/955	no	9.1 (2.4, 34.6)	0.001
Planting_time (>66 days)	465/955	≤66 days	2.6 (1.3, 5.2)	0.008
Farm environmental factor				
Terrain, buffer zone, and proximity				
terrain (sloped)	165/955	flat	8.3 (2.5, 27.3)	< 0.001
buffer_fence (yes)	165/895	no	4.8 (1.9, 12.0)	0.001
proximity_beef (yes)	120/955	no	6.0 (0.5, 79.1)	0.174
proximity_poultry (yes)	110/955	no	8.7 (0.9, 88.0)	0.067
proximity_forest (yes)	60/955	no	0.11 (0.03, 0.43)	0.002
proximity_roadways (yes)	895/955	no	0.07 (0.02, 0.28)	< 0.001
Domestic/wild animal				
wildlife_control (yes)	505/910	no	5.0 (1.9, 13.2)	0.001
domestic_animal (yes)	25/935	no	11.8 (1.1, 122.9)	0.039
Farm_location (Southwestern US)	480/955	Western US	4.4 (0.8, 25.5)	0.096

[a] frequency = number of observations with the comparison level/total number of recorded observations for the variable; [b] OR (95% CI) = odds ratio with 95% confidence interval; [c] Only variables with *P* value <0.2 are shown; [d] the estimated OR (95% CI) value applies to each factor in the "hygiene-field status" group: toilet_training (yes vs. no), washing_stations (yes vs. no), field_grazed (no vs. yes), before_hay (no vs. yes).

The variables listed in Table 7 were tested further for inclusion in the multivariable model. The final multivariable mixed-effect model (Table 8) had 110 missing observations, all of which were for the irrigation time variable. Based on this model, the odds of spinach contamination were reduced to approximately 1 in 4 (odds ratio [OR] = 0.24) when the time since the last irrigation was longer than 5 days. Similarly, the odds of contamination were reduced to approximately 1 in 7 (OR = 0.15) when the field used portable toilets. As stated above, the use of portable toilets represents the "hygiene-field status" group of factors, meaning that the odds of spinach contamination would be equally reduced if any of the factors in this group were considered in the final model instead of the portable toilet use factor. The final model indicated that the odds of spinach contamination were increased to approximately 3 in 1 (OR = 2.7) when spinach was grown for longer than 66 days before sampling. Interestingly, the odds of contamination were considerably higher for farms located in the southwest (Texas) than in the west (Colorado) (OR = 60.7), for fields that used pond water for irrigation (OR = 64.4), and for fields in proximity (within 10 miles) of a poultry farm (OR = 172.1). While the 95% confidence intervals (CIs) for these variables did not includ 1, which indicates strong evidence of an increased risk in the presence of these factors, the CIs were very wide, indicating a high level of uncertainty in the true value of their respective ORs. The proportions of variation explained at the visit and farmlevels were 9.9% and 32.6%, respectively, in the intercept-only model and 13.9% and almost 0%, respectively, in the final model.

Table 8. Association of generic *Escherichia coli* prevalence with risk factors based on the final multivariable mixed-effects logistic regression model with farm and visit as random effects.

Variable (comparison level)	Reference	OR (95% CI) [a]	*P* value
toilets (yes) [b]	no	0.15 (0.05, 0.45)	<0.001
irrigation_time (>5 days)	≤5 days	0.24 (0.09, 0.67)	0.006
planting_time (>66 days)	≤66 days	2.7 (1.2, 6.1)	0.018
farm_location (Southwestern US)	West US	60.7 (7.1, 516.6)	<0.001
irrigation_source_pond (yes)	no	64.4 (4.9, 855.3)	0.002
proximity_poultry (yes)	no	172.1 (21.1, 1402.8)	<0.001
Variance components [c]		Var (StD)	
Farm		$7.2e^{-11}$ ($8.5e^{-6}$)	
Farm visit		0.53 (0.73)	

[a] OR (95% CI) = odds ratio with 95% confidence interval; [b] the estimated OR (95% CI) value applies to each factor in the "hygiene-field status" group: toilet_training (yes vs. no), washing_stations (yes vs. no), field_grazed (no vs. yes), before_hay (no vs. yes); [c] Variance component with standard deviation [Var (StD)] for intercept - only model: Farm = 1.87 (1.37), Farm visit = 0.57 (0.75).

We additionally tested potential 2-way interactions between factors in the final model. Only one interaction term, between "irrigation_time" and "planting_time," had a significant effect on the probability of spinach contamination. It indicated that if spinach was planted >66 days ago and irrigation was applied <5 days ago, the probability of spinach contamination increased by approximately 3%. The predictive performance of the model with the interaction term was comparable to the predictive performance of the simpler model without it, and therefore, the model without the interaction term was retained as the final model.

In terms of predictive performance, our final model had prefect Sp (100%; SE, 0%), while its Se was quite low (33.9%; SE, 6.2%) at the cutoff value of 0.699 that was used to dichotomize the predictions of the logistic regression model. However, because spinach contamination with generic *E. coli* was relatively rare, the NPV of the model

was relatively high (95.3%), meaning that the probability that a negative prediction is truly negative is quite high. Since no false-positive predictions were expected, the PPV was 100%. It should be noted here that the model predictive performance was assessed on the data used for model bundling, and thus, generalization of the results to independent data should be done with caution.

3.4 Discussion

The study described here undertook a comprehensive and organized approach to identify the farm management and environmental factors affecting microbial contamination of produce at the preharvest level. The results indicate that both farm management and environmental factors can affect the risk of spinach contamination with generic *E. coli*.

Our study identified the "hygiene-field status" group of factors to have a strong protective effect on spinach contamination. These factors were the use of portable toilets and hand-washing stations, training in the use of portable toilets, and not the use of the spinach field for grazing or hay production before spinach planting. Because these factors occurred jointly, inference based on their individual effects has to be done with caution. Within the group, the most intriguing result is the potential role that field workers' personal hygiene may play in generic *E. coli* contamination of produce at the preharvest level. Poor personal hygiene of workers is a well-known risk factor for the microbial contamination of produce growing in fields, or during harvest, postharvest processing, and distribution *(22)*. However, to our knowledge, no published epidemiological study has shown the association between workers' hygiene practices and

produce contamination rates at the preharvest level. We found that produce contamination was significantly reduced when workers used hand-washing stations or when the farm provided portable toilets for workers and trained the workers on how to use them. As indicated by the hygiene-field status group, produce contamination was also significantly reduced if the spinach field was not used for hay production or for grazing prior to spinach planting. While the role of these factors in produce contamination is intuitive, surprisingly, only limited published information on these factors exists. One study *(15)* showed tomato contamination with *Salmonella* after planting of tomatoes in soil mixed with debris of tomato plants grown on *Salmonella*-inoculated soil. Grazing on or near fields used for growing of produce is considered a food safety hazard *(87, 123)*. Surface runoff from grazing areas onto cultivated fields has been previously recognized as a risk factor for produce contamination *(75)*. Collectively, conclusions about the role of each individual factor from the hygiene-field status group are valuable because they are either intriguing or intuitive. However, due to their joint appearance (likely due to the small number of enrolled farms), it is impossible to determine which (if not all) of these hygiene-field status factors was truly protective or whether they all were just proxies for another unmeasured but true protective factor. With these limitations in mind, and considering the importance and novelty of our findings, we suggest that personal hygiene may be considered a potential factor for controlling microbial contamination of produce at the postharvest level. Future controlled trials should be conducted to elucidate the role of workers' personal hygiene

in relation to the history of field use and in conjunction with other factors not measured in our study (such as weather).

Farms using manure had a significantly higher proportion of generic *E. coli*-positive samples than did farms not using it (15.6% versus 4.8%). This is consistent with results of previous studies *(147, 148)*. In our study, 60% of farms used manure from dairy cows, and the others used manure from poultry. Studies by Islam et al., who inoculated the same concentrations of microorganisms into manure, showed inconsistent results regarding the survival rates of *E. coli* O157:H7 *(101, 102)* and *Salmonella (104, 105)* in vegetables grown in soil mixed with manure from cows and from poultry. In our study, spinach samples grown in soil mixed with poultry manure had a significantly higher risk of generic *E. coli* contamination than did those grown with cattle manure ($P = 0.045$) (Table 7). However, this factor was not retained in the final multivariable model. Therefore, while the use of manure, particularly poultry manure, on the farm seems to increase the probability of spinach contamination, after controlling for other risk factors, we did not find evidence that manure in general, or poultry manure specifically, significantly increased the probability of spinach contamination with generic *E. coli*.

The odds of spinach contamination with generic *E. coli* was higher in organic than in conventional farms (OR = 2.4), although this difference was not significant ($P = 0.340$) (data not shown). In a study by Mukherjee et al. *(148)*, organic produce showed a significantly greater risk of *E. coli* contamination than conventional produce. It is possible that our study was unable to detect a significant association between the type of

farming (organic versus conventional) and produce contamination due to the relatively small number of enrolled farms. However, it is also possible that the type of farming does not significantly affect the probability of produce contamination, which would support the results of another study that showed that the type of farm (organic, semiorganic, or conventional) was less likely than produce type to affect the risk of *E. coli* contamination *(149)*. Interestingly, in the analysis restricted to organic farms only, the spinach from certified organic farms was less likely to be contaminated with generic *E. coli* than spinach from noncertified organic farms (OR = 0.05) (P = 0.022). This low risk of spinach contamination with generic *E. coli* in the certified farm environment might be attributed to the strict implementation of national organic regulations *(188)*.

Previous studies have shown no apparent effect of time since the last manure spreading, in the range from 90 to 120 days, on produce contamination with *E. coli* (*98, 147)*. Interestingly, our univariate analysis showed a significant association between this factor and spinach contamination when a different cutoff interval (of 200 days) was used. Nevertheless, this factor was not retained in the final model. According to national organic regulations, raw animal manure should be applied at least 90 days prior to harvesting of edible produce that does not come into contact with the soil or soil particles *(188)*. Several studies have assessed the role of manure aging before spreading on produce contamination. A study by Mukherkjee et al. showed the nonsignificant association between manure age (≥6 months) and *E. coli* prevalence in noncertified organic produce *(147)*. However, those authors also showed that manure aged longer than 6 months in certified organic farms *(147)* or 1 year in organic farms *(148)*

significantly decreased the risk of *E. coli* contamination. An experimental study also showed that they dramatically lowered *E. coli* levels by >99% after 90 days of manure storage *(136)*. Our study did not detect any association between manure age and spinach contamination. This may be due to a true lack of association. Alternatively, it may be due to the farmers' poor recall (or record keeping) of the manure age.

At the preharvest level, irrigation is considered one of the most important modes for transmission of microorganisms from their reservoirs to produce *(155)*. Consistent with this, our final model found that the use of pond water for irrigation was a strong predictor of spinach contamination (OR = 64.4) (Table 8). While this association was expected, care is needed in generalization of the results because only one farm in our study used for irrigation water from a pond (approximately 12,000 m^2) located on the field. In the 2002 and 2005 outbreaks of salmonellosis associated with tomatoes, *Salmonella* enterica serovar Newport isolates from two outbreaks had the same genotype profile as isolates from pond water that was used for irrigation *(79)*.

A time of >5 days since the last irrigation was associated with a decreased risk of generic *E. coli* contamination of spinach. Intuitively, this might be because irrigation near sampling with potentially contaminated water increased the risk of produce contamination. A previous study showed the persistence of *E. coli* O157:H7 on lettuce leaves for up to 20 days, after a single exposure to 100 ml of a solution with the pathogen at a concentration of 10^2 CFU/ml *(178)*. Another study showed the persistence of *E. coli* O157:H7 in lettuce phyllospheres over 45 days after irrigation of seedlings with water inoculated with the pathogen (density of 10^7 *E. coli* O157:H7 bacteria·$liter^{-1}$)

on transplanting day and 15 days later *(95)*. The US Food and Drug Administration recommends that the quality of water directly contacting the edible parts of produce should be better than the quality of water that minimally contacts the edible parts of produce *(192)*. Thus, when farmers irrigate leafy green or fruit vegetables with water that could potentially be contaminated with pathogens, they should irrigate the field >5 days before harvest, or they should use furrow or surface irrigation methods rather than overhead or spray irrigation.

In our study, the history of farm intrusion by wildlife was not associated with the presence of *E. coli* contamination of spinach. However, Orozco et al. *(152)* suggested that wildlife is an important vector for *Salmonella* transmission to tomatoes. At the univariate level, there was a significant association between the history of domestic animal intrusion and an increased risk of spinach contamination. This factor was not included into the final model due to its high correlation with the hygiene-field status factors, including field use for grazing before spinach planting. However, it is reasonable to suggest that domestic animal intrusion is one of the important risk factors for *E. coli* spinach contamination. The presence of wildlife might have gone unobserved, as farmers do not stay on the fields all the times. Thus, both wild and domestic animals could have contaminated spinach with *E. coli* in this study. A previous study *(97)* suggested that wildlife intrusion can be an important vehicle for *E. coli* contamination of produce, particularly when there is a noncomposted manure piled on the farm. Interestingly, the proximity (10 miles) of a poultry farm increased the risk of spinach contamination (Table 8). This result may be just a statistical artifact caused by a high correlation

between this variable and farm use of poultry manure. However, spinach could have truly become contaminated by wild birds that are known to be drawn to the poultry barns (fully enclosed housing) and the surrounding habitats *(29)*. Because *E. coli* can grow in soil *(30, 100)*, animal intrusion into a produce farm cumulatively increases the risk of microbial contamination of produce. Thus, practices to prevent or repel wildlife and domestic animal intrusion should be considered as a means to prevent microbial contamination of produce.

Spinach contamination with generic *E. coli* increased if the time since planting of spinach was >66 days. This result is in line with previous studies that observed an increase of produce contamination in mature lettuce *(21, 145)* or spinach *(158)*. Mootian et al. *(145)* proposed that a well-developed secondary root system of lettuce might increase microbial contamination of produce. We suspected that a longer exposure of mature spinach to *E. coli* resulted in more contamination in mature produce than in young produce *(155)*. Spinach usually takes 6 or 7 weeks until its first harvest *(162)*, and it will often be cut 2 or 3 additional times in intervals of 20 to 30 days after the first harvest. Our results suggest that the first cut of spinach crop may be considered microbiologically safer.

Our final statistical model had a perfect Sp but a very low Se (33.9%). However, when the cost of a false negative was set to 14, the Se and Sp of the final model were 71.2% and 75.4%, respectively. Therefore, our model may be practically manipulated depending on our objective (i.e., whether a better Se or Sp is of interest). Therefore, while it is subject to future model assessments on an independent data set, our statistical

model may be a promising tool for the prediction of generic *E. coli* contamination in the field. Moreover, to our knowledge, this is the only published study that investigated the predictive performance of the developed statistical model for the considered farm management and environmental risk factors.

Generic *E. coli* is commonly used as an indicator of environmental fecal contamination. For example, *E. coli* has been recommended as a reliable indicator organism for the potential presence of *S.* Typhimurium in manure-fertilized soil and on vegetables grown in such a soil *(150)*. Thus, the absence of *E. coli* from a produce sample may be taken as a strong indication of the absence of other fecal contaminants *(150)*. However, the presence of *E. coli* on a produce sample as an indication of contamination of fecal origin has to be considered with a grain of salt. *E. coli* has been shown to be able to maintain stable populations in temperate soil and water *(100)*. Thus, while *E. coli* on produce most often originates from recent fecal contamination, it could also be from an environmentally stable population of the microorganism.

The evaluation of spinach contamination with generic *E. coli* described in this paper was part of a larger unpublished study involving the same spinach farms where, in addition to collection of spinach, we also collected a total of 191 drag samples of soil and 26 samples of irrigation water. The original intent of that study was to elucidate the effect of management and environmental factors on the contamination of spinach with foodborne pathogens. However, foodborne pathogens were detected at a very low frequency, which precluded their statistical evaluation and indicators of fecal contamination had to be used instead. Briefly, in addition to testing of spinach for

contamination with *E. coli*, all spinach, soil, and water samples were tested for contamination with *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7, and *Listeria* spp. With spinach contamination being the main focus of the study, soil and water samples were not tested for generic *E. coli* contamination and thus, no inference on the source of *E. coli* detected in spinach could be attempted. Regarding contamination with foodborne pathogens, no *L. monocytogenes* or *E. coli* O157:H7 was detected and only 1 out of 955 samples was contaminated with *Salmonella* spp. Similarly, 5 out of 191 soil drag samples tested positive for *Salmonella* spp; they were detected on two farms (with 1 and 4 positive samples out of 25 samples tested per farm). Interestingly, the *Salmonella* species- positive spinach sample was collected on the farm that had 20% of spinach samples positive for generic *E. coli*, which was the second highest farm level prevalence detected during the study. This somewhat supports previous reports *(150)* about the usefulness of generic *E coli* as an indicator microorganism. On the same farm, one soil drag sample also tested positive for *Salmonella* spp., and one tested positive for *Listeria* spp. (for *Listeria* spp., this was the only positive sample detected during the course of study). These results suggest that preharvest foodborne pathogen contamination of spinach does occur albeit at a low frequency, indicating that high resources would be needed to obtain a sample size sufficiently large for evaluation of management and environmental factors affecting pathogen contamination of spinach. Therefore, studies of produce contamination using indicator organisms, such as the current study, still provide a valuable alternative.

Several studies *(18, 45, 120, 147-149)* have evaluated the impact of farm management practices on produce contamination. Most of those studies focused on factors related to farm management. Compared to those studies, our study comprehensively assessed a large number of farm management and environmental factors, several of which were investigated for the first time. Nevertheless, our study did have several limitations. First, our study was based on a repeated cross-sectional study design, precluding conclusions about causality for produce contamination. Second, because we studied only spinach, caution should be exercised in extrapolating these results to other vegetables or fruits. Third, soil and irrigation water were not tested for contamination with generic *E. coli*. Finally, our study was limited to only 12 farms, and thus, some findings may have been coincidental. Future prospective longitudinal studies should be conducted in order to validate the plausibility of the identified risk factors including a variety of farm settings (e.g., greenhouse conditions) and climate environments (e.g., Northern or Eastern United States). Likewise, intervention trials should be conducted to investigate the effects of measures such as irrigation with good quality water, stopping irrigation up to 5 days before harvest, and improving workers' personal hygiene to validate the findings that these interventions could reduce produce contamination with *E. coli* in the field.

In conclusion, microbial contamination of produce is influenced by farm management and environmental factors. Specifically, microbial contamination of produce seems strongly influenced by the time since last irrigation, the workers' personal hygiene, and the use of the field prior to planting. Our study may serve as a template to

investigate the role of farm and environmental factors in contamination of other produce with generic *E. coli* and other microorganisms relevant to food safety.

CHAPTER IV

FARM MANAGEMENT, ENVIRONMENT AND WEATHER FACTORS JOINTLY AFFECT THE PROBABILITY OF SPINACH CONTAMINATION WITH GENERIC *ESCHERICHIA COLI* AT THE PREHARVEST LEVEL

The National Resources Information (NRI) databases provide underutilized information on the local farm conditions that may predict microbial contamination of leafy-greens at preharvest. Our objectives were to identify NRI weather and landscape factors affecting spinach contamination with generic *Escherichia coli* individually and jointly with farm management and environmental factors. For each of the 955 georeferenced spinach samples (including 63 *E. coli* positive samples) collected between 2010 and 2012 on 12 farms in Colorado and Texas, from the NRI databases we extracted variables describing the local weather (ambient temperature, precipitation, and wind speed) and landscape (soil characteristics and proximity to roads and water bodies). Variables describing farm management and environment were obtained from a survey of the enrolled farms. The variables were evaluated using a mixed-effect logistic regression model with random effects for farm and date. The final model identified precipitation as a single NRI predictor of spinach contamination with generic *E. coli* indicating that the contamination probability increases with an increasing average amount of rain (mm) over the past 29 days (odds ratio [OR] =3.5). The model also identified the farm's hygiene practices as a protective factor (OR=0.06) and manure application (OR=52.2) and state (OR=108.1) as risk factors. In a 5-fold cross-validation the model showed a solid predictive

performance with the area under the ROC curve of 81%. Overall, the findings highlighted the utility of NRI precipitation data in the preharvest produce food safety and demonstrated that farm management, environment and weather factors jointly affect the probability of spinach contamination.

4.1 Introduction

In the United States (US) alone, foodborne pathogens cause an estimated 48 million illnesses annually *(78)*, including 9.4 million of illnesses and 1,351 deaths with known etiology *(169)*. Not only are these foodborne pathogens of concern to public health but they also present a considerable economic burden to the society. For example, the total health-related annual cost of illness due to infections from *Listeria monocytogenes*, non-typhoidal *Salmonella*, *Escherichia coli* O157:H7 was estimated as $2.0 billion, 11.4 billion, and 607 million, respectively *(170)*.

Food safety concerns related to produce have been on the rise as the reported large-scale outbreaks related to contaminated produce, including leafy greens, have been making headlines. The exact number of foodborne illnesses and outbreaks attributable to produce is unknown due to underreporting and difficulties in attributing foodborne illnesses to a particular food commodity. However, based on the data on foodborne disease outbreaks reported to the US Centers for Disease Control and Prevention, among almost 68,000 illnesses in outbreaks assigned to one of the 17 considered food commodities, the commodities associated with the most outbreak related illnesses were poultry (17%), leafy vegetables (13%), beef (12%), and fruits/nuts (11%) *(78)*. Not only

were leafy greens responsible for a considerable proportion of foodborne illnesses, the mean percentage of outbreaks attributed to leafy greens has been on the rise; it increased from 6% (1998-1999) to 11% (2006-2008) *(78)*. Therefore, the reduction in the number of human foodborne cases attributable to leafy greens is of timely importance.

Enteric foodborne pathogens are shed into the environment through the feces of colonized or infected hosts, and *L. monocytogenes* is naturally found in soil. Therefore, contamination of produce, including leafy greens, with these foodborne pathogens is affected by contamination events and pathogens' survivability. Contamination events may occur through routes such as application of raw or inadequately composted manure *(147, 148)*, exposure to contaminated water through irrigation *(137, 184)* or flooding *(152)*, and deposition of feces by infected or carrier wild animals *(152, 184)*. A pathogen's survivability is an inherent pathogen characteristic *(143)* that also varies depending on the environmental and weather conditions. For example, it has been reported that inactivation of enteric bacterial, viral, and protozoan pathogens in the environment may be affected by predation, competition, water stress/osmotic potential, temperature, UV radiation, pH, inorganic ammonia, and organic nutrients *(165)*. In order to effectively control foodborne pathogens in leafy greens at the preharvest level, both the contamination routes and weather and environmental factors affecting pathogens' survivability should be considered.

While produce contamination with enteric foodborne pathogens is of high public health and economic concern, the contamination events are relatively rare *(84, 120, 148)*, thus requiring intensive but also expensive sampling efforts. Therefore, a common

practice in research and control of enteric pathogens is to use generic *Escherichia coli* as an indicator of fecal contamination of produce. Indeed, our previous study reported the potential usefulness of generic *E. coli* as an indicator organism for the presence of *Salmonella* spp *(154)*. Other studies also suggested the usefulness of generic *E. coli* as an indicator of contamination with *E. coli* O157: H7 *(151)* and *Salmonella enterica* serovar Typhimurium *(150)*. Moreover, the utility of generic *E. coli* in evaluating the efficacy of the process to reduce the population of *E. coli* O157: H7 and *Salmonella* spp. was demonstrated *(56)*. Thus, to improve the control of foodborne illnesses related to fresh leafy greens, it is meaningful to identify the risk factors for their contamination with generic *E. coli*.

For research and control of foodborne pathogens (and indicators of fecal contamination) in produce, Geographic Information Systems (GIS) integrated with standard statistical and epidemiological methods provide tremendous opportunities *(41)*. Nevertheless, limited research and application efforts *(109, 183)* have been underway in the US and elsewhere to help facilitate broad implementation of geospatial databases, methods and technologies to improve produce food safety. As indicated above, the role of the local weather and environmental factors in pathogen contamination of produce should be considered in order to effectively control it. Regarding that, the freely available national resource information (NRI) databases developed using GIS, may provide a good and abundant data source on the local landscape (topography, hydrography, soil characteristics, and road network) and weather conditions. Indeed, NRI databases have been used to study the epidemiology of foodborne diseases,

including to identify determinants of the occurrence of foodborne pathogens in the environment (5, 25). It is therefore of interest to determine if NRI information may be useful in determining the probability of leafy greens contamination, whether considered in isolation or jointly with farm management and environmental factors.

In this study we used spinach as a representative of leafy greens produce and generic *E. coli* as an indicator of fecal contamination with objectives to: (i) identify NRI weather and landscape factors that are associated with the probability of spinach contamination with generic *E. coli* at the preharvest level and (ii) determine how these and farm management and environmental factors on a particular farm jointly affect the probability of spinach contamination. In order to address these objectives, by application of spatial and statistical modeling, our previously described data on *E. coli* contamination of spinach on 12 spinach farms in Colorado and Texas and the farm management and environmental factors on the farms obtained through a questionnaire survey *(154)* were integrated with newly obtained data from the existing NRI databases.

4.2 Materials and methods

4.2.1 Spinach contamination data

The collection and microbiological testing of spinach samples have been described in detail in our previous study *(154)*. Briefly, using a repeated cross-sectional study design a total of 955 spinach samples was collected on 12 enrolled farms (4 in Colorado and 8 in Texas) during two spinach growing seasons between June 2010 and February 2012. Colorado and Texas were chosen as representative states of the Western and

Southwestern US, respectively. Spinach is best grown under relatively cool and dry conditions (18 - 24°C days, 4 - 7°C nights) *(134)* and so the spinach growing season in Texas is between November and March while it is from April to September in Colorado. Due to the different timing of spinach growing seasons in these two states, the two states could also be viewed as representative of the spinach produced year round in the US. In Texas, the enrolled farms were located in Cameron, Hidalgo, and Uvalde counties, while in Colorado they were in Adams, Boulder, Larimer, and Saguache counties. Each farm was visited on up to 5 days per growing season for a total of 2 to 8 sampling dates over the study period (Table 9). During each farm visit we collected 5 spinach samples (each consisting of at least 10 randomly selected spinach leaves) from each of 1 to 6 spinach fields per farm. The GPS coordinates for the exact locations of spinach sample collections were recorded using a handheld GPS device (Garmin 12XL; Garmin Ltd, Olathe, KS). Samples were collected and placed into sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI) using sterile gloves. They were shipped in coolers with ice-packs to a laboratory and processed within 48 hours. Twenty-five grams of spinach leaves were suspended in 75 ml phosphate-buffered saline (PBS) placed in stomacher bags. The content of each bag was crushed using a blender (Smasher Lab Blender; AES-Chemunex, France) and then a 1-ml aliquot from the sample bag, followed by 1 ml of each of five 1:10 serial dilutions, was plated directly onto Petrifilm *E. coli*/coliform count plates (3 M Microbiology, St. Paul, MN). After incubation at 37°C for 48 h, the plates were visually assessed by counting blue colonies with gas bubbles (according to the standard *E. coli* Petrifilm enumeration method). A spinach sample was considered

contaminated with generic *E. coli* if at least one colony was observed. The approach had a detection limit of 4 CFU/ml of the plated dilution.

Table 9. Description of spinach sample collection scheme.

State	Farm	Sampling dates	Samples collected	Samples positive
Colorado	1	2010: Jul 26, Aug 24, Sep 7; 2011: May 31, Jul 11, Aug 11	115	0
	2	2010: Jun 7, Jul 6, Aug31; 2011: May 24, May 29, Jun 13, Jun 14, Jul 11	120	24
	3	2010: May 21, Jul 12, Aug 16; 2011:May 29, Jun 20, Jul 18	120	2
	4	2010: Jun 13, Aug 9, Sep 25; 2011: Jun 4, Jul 4, Aug 1	120	1
Texas	1	2010: Nov 19; 2011: Jan 7, Feb 7, Nov 11, Dec 2; 2012: Jan 6	120	11
	2	2010: Nov 19; 2011: Jan 7, Feb 7, Nov 11, Dec 2; 2012: Jan 6	120	4
	3	2010: Dec 3; 2011: Jan 21	10	1
	4	2010: Dec 3; 2011: Jan 21, Feb 18, Mar 4, Dec 16; 2012: Jan 20, Feb 10	95	7
	5	2010: Dec 3; 2011: Jan 21, Feb 18	25	7
	6	2011: Jan 21, Feb 18, Mar 4, Dec 16; 2012: Jan 20, Feb 10	60	1
	7	2010: Dec 3; 2011: Jan 21	10	1
	8	2011: Dec 16; 2012: Jan 20	40	4

4.2.2 Spatial modeling of weather and landscape data

For each geo-referenced location where spinach samples were collected we obtained information from freely available NRI databases on potentially relevant weather and landscape factors following the general approach described in a study by Ivanek et al. *(109)*, In total, we obtained information on 90 variables grouped under ambient temperature, precipitation, wind speed, soil properties and distances to the nearest water body or road (Table 10). Regarding ambient temperature, it is unclear if the average, minimum or maximum daily temperature would be a better predictor of the probability of spinach contamination and therefore we explored the potential role of all three of these temperature characteristics. Data on weather factors (temperature,

precipitation, and wind speed) were obtained through the National Climatic Data Center (http://www.ncdc.noaa.gov/) based on information recorded at land-based weather stations. Specifically, for each sampled location and date of sampling we used the nearest weather station that had recorded the particular weather information for the day or period of interest. Altogether, we used data from 22 weather stations (temperature: 10, wind speed: 10, and precipitation: 10), which were located on average 11.9 km (range 1.5 km to 34.7 km) away from the sampling locations. The effect of a considered weather factor on the probability of spinach contamination may occur instantly or it may accumulate gradually over a period of time *(109)*. If the effect occurs instantly it is unknown if we should be interested in the weather characteristics on the day of sample collection or on any particular day before that. Therefore, for each of the considered weather factors we created 4 variables describing the particular weather factor on the day of sample collection and on day 1, 2, and 3 prior to sample collection. Likewise, if the effect of a weather factor accumulates over a period of time, it is unknown how long period we should consider. Thus, we created additional 14 variables explaining the mean level of a particular weather factor for a period of time between the day of sample collection and day 1, 2,…, 10, 15, 20, 25, and 29 prior to sample collection. That means that for each considered weather factor we created and examined a total of 18 variables. For the analyses, the temperature measurements were converted from degrees Fahrenheit (°F) to degree Celsius (°C) using conversion equation °C = [°F – 32] × [5/9]. The precipitation measurements were converted from inches to millimeters (mm = inch × 25.4). The amount of rain recorded as “trace” was assigned a value of 0.0001 mm. Wind

speed measurements were converted from knots to meter per second (m/s) using equation m/s = knot × 0.514. The weather variables and their notations are defined in Table 10. Additionally, we extracted information about wind gust using the approach described for wind speed, however because of many missing values, the gust variables were not considered in statistical analyses. There were no missing values for wind speed, temperature and precipitation variables.

NRI databases were also used to obtain information on landscape factors at the spinach sampling locations (Table 10). Landscape databases and GPS coordinates of spinach sample collection locations were imported into ArcGIS 10 (ESRI, Redland, CA) and reprojected into the Universal Transverse Mercator, North American Datum of 1983. From the overlay between the GPS coordinates and landscape layers we extracted information about the local soil properties and distances to the nearest water bodies and roads for each sampled location. The soil properties (4 variables) were obtained from the Soil Survey Geographic (SSURGO) database (http://soildatamart.nrcs.usda.gov/). Distances (2 variables) to the nearest water body and road for locations in Texas were extracted from the National Hydrography and TxDOT Roadways datasets, respectively, obtained through the Texas Natural Resources Information System (http://www.tnris.org/). For locations in Colorado information was extracted from the Hydrography-1M and Transportation-1M datasets, respectively, obtained through the Colorado Department of Natural Resources (http://data.geocomm.com/catalog/US/61076/datalist.html). Some of the landscape variables had missing observations. Specifically, 35 observations were missing for the

organic matter variable and 25 observations were missing for each of the variables: soil acidity, soil texture, and slope. Handling of missing observation is described under statistical analysis.

4.2.3 Farm management and environmental factors

A survey of farm management and environmental factors has been described in detail in our previous study *(154)*. Briefly, at the time of spinach sample collection, we used a questionnaire to survey farmers about the general farm-related management and environmental factors that were subsequently coded into a total of 76 explanatory variables (listed in Table 5). In the current study, a univariate statistical analysis was performed on 71 of these variables. The variables with suspected misinterpreted questions in the questionnaire survey as explained in *(154)* ("portable toilet distances from the work area," "wildlife control," and "buffer zone with fence") were excluded. Likewise, the variables for "terrain" and "proximity to the nearest road within 10-mile radius" from the survey *(154)* were replaced by variables describing the same landscape characteristics albeit obtained from the NRI databases). For brevity, out of the 71 considered variables in the current study in Table 10 we list and describe only variables that were significant at 20% level in univariate statistical analyses and were therefore eligible for further statistical consideration. Among variables in Table 10, three had missing values: "time since the last workers' visit" = 265, "organic farming certified by the National Organic Program" = 750, and "wildlife control by fences" = 450 missing values. Handling of these missing observations is described under statistical analysis.

Table 10. Description of the considered explanatory variables obtained from the NRI databases (weather and landscape factors) and from a survey of produce farmers (farm management and environmental factors).

Variable	Description and levels	Unit
Weather factors [c]		
Temperature		
tmX[a]	Mean daily temperature on the day of SC (X=0) or day X prior to SC (X=1, 2, 3)	°C
tmdX[b]	Mean of the average daily temperatures in the period between the day of SC and day X prior to SC (X=1, 2,…, 10, 15, 20, 25, 29)	°C
tiX	Minimum daily temperature on the day of SC (X=0) or day X prior to SC (X=1, 2, 3)	°C
tidX	Mean of the minimum daily temperature between the day of SC and day X prior to SC (X=1, 2,…, 10, 15, 20, 25, 29)	°C
txX	Mean of the maximum daily temperature on the day SC (X=0) or day X prior to SC (X=1, 2, 3)	°C
txdX	Mean of the maximum daily temperature between the day of SC and day X prior to SC (X=1, 2,…, 10, 15, 20, 25, 29)	°C
Precipitation		
pX	Total amount of rain on the day of SC (X=0) or day X prior to SC (X=1, 2, 3)	mm
pdX	Mean amount of rain between the day of SC and day X prior to SC (X=1, 2,…, 10, 15, 20, 25, 29)	mm
Wind speed		
wsX	Mean wind speed on the day of SC (X=0) or day X prior to SC (X=1, 2, 3)	m/s
wsdX	Mean wind speed between the day of SC and day X prior to SC (X=1, 2,…, 10, 15, 20, 25, 29)	m/s
Landscape factors		
Soil properties		
soil_acidity	The relative acidity or alkalinity of soil at SL (6.1-7.9/7.9-9.0)	pH
soil_texture	Soil texture at SL (loam/clay loam/silty clay loam/other)	
slope	The direction toward which the surface of the soil faces at SL (0.4-0.5/1-2/4-7)	degree
organic_matter	The weight of decomposed plant and animal residue at SL (0.5-2.0/2.0-4.0)	%
Distance [c]		
d_road	Distance to the nearest road from SL	m
d_water	Distance to the nearest body of water from SL	m
Farm management factors [d]		
workers_time	Time since the last workers' visit during CGS [c]	Days
hygiene-field status [e]	A composite variable coded with 1 indicating the use of portable toilets and washing stations in the field, training to use portable toilets to staff/temporary workers, and absence of grazing and hay production in the field before spinach planting and 0 otherwise (1/0)	
organic	Organic farming practices currently applied on the farm (yes/no)	
organic_certified	Organic farming certified by the National Organic Program (yes/no)	
before_fallow	Field condition before planting of the spinach during CGS: fallow (yes/no)	
manure_application	Manure spread on the field for CGS (yes/no)	
planting_time	Time since planting spinach [c]	Days
Environmental factors [d]		
proximity_beef	Proximity within 10 mile radius: dairy farm (yes/no)	
proximity_poultry	Proximity within 10 mile radius: poultry farm (yes/no)	
domestic_animal	Domestic animal intrusion of the field for CGS (yes/no)	
wild_control_fences	Wildlife control methods of the farm: fences (yes/no)	
state	Farm location (Texas/Colorado as representative states of Southern US/Southwestern US)	

[a] for example, tm0 denotes the mean daily temperature on the day of sample collection, while tm3 denotes the mean daily temperature on day 3 prior to the day of sample collection; [b] for example, tmd20 denotes the mean of the average daily temperatures recorded for the period between the day of sample collection and day 20 prior to the day of sample collection; [c] continuous variable; [d] farm management and environmental factors obtained through a survey in our previous study *(154)*; [e] Due to the simultaneous occurrence of the listed variables, a composite "hygiene-field status" variable was created and used to evaluate the effect of these factors; SC = sample collection; SL = the sampling location; CGS = the current growing season.

4.2.4 Statistical analyses

Statistical analyses were conducted using R (the R Project for Statistical Computing, http://www.r-project.org/). Except in univariate analyses, *P* values < 0.05 were considered significant. A liberal significance cut-off of 20% was used in univariate analyses to assure that all potentially influential variables (including potential confounders) were evaluated in the multivariable analysis. For the same reason a correction for multiple testing was not conducted. In univariate and multivariable modeling, associations between generic *E. coli* contamination of spinach (dependent variable) and individual explanatory (independent) variables were assessed using a mixed-effect logistic regression model with random effects for farm and date, implemented through "lmer" function in the "lme4" package *(19)*. As a measure of association odds ratio (OR) was used. To check for collinearity between two individual explanatory categorical variables we used the phi coefficient. Spearman's rank correlation analyses were performed to assess correlations between individual explanatory variables when one or both of the explanatory variables were continuous. To assess similarity of the weather and landscape factors between the two states, chi-square test for categorical data and Wilcoxon rank-sum test for continuous data were used. When any two independent variables considered for multivariable modeling had high collinearity or correlation (>60%), these variables were considered one at a time in multivariable modeling.

To better understand the type and overlap of information contained in the weather variables we subjected the weather variables listed in Table 11 to the principal

component analysis (PCA) after standardizing each variable by subtracting the mean and dividing by the standard deviation for the variable. In order to determine the number of meaningful components to retain, we considered the proportion of variance accounted for, the scree test and the interpretability criteria. According to the interpretability criteria, (i) each retained component had to contain at least three variables with major loadings, (ii) the variables loading on a retained component had to share the same conceptual meaning, and (iii) the rotated pattern had to show a simple structure (meaning that (a) most of the variables had relatively high factor loadings on only one component, and near zero loadings on the other components and (b) most components had relatively high factor loadings for some variables, and near-zero loadings for the remaining variables) *(83)*. The results of PCA were used in two ways. First, a representative weather variable was chosen for each retained principal component for consideration in the multivariable modeling. The variable choice was based on the *P* value from the univariate analysis (this was usually the variable with the lowest *P* value) and interpretability and robustness of a conclusion from the multivariable analysis. Second, we predicted the principal component scores for each retained principal component and used the scores as new explanatory variables representing the whole group of weather variables of the corresponding principal component in the univariate and, if applicable, in the multivariable modeling.

The final multivariable mixed-effect logistic regression model was manually selected by conducting a backward elimination process until only significant variables remained ($P < 0.05$ based on the Wald Z test), where each term deletion was followed by

a likelihood ratio (LR) test and comparison based on the Akaike information criterion (AIC). To assure comparability of nested models required for the LR test, the dataset was reduced to observations with complete information (i.e., observations with missing values were excluded) for the part of analysis that evaluated the variables with missing data. Because farm- and weather- related factors differed by state, suggesting a potential confounding effect by state, the effect of state factor was examined in all considered multivariable models by comparing the fit of the models with and without the state factor. The presence of confounding was determined based on the >20-30% change in ln(OR) between the estimate obtained in the model without state (crude estimate) and the estimate obtained after controlling for the effect of state (adjusted estimate) *(49)*.Two-way interactions of explanatory variables were also considered. The goodness of fit was evaluated based on how much the observed proportions agreed with the mean predicted probabilities using "plot.logistic.fit.fnc" function in the "language" package *(11)*. Latent variable approach was used to estimate the percent of variation explained by random effects (farm and date) for the final model *(49)*. To diagnose collinearity in the final model we used variance inflation factor (VIF). Locally weighted scatter plot smoothing was used to assess the linearity assumption between the logit of outcome (log ['probability of contamination'/(1-'probability of contamination')]) and individual continuous explanatory variables in the final model *(49)*.

To evaluate the utility of weather and landscape information from the NRI databases in predicting spinach contamination when these factors are considered alone or jointly with a survey of farm management and environmental factors we compared the

final statistical model developed based on the consideration of weather and landscape data only ("NRI model") with the final statistical model in which NRI data were considered alongside with the survey data ("NRI-survey model"). The predictive performances of the final NRI and NRI-survey models were compared by examining the receiver operating characteristic curve (ROC) and quantifying the area under the curve (AUC). Statistical testing of the difference between the AUCs was conducted using the "roc.test" function in the "pROC" package *(163)*. In this assessment of a model's predictive performance, the data used for model development were also used for testing of the model's predictive performance and it thus served as an internal validation of the developed statistical models. Independent dataset for external validation of the developed models was not available. However, to asses robustness of a model's predictive ability, we conducted a 5-fold cross-validation where the dataset was randomly divided into 5 subsets and then 4 subsets were used for estimation of the model's coefficients while the 5^{th} subset was used to test the model's predictive ability; this process was repeated 5 times, every time with a different test subset. The mean (and range) AUC from cross-validation was recorded and used for comparison of models.

4.3 Results

In total, 955 spinach samples were collected on 37 days during the period between June 7, 2010 and February 10, 2012 (Table 9). The overall median temperature on the day of sample collection was 17.4°C (range 8.6°C to 26.2°C). On 10 out of 37 sample collection days it rained. The weather conditions in Texas and Colorado were different.

For example, the median temperature on the sampling days in Texas was 13.5°C (range 8.6°C to 23.4°C), which was significantly lower than the temperature of 18.4°C (range 9.6°C to 26.2°C) in Colorado (*P* value <0.001). The occurrence of rain on the day of sample collection was similar in Texas and Colorado; on 2 out of 13 sample collection days it rained in Texas while it rained on 8 out of 24 sample collection days in Colorado (*P* value = 0.432). However, the median amount of rain on the rainy sampling days in Texas (4.3 mm) and Colorado (2.8 mm) were borderline significantly different (*P* value = 0.07). The soil texture was significantly different between the farms enrolled in Texas and Colorado (*P* values <0.001); most of sampled spinach was grown on silty clay loam (63%) in Texas while it was grown on loam (70%) soil in Colorado. In Texas, a total of 93% sampled spinach was grown on a relatively flat terrain (0.4 - 0.5°), while in Colorado all sampled spinach was grown on a steeper terrain (1 - 2°: 73%; 4 - 7°: 27%). In terms of the proximities to the nearest water body and road, the enrolled farms in Texas and Colorado were significantly different with the median distance to the nearest water body of 1,607 m (range 341 m to 8,156 m) in Texas and 352 m (range 7 m to 1,153 m) in Colorado. Likewise, the median distance to the nearest road was 267 m (range 3 m to 864 m) in Texas and 393m (range 21 m to 3,823 m) in Colorado.

Generic *E. coli* was detected on 6.6% (63/955) of spinach samples. Using a *P* value of 20% as a significance level cutoff, in the univariate mixed-effect models (Table 11), the spinach contamination with generic *E. coli* was reduced when spinach was exposed to a higher average, minimum or maximum temperature for several days before sample collection. On the other hand, spinach contamination was increased when

spinach was exposed to a higher amount of rain for several days before sample collection.

Table 11. Significant associations between the individual weather variables and spinach contamination with generic *Escherichia coli* based on the univariate mixed-effects logistic regression models with farm and date as random effects.

Variable	OR (95% CI) [a]	*P* value [b]
Temperature		
tmd5	0.85 (0.67, 1.08)	0.190
tmd7	0.81 (0.61, 1.09)	0.164
tmd8	0.78 (0.58, 1.06)	0.118
tmd9	0.77 (0.56, 1.05)	0.095
tmd10	0.77 (0.56, 1.04)	0.091
tmd15	0.76 (0.57, 1.03)	0.077
tmd20	0.76 (0.56, 1.02)	0.065
tmd25	0.75 (0.56, 1.01)	0.061
tmd29	0.76 (0.57, 1.01)	0.055
tid10	0.82 (0.61, 1.10)	0.181
tid15	0.81 (0.60, 1.08)	0.148
tx2	0.87 (0.70, 1.08)	0.196
tx3	0.86 (0.73, 1.03)	0.104
txd3	0.84 (0.66, 1.08)	0.183
txd4	0.84 (0.67, 1.05)	0.127
txd5	0.84 (0.68, 1.04)	0.116
txd6	0.83 (0.66, 1.05)	0.126
txd7	0.81 (0.63, 1.05)	0.111
txd8	0.80 (0.61, 1.04)	0.089
txd9	0.80 (0.61, 1.03)	0.084
txd10	0.80 (0.62, 1.04)	0.096
txd15	0.80 (0.61, 1.04)	0.089
txd20	0.77 (0.59, 1.01)	0.055
txd25	**0.76 (0.58, 0.99)**	**0.045**
txd29	**0.75 (0.58, 0.97)**	**0.028**
Precipitation		
pd5	1.18 (0.96, 1.45)	0.113
pd6	1.19 (0.95, 1.48)	0.124
pd7	1.23 (0.95, 1.59)	0.113
pd8	1.28 (0.96, 1.71)	0.096
pd9	1.36 (0.99, 1.85)	0.057
pd10	1.32 (0.94, 1.84)	0.106
pd15	1.37 (0.86, 2.18)	0.179
pd25	1.78 (0.91, 3.47)	0.091
pd29	**2.37 (1.16, 4.87)**	**0.018**
PCA scores [c]		
PC1 (temperature)	1.30 (0.99, 1.71)	0.062
PC2 (precipitation)	1.19 (0.95, 1.49)	0.122

[a] OR (95% CI) = odds ratio with 95% confidence interval; [b] Only variables with *P* value <0.20 are shown.
c Principal component (PC) scores were estimated for the variables identified through the principal component analysis in Table 12

In PCA only two components explained 87.9% of the total variability (Table 12). Twenty-two variables, describing the mean, minimum, and maximum daily or period temperatures, loaded on the first component (labeled as the temperature component). Nine variables describing the precipitation loaded on the second component (labeled as the precipitation component). The principal component scores for the temperature and precipitation components were examined as predictors of spinach contamination in univariate analysis, and they were both significant at the 20% level (Table 11).

The final multivariable NRI model had a single NRI predictor, the mean amount of rain between the day of sample collection and day 29 prior. However, adding the state factor into the NRI model with pd29 only (AIC = 366) improved the model fit (LR =5.6, degrees of freedom (df) =5-4 = 1, χ^2 p-value =0.02; AIC = 362) and the model with pd29 and state was considered as the final NRI model (Table 13). The model showed that, after controlling for the effect of state, the OR describing the effect of rain on the probability of spinach contamination increased by 227% (adjusted OR = 16.9 vs. crude OR = 2.8). No interaction between the rain and state factors was detected. The NRI model had a low predictive ability in internal validation (AUC = 69%). The cross-validation results, with mean AUC = 68% (range 64% - 74%), indicated weak repeatability (Figure 5). When we attempted to use the principal component scores for temperature and precipitation instead of the actual temperature and precipitation variables, the final model could not be selected at the 5% significance level (including after controlling for the effect of state).

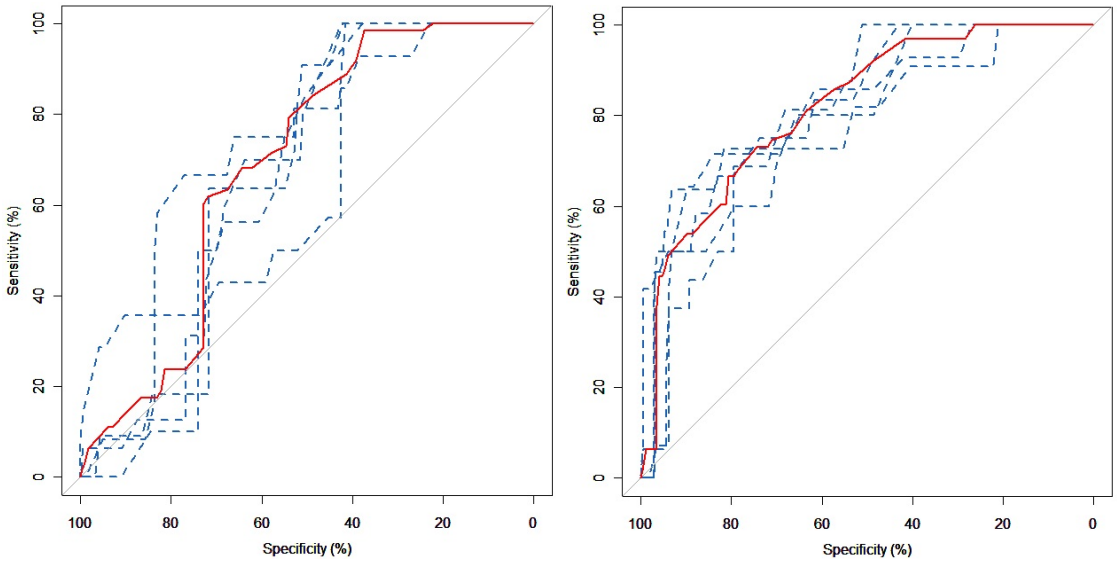
Table 12. Principal component analysis weather factors in Table 11.

	PC1	PC2
tmd5	**-0.20**	0.04
tmd7	**-0.22**	0.06
tmd8	**-0.22**	0.04
tmd9	**-0.22**	0.03
tmd10	**-0.22**	0.02
tmd15	**-0.22**	0.06
tmd20	**-0.21**	0.07
tmd25	**-0.20**	0.09
tmd29	**-0.20**	0.08
tx3	**-0.20**	0.03
txd3	**-0.20**	-0.01
txd4	**-0.21**	-0.02
txd5	**-0.21**	-0.02
txd6	**-0.22**	-0.02
txd7	**-0.22**	-0.01
txd8	**-0.22**	-0.02
txd9	**-0.22**	-0.04
txd10	**-0.21**	-0.04
txd15	**-0.22**	0.01
txd20	**-0.22**	0.03
txd25	**-0.21**	0.05
txd29	**-0.21**	0.04
pd5	0.03	**0.34**
pd6	0.03	**0.34**
pd7	0.02	**0.34**
pd8	0.02	**0.35**
pd9	0.02	**0.35**
pd10	0.02	**0.35**
pd15	0.05	**0.33**
pd25	0.05	**0.27**
pd29	0.06	**0.25**
StD (% of variance; cumulative %)	4.45 (0.64; 0.64)	2.73 (0.24; 0.88)

Table 13. The final NRI multivariable mixed-effects logistic regression model with farm and date as random effects [b].

Variable (comparison level or unit)	Reference	OR (95% CI) [a]	*P* value [b]
pd29 (mm)		2.9 (1.3, 6.3)	0.008
state (Texas)	Colorado	16.9 (1.4, 206.2)	0.027

[a] OR (95% CI) = odds ratio with 95% confidence interval; [b] Variance component values (standard deviation) were 1.145 (1.070) for farm and 4.298 (2.073) for date. For the intercept-only model, variance component values were 0.938 (0.968) for farm and 6.106 (2.471) for date.



(a) (b)

Figure 5. The receiver operating characteristics (ROC) curves from each of the five runs of the 5-fold cross-validation (dashed lines) and from the internal validation (solid line). (a) NRI model, including state and pd29; (b) NRI-survey model, including state, pd29, hygiene-field status, and the use of manure fertilizer.

Univariate mixed effect logistic regression analysis (with farm and date as random effects) of survey variables indicated associations between the probability of spinach contamination and several farm management and environmental factors (Table 14). To assess if weather data could complement the farm management and environmental factors in explaining the probability of spinach contamination, the variables listed in Tables 11 and 14 were considered in a multivariable mixed-effect logistic regression model. Several variables in Table 14 were highly correlated (e.g., the proximity of a beef farm and poultry farm and domestic animal intrusion) and thus in the multivariable modeling they were considered one at a time. The final NRI-survey model is shown in Table 15. Based on this model, the odds of spinach contamination with generic *E. coli* were reduced to approximately 1 in 17 (OR = 0.06) in the presence of

"hygiene-field status" factors on the sampled field. However, the odds of contamination were increased (OR = 3.5) for every mm increase in the mean amount of rain between the day of sample collection and day 29 prior. The odds of contamination were elevated to approximately 52 in 1 (OR = 52.2) if manure fertilizer was applied onto the field before the current growing season. Likewise, the probability of spinach contamination was higher in Texas than in Colorado (OR=108.1). Regarding the effect of state, it is interesting to note that the final NRI–survey model in Table 15 with state included (AIC = 346) had a statistically better fit than the model without state (LR =14.8, df =7-6 = 1, χ^2 p-value =0.0001; AIC = 359). None of the possible 2-way interactions in the NRI-survey model was found significant. However, there was an indication of a possible confounding effect of state on: (i) the association between rain and spinach contamination (crude OR = 2.4 vs. adjusted OR = 16.9) and (ii) association between the use of manure fertilizer and spinach contamination (crude OR = 10.4 vs. adjusted OR = 68.9). Additionally, the association between the hygiene-field status factors and spinach contamination seemed to have been confounded by the effect of rain (crude OR = 0.14 vs. adjusted OR = 0.05). Causal diagram in Figure 6 depicts the identified determinants of spinach contamination in the final NRI-survey model and their relationships. In internal validation, the predictive ability of the final NRI-survey model (AUC = 82%) was significantly better than that of the final NRI model (P < 0.001). In cross-validation, the mean AUC was 81% (range 80% - 84%) (Figure 5). The proportions of variation accounted for in the NRI-survey model were 1.4% and 51.1% at the farm and date

levels, respectively, in the multi-level intercept-only model and 9.1% and 59.1%, respectively, in the multi-level final model.

Table 14. Farm management and environmental factors identified through analysis of the variables from a survey of spinach farmers that were significantly associated with spinach contamination with generic *Escherichia coli* based on the univariate mixed-effects logistic regression models with farm and date as random effects.

Variable	Level	Frequency [a]	OR (95% CI) [b]	*P* value [c]
Farm management factor				
workers_time	>3days	13/330	0.31 (0.09, 1.04)	0.057
	≤3days	32/360	Reference	
hygiene-field status [d]	1	56/930	0.14 (0.02, 0.86)	0.034
	0	7/25	Reference	
organic	yes	31/260	3.7 (0.8, 16.4)	0.089
	no	32/695	Reference	
organic_certified	yes	4/175	0.01 (0.00, 0.98)	0.049
	no	7/25	Reference	
before_fallow	yes	15/165	5.8 (1.2, 27.6)	0.027
	no	48/790	Reference	
manure_application	yes	25/160	10.4 (1.4, 78.4)	0.024
	no	38/795	Reference	
planting_time	>66days	46/465	2.2 (0.8, 6.2)	0.144
	≤66days	17/490	Reference	
Farm environmental factor				
proximity_beef	yes	24/120	9.0 (0.6, 145.1)	0.120
	no	39/835	Reference	
proximity_poultry	yes	24/110	11.4 (0.8, 168.6)	0.077
	no	39/845	Reference	
domestic_animal	yes	7/25	7.09 (1.20, 42.02)	0.031
	no	56/910	Reference	
wild_control_fences	yes	19/325	0.16 (0.01, 2.16)	0.168
	no	28/180	Reference	
state	Texas	36/480	12.6 (0.9, 180.1)	0.061
	Colorado	27/475	Reference	

[a] frequency = number of observations with generic *E. coli* contaminated spinach/total number of recorded observations for the variable; [b] OR (95% CI) = odds ratio with 95% confidence interval; [c] Only variables with *P* value < 0.20 are shown; [d] the estimated OR (95% CI) value applies to all factors within the composite variable "hygiene-field status": level 1 indicates presence of toilet training and use of toilets and washing stations but absence of field grazing and hay production before planting of the spinach during the current growing season.

Table 15. The final NRI-survey multivariable mixed-effects logistic regression model with farm and date as random effects [c].

Variable (comparison level or unit)	Reference	OR (95% CI) [a]	*P* value
hygiene-field status (1) [b]	0	0.06 (0.01, 0.30)	0.001
pd29 (mm)		3.5 (1.7, 7.3)	0.001
manure_application (yes)	no	52.2 (2.8, 968.0)	0.008
state (Texas)	Colorado	108.1 (4.8, 2447.3)	0.003

[a] OR (95% CI) = odds ratio with 95% confidence interval; [b] the estimated OR (95% CI) value applies to all factors within the composite variable "hygiene-field status" group: level 1 indicates presence of toilet training and use of toilets and washing stations but absence of field grazing and hay production before planting of the spinach during the current growing season; [c] Variance component values (standard deviation) were 0.100 (0.316) for farm and 3.534 (1.880) for date. For the intercept-only model, variance component values were 0.938 (0.968) for farm and 6.106 (2.471) for date.

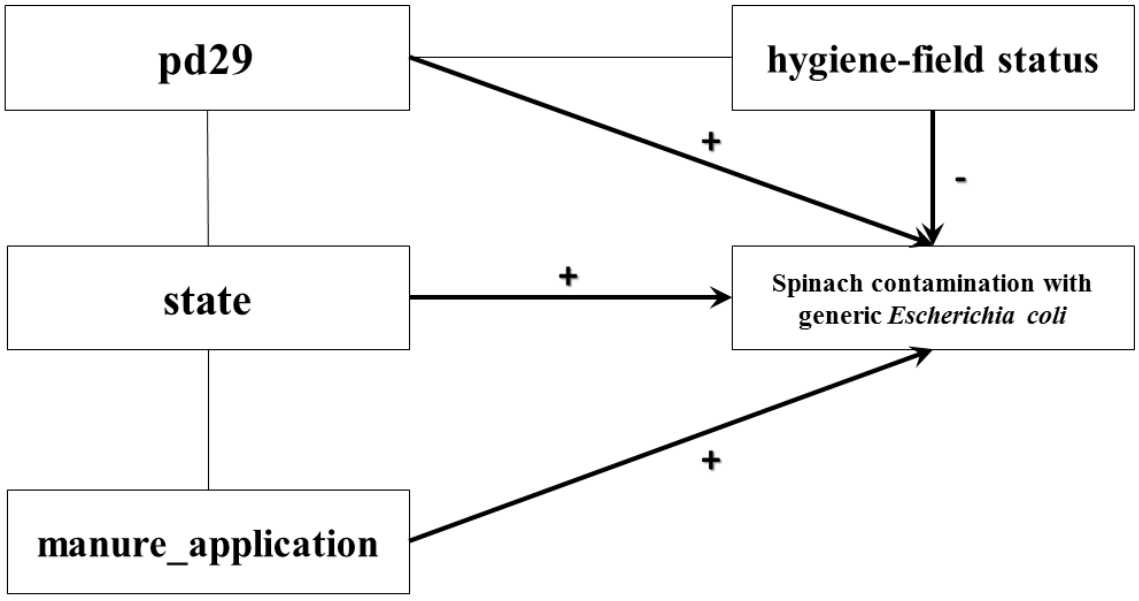


Figure 6. Proposed causal diagram of how farm management, environmental, and weather factors jointly influence spinach contamination with generic *Escherichia coli*.
Thick arrow indicates an exposure effect; Thin line indicates a confounding effect; Plus and negative signs indicate positive and negative associations, respectively, between the exposure variables and spinach contamination.

In multivariable modeling of temperature and precipitation component scores jointly with the farm management and environmental factors, the final model included the same variables as the final NRI-survey model counterpart described above. The two models also had equal predictive performance (results not shown). However, the model coefficients were different indicating slightly different magnitudes of association (for example for the precipitation component scores the estimated OR = 1.26; 95% confidence interval = [1.01, 1.56]). Because the models had equal structure and predictive performance, and because the principal component scores were difficult to interpret in the model with precipitation component scores, we are not showing this model here.

4.4 Discussion

This study evaluated the utility of easily accessible weather and landscape data from the NRI databases in explaining the probability of spinach contamination with generic *E. coli* at the preharvest level when used alone or together with a survey of produce farmers about the farm management and environmental factors on their farms. The study results demonstrated that NRI databases could be used relatively easy to obtain information on precipitation as a determinant of spinach contamination with generic *E. coli*. However, when precipitation was considered together with the farm management and environmental factors provided by surveyed farmers the predictive ability of the developed statistical model was significantly improved supporting that farm

management, environmental, and weather factors should be considered together to predict spinach contamination and design novel control strategies.

The study identified that the risk of spinach contamination with generic *E. coli* increased with the mean amount of rain between the day of sample collection and day 29 prior to sample collection. Consistent with our finding, previous studies have shown that storms and rain increased produce contamination *(37, 38, 131)*. They identified that rain splashed *Salmonella* Typhimurium onto tomato plants *(37)*, splashed *Colletotrichum acutatum* onto strawberry *(131)*, and lead to the transport of *Salmonella* Typhimurium to tomato fruits by aerosols *(38)*. A modeling study showed that the probability of lettuce contamination with *E. coli* O157:H7 from manure-amended soil was significantly correlated with the number of times it rained *(67)*. If microorganisms persist on produce prior to rain, high humidity itself was suggested to contribute to the better survival or growth of the microorganisms on produce. *(24, 50, 155)*. A study by Strawn et al. *(183)* identified precipitation as an important factor influencing the isolation of *Salmonella* on produce farms. The results from our study specify that, after controlling for the effect of manure application, state and "hygiene-field status" factors, for every mm of rainfall during the 29 period before sample collection, the odds of spinach contamination increase by 3.5 (95%CI 1.7-7.3). This may prompt farmers to use NRI weather databases to monitor the amount of rain on their spinach fields during a growing season and use that information, in conjunction with information on their particular farm management practices, to predict the microbial quality of harvested spinach. That being said, our study also identified a possible distorting confounding effect of state *(49)* on the

association between the amount of rain during the period before sample collection and spinach contamination by making the association seem weaker than it actually was. It is unknown whether the state effect is a true population or sample confounder *(49)*. Nevertheless, the fact controlling for the effect of state significantly improved the model fit and dramatically enhanced the effect of identified risk factors suggest that the effect of state should be considered in multistate (or, for that matter, multi-country) field studies of produce contamination. The likely explanation for the confounding effect of state may be in the different distributions of weather, landscape and management practices across the states. For that same reason, generalizing our results to produce farms in other states should be done with caution.

At the univariate level, the probability of spinach contamination with generic *E. coli* decreased significantly (at 5% significance level) with an increasing average of the maximum daily temperatures between the day of sample collection and day 25 or 29 prior to sample collection. Such finding was surprising since the growth rate of *E. coli* is optimal at warm temperatures between mid-20 and high-30 °C *(44, 99)*. However, after controlling for the effect of state in multivariable modeling, these temperature variables were no longer significantly associated with spinach contamination supporting that state acted as a distorter variable *(49)*. While temperature may be a true predictor of spinach contamination with generic *E. coli*, based on the obtained NRI temperature data we were unable to confirm its effect. Interestingly, the most significant precipitation variable in the univariate analysis (Table 11) was that describing average during the 29 day period before sample collection. That result might be attributed to the long-term, or even

seasonal, effect of weather on the survival and growth of generic *E. coli* on spinach at the preharvest level. The result also indicate that weather over a longer period of time before sample collection than the longest period of 29 days considered in the current study may be an even more informative predictor of spinach contamination. Thus, future studies could be designed to elucidate the long-term and seasonal effects of weather on the probability of produce contamination.

Spinach contamination with generic *E. coli* was significantly elevated if the farm applied manure fertilizer (OR = 52.2). A previous study *(147)* showed similar results: the use of manure fertilizer increased the produce contamination with *E. coli* in organic (OR = 13.2) and semiorganic (OR = 12.9) farms. A recent study by Strawn et al. showed that manure application within a year prior to sample collection increased the likelihood *Salmonella* being detected in a produce field *(184)*. However, the final model of our previous study, that used the same survey (albeit not NRI) data, did not include the use of manure fertilizer *(154)*. A possible reason for that may be a confounding effect of state on the association between the manure use and spinach contamination identified in the current study and forward selection of the final model used in *(154)*. Indeed, after controlling for the effect of state, the association between the use of manure fertilizer and spinach contamination became much stronger.

The "hygiene-field status" group was identified to have a protective effect (OR = 0.06) on spinach contamination with generic *E. coli* when considered along with weather and landscape factors as well as with the other farm management and environmental factors. This is in agreement with the results from our previous study where "hygiene-

field status" was the strongest protective factor (OR = 0.15) among the 76 surveyed farm management and environmental factors *(154)*. The repeatability of the finding reconfirms the potential importance of this group of factors in produce food safety highlighting the need to further elucidate their role in produce contamination. The association between the spinach contamination and "hygiene-field status" group of factors seem to have been confounded by the amount of rainfall during the period prior to sample collection by making the association seem weaker than it actually was. A closer examination of the data suggests that confounding may be explained by an uneven distribution of the amount of rainfall with respect to the presence of "hygiene-field status" group factors on the sampled fields. Thus, it seems more likely that the identified confounding was a statistical artifact rather than a biologically meaningful effect of rain on the association between the "hygiene-field status" group of factors and spinach contamination.

In addition to the "hygiene-field status" group of factors, our previous study *(154)* identified association between spinach contamination with generic *E. coli* and the following risk factors: an irrigation lapse time of >5 days, a >66-day period since the planting of spinach, the farm location in Texas as representative of the Southwestern US , the use of pond water for irrigation, and the proximity (within 10 miles) of a poultry farm. However, these factors were not identified as significant in the final NRI-survey model developed in the current study. There may be two explanations for that. First, the previous and the current study considered slightly different random structures of the data. The previous study considered "farm" and "farm visit" as random variables

whereas in the current study "farm" and "date" were considered as random factors, because "date" explained the highest proportion of variation for weather factors. For example, in the univariate analyses for the mean temperature of the day of sample collection, "farm" and "date" explained 8.4% and 60.6% of variation, respectively, whereas "farm" and "farm visit" explained 32.9% and 12.1%, respectively. The difference in the considered random effects explains the differences between the results shown Table 14 and those in Table 7 of our previous study *(154)*. The second reason for a different structure of the final model between the two studies may be consideration of additional explanatory variables (e.g., weather factors) in the current study. Nevertheless, the fact that two models commonly retained the "hygiene-field status" group of factors is important, because farmers can improve the produce safety by supporting workers' hygiene and by managing the field condition before planting spinach.

Previous studies showed the difference of persistence and growth of microorganisms according to soil acidity *(116)* and the degree of organic matter in soil *(115)*. However, the quality of data obtained from NRI databases for those variables was not satisfactory. For example, the soil acidity data had three overlapping levels (pH "6.1 – 7.9", "6.6 – 8.4", and "7.9 – 9.0"). We encountered a similar problem with the degree of organic matter. Thus, the statistical analysis of these factors was not meaningful supporting that future evaluation of these soil property data should consider sources other than the NRI databases considered here. Several previous studies conducted under the controlled conditions showed that the survivability of microorganisms in soil or on

produce was significantly affected by slope *(2)* and soil texture *(92)*. However, our findings did not support the results of those controlled trials. The inconsistencies between the controlled trials and our observational study may be attributed to the farm management or weather factors which may have obscured the relationship between these soil factors and spinach contamination. We additionally tested soil salinity and soil type (e.g., classified as entisols, inceptisols, and mollisols) to determine whether they influenced the probability of spinach contamination with *E. coli*, but the results were not significant.

Spinach samples collected in close proximity (i.e., on the same farm or even in the same county) were likely to be more similar to each other than samples collected in distant locations. Such autocorrelation, if not accounted for, may bias the results of statistical analysis by deflating standard errors *(172)*. In the current study, the spatial autocorrelation was accounted for, at least partially, by considering farm as a random effect *(73)*.

The ability of the final NRI-survey model to correctly identify spinach contamination with generic *E. coli* was better than that of the final NRI model based on the models' estimates of AUC. However, the final NRI-survey model was not significantly better than the model based on survey data only from our previous study *(154)* (results not shown). This suggests that the predictor variables from a survey are equally good indicators of spinach contamination whether used alone or together with weather variables obtained from NRI databases. This makes sense because farm management practices will tend to adapt to the local weather events. While the final

NRI-survey model showed a relatively high and repeatable predictive performance in cross-validation caution is needed in generalizing model results to other locations before the model is evaluated on an independent dataset.

Our results suggest novel approaches to improve food safety of fresh produce at the preharvest level. For example, farmers could adjust farm management practices (e.g., harvest time) according to the rainfall conditions with the goal of producing microbiologically safer fruits and vegetables. Avoiding application of animal manure would significantly reduce produce contamination. Most of all, we propose that portable toilets and hand-washing stations should be provided in the fields for workers, and the farmers should train their field workers on how to use them in order to reduce the produce contamination with microorganisms.

To our knowledge, this is the first study on the association between produce contamination and the combination of farm management, environmental, and weather factors. Previous field studies have evaluated the effects of only a subset of these factors on produce contamination *(147-149, 154).* Although a recent study *(183)* identified risk factors (e.g., temperature, precipitation, available water storage in soil) among landscape and meteorological factors for the isolation of *Salmonella* and *L. monocytogenes* on fruit and vegetable farms, they only collected soil, water, feces, and drag swabs samples, and not crop samples as it was done in the current study. Nevertheless, the current study has some limitations. First, the causes of spinach contamination with generic *E. coli* could not be determined explicitly due to the cross-sectional nature of the study. Second, measurement error in the weather data may be considerable considering that some of the

nearest weather stations were up to 35 km away from the enrolled farms. Third, caution is needed in generalizing the results to all spinach farms in the US, because our study was based on only 12 spinach farms located in Colorado and Texas as representative of the Western and Southwestern US, respectively. While the study focused on spinach, some of the findings are likely to be generalizable to other leafy greens due to the common properties of leafy greens, such as similar cultivation and harvest methods, less direct contact of the edible portion with soil, and direct exposure to irrigation water. Furthermore, the developed geospatial and statistical modeling framework is adaptable to study determinants of produce contamination with other foodborne pathogens and on other produce commodities at the preharvest level.

In conclusion, farm management, environmental and weather factors jointly affect the produce contamination with generic *E. coli*. Spinach contamination was significantly associated with the "hygiene-field status" group of factors, rainfall, and the use of manure fertilizer with the effect of the latter two factors likely being confounded by state. Thus, future studies of microbial contamination of leafy greens need to focus on these factors.

CHAPTER V

CONCLUSIONS

L. monocytogenes, Salmonella, and *E. coli* O157:H7 are important foodborne pathogens of concern to safety of US food supply. Contamination of fruits and vegetables with these pathogens have caused numerous foodborne outbreaks *(169)* and imposed a considerable economic burden to the society *(170)*. Produce contamination may occur on the farm from numerous sources, such as soil, manure fertilizer, irrigation water, and intrusions of wild or domestic animals *(66)*. This dissertation provides a systematic review of the current knowledge about the risk factors for the contamination of preharvest fruits and vegetables with *L. monocytogenes*, *Salmonella,* and *E. coli* O157:H7 and provides valuable information for better understanding of the effects of farm management, environmental, and meteorological factors on the produce contamination with generic *E. coli*.

Most reported epidemiologic studies interested in the role of farm management factors in microbial contamination of produce focused on only a subset of those factors, primarily soil and irrigation water. Thus, it was important to summarize the existing knowledge about the risk factors for produce contamination so that more comprehensive and advanced epidemiological studies could be conducted. With this need, a comprehensive systematic review, in Chapter II, was conducted to identify risk factors for microbial contamination of produce, and those factors were grouped by the elements

of the epidemiological triad: animal hosts, pathogens, and the local environment (produce, water, soil, and local ecological factors).

The systematic review study highlighted the importance of soil and irrigation water in contamination of produce and identified additional risk factors, including growing produce on clay-type soil, the use of contaminated or non-pH-stabilized manure fertilizer, and the use of spray irrigation with contaminated water, with a particular risk of contamination on the lower leaf surface. However, the systematic review showed that only a few field studies have taken into account the effect of field workers' personal hygiene on produce contamination, although that is a well-known risk factor during preharvest and postharvest *(22)*. Our Chapter III addressed that knowledge gap. The review study also indicated that very few of the published studies had statistically evaluated the associations between weather factors and produce contamination. Thus, Chapter IV evaluated the effect of weather factors together with farm management and environmental factors on the probability of spinach contamination with generic *E. coli*.

To determine the effect of farm management factors on produce contamination, two epidemiology studies were performed in Chapter III and IV. In terms of farm management practices, the models showed that spinach contamination with generic *E. coli* was significantly reduced in the presence of "hygiene-field status" group of factors (These factors were the use of portable toilets and hand-washing stations, training in the use of portable toilets, and not use of the spinach field for grazing or hay production before spinach planting) and an irrigation lapse time of >5 days. Spinach contamination was significantly increased with the use of pond water for irrigation, a >66-day period

since the planting of spinach, and the use of manure fertilizer. The “Hygiene-field status” group of factors was significant in both statistical models. The repeatability of the finding confirms the potential importance of this group of factors in produce food safety highlighting the need to further elucidate their role in produce contamination.

Regarding to farm environment factors, only two variables, farm location (i.e., state) and the proximity of a poultry farm, were retained in our statistical models. A previous study showed the significantly different *E. coli* contamination between regions within Minnesota and Wisconsin *(147)*. The differences of climate, soil, and some farm management and environmental patterns between regions might contribute to the difference in the frequency of produce contamination between the two regions. Although wild birds are known to be drawn to the poultry barns *(29)*, it was not clear whether the association between produce contamination and the proximity of a poultry farm was a statistical artifact. Thus, future studies should be conducted to evaluate whether poultry farm environment could influence on produce contamination.

In addition to farm environment, weather also had an effect on the probability of spinach contamination (Chapter IV). Specifically, the probability of spinach contamination was increased with the mean amount of rain between the day of sample collection and day 29 prior to sample collection. However, after controlling for the confounding effect of state, the results indicated that ambient temperature does not have any effect on the probability of spinach contamination. The effect of rain could be twofold. First, the splashing effect of rain may have represented the contamination event by transporting the microorganisms from soil onto the spinach. Secondly, wet spinach

surface may have represented favorable conditions for the survival and growth of microorganisms.

As the first systematic review in the field of plant agriculture, Chapter II may provide a cornerstone for developing systematic review methodology in produce safety field. Moreover, the summarized current knowledge may be used as guidelines for decision making by policy makers and farmers. For example, potentially contaminated water can be prohibited or avoided to irrigate produce using spray method. This review may also provide an outline of topics needing future research. For example, observational field study should be conducted to identify the risk factors related to produce contamination in the natural environment.

The results of Chapter III and IV suggest novel approach to predict produce contamination in the field and to improve food safety of fresh produce at the preharvest level. For example, when the produce field is irrigated with pond water or under high rainfall conditions, the farmers may predict a higher risk of microbial contamination of produce and delay their harvest time. If the use of manure fertilizer is not necessary, the farmers could avoid it to reduce produce contamination. Although numerous field studies were performed to investigate risk factors of produce contamination, to our knowledge, this is the only published study that evaluated predictive performance of the developed model and compared the models based on different risk factor categories (e.g., models based on farm management and environmental factors vs. on farm management, environment and weather factors). This approach can be applied to future studies.

In conclusion, microbial contamination of produce is influenced by farm management, environmental, and weather factors. This dissertation may serve as an example to identify and characterize known risk factors for produce contamination and investigate the role of risk factors in the field.

REFERENCES

1. Abreu, I. M. O., A. M. R. Junqueira, J. R. Peixoto, and S. A. Oliveira. 2010. Microbiological quality and productivity of lettuce under chemical and organic fertilization. *Ciênc. Tecnol. Aliment.* 30:108-118.
2. Abu-Ashour, J., and H. Lee. 2000. Transport of bacteria on sloping soil surfaces by runoff. *Environ. Toxicol.* 15:149-153.
3. Adams, M., and M. Moss. 2000. Food microbiology. The Royal Society of Chemistry, Cambridge.
4. al-Ghazali, M. R., and S. K. al-Azawi. 1990. *Listeria monocytogenes* contamination of crops grown on soil treated with sewage sludge cake. *J. Appl. Bacteriol.* 69:642-647.
5. Alam, M. J., and L. Zurek. 2004. Association of *Escherichia coli* O157:H7 with houseflies on a cattle farm. *Appl. Environ. Microbiol.* 70:7578-7580.
6. Anderson, G. L., K. N. Caldwell, L. R. Beuchat, and P. L. Williams. 2003. Interaction of a free-living soil nematode, *Caenorhabditis elegans*, with surrogates of foodborne pathogenic bacteria. *J. Food Prot.* 66:1543-1549.
7. Armon, R., C. G. Dosoretz, Y. Azov, and G. Shelef. 1994. Residual contamination of crops irrigated with effluent of different qualities: a field study. *Water Sci. Technol.* 30:239-248.

8. Arthurson, V., A. Sessitsch, and L. Jaderlund. 2011. Persistence and spread of *Salmonella enterica* serovar Weltevreden in soil and on spinach plants. *FEMS Microbiol. Lett.* 314:67-74.

9. Aruscavage, D., S. A. Miller, M. L. Ivey, K. Lee, and J. T. LeJeune. 2008. Survival and dissemination of *Escherichia coli* O157:H7 on physically and biologically damaged lettuce plants. *J. Food Prot.* 71:2384-2388.

10. Aruscavage, D., P. L. Phelan, K. Lee, and J. T. LeJeune. 2010. Impact of changes in sugar exudate created by biological damage to tomato plants on the persistence of *Escherichia coli* O157:H7. *J. Food Sci.* 75:M187-192.

11. Baayen, R. H. 2012. languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics". Available at: http://cran.r-project.org/web/packages/languageR/languageR.pdf. Accessed 5 January 2013.

12. Balshem, H., M. Helfand, H. J. Schunemann, A. D. Oxman, R. Kunz, J. Brozek, G. E. Vist, Y. Falck-Ytter, J. Meerpohl, S. Norris, and G. H. Guyatt. 2011. GRADE guidelines: 3. Rating the quality of evidence. *J. Clin. Epidemiol.* 64:401-406.

13. Barak, J. D., S. T. Koike, and R. L. Gilbertson. 2001. Role of crop debris and weeds in the epidemiology of bacterial leaf spot of lettuce in California. *Plant Dis.* 85:169-178.

14. Barak, J. D., A. Liang, and K. E. Narm. 2008. Differential attachment to and subsequent contamination of agricultural crops by *Salmonella enterica*. *Appl. Environ. Microbiol.* 74:5568-5570.

15. Barak, J. D., and A. S. Liang. 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. *PLoS One*. 3:e1657.

16. Barak, J. D., L. C. Whitehand, and A. O. Charkowski. 2002. Differences in attachment of *Salmonella enterica* serovars and *Escherichia coli* O157:H7 to alfalfa sprouts. *Appl. Environ. Microbiol.* 68:4758-4763.

17. Bardet, A. 2007. Organic products and irrigation water. Transfer of human pathogens to vegetables. *Infos-Ctifl.* 233:49-52.

18. Barker-Reid, F., D. Harapas, S. Engleitner, S. Kreidl, R. Holmes, and R. Faggian. 2009. Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. *J. Food Prot.* 72:458-464.

19. Bates, D., M. Maechler, and B. Bolker. 2012. lme4: Linear mixed-effects models using S4 classes. Available at: http://cran.r-project.org/web/packages/lme4/lme4.pdf. Accessed 5 January 2013.

20. Baudišová, D. 1997. Evaluation of *Escherichia coli* as the main indicator of faecal pollution. *Water Sci. Technol.* 35:333-336.

21. Bernstein, N., S. Sela, and S. Neder-Lavon. 2007. Assessment of contamination potential of lettuce by *Salmonella enterica* serovar Newport added to the plant growing medium. *J. Food Prot.* 70:1717-1722.

22. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204-216.

23. Beuchat, L. R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microb. Infect.* 4:413-423.

24. Beuchat, L. R. 2006. Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *Br. Food J.* 108:38-53.

25. Beuchat, L. R., A. J. Scouten, R. I. Allen, and R. S. Hussey. 2003. Potential of a plant-parasitic nematode to facilitate internal contamination of tomato plants by *Salmonella. J. Food Prot.* 66:1459-1461.

26. Brandl, M. T., and R. Amundson. 2008. Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica. Appl. Environ. Microbiol.* 74:2298-2306.

27. Brandl, M. T., and R. E. Mandrell. 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. *Appl. Environ. Microbiol.* 68:3614-3621.

28. Burnett, S. L., and L. R. Beuchat. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *J. Ind. Microbiol. Biotechnol.* 27:104-110.

29. Burns, T. E., C. Ribble, C. Stephen, D. Kelton, L. Toews, J. Osterhold, and H. Wheeler. 2012. Use of observed wild bird activity on poultry farms and a literature review to target species as high priority for avian influenza testing in 2 regions of Canada. *Can. Vet. J.* 53:158-166.

30. Byappanahalli, M. N., R. L. Whitman, D. A. Shively, M. J. Sadowsky, and S. Ishii. 2006. Population structure, persistence, and seasonality of autochthonous

Escherichia coli in temperate, coastal forest soil from a Great Lakes watershed. *Environ. Microbiol.* 8:504-513.

31. Cai, S. W. 1986. A bacteriological and helminthological investigation on a sewage-irrigated area in Beijing suburb. *Zhonghua Yu Fang Yi Xue Za Zhi*. 20:280-282.

32. Cai, S. W., S. Y. Zhou, J. Q. Wang, S. Y. Li, X. L. Zhu, J. J. Wang, and J. R. Xue. 1988. A bacteriological and helminthological investigation of a sewage-irrigated area in a Beijing suburb. *Biomed. Environ. Sci.* 1:332-338.

33. Caldwell, K. N., G. L. Anderson, P. L. Williams, and L. R. Beuchat. 2003. Attraction of a free-living nematode, *Caenorhabditis elegans*, to foodborne pathogenic bacteria and its potential as a vector of *Salmonella* poona for preharvest contamination of cantaloupe. *J. Food Prot.* 66:1964-1971.

34. Calvin, L. 2007. Outbreak linked to spinach forces reassessment of food safety practices. Available at: http://www.ers.usda.gov/AmberWaves/June07/Features/Spinach.htm. Accessed 26 Feburary 2012.

35. Centers for Disease Control and Prevention. 2011. Investigation update: Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen farms, Colorado. Available at: http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html. Accessed 26 Feburary 2012.

36. Centre for Reviews and Dissemination. 2009. Systematic Reviews: CRD's guidance for undertaking reviews in health care. Available at:

http://www.york.ac.uk/inst/crd/SysRev/!SSL!/WebHelp/SysRev3.htm. Accessed 26 Feburary 2012.

37. Cevallos-Cevallos, J. M., M. D. Danyluk, G. Gu, G. E. Vallad, and A. H. van Bruggen. 2012. Dispersal of Salmonella Typhimurium by rain splash onto tomato plants. *J. Food Prot.* 75:472-479.

38. Cevallos-Cevallos, J. M., G. Gu, M. D. Danyluk, N. S. Dufault, and A. H. van Bruggen. 2012. Salmonella can reach tomato fruits on plants exposed to aerosols formed by rain. *Int J Food Microbiol*. 158:140-146.

39. Chale-Matsau, J. R., and H. G. Snyman. 2006. The survival of pathogens in soil treated with wastewater sludge and in potatoes grown in such soil. *Water Sci. Technol.* 54:163-168.

40. Choi, S., J. Bang, H. Kim, L. R. Beuchat, and J. H. Ryu. 2011. Survival and colonization of *Escherichia coli* O157:H7 on spinach leaves as affected by inoculum level and carrier, temperature and relative humidity. *J. Appl. Microbiol.* 111:1465-1472.

41. Clarke, K. C., S. L. McLafferty, and B. J. Tempalski. 1996. On epidemiology and geographic information systems: a review and discussion of future directions. *Emerg. Infect. Dis.* 2:85-92.

42. Cooley, M. B., D. Chao, and R. E. Mandrell. 2006. *Escherichia coli* O157:H7 survival and growth on lettuce is altered by the presence of epiphytic bacteria. *J. Food Prot.* 69:2329-2335.

43. Cooley, M. B., W. G. Miller, and R. E. Mandrell. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae. Appl. Environ. Microbiol.* 69:4915-4926.

44. Cooper, V. S., A. F. Bennett, and R. E. Lenski. 2001. Evolution of thermal dependence of growth rate of *Escherichia coli* populations during 20,000 generations in a constant environment. *Int. J. Org. Evol.* 55:889-896.

45. Cote, C., and S. Quessy. 2005. Persistence of *Escherichia coli* and *Salmonella* in surface soil following application of liquid hog manure for production of pickling cucumbers. *J. Food Prot.* 68:900-905.

46. Critzer, F. J., and M. P. Doyle. 2010. Microbial ecology of foodborne pathogens associated with produce. *Curr. Opin. Biotechnol.* 21:125-130.

47. Delaquis, P., S. Bach, and L. D. Dinu. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. *J. Food Prot.* 70:1966-1974.

48. Dewaal, C. S., G. Hicks, K. Barlow, L. Alderton, and L. Vegosen. 2006. Foods associated with foodborne illness outbreaks from 1990 through 2003. *Food Prot. Trends*. 26:466-473.

49. Dohoo, I. R., W. Martin, and H. Stryhn. 2010. Veterinary epidemiologic research. VER Incorporated, Prince Edward Island.

50. Doyle, M. P., and M. C. Erickson. 2008. Summer meeting 2007 - the problems with fresh produce: an overview. *J. Appl. Microbiol.* 105:317-330.

51. Dreux, N., C. Albagnac, F. Carlin, C. E. Morris, and C. Nguyen-The. 2007. Fate of *Listeria* spp. on parsley leaves grown in laboratory and field cultures. *J. Appl. Microbiol.* 103:1821-1827.

52. Dreux, N., C. Albagnac, M. Federighi, F. Carlin, C. E. Morris, and C. Nguyen-the. 2007. Viable but non-culturable *Listeria monocytogenes* on parsley leaves and absence of recovery to a culturable state. *J. Appl. Microbiol.* 103:1272-1281.

53. Duffy, B. 2003. Tomato hybrid differences as hosts for human pathogenic *Escherichia coli* O157:H7 and *Salmonella* Thompson. *Tests Agrochem. Cultiv.* 24:16-17.

54. Duffy, B., S. Ravva, and L. Stanker. 2008. Cantaloupe cultivar differences as opportunistic hosts for human pathogenic *Escherichia coli* O157:H7 and *Salmonella. Eur. J. Horti. Sci.* 73:73-75.

55. Dunn, R. R., T. J. Davies, N. C. Harris, and M. C. Gavin. 2010. Global drivers of human pathogen richness and prevalence. *Proc. Biol. Sci.* 277:2587-2595.

56. Eblen, D. R., B. A. Annous, and G. M. Sapers. 2005. Studies to select appropriate nonpathogenic surrogate *Escherichia coli* strains for potential use in place of *Escherichia coli* O157:H7 and *Salmonella* in pilot plant studiest. *J. Food Prot.* 68:282-291.

57. Erickson, M. C., J. Liao, A. S. Payton, D. G. Riley, C. C. Webb, L. E. Davey, S. Kimbrel, L. Ma, G. Zhang, I. Flitcroft, M. P. Doyle, and L. R. Beuchat. 2010. Preharvest internalization of *Escherichia coli* O157:H7 into lettuce leaves, as affected by insect and physical damage. *J. Food Prot.* 73:1809-1816.

58. Erickson, M. C., C. C. Webb, J. C. Diaz-Perez, S. C. Phatak, J. J. Silvoy, L. Davey, A. S. Payton, J. Liao, L. Ma, and M. P. Doyle. 2010. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. *J. Food Prot.* 73:500-506.

59. Erickson, M. C., C. C. Webb, J. C. Diaz-Perez, S. C. Phatak, J. J. Silvoy, L. Davey, A. S. Payton, J. Liao, L. Ma, and M. P. Doyle. 2010. Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *J. Food Prot.* 73:1023-1029.

60. Escaff, G. M., C. A. Urbina, and G. J. Mery. 1979. Contamination of cabbages irrigated with polluted water. *Agric. Tec.* 39:59-62.

61. European Commission. 2007. Agricultural commodity markets past developments fruits and vegetables, An analysis of consumption, production and trade based on statistics from the Food and Agriculture Organization (FAO). Available at: http://ec.europa.eu/agriculture/analysis/tradepol/worldmarkets/fruitveg/072007_en.pdf. Accessed 5 January 2013.

62. Fasciolo, G., M. I. Meca, E. Calderon, and M. Rebollo. 2005. Microbial contamination of garlics and soils irrigated with treated domestic wastewater in Mendoza (Argentina). *Rev. Fac. Cienc. Agrar. Univ. Nac. Cuyo.* 37:31-40.

63. Fasciolo, G. E., M. I. Meca, E. Gabriel, and J. Morabito. 2002. Effects on crops of irrigation with treated municipal wastewaters. *Water Sci. Technol.* 45:133-138.

64. Ferens, W. A., and C. J. Hovde. 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog. Dis.* 8:465-487.

65. Fischer-Arndt, M., D. Neuhoff, L. Tamm, and U. Kopke. 2010. Effects of weed management practices on enteric pathogen transfer into lettuce (*Lactuca sativa* var. *capitata*). *Food Control*. 21:1004-1010.

66. Food and Agriculture Organization, and World Health Organization. 2008. Microbiological hazards in fresh fruits and vegetables: meeting report, microbiological risk assessment series. Available at: http://www.who.int/entity/foodsafety/publications/micro/MRA_FruitVeges.pdf. Accessed 15 September 2013.

67. Franz, E., A. V. Semenov, and A. H. van Bruggen. 2008. Modelling the contamination of lettuce with *Escherichia coli* O157:H7 from manure-amended soil and the effect of intervention strategies. *J. Appl. Microbiol.* 105:1569-1584.

68. Franz, E., and A. H. van Bruggen. 2008. Ecology of *E. coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. *Crit. Rev. Microbiol.* 34:143-161.

69. Franz, E., A. D. van Diepeningen, O. J. de Vos, and A. H. van Bruggen. 2005. Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar typhimurium in manure, manure-amended soil, and lettuce. *Appl. Environ. Microbiol.* 71:6165-6174.

70. Franz, E., A. A. Visser, A. D. Van Diepeningen, M. M. Klerks, A. J. Termorshuizen, and A. H. van Bruggen. 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. *Food Microbiol.* 24:106-112.

71. Gagliardi, J. V., and J. S. Karns. 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. *Appl. Environ. Microbiol.* 66:877-883.

72. Gagliardi, J. V., and J. S. Karns. 2002. Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. *Environ. Microbiol.* 4:89-96.

73. Garber, S. M., and D. A. Maguire. 2003. Modeling stem taper of three central Oregon species using nonlinear mixed effects models and autoregressive error structures. *Forest Ecol. Manag.* 179:507-522.

74. Ge, C., C. Lee, and J. Lee. 2012. The impact of extreme weather events on Salmonella internalization in lettuce and green onion. *Food Res. Int.* 45:1118-1122.

75. Gelting, R. J., M. A. Baloch, M. A. Zarate-Bermudez, and C. Selman. 2011. Irrigation water issues potentially related to the 2006 multistate *E. coli* O157:H7 outbreak associated with spinach. *Agr. Water Manage.* 98:1395-1402.

76. Gonzalez, C. J., T. M. Lopez-Diaz, M. L. Garcia-Lopez, M. Prieto, and A. Otero. 1999. Bacterial microflora of wild brown trout (*Salmo trutta*), wild pike (*Esox lucius*), and aquacultured rainbow trout (*Oncorhynchus mykiss*). *J. Food Prot.* 62:1270-1277.

77. Gorski, L., J. D. Palumbo, and R. E. Mandrell. 2003. Attachment of *Listeria monocytogenes* to radish tissue is dependent upon temperature and flagellar motility. *Appl. Environ. Microb.* 69:258-266.

78. Gould, L. H., K. A. Walsh, A. R. Vieira, K. Herman, I. T. Williams, A. J. Hall, and D. Cole. 2013. Surveillance for foodborne disease outbreaks - United States, 1998-2008. *MMWR Morb. Mortal. Wkly. Rep.* 62:1-34.

79. Greene, S. K., E. R. Daly, E. A. Talbot, L. J. Demma, S. Holzbauer, N. J. Patel, T. A. Hill, M. O. Walderhaug, R. M. Hoekstra, M. F. Lynch, and J. A. Painter. 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. *Epidemiol. Infect.* . 136:157-165.

80. Guan, T. T., G. Blank, and R. A. Holley. 2005. Survival of pathogenic bacteria in pesticide solutions and on treated tomato plants. *J. Food Prot.* 68:296-304.

81. Guan, T. Y., G. Blank, A. Ismond, and R. Van Acker. 2001. Fate of foodborne bacterial pathogens in pesticide products. *J. Sci. Food Agric.* 81:503-512.

82. Guo, X., J. Chen, R. E. Brackett, and L. R. Beuchat. 2001. Survival of *Salmonellae* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Appl. Environ. Microbiol.* 67:4760-4764.

83. Hatcher, L. 1994. A step-by-step approach to using the SAS system for factor analysis and structural equation modeling. Sas Institute.

84. Hilborn, E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, M. A. Lambert-Fair, J. A. Farrar, M. K.

Glynn, and L. Slutsker. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.* 159:1758-1764.

85. Hintz, L. D., R. R. Boyer, M. A. Ponder, R. C. Williams, and S. L. Rideout. 2010. Recovery of *Salmonella enterica* Newport introduced through irrigation water from tomato (*Lycopersicum esculentum*) fruit, roots, stems, and leaves. *HortScience*. 45:675-678.

86. Hoar, B., E. Atwill, L. Carlton, J. Celis, J. Carabez, and T. Nguyen. 2013. Buffers between grazing sheep and leafy crops augment food safety. *Calif. Agr.* 67:104-109.

87. Hoar, B. R. 2010. Food safety risks associated with sheep grazing in vegetable stubble fields. p. 37-38. Center for produce safety 2010 research symposium, Davis, CA, 23 June 2010. Center for produce safety, Davis, CA.

88. Hopewell, S., S. McDonald, M. Clarke, and M. Egger. 2007. Grey literature in meta-analyses of randomized trials of health care interventions. *Cochrane Database Syst. Rev.*:MR000010.

89. Hopkins, B. A., J. K. Skeeles, G. E. Houghten, D. Slagle, and K. Gardner. 1990. A survey of infectious diseases in wild turkeys (*Meleagridis gallopavo silvestris*) from Arkansas. *J. Wildl. Dis.* 26:468-472.

90. Hora, R., K. Warriner, B. J. Shelp, and M. W. Griffiths. 2005. Internalization of *Escherichia coli* O157:H7 following biological and mechanical disruption of growing spinach plants. *J. Food Prot.* 68:2506-2509.

91. Hutchison, M. L., S. M. Avery, and J. M. Monaghan. 2008. The air-borne distribution of zoonotic agents from livestock waste spreading and microbiological risk to fresh produce from contaminated irrigation sources. *J. Appl. Microbiol.* 105:848-857.

92. Ibekwe, A. M., C. M. Grieve, S. K. Papiernik, and C. H. Yang. 2009. Persistence of *Escherichia coli* O157:H7 on the rhizosphere and phyllosphere of lettuce. *Lett. Appl. Microbiol.* 49:784-790.

93. Ibekwe, A. M., C. M. Grieve, and C. H. Yang. 2007. Survival of *Escherichia coli* O157:H7 in soil and on lettuce after soil fumigation. *Can. J. Microbiol.* 53:623-635.

94. Ibekwe, A. M., S. K. Papiernik, C. M. Grieve, and C. H. Yang. 2011. Quantification of persistence of *Escherichia coli* O157:H7 in contrasting soils. *Int. J. Microbiol.* 2011.

95. Ibekwe, A. M., P. M. Watt, P. J. Shouse, and C. M. Grieve. 2004. Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. *Can. J. Microbiol.* 50:1007-1014.

96. Ilic, S., A. Rajic, C. J. Britton, E. Grasso, W. Wilkins, S. Totton, B. Wilhelm, L. Waddell, and J. T. LeJeune. 2011. A scoping study characterizing prevalence, risk factor and intervention research, published between 1990 and 2010, for microbial hazards in leafy green vegetables. *Food Control*. 23:7-19.

97. Ingham, S. C., M. A. Fanslau, R. A. Engel, J. R. Breuer, J. E. Breuer, T. H. Wright, J. K. Reith-Rozelle, and J. Zhu. 2005. Evaluation of fertilization-to-

planting and fertilization-to-harvest intervals for safe use of noncomposted bovine manure in Wisconsin vegetable production. *J. Food Prot.* 68:1134-1142.

98. Ingham, S. C., J. A. Losinski, M. P. Andrews, J. E. Breuer, J. R. Breuer, T. M. Wood, and T. H. Wright. 2004. *Escherichia coli* contamination of vegetables grown in soils fertilized with noncomposted bovine manure: garden-scale studies. *Appl. Environ. Microbiol.* 70:6420-6427.

99. Ingraham, J., and A. Marr. 1996. Effect of temperature, pressure, pH, and osmotic stress on growth. *Escherichia coli and Salmonella*. 2:1570-1578.

100. Ishii, S., W. B. Ksoll, R. E. Hicks, and M. J. Sadowsky. 2006. Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl. Environ. Microbiol.* 72:612-621.

101. Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J. Food Prot.* 67:1365-1370.

102. Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and J. Xiuping. 2005. Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. *Food Microbiol.* 22:63-70.

103. Islam, M., J. Morgan, M. P. Doyle, and X. Jiang. 2004. Fate of *Escherichia coli* O157:H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. *J. Food Prot.* 67:574-578.

104. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Appl. Environ. Microbiol.* 70:2497-2502.

105. Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang. 2004. Persistence of *Salmonella enterica* serovar typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog. Dis.* 1:27-35.

106. Iturriaga, M. H., E. F. Escartin, L. R. Beuchat, and R. Martinez-Peniche. 2003. Effect of inoculum size, relative humidity, storage temperature, and ripening stage on the attachment of *Salmonella* Montevideo to tomatoes and tomatillos. *J. Food Prot.* 66:1756-1761.

107. Iturriaga, M. H., M. L. Tamplin, and E. F. Escartin. 2007. Colonization of tomatoes by *Salmonella* montevideo is affected by relative humidity and storage temperature. *J. Food Prot.* 70:30-34.

108. Ivanek, R., Y. T. Grohn, L. W. Tauer, and M. Wiedmann. 2004. The cost and benefit of Listeria monocytogenes food safety measures. *Crit. Rev. Food Sci. Nutr.* 44:513-523.

109. 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.

110. Ivanek, R., Y. T. Grohn, and M. Wiedmann. 2006. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modeling. *Foodborne Pathog. Dis.* 3:319-336.

111. Izumi, H., J. Poubol, K. Hisa, and K. Sera. 2008. Potential sources of microbial contamination of satsuma mandarin fruit in Japan, from production through packing shed. *J. Food Prot.* 71:530-538.

112. Izumi, H., Y. Tsukada, J. Poubol, and K. Hisa. 2008. On-farm sources of microbial contamination of persimmon fruit in Japan. *J. Food Prot.* 71:52-59.

113. Jablasone, J., L. Y. Brovko, and M. W. Griffiths. 2004. A research note: the potential for transfer of *Salmonella* from irrigation water to tomatoes. *J. Sci. Food Agric.* 84:287-289.

114. Jaeger, J., D. Harapas, P. Franz, K. Wilkinson, and R. Premier. 2003. Safe use of poultry litter in vegetable production. p. 200-203. *In*, Australian postharvest horticulture conference, Brisbane, Australia, 1-3 October, 2003 Queensland Government, Department of Primary Industries, Brisbane.

115. Jamieson, R., R. Gordon, K. Sharples, G. Stratton, and A. Madani. 2002. Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Can. Biosyst. Eng.* 44:1-9.

116. Jiang, X., J. Morgan, and M. P. Doyle. 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl. Environ. Microbiol.* 68:2605-2609.

117. Jimenez, B., A. Austin, E. Cloete, and C. Phasha. 2006. Using Ecosan sludge for crop production. *Water Sci. Technol.* 54:169-177.

118. Jimenez, B., A. Austin, E. Cloete, C. Phasha, and N. Beltran. 2007. Biological risks to food crops fertilized with Ecosan sludge. *Water Sci. Technol.* 55:21-29.

119. Johannessen, G. S., G. B. Bengtsson, B. T. Heier, S. Bredholt, Y. Wasteson, and L. M. Rorvik. 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl. Environ. Microbiol.* 71:2221-2225.

120. Johannessen, G. S., R. B. Froseth, L. Solemdal, J. Jarp, Y. Wasteson, and M. R. L. 2004. Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. *J. Appl. Microbiol.* 96:787-794.

121. Kenney, S. J., G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat. 2005. Persistence of *Escherichia coli* O157:H7, *Salmonella* Newport, and *Salmonella* Poona in the gut of a free-living nematode, *Caenorhabditis elegans*, and transmission to progeny and uninfected nematodes. *Int. J. Food Microbiol.* 101:227-236.

122. Kenney, S. J., G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat. 2006. Migration of *Caenorhabditis elegans* to manure and manure compost and potential for transport of *Salmonella* newport to fruits and vegetables. *Int. J. Food Microbiol.* 106:61-68.

123. Kitinoja, L., and J. R. Gorny. 1999. Postharvest technology for small-scale produce marketers: Economic opportunities, quality and food safety. Available at: http://extension.psu.edu/food-safety/farm/resources/packing/p/at_download/file. Accessed 5 January 2013.

124. Klerks, M. M., E. Franz, M. van Gent-Pelzer, C. Zijlstra, and A. H. van Bruggen. 2007. Differential interaction of *Salmonella enterica* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency. *ISME J.* 1:620-631.

125. Kroupitski, Y., D. Golberg, E. Belausov, R. Pinto, D. Swartzberg, D. Granot, and S. Sela. 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Appl. Environ. Microbiol.* 75:6076-6086.

126. Kupriyanov, A. A., N. N. Kunenkova, A. H. C. v. Bruggen, and A. M. Semenov. 2009. Translocation of bacteria from animal excrements to soil and associated habitats. *Eurasian Soil Sci.* 42:1263-1269.

127. Larney, F. J., D. M. Sullivan, K. E. Buckley, and B. Eghball. 2006. The role of composting in recycling manure nutrients. *Can. J. Soil Sci.* 86:597-611.

128. Liao, C. H., C. W. Honeycutt, T. S. Griffin, and J. M. Jemison. 2003. Occurrence of gastrointestinal pathogens in soil of potato field treated with liquid dairy manure. *J. Food Agric. Environ.* 1:224-228.

129. Loncarevic, S., G. S. Johannessen, and L. M. Rorvik. 2005. Bacteriological quality of organically grown leaf lettuce in Norway. *Lett. Appl. Microbiol.* 41:186-189.

130. Machado, D. C., C. M. Maia, I. D. Carvalho, N. F. d. Silva, M. C. D. P. B. Andre, and A. B. Serafini. 2006. Microbiological quality of organic vegetables

produced in soil treated with different types of manure and mineral fertilizer. *Braz. J. Microbiol.* 37:538-544.

131. Madden, L., X. Yang, and L. Wilson. 1996. Effects of rain intensity on splash dispersal of Colletotrichum acutatum. *Phytopathology*. 86:864-874.

132. Mallmann, W. L., and W. Litsky. 1951. Survival of selected enteric organisms in various types of soil. *Am. J. Public Health Nations Health*. 41:38-44.

133. Manas, P., E. Castro, and J. de Las Heras. 2009. Irrigation with treated wastewater: effects on soil, lettuce (*Lactuca sativa* L.) crop and dynamics of microorganisms. *J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng.* 44:1261-1273.

134. Masabni, J. Spinach. Available at: http://aggie-horticulture.tamu.edu/vegetable/files/2011/10/spinach.pdf. Accessed 15 September 2013.

135. Materon, L. A. 2003. Survival of *Escherichia coli* O157:H7 applied to cantaloupes and the effectiveness of chlorinated water and lactic acid as disinfectants. *World J. Microbiol. Biotechnol.* . 19:867-873.

136. Meals, D. W., and D. C. Braun. 2006. Demonstration of methods to reduce *E. coli* runoff from dairy manure application sites. *J. Environ. Qual.* 35:1088-1100.

137. Melloul, A. A., L. Hassani, and L. Rafouk. 2001. *Salmonella* contamination of vegetables irrigated with untreated wastewater. *World J. Microbiol. Biotechnol.* 17:207-209.

138. Melotto, M., W. Underwood, J. Koczan, K. Nomura, and S. Y. He. 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell*. 126:969-980.

139. Merlin, T., A. Weston, R. Tooher, P. Middleton, R. Tooher, J. Salisbury, K. Coleman, S. Norris, K. Grimmer-Somers, and S. Hillier. 2009. NHMRC levels of evidence and grades for recommendations for developers of guidelines. Available at: http://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/evidence_statement_form.pdf. Accessed 26 Feburary 2012.

140. Michino, H., K. Araki, S. Minami, T. Nakayama, Y. Ejima, K. Hiroe, H. Tanaka, N. Fujita, S. Usami, and M. Yonekawa. 1998. Recent outbreaks of infections caused by *Escherichia coli* O157: H7 in Japan. p. 73-81. *In* J.B. Kaper, and A.D. O'Brein (ed.), *Escherichia coli* O157: H7 and other shiga toxin-producing *E. coli* strains, Washington, DC.

141. Miles, J. M., S. S. Sumner, R. R. Boyer, R. C. Williams, J. G. Latimer, and J. M. McKinney. 2009. Internalization of *Salmonella enterica* serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. *J. Food Prot.* 72:849-852.

142. Mitra, R., E. Cuesta-Alonso, A. Wayadande, J. Talley, S. Gilliland, and J. Fletcher. 2009. Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen *Escherichia coli* O157:H7 in spinach. *J. Food Prot.* 72:1521-1530.

143. Mitscherlich, E., and E. H. Marth. 1984. Microbial survival in the environment. Bacteria and rickettsiae important in human and animal health. Springer-Verlag, New York.

144. Moher, D., P. Fortin, A. R. Jadad, P. Juni, T. Klassen, J. Le Lorier, A. Liberati, K. Linde, and A. Penna. 1996. Completeness of reporting of trials published in languages other than English: implications for conduct and reporting of systematic reviews. *Lancet*. 347:363-366.

145. Mootian, G., W. H. Wu, and K. R. Matthews. 2009. Transfer of *Escherichia coli* O157:H7 from soil, water, and manure contaminated with low numbers of the pathogen to lettuce plants. *J. Food Prot.* 72:2308-2312.

146. Mukherjee, A., S. Cho, J. Scheftel, S. Jawahir, K. Smith, and F. Diez-Gonzalez. 2006. Soil survival of *Escherichia coli* O157:H7 acquired by a child from garden soil recently fertilized with cattle manure. *J. Appl. Microbiol.* 101:429-436.

147. Mukherjee, A., D. Speh, and F. Diez-Gonzalez. 2007. Association of farm management practices with risk of *Escherichia coli* contamination in pre-harvest produce grown in Minnesota and Wisconsin. *Int. J. Food Microbiol.* 120:296-302.

148. Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez. 2004. Preharvest evaluation of *coliforms, Escherichia coli, Salmonella,* and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* 67:894-900.

149. Mukherjee, A., D. Speh, A. T. Jones, K. M. Buesing, and F. Diez-Gonzalez. 2006. Longitudinal microbiological survey of fresh produce grown by farmers in the upper midwest. *J. Food Prot.* 69:1928-1936.

150. Natvig, E. E., S. C. Ingham, B. H. Ingham, L. R. Cooperband, and T. R. Roper. 2002. *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Appl. Environ. Microbiol.* 68:2737-2744.

151. Ogden, L. D., D. R. Fenlon, A. J. Vinten, and D. Lewis. 2001. The fate of *Escherichia coli* O157 in soil and its potential to contaminate drinking water. *Int. J. Food Microbiol.* 66:111-117.

152. Orozco, R. L., M. H. Iturriaga, M. L. Tamplin, P. M. Fratamico, J. E. Call, J. B. Luchansky, and E. F. Escartin. 2008. Animal and environmental impact on the presence and distribution of *Salmonella* and *Escherichia coli* in hydroponic tomato greenhouses. *J. Food Prot.* 71:676-683.

153. Palese, A. M., S. Masi, C. Xiloyannis, G. Figliuolo, V. Pasquale, and G. Celano. 2009. Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. *Agric. Ecosyst. Environ.* 129:43-51.

154. Park, S., S. Navratil, A. Gregory, A. Bauer, I. Srinath, M. Jun, B. Szonyi, K. Nightingale, J. Anciso, and R. Ivanek. 2013. Generic *Escherichia coli* contamination of spinach at the preharvest level: The role of farm management and environmental factors. *Appl. Environ. Microbiol.*

155. Park, S., B. Szonyi, R. Gautam, K. Nightingale, J. Anciso, and R. Ivanek. 2012. Risk factors for microbial contamination in fruits and vegetables at the preharvest level: a systematic review. *J. Food Prot.* 75:2055-2081.

156. Persaud, N., and M. M. Mamdani. 2006. External validity: the neglected dimension in evidence ranking. *J. Eval. Clin. Pract.* 12:450-453.

157. Prazak, A. M., E. A. Murano, I. Mercado, and G. R. Acuff. 2002. Prevalence of *Listeria monocytogenes* during production and postharvest processing of cabbage. *J. Food Prot.* 65:1728-1734.

158. Pu, S., J. C. Beaulieu, W. Prinyawiwatkul, and B. Ge. 2009. Effects of plant maturity and growth media bacterial inoculum level on the surface contamination and internalization of *Escherichia coli* O157:H7 in growing spinach leaves. *J. Food Prot.* 72:2313-2320.

159. Pueppke, S. G., M. C. Bolanos-Vasquez, D. Werner, M. P. Bec-Ferte, J. C. Prome, and H. B. Krishnan. 1998. Release of flavonoids by the soybean cultivars McCall and peking and their perception as signals by the nitrogen-fixing symbiont sinorhizobium fredii. *Plant Physiol.* 117:599-606.

160. Renter, D. G., and J. M. Sargeant. 2002. Enterohemorrhagic *Escherichia coli* O157: epidemiology and ecology in bovine production environments. *Anim. Health Res. Rev.* 3:83-94.

161. Rhoades, J. R., G. Duffy, and K. Koutsoumanis. 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and

Listeria monocytogenes in the beef production chain: a review. *Food Microbiol.* 26:357-376.

162. Robbins, A. R. 1974. 25 vegetables anyone can grow. Dover, New York.

163. Robin, X., N. Turck, A. Hainard, N. Tiberti, F. Lisacek, J. Sanchez, and M. Müller. 2013. Package 'pROC'. Available at: http://cran.r-project.org/web/packages/pROC/pROC.pdf. Accessed.

164. Rodriguez, D. M., F. E. Torres, E. V. Gutierrez, M. P. Lopez, M. M. Martinez, and A. K. Carrascal. 2008. *Salmonella* Typhimurium determination in compost artificially inoculated in a lettuce crop. *Acta Biol. Colomb.* 13:61-72.

165. Rogers, S., and J. Haines. 2005. Detecting and mitigating the environmental impact of fecal pathogens originating from confined animal feeding operations: review. Available at: http://storage.globalcitizen.net/data/topic/knowledge/uploads/2012012015737302.pdf. Accessed 15 September 2013.

166. Sargeant, J. M., M. R. Amezcua, A. Rajic, and L. Waddell. 2005. A guide to conducting systematic reviews in agri-food public health. Available at: http://www.fsrrn.net/UserFiles/File/conductingsysreviewsenglish%5B1%5D.pdf. Accessed 26 Feburary 2012.

167. Sargeant, J. M., A. Rajic, S. Read, and A. Ohlsson. 2006. The process of systematic review and its application in agri-food public-health. *Prev. Vet. Med.* 75:141-151.

168. Scallan, E., P. M. Griffin, F. J. Angulo, R. V. Tauxe, and R. M. Hoekstra. 2011. Foodborne illness acquired in the United States--unspecified agents. *Emerg. Infect. Dis.* 17:16-22.

169. 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.

170. Scharff, R. L. 2012. Economic burden from health losses due to foodborne illness in the United States. *J. Food Prot.* 75:123-131.

171. Scheelings, T. F., D. Lightfoot, and P. Holz. 2011. Prevalence of *Salmonella* in Australian reptiles. *J. Wildl. Dis.* 47:1-11.

172. Segurado, P., M. B. AraÚJo, and W. E. Kunin. 2006. Consequences of spatial autocorrelation for niche-based models. *J. Appl. Ecol.* 43:433-444.

173. Semenov, A. V., A. H. van Bruggen, L. van Overbeek, A. J. Termorshuizen, and A. M. Semenov. 2007. Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. *FEMS Microbiol. Ecol.* 60:419-428.

174. Sharma, M., D. T. Ingram, J. R. Patel, P. D. Millner, X. Wang, A. E. Hull, and M. S. Donnenberg. 2009. A novel approach to investigate the uptake and internalization of *Escherichia coli* O157:H7 in spinach cultivated in soil and hydroponic medium. *J. Food Prot.* 72:1513-1520.

175. Shi, X., A. Namvar, M. Kostrzynska, R. Hora, and K. Warriner. 2007. Persistence and growth of different *Salmonella* serovars on pre- and postharvest tomatoes. *J. Food Prot.* 70:2725-2731.

176. Shi, X., Z. Wu, A. Namvar, M. Kostrzynska, K. Dunfield, and K. Warriner. 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with *Salmonella* typhimurium or *Salmonella* montevideo. *J. Appl. Microbiol.* 107:329-338.

177. Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67:2342-2353.

178. Solomon, E. B., H. J. Pang, and K. R. Matthews. 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *J. Food Prot.* 66:2198-2202.

179. Solomon, E. B., C. J. Potenski, and K. R. Matthews. 2002. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. *J. Food Prot.* 65:673-676.

180. Solomon, E. B., S. Yaron, and K. R. Matthews. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Appl. Environ. Microbiol.* 68:397-400.

181. Steele, M., and J. Odumeru. 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. *J. Food Prot.* 67:2839-2849.

182. Stine, S. W., I. Song, C. Y. Choi, and C. P. Gerba. 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *J. Food Prot.* 68:913-918.

183. 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.

184. 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. *In Press. Appl. Environ. Microbiol.*

185. Takeuchi, K., C. M. Matute, A. N. Hassan, and J. F. Frank. 2000. Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* typhimurium, and *Pseudomonas fluorescens* to lettuce leaves. *J. Food Prot.* 63:1433-1437.

186. Talley, J. L., A. C. Wayadande, L. P. Wasala, A. C. Gerry, J. Fletcher, U. DeSilva, and S. E. Gilliland. 2009. Association of *Escherichia coli* O157:H7 with filth flies (*Muscidae* and *Calliphoridae*) captured in leafy greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera: Muscidae). *J. Food Prot.* 72:1547-1552.

187. Tortorello, M. L. 2003. Indicator organisms for safety and quality--uses and methods for detection: minireview. *J. AOAC Int.* 86:1208-1217.

188. U.S. Department of Agriculture. 2000. National organic program; Final rule. 7 CFR Part 205. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5087165. Accessed 5 January 2013.

189. U.S. Department of Agriculture. 2008. Fruit and tree nuts situation and outlook yearbook 2008. Available at: http://usda.mannlib.cornell.edu/usda/current/FTS-yearbook/FTS-yearbook-10-30-2008.pdf. Accessed.

190. U.S. Department of Agriculture. 2011. Vegetables and melons yearbook. Available at: http://usda01.library.cornell.edu/usda/ers/89011/89011.pdf. Accessed 26 Feburary 2012.

191. U.S. Department of Agriculture. 2012. Vegetables 2011 Summary. Available at: http://usda01.library.cornell.edu/usda/current/VegeSumm/VegeSumm-01-26-2012.pdf. Accessed 5 January 2013.

192. U.S. Food and Drug Administration. 1998. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. Available at: http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf. Accessed 5 January 2013.

193. U.S. Food and Drug Administration. 2008. Guide to minimize microbial food safety hazards for fresh fruits and vegetables; Request for comments and for scientific data and information [Docket No. FDA–2008–N–0455], Federal Register, 73, no 170: 51306-51309. Available at:

http://www.gpo.gov/fdsys/pkg/FR-2008-09-02/pdf/E8-20187.pdf. Accessed 15 September 2013.

194. U.S. Food and Drug Administration. 2009. Guidance for industry: Evidence-based review system for the scientific evaluation of health claims - final. Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/ucm073332.htm. Accessed 26 Feburary 2012.

195. U.S. National Oceanic and Atmospheric Administration. 2011. Climatological data. Annual summary. Colorado. 2010. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-A3DFB510-90D9-448B-80DE-C3F0419777F4.pdf. Accessed 23 April 2013.

196. U.S. National Oceanic and Atmospheric Administration. 2011. Climatological data. Annual summary. Texas. 2010. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-060FA273-ED2B-4F1F-94F4-9B28478A4D75.pdf. Accessed 23 April 2013.

197. U.S. National Oceanic and Atmospheric Administration. 2012. Climatological data. Annual summary. Colorado. 2011. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-2F9C4C7E-2025-4EA1-82F1-FA4C88DC52BE.pdf. Accessed 23 April 2013.

198. U.S. National Oceanic and Atmospheric Administration. 2012. Climatological data. Annual summary. Texas. 2011. Available at:

http://www1.ncdc.noaa.gov/pub/orders/IPS-B5DF9D91-B12A-44FA-B746-1835E87E6D21.pdf. Accessed 23 April 2013.

199. U.S. National Oceanic and Atmospheric Administration. 2013. Climatological data. Annual summary. Texas. February 2012. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-DFDA7087-CB33-41A4-AA70-C84A01ADC14C.pdf. Accessed 23 April 2013.

200. U.S. National Oceanic and Atmospheric Administration. 2013. Climatological data. Annual summary. Texas. January 2012. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-F072DAAF-076A-46D6-9087-CCDAC68069D2.pdf. Accessed 23 April 2013.

201. U.S. National Oceanic and Atmospheric Administration. 2013. Climatological data. Annual summary. Texas. March 2012. Available at: http://www1.ncdc.noaa.gov/pub/orders/IPS-C6ED4CF8-5B40-498A-83F5-BA7C0B3031F6.pdf. Accessed 23 April 2013.

202. U.S. Natural Resources Conservation Service. Soil Data Mart. Available at: http://soildatamart.nrcs.usda.gov. Accessed 23 April 2013.

203. Ukuku, D. O., and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J. Food Prot.* 65:1093-1099.

204. Van Renterghem, 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.

205. Vengust, G., Z. Valencak, and A. Bidovec. 2006. A serological survey of selected pathogens in wild boar in Slovenia. *J. Vet. Med. B. Infect. Dis. Vet. Public. Health.* 53:24-27.

206. Wallace, J. S., T. Cheasty, and K. Jones. 1997. Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds. *J. Appl. Microbiol.* 82:399-404.

207. Wiessner, S., B. Thiel, J. Kraemer, and U. Koepke. 2009. Hygienic quality of head lettuce: effects of organic and mineral fertilizers. *Food Control*. 20:881-886.

208. Wong, J. W., and A. Selvam. 2009. Reduction of indicator and pathogenic microorganisms in pig manure through fly ash and lime addition during alkaline stabilization. *J. Hazard Mater.* 169:882-889.

209. Yang, C. H., D. E. Crowley, J. Borneman, and N. T. Keen. 2001. Microbial phyllosphere populations are more complex than previously realized. *Proc. Natl. Acad. Sci. U. S. A.* 98:3889-3894.

210. Zhang, G., L. Ma, L. R. Beuchat, M. C. Erickson, V. H. Phelan, and M. P. Doyle. 2009. Lack of internalization of *Escherichia coli* O157:H7 in lettuce (*Lactuca sativa L.*) after leaf surface and soil inoculation. *J. Food Prot.* 72:2028-2037.

APPENDIX A

CHECKLIST FOR APPRAISING THE REVIEWED STUDIES*

A-1 Checklist for appraising the controlled trial

Questions	Answers
Objectives	
1. Do the objectives address the systematic review question?	yes/no
Treatment (Inoculation, treatment allocation)	
2. Prior to the inoculation, were the sampling units tested for the outcome pathogen?	yes/no
3. Was an appropriate control group used?	yes/no
4. Were sampling units randomly allocated to the treatment and control groups?	yes/no
5. Were sampling units randomly allocated to the experiment locations?	yes/no
6. Were the treatment protocols adequately described?	yes/no
Outcome assessment	
7. Were laboratory tests to determine the outcome described and adequate?	yes/no
Withdrawals and loss to follow-up	
8. Were withdrawals and/or losses to follow-up reported?	yes/partial/no
9. Was the proportion of lost to follow-up adequate?	yes/no
Data analysis	
10. Was the statistical analysis used?	yes/no
11. Was the statistical analysis appropriate?	yes/no
12. Were the estimates and measures of variability used to address the research question presented adequately?	yes/no
13. Were confounders appropriately considered?	yes/no
Conclusions	
14. Were conclusions supported by the results?	yes/no

* Modified version of the checklists for quality appraisal designed by Sargeant et al (2005).

A-2 Checklist for appraising the observational study

Questions	Answers
Objectives	
1. Do the objectives address the systematic review question?	yes/no
Treatment (treatment, treatment allocation)	
2. Prior to planting, were the sampling units tested for the outcome pathogen?	yes/no
3. Was an appropriate control group used?	yes/no
4. Were sampling units randomly selected?	yes/no
5. Were the treatment protocols adequately described?	yes/no
Outcome assessment	
6. Were laboratory tests to determine the outcome described and adequate?	yes/no
Withdrawals and loss to follow-up	
7. Were withdrawals and/or losses to follow-up reported?	yes/partial/no
8. Was the proportion of lost to follow-up adequate?	yes/no
Data analysis	
9. Was the statistical analysis used?	yes/no
10. Was the statistical analysis appropriate?	yes/no
11. Were the estimates and measures of variability used to address the research question presented adequately?	yes/no
12. Were confounders appropriately considered?	yes/no
Conclusions	
13. Were conclusions supported by the results?	yes/no

APPENDIX B

QUESTIONNAIRE

Farm ID _____

Visit # _____

Date _____

Questionnaire Completed by _____

A. GENERAL farm and management information

A.1. **Size of farm:**_____ Acres

A.2. **Has the farm ever been organic?**

① YES →
② NO

From (month/year) _____ To (month/year) _____
What National Organic Program certification agent did you use
_____

A.3. **What crops were grown on this farm in the previous 3 years** (if needed use Interviewer guide in the end of this questionnaire as a reminder)**?**

_____

A.4. **What is your crop rotation cycle?** _____

A.5. **Was the field ever used for grazing?**

① YES →
② NO

Field 1	**Field 2**	**Field 3**	**Field 4**
From (month/year) _____	From (month/year) _____	From (month/year) _____	From (month/year) _____
To (month/year) _____	To (month/year) _____	To (month/year) _____	To (month/year) _____

A.6. Do you own your own farm equipment for all operations?

① YES
② NO →

① Borrow
② Lease/rent
③ Other _____

A.7. Do you clean farm equipment?

① YES
② NO

A.8. Do you have a staff year-round?

① YES, approximately _____
② NO

A.9. Do you have temporary workers?

① YES, approximately _____
② NO

A.10. Are portable toilets provided to staff/workers in the field?

① YES →
② NO

How far are the toilets from the work area where the workers are located? List approximate distance _____

A.11. Do you train staff/workers to use portable toilets?

① YES
② NO

A.12. Are there portable hand washing stations provided to staff/workers in the field?

① YES
② NO

A.13. Does the farm irrigate?

① YES
② NO
③ Weather dependent

i. What type of irrigation is used (if applicable)**?**

Field 1	**Field 2**	**Field 3**	**Field 4**
Drip	Drip	Drip	Drip
Overhead	Overhead	Overhead	Overhead
Spray	Spray	Spray	Spray
Flood	Flood	Flood	Flood
Other _____	Other _____	Other _____	Other _____

ii. What is the source of water for irrigation (circle all that apply)**?**

Field 1	**Field 2**	**Field 3**	**Field 4**
Pond	Pond	Pond	Pond
Well	Well	Well	Well
Municipal	Municipal	Municipal	Municipal
River/stream/creek	River/stream/creek	River/stream/creek	River/stream/creek
Man Made Reservoirs	Man Made Reservoirs	Man Made Reservoirs	Man Made Reservoirs
Other _____	Other _____	Other _____	Other _____

A.14. Do you use any wildlife control (circle all that apply)**?**

① YES →
② NO

Field 1	**Field 2**	**Field 3**	**Field 4**
Fences	Fences	Fences	Fences
Scarecrows	Scarecrows	Scarecrows	Scarecrows
Traps	Traps	Traps	Traps
Poison	Poison	Poison	Poison
Hunting	Hunting	Hunting	Hunting
Bombs, woodchuck	Bombs, woodchuck	Bombs, woodchuck	Bombs, woodchuck
Other _____	Other _____	Other ____	Other ____

A.15. The farm is located on what kind of terrain?

Field 1	Field 2	Field 3	Field 4
Flat	Flat	Flat	Flat
Valley	Valley	Valley	Valley
Sloped	Sloped	Sloped	Sloped
Steep	Steep	Steep	Steep
Hill	Hill	Hill	Hill
Don't know	Don't know	Don't know	Don't know

A.16. Does the farm have a buffer zone from neighbors, road ways, etc?

① YES

② NO

i. If yes, what type of buffer zone?

Field 1	Field 2	Field 3	Field 4
Fence	Fence	Fence	Fence
Tree Line	Tree Line	Tree Line	Tree Line
Ditch	Ditch	Ditch	Ditch
Shrubbery	Shrubbery	Shrubbery	Shrubbery
Other _____	Other _____	Other _____	Other _____
Don't know	Don't know	Don't know	Don't know

A.17. Is the field in the general proximity (within 10 mile radius) of any of the following? If so, approximately how close is the nearest:

	Field 1	Field 2	Field 3	Field 4
Dairy Farm				
Beef Farm				
Water Sources (running or standing)				
Landfill				
Residential				
Poultry Farm				
Swine Farm				
Forest				
Roadways				
Other				
Don't know				

B. CHANGEABLE management actions and routine surveillance

B.1. In what condition were the fields before planting of the spinach crop this season?

Field 1	**Field 2**	**Field 3**	**Field 4**
Fallow	Fallow	Fallow	Fallow
Rotavated	Rotavated	Rotavated	Rotavated
Tilled	Tilled	Tilled	Tilled
Cover crop (type)	Cover crop (type)	Cover crop (type)	Cover crop (type)
Hay	Hay	Hay	Hay
Other _____	Other _____	Other _____	Other _____

B.2. When was the current spinach crop planted?

	Field 1	**Field 2**	**Field 3**	**Field 4**
Date				

B.3. During the period since our last visit to the farm or during the previous 2 months, whichever is shorter,

i. has the soil been tilled, rotavated or aerated?

Field 1	**Field 2**	**Field 3**	**Field 4**
Yes, _ days ago	Yes, _days ago	Yes, _days ago	Yes, _days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

ii. has the farm irrigated its fields?

Field 1	**Field 2**	**Field 3**	**Field 4**
Yes, _ days ago	Yes, _days ago	Yes, _days ago	Yes, _days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

iii. has manure been applied to farm fields?

Field 1	**Field 2**	**Field 3**	**Field 4**
Yes, _ days ago	Yes, _days ago	Yes, _days ago	Yes, _days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

a. If yes, what was the source (circle all that apply)?

Field 1	**Field 2**	**Field 3**	**Field 4**
Dairy farm	Dairy farm	Dairy farm	Dairy farm
Feedlot	Feedlot	Feedlot	Feedlot
Swine farm	Swine farm	Swine farm	Swine farm
Poultry farm	Poultry farm	Poultry farm	Poultry farm
Other	Other	Other	Other
Don't know	Don't know	Don't know	Don't know

b. If yes, how long was the applied manure aged before spreading?

Field 1	**Field 2**	**Field 3**	**Field 4**
___weeks	___weeks ago	___weeks	___weeks
Don't know	Don't know	Don't know	Don't know

iv. has compost been applied to farm fields?

Field 1	Field 2	Field 3	Field 4
Yes, _days ago	Yes, _days ago	Yes, _days ago	Yes, _days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

v. has chemical/synthetic fertilizer been applied to farm fields?

Field 1	Field 2	Field 3	Field 4
Yes, ____type ____days ago	Yes, ____type ____days ago	Yes, ____type ____days ago	Yes, ____type ____days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

a. If yes, how was the fertilizer applied to the fields?

Field 1	Field 2	Field 3	Field 4
Fertigation	Fertigation	Fertigation	Fertigation
Foliar spray	Foliar spray	Foliar spray	Foliar spray
Ground application	Ground application	Ground application	Ground application
Other _____	Other _____	Other _____	Other _____

vi. have pesticides been applied to farm fields?

Field 1	Field 2	Field 3	Field 4
Yes, _days ago	Yes, _days ago	Yes, _days ago	Yes, _days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

a. If yes, what was the type of pesticide applied?

Field 1	Field 2	Field 3	Field 4
Herbicide	Herbicide	Herbicide	Herbicide
Fungicide	Fungicide	Fungicide	Fungicide
Insecticide	Insecticide	Insecticide	Insecticide
Other _____	Other _____	Other _____	Other _____

b. If yes, how was the pesticide applied to fields?

Field 1	Field 2	Field 3	Field 4
Low volume spray	Low volume spray	Low volume spray	Low volume spray
High volume spray	High volume spray	High volume spray	High volume spray
Fog	Fog	Fog	Fog
Foliar	Foliar	Foliar	Foliar
Soil	Soil	Soil	Soil
Other _____	Other _____	Other _____	Other _____

vii. have domestic animals been observed in the farm fields?

Field 1	Field 2	Field 3	Field 4
Yes, ____species ___days ago	Yes, ____species ___days ago	Yes, ____species ___days ago	Yes, ____species ___days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

viii. has wildlife been observed in the farm fields?

Field 1	Field 2	Field 3	Field 4
Yes, ____species ___days ago	Yes, ____species ___days ago	Yes, ____species ___days ago	Yes, ____species ___days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

ix. have staff members/temporary workers been in the farm field?

Field 1	Field 2	Field 3	Field 4
Yes, staff ___days ago	Yes, staff ___days ago	Yes, staff ___days ago	Yes, staff ___days ago
Yes, workers ___days ago	Yes, workers ___days ago	Yes, workers ___days ago	Yes, workers ___days ago
Not during this period	Not during this period	Not during this period	Not during this period
No, never	No, never	No, never	No, never
Don't know	Don't know	Don't know	Don't know

x. has training on food safety been provided to staff members/temporary workers?

① YES
② NO

xi. has any routine microbial testing been done on your farm?

① YES
② NO

a. Who requires it to be completed?

① Owner
② Fresh cut processing buyers
③ Retail buyers
④ Co-Op buyers
⑤ Other _____

b. What organisms were tested for and when; the results of the testing if known?

	Field 1		**Field 2**		**Field 3**		**Field 4**	
	date	result	date	result	date	result	date	result
Coliforms								
E. coli generic								
E. coli O157								
E. coli STEC								
Listeria monocytogenes								
Salmonella								
Other _____								

c. What was tested on the farm?

Area 1	**Area 2**	**Area 3**	**Area 4**
Produce	Produce	Produce	Produce
Irrigation Water	Irrigation Water	Irrigation Water	Irrigation Water
Spray Water	Spray Water	Spray Water	Spray Water
Soil	Soil	Soil	Soil
Equipment	Equipment	Equipment	Equipment

Interviewer guide

1. Crop Kind

□ Alfalfa
□ Apples
□ Arugula
□ Baby Leaf Lettuce
□ Barley
□ Beans
□ Beats
□ Berries
□ Broccoli
□ Buttered Lettuce
□ Cabbage
□ Chard
□ Celery
□ Cherries
□ Corn (sweet)
□ Cucumbers
□ Endives
□ Escarole
□ Garlic
□ Green Leaf Lettuce
□ Green Onions
□ Herbs
□ Iceberg Lettuce
□ Kale
□ Mango
□ Melons
□ Nuts
□ Oats
□ Onions
□ Oranges
□ Papaya
□ Peaches
□ Peas
□ Peppers
□ Pineapples
□ Potatoes
□ Pumpkins
□ Radish
□ Red Leaf Lettuce
□ Romaine Lettuce
□ Spinach
□ Spring Mix
□ Sprouted Seeds
□ Squash
□ Tomatoes
□ Turnip
□ Wheat
□ Other
(Please list below)