**Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Jacquelyn Sunshine Lickness Date

Assessing microbial contamination on produce, environmental sources, and farm worker hands throughout the production process on farms and packing sheds in northern Mexico

By

Jacquelyn Sunshine Lickness
MPH

Global Health

_____ [Chair's signature]
Juan Leon, PhD, MPH
Committee Chair

_____ [Member's signature]
Faith Bartz, PhD
Committee Member

# Assessing microbial contamination on produce, environmental sources, and farm worker hands throughout the production process on farms and packing sheds in northern Mexico

By

Jacquelyn Sunshine Lickness

B.A., University of California, Los Angeles, 2010

Thesis Committee Chair: Juan Leon, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2014

# Abstract

Assessing microbial contamination on produce, environmental sources, and farm worker hands throughout the production process on farms and packing sheds in northern Mexico

By Jacquelyn Sunshine Lickness

Produce-related foodborne illnesses are a significant public health burden. It is critical to identify routes of fecally-associated contamination in produce in the agricultural production environment to design appropriate interventions aimed at preventing the introduction of microbial contamination on farms. The study goals were to quantify microbial contamination in soil, water, hand rinse, and produce rinse samples for four microbial indicators (*E. coli, Enterococcus spp.*, fecal coliforms, and somatic coliphages) and to assess the relationship between microbial contamination in produce rinses and soil, water, and hand rinse samples. Produce rinse samples (N=279) were collected from farms and packing sheds and matched to soil (N=81), water (N=164) and hand rinse samples (N=196) during the 2011 and 2012 growing seasons. Samples were processed by enumerative methods for *E. coli*, *Enterococcus spp.*, fecal coliforms, and somatic coliphages. We used bivariate analysis, multivariate linear models, and logistic models to evaluate the relationship between produce rinse samples and environmental samples for all four microbial indicators. Our findings showed low levels of contamination in soil and water samples and a lack of significant association between soil and water contamination and produce contamination. We also found a high proportion of positive samples in hand rinses and a significant association between concentration of microbial indicators in hand rinse samples and concentration in produce rinse samples ($\beta$=0.17-0.57, 95% CI=0.03-0.69). Consistent with prior studies, farms in this study employed techniques that carry a lower risk of microbial contamination including the irrigation of produce with well water from irrigation drip-tape hoses and the use of synthetic fertilizer covered by plastic mulch. Mechanistically, the relationship between hand and produce contamination may be explained by effective microbial adherence and transfer as well as repeated contact between hands and produce. These results highlight the need for interventions surrounding farmworker hygiene and sanitation to interrupt microbial adherence and persistence on farmworkers hands.

# Assessing microbial contamination on produce, environmental sources, and farm worker hands throughout the production process on farms and packing sheds in northern Mexico

By

Jacquelyn Sunshine Lickness

B.A., University of California, Los Angeles, 2010

Thesis Committee Chair: Juan Leon, PhD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2014

**Acknowledgements**:

I am incredibly fortunate to have had Juan Leon as my mentor over the past two years. He offered invaluable guidance and encouragement that allowed me to develop both professionally and personally. Faith Bartz was instrumental during my two years at Clean Greens. Her willingness to offer knowledgeable support and direction cannot be overstated. I deeply appreciate the support of many others, especially Domonique Watson, Anna Aceituno, and the entire Clean Greens team.

Many thanks to my family—Barbie, Tim, Becky, Billy, Rigo, and Delilah—for their unconditional love. They are a constant source of inspiration and my success is due in large part to their support.

## Table of Contents

# Chapter 1: Comprehensive Review of the Literature

## Burden of Produce-Related Foodborne Illnesses

The burden of foodborne illnesses to society is substantial and produce contamination plays an important role. With as many as 48 million new cases of foodborne illnesses annually in the U.S. resulting in 128,000 hospitalizations and 3,000 deaths, the economic burden and the public health implications are extensive. The total health-related cost per year due to infections by foodborne pathogens amounts to a $14 million loss to the U.S. economy, approximately $1.4 million resulting from contaminated produce [1]. While foodborne illnesses have traditionally been linked to animal products, produce is increasingly being recognized for contributions to the overall number of foodborne illness (reviewed in [2]). The proportion of foodborne outbreaks associated with produce has been on the rise (reviewed in [3, 4]). In the 1970s it was estimated that in outbreaks with a known food vehicle, fresh produce was attributed to <1% compared to 6% in the 1990s [5]. It is currently estimated that every year there are 9 million cases foodborne illnesses attributable to a known pathogen, 46% of which are attributable to produce [6]. Produce caused the second highest number of foodborne disease outbreaks and accounted for the highest proportion of disease cases [7]. An upward trend in the number of outbreaks due to contaminated produce poses a serious public health threat.

Foodborne illnesses associated with fresh produce have gained increasing recognition in recent decades due to a confluence of factors including an increase in the consumption of produce, changes to the produce production process, and greater surveillance and detection methods in the

public health field (reviewed in [8]). One reason consumption of produce has risen is because fresh produce is more available for purchase. From 1970 to 2008, there was a 30% rise in per capita availability of fruit and 20% rise of vegetables [9]. In addition, consumers are increasingly health conscious and encouraged to increase the proportion of produce in their meals [10]. As a result, many Americans eat larger quantities of fresh, minimally processed produce compared to heat-treated or other variations of preparation [11]. During 1997 to 1999 the average American consumed 741 pounds of fresh produce a year, a 25% rise over consumption during 1977-1979 [10]. Evidence of greater produce consumption, produce import shares rose from 16.8% in 1990 to 25.6% in 2009 out of total U.S. food consumption [9]. The per capita consumption of produce is one trend that plays a role in an increase of produce-related foodborne illnesses [12]. Changes in food production, global food trade, and monitoring of reportable diseases have also contributed to the rise in foodborne illnesses in the U.S. (reviewed in [8]). The result is that there has been an overall upward trend in the number and proportion of produce-attributable outbreaks in the U.S. (reviewed in [5, 13]).

The number and severity of produce-associated foodborne outbreaks demonstrates the widespread distribution and effects of produce contamination. Many well-known and highly publicized outbreaks have implicated produce as the single food vehicle. In 2006, for example, spinach contaminated with *E. coli* sickened more than 200 people in a multi-state outbreak [14]. Jalapeño peppers, serano peppers, and tomatoes were implicated in a Salmonella Saintpaul outbreak in 2008 causing 1,442 illnesses and resulting in the Food and Drug Administration (FDA) issuing advice to avoid tomatoes and jalapeños of Mexican origin [15]. Cantaloupes from a farm in Colorado were implicated in a 2011 multi-state listeriosis outbreak that caused 146

illnesses and 30 deaths [16]. In 2011, approximately 5,000 cartons of cantaloupes were recalled as a result of contamination by Salmonella Panama that caused a multistate outbreak [15]. In response to produce contamination and subsequent outbreaks, the U.S. Food and Drug Administration recommends farms to follow Good Agricultural Practices (GAPs) in an effort to minimize microbial food safety hazards in fresh produce. To demonstrate the need to follow these guidelines, especially as outbreaks continue to pose a serious public health threat, it is important to understand foodborne pathogens and their behavior in the environment in which produce is grown and harvested.

## Common Foodborne Pathogens Associated With Produce

There are many pathogens that cause foodborne illness, however a relatively small number are responsible for the majority of produce-related illnesses. There are 1,400 potential food-contaminating species of pathogens, but 31 are well known and commonly recognized, reviewed in [17, 18]. It is estimated that in the U.S. eight known pathogens constitute 95% of foodborne illnesses, hospitalizations, and deaths [18, 19]. Among all foodborne pathogens, norovirus and hepatitis A are epidemiologically important due to the number and severity of cases (reviewed in [20]). However, Salmonella, *E. coli*, and clostridium are the most common produce-related pathogens with Salmonella alone accounting for 48% of produce-related cases [5, 21]. Salmonella in tomatoes and salmonella in melons are both major food-pathogen combinations commonly implicated in outbreaks (reviewed in [21]). Norovirus and Salmonella are considered diseases of high burden and produce is an important vehicle in the transmission of these diseases [18].

## Fecal Indicators as a Proxy for Foodborne Pathogen Contamination

Pathogens are harmful to humans, however they are relatively rare, sporadically distributed, and challenging to detect in the laboratory. For example, in a study seeking to detect Salmonella spp. in raw produce, Sant'Ana *et al.* [22] found that there were only four samples that tested positive for Salmonella out of 512 samples total. Similar to Salmonella, *E. coli* 0157:H7 is often absent or undetectable in most tested meat, produce, and juice samples [23]. Since pathogens are rare in the environment, to accurately and sensitively detect pathogens, many samples and numerous assays are required, posing financial and technical barriers (reviewed in [24]). It is difficult, time consuming, and ineffective to test environmental samples for the presence of pathogens and thus an imperfect method in studies seeking to effectively characterize fecal contamination.

An alternative to monitoring for the presence of pathogens is assaying for presence of fecal indicator organisms. Fecal indicator organisms, known as indicators, are non-pathogenic organisms that occur naturally in human and animal feces and are ecologically similar to food pathogens (reviewed in [25, 26]). Indicators of fecal contamination therefore signal an increased likelihood of that a pathogen may originate from the same source (reviewed [25, 27]). Both enteric pathogens and ecologically similar indicators are found in feces. When testing food for these indicators, indicator organisms are indicative of whether that item has been exposed to conditions of riskier environmental conditions that may be fit for pathogens to thrive (reviewed [25]). Moreover, indicators are easier to monitor than pathogens because they exist in higher numbers, are easily detected, quickly measureable, and require less expensive laboratory methods [25].

There are several organisms that are commonly used to indicate fecal contamination and monitor food quality, including fecal coliforms, *E. coli*, coliphages, and *Enterococcus spp*. The *Enterobacteriaceae* family consists of facultatively anaerobic gram-negative bacilli that ferment glucose [27, 28]. Both pathogenic and non-pathogenic forms of the organism comprise a family such as *Enterobacteriaceae*. The *Enterobacteriaceae* family contains many familiar pathogens such as *E. coli* , Shigella, and Salmonella in addition to many harmless bacteria (reviewed in [17]). Both fecal coliforms and *E. coli* are microbes commonly found in the digestive tracts of animals and humans [27, 28] and indicate the presence of feces [28]. Coliphages are bacterial viruses, known as bacteriophage, that infect coliforms. They resemble enteroviruses of both humans and animals by survivability and patterns of persistence [29, 30]. Coliphages have been proposed as indicators for the possible presence of *E. coli*. *Enterococcus spp*., a member of the enterococci gram-positive bacteria genus, is known to colonize the gastrointestinal tract of warm-blooded mammals and is thus recognized as an indicator of fecal contamination (reviewed in [31]). Enterococcus and coliphage are associated with the increased likelihood of enteric pathogens (reviewed in [31]). All of these indicators suggests the possibility of fecal contamination and are therefore helpful in understanding contamination levels in the farm environment.

Fecal coliforms, *E. coli*, somatic coliphages, and *Enterococcus spp*. persist in the environment and are often characterized as hardy. Coliforms have the potential to survive and reproduce in the environment without a human host. Additionally, they are resistant to freezing temperatures, but may not survive in hot conditions (reviewed in [27]). Enterococcus is hardier than *E. coli* , tolerant to heat and resistant to freezing (reviewed in [27]). Coliphages provide a conservative

estimate of viral load since they are more environmentally resistant as compared to *E. coli* [30]. These characteristics of these microbial indicators make them ideal for continued and sustained propagation in the environment.

There are several limitations of using indicators to test for the presence of pathogens. Many indicator organisms do not require an animal host's digestive tract to live and may persist instead in the natural environment (reviewed in [27]). Increased levels of indicators are sometimes associated with an increased probability of detecting a pathogen and the presence of an indicator may indicate the presence of a pathogen. However, the absence of indicator organisms does not eliminate the possibility of contamination by enteric pathogens (reviewed in [27]). Furthermore, levels of fecal indicators do not correlate precisely with levels of fecal contamination in food products, water samples, and soil (reviewed in [27, 32]). The absence of a correlation between indicators and pathogens makes indicators an imperfect tool for assessing the prevalence of pathogens [33]. Despite these limitations, the presence of indicators is the best alternative to understanding the risk of fecal contamination.

## Sources of Produce Contamination on Farms

It is critical to understand the source of contamination in order to employ safer methods in the produce production process and reduce the number of produce-related illnesses. Fecal contamination of produce occurs when animal or human fecal matter adheres to fruits and vegetables. It is typical of fecal matter to contain viruses, bacteria, and parasites, some of which are pathogenic. There are many points in the farm environment that produce may come into contact and be contaminated by fecal material containing potentially harmful bacteria, viruses,

and microbes (reviewed in [8]). These pathogens and others can be introduced to produce through routes including contaminated soil, water, equipment, human handling, and insect and animal excrement.

There are multiple opportunities throughout the farm production process in which fecal matter may come into contact with produce. A range of interactions of fruits and vegetables with the environment during growth, harvest, transport, food preparation and consumption have the potential to introduce microbes. In this farm-to-fork continuum, the farm is the first source of contamination and is a logical place to ensure that food safety measures are enforced in order to reduce the spread of foodborne illnesses. Federal legislation has been proposed that establishes standards to prevent and mitigate the spread of pathogens through rules aimed at farmworker hand hygiene, agricultural water, equipment, wild animals, and soil amendments [34]. Therefore, it is important to understand the potential sources of contamination on the farm.

Worker's hygienic practices affect levels of contamination on produce. Workers make contact with produce throughout the production process from growing and harvesting to packing and processing (reviewed in [35]). According to a survey of over 3,000 American farms, 94% of all fruit acres and 87% of all vegetable acres were harvested by hand [20] . Incidentally, humans serve as the primary reservoir for several diseases such as norovirus, hepatitis A, and Shigella, (reviewed in [8]). In addition, enteric pathogens from other animals can be transferred using humans as the vehicle of microbes from the environment to produce. The spread of these pathogens may occur as a result of ineffective or non-existent hand hygiene (reviewed in [8]). Hand washing is recognized as an effective Good Agricultural Practice (GAP) to reduce the

spread of enteric pathogens that may contaminate produce at the farm-level [36]. In addition, available and easily accessible hand washing stations help to promote hand washing [37]. Availability of hygiene facilities on farms, labor policies that encourage hygienic practices, and farmworker education on good hygiene practices are effective ways in which hygiene compliance reduce the risk of produce contamination by farmworkers.

The presence of wild and domestic animals as well as insects in the farm environment directly expose produce to pathogenic organisms (reviewed in [35]). An outbreak of Salmonella due to contaminated sprouts caused 500 illnesses and an investigation revealed the presence of flies, rodent droppings, and livestock in close proximity to the harvesting field [31]. Cattle are a primary reservoir for *E. coli* 0157:H7 and the pathogen is known to persist for up to 70 days in bovine feces [26, 35, 38]. Wild birds and flies may also carry *E. coli* 0157:H7 [2, 35]. Such wildlife is difficult to control since the construction of fences would not provide a barrier to entrance, posing a threat to the safety of produce (reviewed in [35, 39]). Insects may also be a source of contamination, Iwasa *et al.* [40] demonstrated in the laboratory the direct transfer of bacteria from contaminated flies to plants and fruits. In both laboratory-controlled experiments and field investigations, there is evidence that animal and insect fecal matter may contaminate produce.

Water comes into contact with produce during both the pre- and post- agricultural production process. During pre-harvest, water is an important source of contamination of produce because it can serve as a reservoir for microbes that can then be transferred to produce through methods including irrigation [41, 42]. Generally, source water is derived from surface water or

groundwater (reviewed in [20]). It is possible that animal or human feces may directly or indirectly enter surface water through rainwater or runoff, which may then contaminate produce if the surface water is directly applied to crops [38]. Irrigation water is delivered from the source to produce using drip or ground water systems. Irrigation water that employs sewage water is an important environmental source for Salmonella and other harmful pathogens on produce [43, 44]. This is of particular concern if water is contaminated and does not undergo treatment to remove or inactivate contaminants because its use during pre-harvest production practices may aid in spreading enteric pathogens to produce [45, 46]. Microbes delivered via water, even in trace amounts, then have the potential to multiply in soil or cross contaminate the surface of produce (reviewed in [47]). The type, treatment, and application of water to produce either mitigate or reduce the risk of produce contamination.

The application of manure, organic fertilizer, and water to soil provides opportunity for contamination by fecal matter [46]. Produce becomes contaminated by direct defecation of farmworkers or by its exposure to untreated or inadequately treated sewage effluents. Subsequent growth and survival of pathogens in soil fluctuates according to soil composition and environmental conditions such as temperature and moisture (reviewed in [38]). While some pathogens such as *Clostridium botulinum, Listeria monocytogenes,* and *Bacillus cereus* occur naturally in soil, other pathogens may enter soil through purposeful activities like the application of manure fertilizers (reviewed in [9, 35]). Soil contaminated with *Salmonella sp.* thrived for months after using manure-based fertilizer (reviewed in [9]). Similarly, Islam et. al [43] found that *E. coli* O157:H7 persisted in soil and on lettuce for several months after contaminated manure was applied to plant seedlings. Salmonella, *E. coli* O157:H7, *Campylobacter jejuni,*

*Vibrio cholerae*, parasites, and viruses have also been found to infect other mediums such as feces, manure, and water that deposit in soil (reviewed in [35]). Precautionary measures to mitigate the application of material contaminated with fecal matter should be emphasized in pre-harvest production practices.

During the post-harvest phase, produce moves from the field to the packing shed where it may be exposed to water during washing, flumes, dunk tanks, conveying, or cooling. In addition to potential exposure to contaminated water at the pre harvest phase, Akins *et al.* [48] found that water used in the postharvest stage may also be a source of contamination. Plants may be particularly susceptible at this stage due to exterior damage, interaction of the plant surface with production facility material, unnatural flora, lack of nutrients, and increased contact with contaminated crops (reviewed in [17]). Pathogens have the potential to survive in post-harvest operations, especially where there is minimal processing [49]. In addition, changes to the processing and distribution of agricultural products that allow for a greater supply and range of products to be consumed by the public, such as triple-washing of pre-packaged leafy greens, may heighten the risk of more widespread outbreaks. The introduction and growth of pathogens on produce during the post-harvest production stage is yet another aspect of contamination in the farm environment.

Another potential mechanism for contamination includes the use of fecally-contaminated tools and equipment during pre- and post-harvest activities. Harvesting equipment, transport containers and vehicles, and other processing equipment may directly transfer microbes from their surface to produce (reviewed in [35]). During harvest, farm machinery that is driven from

one field to another may indirectly contaminate produce if movement aerosolizes contaminated dirt. After harvest, produce travels to packinghouses where equipment and surfaces make repeated physical contact with produce and pose a risk of cross contaminating produce surfaces. The transport and treatment of produce exposes it to a range of human and mechanical activities that have been found in some cases to increase levels of contamination and in other instances, to decrease the amount of microbes on produce. For example, concentrations of generic *E. coli* were found higher at the final stages of preparation compared to field samples [50]. Johnston *et al.* [51] found that contamination depends on the type of produce being processed since belts and other surfaces in packing houses were found to be relatively clean. Sterilization of farming equipment and packing shed surfaces is a means to address the transfer of pathogens and fecal matter between produce and other surfaces.

## Detection Methods

Testing the microbial quality of produce is necessary in order to enhance food safety. There are several methods used to identify pathogens and indicators including culture-based, biochemical, genetic, and serological testing with each method serving a different purpose [52, 53]. Presumptive identification, known as screening, is used for indicator organisms due to its sensitivity, reliability, cost, and speed. In addition, other criterion including validity, reliability, feasibility and effectiveness are important to recognize when choosing an appropriate method of testing [53]. Culture-based methods are considered the gold standard for food microbiological testing because of the degree of validity [52]. Culture-based methods primarily include three important steps, cultural enrichment followed by selective-differential plating and confirmation. The first step in preparing samples is the recovery of microbes from produce followed by plating

or enrichment in the laboratory [54]. In the process of preparing samples, the effective recovery of microbes is important for an accurate and measured understanding of the extent of contamination. Cultural enrichment is necessary to bring the microorganism of interest to detectable limits. After the cultural enrichment process, selective and differential plating, confirmation, and sometimes subtypying occur. The selection of a method for testing microbes is critical to gaining an accurate estimate of the levels of contamination. While there are other emerging technologies, the culture-based method is most highly regarded and most commonly used in public health food safety testing.

## Goal

Soil, irrigation water, source water, and farmworkers hands are potential routes of contamination of produce during the production process. Little is known about the relative contributions of each of these environmental factors on produce microbial levels. There is a need to understand whether there is an association between microbial indicator concentrations in the environment and on produce in order to reduce fecal contamination on farms and lower the burden of foodborne disease. The goal of this research is to quantify the relationship between fecal indicator levels in the environment and on produce. To achieve this goal, we aim to 1) quantify the prevalence and concentration of *E. coli*, *Enterococcus spp*., fecal coliforms, and somatic coliphages on environmental samples (hands, soil, irrigation water, and source water) and produce; 2) determine whether there is an association between environmental contamination and produce contamination by generating linear and logistic models and conducting correlation analysis; and 3) based on the identified associations, determine the routes of fecal contamination that present the greatest risk to produce safety on farms and packing sheds.

## Significance

Quantifying the relationship between microbial indicator contamination in environmental sources and microbial indicator contamination in produce will help identify sources of contamination in the produce production process. Beta estimate from linear models and odds ratios from logistic models will allow us to determine the magnitude and significance of these relationships. It is important to understand routes of contamination in the farm environment so that steps can be taken to mitigate the introduction of fecally-associated microorganisms. We will be able to explore possible mechanisms of transfer of microbial indicator by assessing the relationships between soil, water, and hand contamination and produce contamination. This is critical because it will provide new direction for future studies, potentially impact farm policy and practices, and give us greater insight about farm-level contamination. Food illnesses are a serious public health burden so data ascertained through this epidemiological analysis will enhance the body of knowledge needed to address this issue.

# Chapter 2: Manuscript

## Introduction

Produce-related foodborne illnesses and outbreaks have increased over the past few decades, accounting for 0.7% of all outbreaks in the 1970s to 12% in the 1990s [3, 13, 35]. The upward trend in produce-related foodborne illness and outbreaks are due to several factors including a rise in the consumption of produce, increased surveillance and detection, and changes in the production process and distribution, reviewed in [8, 35]. Although numerous types of produce may serve as the vehicle of transmission of foodborne pathogens, certain types of produce appear more frequently in foodborne outbreaks such as leafy greens, jalapeños, tomatoes, and melons. Melons have been associated with infections of Salmonella, Listeria, Norovirus, and *Escherichia coli* (*E. coli)* [55]; tomatoes and jalapeños have been found as the vehicle of transmission of Salmonella [56]; leafy greens were found to be the source of *E. coli* [8] and Norovirus [12] outbreaks.

There are many potential sources of fecal contamination throughout the farm production process. At the pre- and post- harvest phases, possible sources of contamination of produce include source water and irrigation water [2, 38], soil [9, 35, 38], animal droppings [2, 26, 38], farm workers hands [8, 57], and tools, equipment and other contact surfaces [50, 51]. Under laboratory conditions, it has been demonstrated that produce can acquire pathogens from water [58], surfaces [59], hands [60], and soil [61]. Similarly, epidemiological studies conducted in outbreak investigations have implicated contaminated water [62], surfaces [63], hands[64] , and soil [65]. However, these types of studies have limited public health relevance because studies conducted in the laboratory are artificial and outbreak investigations rely heavily on speculation.

Ultimately, there are no comprehensive studies directly linking produce contamination to water, surfaces, hands or soil in natural settings, which is necessary to identify routes of contamination on farms.

Due to the sporadic distribution and low concentrations of pathogens in the farm environment, laboratory detection methods are difficult, time consuming, and often ineffective [22-24]. Instead, industry and regulators use microbial indicator organisms, known as indicators, as an alternative method to monitor the presence of pathogens because they are ecologically similar to pathogens, yet easier to detect and measure. There are several organisms that are commonly used to indicate fecal contamination and monitor food quality, including *E. coli, Enterococcus spp*., fecal coliforms, and somatic coliphages. These organisms fall under two bacterial groups, coliforms and fecal streptococci, and one virus, somatic coliphage, which infect coliform bacterium. The fecal coliform group includes known pathogens such as Salmonella, Shigella, Yersinia, and *E. coli* and the fecal streptococci group includes enterococci (reviewed in [53]). *E. coli*, *Enterococcus spp*., fecal coliforms, and somatic coliphages live in the guts of warm-blooded organisms and signal a risk of fecal contamination in the environment. Although they are imperfect, many researchers [26, 28, 66] use microbial indictors to characterize fecal contamination in environmental samples.

Despite the growing trend in produce-associated foodborne illnesses, there are virtually no comprehensive studies that examine the mechanisms of fecal contamination in the produce production process. Thus, the goal of our study is to determine the relationship between contamination in soil, water, and hand samples and contamination in produce samples across

microbial indicator (*E. coli*, *Enterococcus spp*., fecal coliforms, and somatic coliphages). During the 2010 and 2011 growing season, we collected matched environmental samples and produce (jalapeño, tomato and melon) rinse samples at different steps in the production process (harvest, distribution, and the packing shed). Samples were processed by enumerative methods and double data entry and reconciliation were used to address discrepancies. The results indicate that the presence of microbial indicators in hand samples was significantly associated with corresponding indicators in produce samples. In contrast, soil and water samples showed no relationship with produce samples across microbial indicators. Greater knowledge about the magnitude and significance of associations between fecally-associated environmental and produce contamination at the farm-level will help to inform targeted interventions and reduce the burden of foodborne illnesses due to minimally-processed produce.

## Methods

### Study area

The study area comprised the Mexican states of Nuevo León and Coahuila on the United States-Mexico border. This region is a major agricultural area that regularly exports to the United States and has high production volumes of some crops that are considered at elevated risk for contamination with enteric pathogens: cantaloupes, tomatoes, and jalapeño peppers [56]. Eleven farms and seven packing sheds participated in this study: five farms produced cantaloupes, five produced jalapeño peppers, and five produced tomatoes (four of which were also included as jalapeño farms). Institutional review board approval was received by the lead institution (Emory University) covering the duration of the study (approval number IRB00035460).

### Sample collection

Samples were collected from May 2011 to December 2012. During each sampling event, 8 to 10 samples consisting of fresh produce rinses obtained before harvest (pre-harvest), during harvest (harvest), and during packing (packing) or just prior to distribution (distribution), of hand rinses from the pickers/packers, and of water from the irrigation source and/or field irrigation lines were collected. For each produce rinse, environmental samples of soil, source water, irrigation water, and hand rinse samples were collected to match that produce sample spatially and temporally. This process was used to match each produce rinse sample to corresponding soil, water, and hand rinse sample. Samples were composited to avoid skewing the data for samples that exhibited extreme microbial counts. Composite sample were prepared using an aseptic

technique in the laboratory in which triplicate samples that were collected from 3 random locations within each field were composited in a sterile 9 oz. whirl-pack bag and thoroughly mixed. They were then placed in the refrigerator to be processed within 24 hours for microbial analysis. This procedure to prepare composite samples was applied uniformly to all produce rinses, hands rinses, soil, and water collected at each site. However, each type of sample had a different sample collection protocol, described below. All samples were placed on ice after collection, driven to the laboratory at the Universidad Autónoma de Nuevo León (UANL), and stored at 4°C until processing for microbial indicator analyses. Samples were processed within 48 h of harvest for peppers and tomatoes and within 72 to 96 h for melons.

### Produce rinses

At harvest, produce rinse samples were collected immediately after a farmworker handled the produce item. Multiple produce items were combined to create a single produce rinse sample. At each step, triplicate produce samples were collected at random locations in the field and composited. Specifically, composite samples represented rinses of 54 tomatoes, 42 jalapeños, or 6 cantaloupes in 1500 milliliters of 0.15% sterile peptone water. The variation in the number of rinses per produce type reflects the number of items needed to standardize the surface area being sampled across produce types. For preparation of the rinses, half of each batch of produce was placed in a Whirl-Pak bag containing 500 ml 0.15% sterile peptone water (PW), shaken for 30 seconds, massaged for 30 seconds, and shaken again for 30 seconds. The first half of the produce batch was removed and replaced with the second half, and the process was repeated. This process was done three times with three different produce batches, and the rinses were combined to create a composite sample of 1,500 ml. The composite sample was divided into smaller

subsamples for microbiological testing. Sample collection was done for produce collected at several points in the production process (pre-harvest, harvest, distribution, and packing).

## Water

Water samples were collected from the well that was used for irrigation water and from the irrigation lines on the field. Well water samples were collected by first disinfecting the pump with 200 ppm hypochlorite. The pump was allowed to run for 30 s before three 1.5-liter water samples were collected in Whirl-Pak bags (Nasco, Ft. Atkinson, WI). Irrigation water samples were collected as close as possible to the harvest row where the drip tape deposited irrigation water or from the center of the distribution system when this was not possible and were collected in the same manner as well water. At the time of collection of produce rinses, water from the closest drip tape hose connection and associated wells were sampled to ensure matched water and hand rinse samples. Three well or in-field irrigation water samples were combined to create a composite sample of ~4.5 liters, which was then re-divided into smaller subsamples for specific microbiological testing of indicators.

## Soil

At the time of produce rinse sample collection, soil samples were taken from the ground area surrounding the stem of that produce item. At each visit, triplicate 100-gram soil samples were collected. At each of the three sampling locations, seven 15 grams soil samples were collected to produce a minimum of 100 grams of soil. The samples were aseptically collected using a scoop within 30 cm of the stems of the sampled plants. The soil sample was then deposited into a

sterile 7 oz. whirl-pack bag and placed on ice for delivery to the testing laboratory.

### Hand rinses

Before sample collection, researchers obtained written consent from farm managers and oral consent from farm workers. Before the farmworker harvested the produce, hand rinse samples were collected. Hand rinse samples were matched to produce rinse samples that were collected after farmworkers harvested the produce item. To collect a hand rinse sample, the worker placed his or her hand in a Whirl-Pak bag containing 750ml PW. The worker was asked to shake the hand for 30 s, and then the hand was massaged for an additional 30 s. The first hand was removed from the rinse solution, the second hand was placed in the same bag, and the process was repeated. Three individual hand rinse samples (representing the hands of three pickers or packers, 750 ml each) were combined to create a composite sample of 2,250 ml that was divided into smaller subsamples for specific microbiological testing.

### Microbial indicator testing

Samples were analyzed at UANL using a membrane filtration method. Sample volumes, ranging from 10 µl to 50 ml for produce and from 10 to 250 ml for water were vacuum filtered through a 47 mm, 0.45 µm pore size S-Pack filter (Millipore, Billerica, MA). Following filtration, the filters were removed and placed in selective plates for microbial quantification. *Enterococcus spp.* were enumerated on KF Streptococcus agar (Oxoid Limited, Basingstoke, Hampshire, UK). Plates were inverted and incubated at 37°C for 48 hours. *E. coli* and fecal coliforms were enumerated on RAPID'E. coli 2 agar (Bio-Rad Laboratories, Inc., Hercules, CA). The plates

were inverted and incubated at 44°C for 24 hours. The color of the colonies was used to distinguish between the presence of *E. coli* and fecal coliforms.

Somatic coliphage was analyzed on a FastPhage MPN Quanti-tray (Charm Sciences, Inc., Lawrence, MA). Samples were combined with fluorescence-based media inoculated with *E. coli* and then divided into Most Probable Number (MPN) partitions. For this technique, compartments with at least one plaque forming unit (PFU) fluoresce when exposed to UV light. This allows for quantification of the number of fluorescing compartments, which is used to determine MPN using a conversion table. According to the concentration of particles in the sample, 100 ml of sample or 10 ml of sample diluted with 90 ml of 0.15% peptone water. Trays were incubated at 37°C for 6 hours.

The number of colony forming units (CFU) per filtered volume was used to quantify mean indicator concentrations (*E. coli* , *Enterococcus*, fecal coliforms) in each sample. The average concentration (number of CFU per volume filtered) of *E. coli* in each sample was determined and standardized to CFU per 100 ml. For statistical analyses, samples below the assay lower limit of detection were reported as 0.5 CFU per greatest volume filtered (1/2 lower limit of detection), and samples above the upper limit of quantification were reported as 500 CFU (2x upper limit of quantification) per smallest volume tested [67]. The most probable number (MPN) was used to quantify somatic coliphage. Indicator concentrations on produce were measured in CFU or MPN per fruit and in CFU or MPN per milliliter. Measuring concentrations per milliliter (equivalent to per 736 $cm^2$) served to correct for differences in fruit surface area. Indicator concentrations in irrigation water were measured in CFU or MPN per 100 milliliters. Based on the observed CFU per plate, samples were assigned types below quantifiable range, within the quantifiable range, or above the quantifiable range. The quantifiable range of CFU or

MPN per plate was 25 to 250 CFU and 1 to 2420 MPN, although in some instances samples below or above this range were counted.

## Statistical Analyses

A total of 715 samples were analyzed using SAS 9.3 (SAS Institute Inc., Cary, N.C.). First, descriptive statistics were used to assess the distribution of the data and to make comparisons of prevalence and concentration across microbial indicators and sample types. Next, linear models and logistic models were used to determine the relationship between microbial contamination in produce and the same microbial indicators in hands, soil, and water. For all models, significance was determined at the 0.05 level. The predictor variables in these models were microbial presence and concentration on hands, water, and soil samples and the response variables were microbial presence and concentration in produce.

We performed multivariate logistic regression modeling to assess significant predictors of the presence of each of the four microbial indicators on produce. The output of the logistic model is an odds ratio that estimates the effect of microbial contamination of a single environmental sample (e.g., hands, soil, or water) adjusted for other variables in the model (e.g., type of produce, point in the chain, or year of collection). The concentrations of microbial indicators were dichotomized at the level of detection to generate logistic models. Models were developed to include environmental sample of interest, produce type, year of collection, and point in the chain. In models with complete separation or quasi-complete separation of data, Firth corrections were used to yield a penalized likelihood ratio estimate [68]. Final models were selected using

Alkaline Information Criterion (AIC), a measure of the relative quality of a model, and likelihood ratio values to compare the full model, most reduced model, and the model achieved through stepwise logistic regression [68].

Correlation analysis was used in combination with linear regression to determine the association between produce rinse concentration and hand rinses, soil, and water concentration for each of the four microbial indicators. Spearman correlation, a non-parametric method, was used to evaluate the direct relationship between produce contamination and soil, water, and hand rinse contamination for each microbial indicator and crop type. General linear regression models were used to quantify the effect of hand and environmental contamination on produce contamination for four microbial indicators. Somatic coliphage, *Enterococcus spp.*, *E. coli*, and fecal coliforms were quantified in hand, soil, and irrigation water, and compared to the levels of the same indicators on produce (e.g., jalapeños, melons, and tomatoes). Mulitvariate linear models for each of the four microbial indicators were used to determine significant predictors in the levels of contamination of produce, adjusting for other variables (e.g., produce type, point in chain, and year of collection). The general linear models included all observations including estimated concentrations from those samples with indicators above or below the limit of quantification. Full models and models selected through the stepwise method were developed for each of the microbial indicators and final models were chosen according to adjusted $R^2$ values and AIC [69]. Final models had lower AIC scores and higher $R^2$ values relative to other versions of model.

## Results

We sought to quantify contamination of several microbial indicators (*E. coli*, *Enterococcus spp.*, fecal coliforms, and somatic coliphages) in environmental samples and produce. To assess the prevalence of contamination across sample types, the percent positive samples (samples with concentrations of microbial indicators above the limit of detection) were calculated for each of the four microbial indicators in produce, hand rinse, soil, irrigation water, and source water samples (Table 1). The prevalence of each indicator organism was compared to the same indicator organism across hand rinse, produce rinse, soil, irrigation water, and source water samples. For each indicator organism, the prevalence of contamination varied greatly in soil, water, hand rinse, and produce rinse samples as seen in Table 1, with the exception of fecal coliforms, which exhibited a high prevalence (>80%) for all sample types. Although fecal coliform contamination was high in all sample types, the indicator organism was found in a higher percent of produce rinses (97%), hand rinses (96%), and soil samples (96%). Similarly, hand rinses (39%) as well as source water (53%) had a higher proportion of samples with detectable levels of *E. coli* compared to other sample types. Somatic coliphage in produce (84%) and hand rinses (66%) had a higher prevalence of positive samples compared to the same microbial indicator in soil, source water, and irrigation water. *Enterococcus spp.* in soil (67%) was the least contaminated, which contrasted considerably with *Enterococcus spp.* in hand rinses (100%), the most contaminated. In general, results showed microbial indicators were more prevalent in hands than in irrigation water, source water, and soil samples.

To identify potential relationships between hand, soil, and irrigation water contamination and contamination of the same indicator organism on produce, logistic regression models were

constructed for each indicator organism. Odds ratios (ORs) and 95% confidence intervals were calculated and are presented in Figure 1, adjusted for produce type, specific step in the production process, and year of sample collection. Models for hand contamination with *Enterococcus spp.* were unable to run due to an insufficient distribution of observations, while models for the other microbial indicators in irrigation water, source water, and soil were able to be run fully but required Firth correction with penalized likelihood estimates to provide appropriate estimates. Among hand rinses, ORs for *E. coli* and somatic coliphage were significant with a positive relationship with produce rinses. For *E. coli*, produce was eight times more likely to be contaminated if hands were contaminated and for coliphage, produce was six times more likely to be contaminated if hands were contaminated. No meaningful relationships were seen among irrigation water, source water, or soil samples. In conclusion, the relative odds of the occurrence of produce contamination was higher given the presence of microbial contamination on hands for *E. coli* and coliphage.

Spearman's rank correlation was also used to measure the statistical dependence of the concentration of each microbial indicator in produce rinses to the same microbial indicator in hand, soil, and water samples (Table 2). Whereas soil and source water samples for some microbial indicators had a significant negative association with produce rinses, overall, hand rinses had a significant positive association with produce rinses as seen in Table 2. For example, we found significant negative associations of concentrations *E. coli* concentrations between produce rinses and "Soil (All)" (Rho=-0.22 p=0.05). Similarly, we found a significant negative association of concentrations of *E. coli* (Rho=-0.21, p=0.05) and *Enterococcus spp.* (Rho=-0.34, p<0.01) between produce rinses and "Source water (All)". Interestingly, the significant

associations found in source water and soil samples and produce rinses were not significant when stratified by produce type. In contrast, we found that generally for jalapeño, tomato, and melon, *E. coli*, *Enterococcus spp*., fecal coliforms, and somatic coliphages hand rinse concentration values were significantly positively correlated to the concentration of the same microbial indicators in produce rinses (Rho= 0.41-0.77, $p<0.01$). These results indicate that only a few bivariate relationships between environmental sample contamination and produce contamination were found to be significant. In conclusion, across all four indicators, contamination in hand rinses showed a significant positive correlation with produce rinse contamination, whereas *E. coli* in soil and *E. coli* and *Enterococcus spp.* in source water samples showed a significant negative correlation.

By generating multivariate linear regression models that adjusted for potential confounders such as crop type, year of data collection, and step in the production process, we sought to improve our understanding of the true relationship between concentrations of microbial indicators on hands, soil, source water, and irrigation water and concentrations of microbial indicators on produce. To undertake these analyses, *E. coli*, *Enterococcus spp*., fecal coliforms, and somatic coliphages were quantified in hand, soil, source water, and irrigation water and were compared to the concentration of the same indicators on matched produce samples (jalapeños, melons, and tomatoes, Figure 2). Multivariate regression models were adjusted for produce type, specific steps in the production process, and year of sample collection. In Figure 2, effect estimates (β) and 95% confidence intervals are presented for each of the indicator organisms within a specific parameter of interest. After employing stepwise regression, the final model included significant predictor variables and all other potential confounders. We found that all four microbial

indicators in hand contamination had a significant, positive relationship with the same microbial indicators in produce contamination. An increase of $\log_{10}$ 1 cfu/hand in *E. coli,* fecal coliforms, *Enterococcus spp.,* and somatic coliphage in hands led to an increase of 0.38 $\log_{10}$ cfu/fruit increase for *E. coli*, 0.55 $\log_{10}$ cfu/fruit for fecal coliforms, 0.57 $\log_{10}$ cfu/fruit for *Enterococcus spp.*, and 0.17 $\log_{10}$ cfu/fruit for somatic coliphage in produce rinses. In contrast to results obtained from correlation analysis that showed a significant negative relationship between *E. coli* in soil and hands, linear models that adjusted for other variables, showed a significant positive relationship between *E. coli* contamination in soil and produce ($\beta$=0.40, $p<0.05$). In summary, we found the risk of contamination of produce is greater if hands are contaminated with *E. coli*, fecal coliforms, and *Enterococcus spp.*, and greater if soil is contaminated with *E. coli*.

## Discussion

The goals for the study were to characterize levels of microbial indicators on environmental samples and produce and determine the relationship between produce rinse samples and environmental samples from farms and packing sheds on the U.S.-Mexico border. Our results showed that the prevalence of fecal contamination was high in hands and detectable, but relatively low in soil and water. We also found that were was a significant association between hand rinses and produce rinses and there was, in general, no association between soil and water samples and produce rinses. There are a variety of mechanisms related to microbe adherence and persistence on environmental surfaces as well as farming practices and conditions that may explain these findings.

We found that hand rinses had a high proportion of positive samples for all four microbial indicators possibly explained by the distinctive flora, texture, surface, grooves and moisture on hands that favor adherence of microbial indicators. As evidence of this hypothesis, transient flora in hands contains viruses, bacteria, and fungi acquired from external surfaces. Although transient flora are found infrequently on hands, they have the ability to survive, multiply and cause disease (reviewed in [70]). Variations in the amount of microbes depends on many factors including skin texture, dryness, moisture, and thickness, and pH [60]. Further evidence of their adherence, microbial indicators survive in various environments, including hands, because they are hardy organisms that are often resistant to harsh and inhospitable elements (reviewed in [27, 71]). Therefore, microbes may thrive on the surfaces of hands, which may explain the high prevalence *E. coli*, *Enterococcus spp.*, fecal coliforms, and somatic coliphage found in hand rinse samples.

Another mechanism that may explain the high proportion of positive hand rinse samples is the lack of good hygiene practices on the farm during harvest that allowed for sustained adherence of microorganisms on hands. Microorganisms adhere to hands after exposure to fecal matter and remain on hands if there is no mechanism, such as hand washing, to remove them. Information on hand washing practices, toilet use, and other farm conditions was gathered in a farm manager and farm worker qualitative questionnaire (data not shown). According to data obtained from the questionnaire, three quarters of farmers reported the presence of hand washing facilities outside of the bathrooms, but reported having to walk an average of 1,135 meters to use bathroom facilities. In terms of larger implications, a nationwide survey of farmworkers showed that 20% are paid by the number of pieces of produce they collected, which, in those conditions, may deter farmworkers from using distant bathroom and hand washing facilities [72]. Furthermore, respondents observed that of those who used the bathroom, many did not use the hand washing facilities, which may arise from inadequate or absent hygiene training. The reason that hand washing is important is because hand washing with soap and water helps removes fecal matter and other contaminants that hands are exposed to after defecation and toilet use [73, 74]. The FDA recommends providing accessible sanitary facilities including toilets and hand washing facilities, located not more than 0.25 mile from all employees, for all field workers during planting, harvesting, and other field activities [36]. Inaccessible facilities and poor hygiene behavior may have contributed to the high proportion of hands that were positive for microbial indicators and suggest that methods for improving hygiene and sanitation conditions on farms would be useful to reduce levels of contamination in the produce production process.

Low proportions of positive samples were detected in soil samples across microbial indicators. Fecal contamination of soil may have occurred because measures to prevent human and animal

defecation near crops were ineffective. Wildlife is inherently difficult to control and the construction of fences may not provide a barrier to entrance (reviewed in [35, 39]). Wildlife intrusion on farms is a source of fecal contamination (reviewed in [35]), which may help explain detectable levels of fecal matter contamination found in soil. Soil contamination may have also occurred as a result of open human defecation. An additional component of our study, examining the human and animal source of fecal matter contamination in produce, identified approximately half of samples positive for fecal contamination as positive for human-specific markers, which suggests the presence of human feces on the farms (data not shown). Human and animal defecation may have contributed to detectable levels of fecal contamination, however, levels of microbial indicators in soil and water remained below standards set by the EPA. The reason that levels of fecal contamination were low on our farms may be due to the use of synthetic fertilizers the use of which was ascertained from interviews with farm and shed managers (data not shown). Synthetic fertilizers are free of organic and manure-based soil amendments and therefore carry a low risk of fecal contamination (reviewed in [61, 66]). Our results, consistent with other studies, underscore the low-risk nature of synthetic fertilizers and also highlight the need to modify pre-harvest farm practices and conditions to reduce instances of open human and animal defecation in the crop harvesting area.

Microbial indicators were found in source water and irrigation water, providing evidence that contamination may have occurred at the well and during transport of water en route to the crops. Although ground water carries a lower risk of fecal contamination than surface water, Abbaszadegan *et al.* [75] verified that groundwater may carry low levels of pathogens and microbes. Pathogens and microbes may infiltrate groundwater via aquifers, damaged well

infrastructure or as a result of inadequate cleaning of the well [76]. Although detectable levels of microbial indicators were found in source water, levels of contamination were found to be below EPA standards, which is congruent with other studies demonstrating that ground water carries a relatively low risk of contamination, compared to surface water [45, 46, 77]. Water may also become contaminated if it is exposed to fecal matter en route to crops, which, for an irrigation hose, may occur if there is damage to the irrigation hose or improper use [78]. In addition, an irrigation hose may have a vacuum-like effect when the hose connections are not properly secured, providing an opportunity for particles from the surrounding environment to infiltrate the irrigation hose water. As noted with source water, microbial loads in irrigation water fell below EPA thresholds, demonstrating that irrigation hoses provided an effective barrier against exposure to fecal matter from the surrounding environment.

Concentrations of indicator organisms on hands were associated with concentrations of indicator organisms on produce, providing evidence there was a transfer of indicator organisms between hands and produce. One hypothesis for this association is that humans may aid in the transfer of fecal contamination to produce, either as the vehicle of transfer from contaminated sources such as soil, water, equipment, or produce or as the reservoir for indicator or pathogenic microorganisms [60, 79]. Studies have shown that hands are important in the spread of pathogens since they commonly come into contact with fluids, surfaces or fomites contaminated with microbes (reviewed in [80]). Microbes persist on the pads of hands, fingers, and fingernails, which aids in spreading microbial organisms [80, 81]. It has been documented that microorganisms on hands can be transferred to other surfaces, including produce items [82, 83]. Cliver *et al*. [84] found that nearly two thirds of porcine enterovirus was recovered from the

surface of a tomato that was touched by a finger artificially contaminated with fecal matter containing the virus. In another study, Bidawid *et al.* [60] demonstrated that hands and finger pads artificially infected with Hepatitis A had a transfer rate of 9.2% ± 0.9% of the virus to lettuce. Transfer efficiency of microorganisms from surfaces to produce varies according to the microbial load, species of virus or bacteria, survival, type and duration of surface contact, and atmospheric conditions [70, 85, 86]. For instance, Mbithi *et al.* [87] found that elevated contact pressure resulted in a dramatic increase of the amount of virus transferred. Taking into account the amount of viral and bacterial microorganisms in fecal matter, even a small amount of fecal matter on hands may contain millions of viruses and bacterium. By extension, even low transfer rates have the ability to effectively transfer high microbial loads of microorganisms from hands to produce. Evidence shows that hands may serve as the vehicle of transfer, which helps in explaining the association of hand contamination and produce contamination.

In the agricultural environment, farmworkers may repeatedly handle produce, allowing for cross contamination of microorganisms and bolstering evidence for the transfer of fecal contamination between produce and hands. There is ample opportunity during the agricultural production process in which farmworkers may handle produce, at which time viruses, bacterium, and other microorganisms may transfer between hand surfaces and produce surfaces. From surveys of farms and sheds, and interviews with farm and shed managers (data not shown), the farms participating in our study had farmworkers manually pick, load, transport, and package produce, which is common practice in produce production. Manually handling produce is so widespread that a 1998 survey of over 2,000 farms found that 94% of all fruit acres and 87% of all vegetable acres were harvested by hand [88]. In addition to a multi-step produce production process that

may increase the frequency of exposure to handling, researcher observation (data not shown) confirmed that there were instances when more than one farmworker handled a single produce item at one step, providing yet more support of high frequencies of physical contact between farmworkers hands and produce. Increased frequency of physical contact between farmworkers hands and produce suggests an increased risk of cross contamination and provides support for the association between fecal contamination in produce and hands.

A final mechanism to understand the association of hand contamination and produce contamination is the transfer of microorganisms from produce to hands. Produce may harbor microorganisms since they are regularly exposed to external factors such as water, soil, and animal droppings that occur as a result of crop growth in the natural environment [57]. Ailes *et al.* [50] examined levels of contamination at different steps in the production process and found that there was baseline contamination on produce in the field prior to harvest. In addition, the study found that at the packing shed, levels of microbial indictors were significant higher than at other steps, suggesting that contact among produce items may allow for cross contamination [50].. This suggests that it is possible that fecal contamination may move from the surface of produce to the surface of hands because there are various other routes of contamination of produce. However, we found the vast majority studies assessing cross contamination from food to hands did not focus on fresh produce but instead focused on foods of animal origin [59, 89, 90]. Future research should investigate transfer rates from contaminated raw vegetables to the surface of hands.

We found the concentration and presence of indicator organisms in soil and water was not associated with concentration and presence in produce rinses. Although bivariate analysis (Table

2) suggested that there was a significant negative correlation between microbial indicator concentration in irrigation water and microbial indicator concentration in produce, this relationship did not remain after adjusting for potential confounders (Figure 2). Crop type may be a confounder if melons farms, for instance, consistently had water with low fecal contamination and high contamination in produce, thus adjusting for melons would reconcile this spurious relationship. Multivariate models proved that there was a lack of association, possibly explained by the hypothesis that certain farm practices inhibited the spread of microbes between water and produce. As evidence of this hypothesis, in our study, farms placed plastic mulch over the soil in order to prevent desiccation of soil from the arid climate, which may have inadvertently prevented soil particles containing microorganisms from aerosolizing. Microorganisms that become airborne may come into contact with and adhere to the exterior of plants [91]. Furthermore, irrigation water was applied directly to the soil surface using perforated hoses that dripped water at the base of the plant without touching the surface of the produce. This is consistent with other studies that have shown drip irrigation prevents water from coming into contact with the surface of produce [92]. Other studies have confirmed a relationship between contamination in soil [93] and water [94] and contamination in produce, however in these studies soil and water had a physical interaction with produce whereas in our study, there were physical barriers or specific mechanisms that prevented potential transfer. These mechanisms limited interaction between soil and water and produce may explain a lack of association between microbial presence and levels on water and that on produce.

## Strengths and Limitations

It is imperative to understand the relationship between fecal contamination in soil, water, and hands and fecal contamination in produce, however one limitation of our study is the use of indicator organisms instead of foodborne pathogen to assess levels of fecal indicators. Indicator organisms are used as surrogates for enteric pathogens to evaluate food microbiological quality and safety, however indicator contamination does not correlate precisely with pathogen or fecal contamination [27, 32]. Since pathogens are present in low concentrations and they are difficult to detect in the environment, indicators are the best alternative to assess fecal contamination. Our study was strengthened because we tested samples for four microbial indicators in order to gain a more comprehensive understanding of the fluctuations of several indicator organisms.

In our study, indicator concentration values were imputed for assays with plate counts that fell below the limit of detection or above the limit of quantification. While this helps to maximize sample size, it weakens the accuracy of our findings. Future analysis could improve statistical techniques by employing methods that better approximate values above the limit of quantification and below the limit of detection.

Although we were able to determine the relationship between contamination in environmental samples and contamination in produce, causality could not be evaluated due to limitations in the study design. To identify causality, the design would have to incorporate a temporal component that measured contamination of the same sample at multiple points in time. However, this type of design would be logistically complicated and time consuming. One of the strengths of this study was the ability to collect hundreds of samples in a relatively short period of time, which would

not have been possible if a temporal relationship were included. Past studies have focused either on understanding routes of contamination in a laboratory setting or by conducting an epidemiological analysis of an outbreak investigation, whereas our study employed epidemiologic modeling as a novel approach to examine routes of contamination in the farm environment. Our study used robust collection methods, took place in the natural environment, employed rigorous laboratory methods, and used epidemiological methods to analyze the collected data. In addition, the ability to match each produce rinse to a corresponding, matched hand rinse is a strength of this study that provides evidence to support a direct relationship between hand contamination and produce contamination.

## Tables and Figures

**Table 1: Concentration and prevalence of indicator organism on produce in the farm environment**

| | ***E. coli*** | | | ***Enterococcus spp.*** | | | **Fecal coliforms** | | | **Somatic coliphage** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample size | Geometric mean and confidence interval | Percent positive | Sample size | Geometric mean and Confidence interval | Percent positive | Sample size | Geometric mean and confidence interval | Percent positive | Sample size | Geometric mean and confidence interval | Percent positive |
| **Produce (All)** | 279 | 1.7 (1.4, 1.9) | 32 | 279 | 5.3(5.0, 5.6) | 86 | 275 | 5.4 (5.1, 5.6) | 97 | 206 | 2.2 (2.0, 2.5) | 84 |
| Jalapeño | 64 | 0.3 (-0.0, 0.6) | 15 | 64 | 3.6 (3.1, 4.0) | 70 | 61 | 3.9 (3.2, 4.5) | 92 | 46 | 1.2 (0.8, 1.7) | 78 |
| Tomato | 87 | 0.2 (-0.0, 0.5) | 20 | 87 | 3.5 (3.2, 3.8) | 77 | 86 | 4.5 (4.1, 5.0) | 98 | 69 | 0.9 (0.6, 1.2) | 81 |
| Melon | 128 | 3.3 (2.9, 3.7) | 49 | 128 | 7.3 (7.0, 7.6) | 99 | 128 | 6.7 (6.4, 6.9) | 100 | 91 | 3.7 (3.4, 4.0) | 90 |
| **Hand Rinse (All)** | 196 | 2.6 (2.3, 2.9) | 39 | 196 | 6.6 (6.4, 6.8) | 100 | 196 | 5.9 (5.6, 6.1) | 96 | 145 | 2.2 (1.9, 2.5) | 66 |
| Jalapeño | 43 | 2.3 (1.8, 2.8) | 42 | 43 | 6.0 (5.5, 6.5) | 100 | 43 | 5.0 (4.4, 5.7) | 93 | 33 | 1.6 (1.1, 2.1) | 61 |
| Tomato | 61 | 1.7 (1.3, 2.0) | 21 | 61 | 6.2 (5.8, 6.5) | 100 | 61 | 5.0 (4.6, 5.5) | 92 | 49 | 1.5 (1.2, 1.9) | 65 |
| Melon | 92 | 3.4 (3.0, 3.8) | 50 | 92 | 7.2 (6.8, 7.5) | 100 | 92 | 6.8 (6.5, 7.1) | 100 | 63 | 3.0 (2.5, 3.5) | 68 |
| **Soil (All)** | 81 | 0.0 (-0.3, 0.3) | 21 | 81 | 1.4 (1.1, 1.6) | 67 | 80 | 2.5 (2.3, 2.8) | 96 | 61 | -0.4 (-0.6, -0.2) | 34 |
| Jalapeño | 18 | 0.4 (-0.1, 0.9) | 6 | 18 | 1.4 (1.1, 1.7) | 56 | 18 | 2.7 (2.0, 3.3) | 94 | 13 | -0.6 (-1.0, -0.2) | 62 |
| Tomato | 25 | 0.6 (0.2, 1.0) | 12 | 25 | 1.4 (1.1, 1.7) | 56 | 24 | 2.3 (2.0, 2.7) | 96 | 18 | -0.7 (-1.3, -0.1) | 56 |
| Melon | 38 | -0.6 (-0.9, -0.2) | 34 | 38 | 1.3 (0.9, 1.7) | 82 | 38 | 2.5 (2.1, 2.9) | 100 | 30 | -0.2 (-0.5, 0.1) | 10 |
| **Irrigation Water (All)** | 76 | -0.1 (-0.3, 0.1) | 41 | 75 | 0.5 (0.2, 0.8) | 84 | 73 | 1.7 (1.4, 2.0) | 93 | 47 | 1.0 (0.5, 1.5) | 45 |
| Jalapeño | 15 | -0.5 (-0.8, -0.2) | 20 | 14 | 0.6 (0.0, 1.2) | 93 | 14 | 1.5 (0.8, 2.3) | 86 | 7 | 1.4 (-0.2, 2.9) | 57 |
| Tomato | 23 | 0.3 (-.05, .7) | 70 | 23 | 0.5 (0.2, 0.8) | 91 | 21 | 1.6 (1.2, 1.9) | 100 | 10 | 1.5 (0.5, 2.5) | 80 |
| Melon | 38 | -0.2 (-0.5, 0.1) | 32 | 38 | 0.5 (0.04, 1.0) | 76 | 38 | 1.7 (1.2, 2.2) | 92 | 30 | 0.7 (0.2, 1.3) | 30 |
| **Source Water (All)** | 83 | 0.0 (-0.2, 0.2) | 53 | 80 | 0.3 (0.1, 0.5) | 90 | 76 | 1.7 (1.4, 1.9) | 93 | 41 | 1.8 (1.2, 2.5) | 59 |
| Jalapeño | 27 | -0.2 (-0.5, 0.1) | 41 | 26 | 0.7 (0.4, 1.0) | 96 | 24 | 1.7 (1.2, 2.2) | 92 | 11 | 2.2 (0.9, 3.6) | 64 |
| Tomato | 25 | 0.3 (0.0, 0.6) | 92 | 23 | 0.3 (0.1, 0.6) | 96 | 21 | 1.4 (1.1, 1.7) | 100 | 13 | 2.3 (1.3, 3.4) | 77 |
| Melon | 31 | -0.1 (-0.6, 0.4) | 32 | 31 | -0.1 (-0.4, 0.2) | 81 | 31 | 1.8 (1.4, 2.3) | 90 | 17 | 1.2 (0.2, 2.2) | 41 |

*Unit measurements: hands=CFU/hand, produce=CFU/produce, soil=CFU/g, and water=CFU/ml

**Somatic coliphage unit measurements are in MPN instead of CF

**Table 2: Spearman correlation coefficient of indictor organism concentrations on environmental samples and produce rinses**

| | *E. coli* | | *Enterococcus spp.* | | Fecal coliforms | | Somatic coliphage | |
|---|---|---|---|---|---|---|---|---|
| | N | Rho (p-value) | N | Rho (p-value) | N | Rho (p-value) | N | Rho (p-value) |
| **Hands (All)** | 196 | 0.60* (>0.01) | 196 | 0.67* (>0.01) | 194 | 0.75* (>0.01) | 143 | 0.41* (>0.01) |
| Jalapeño | 43 | 0.51* (>0.01) | 43 | 0.57* (>0.01) | 41 | 0.64* (>0.01) | 31 | 0.25 (0.18) |
| Tomato | 61 | 0.51* (>0.01) | 61 | 0.35* (0.005) | 61 | 0.58* (>0.01) | 49 | 0.20 (0.16) |
| Melon | 92 | 0.51* (>0.01) | 92 | 0.67* (>0.01) | 92 | 0.77* (>0.01) | 63 | 0.21 (0.10) |
| **Soil (All)** | 80 | -0.22* (0.05) | 80 | 0.03 (0.77) | 78 | -0.11 (0.34) | 58 | 0.25 (0.06) |
| Jalapeño | 18 | 0.38 (0.12) | 18 | -0.25 (0.31) | 18 | -0.29 (0.26) | 11 | -0.31 (0.36) |
| Tomato | 25 | 0.38 (0.06) | 25 | 0.13 (0.53) | 25 | 0.22 (0.31) | 18 | 0.14 (0.58) |
| Melon | 37 | 0.25 (0.14) | 37 | 0.23 (0.17) | 37 | -0.36* (0.03) | 29 | 0.05 (0.78) |
| **Irrigation water (All)** | 75 | -0.17 (0.14) | 74 | -0.14 (0.23) | 72 | 0.009 (0.94) | 46 | -0.25 (0.10) |
| Jalapeño | 15 | 0.50 (0.06) | 14 | 0.06 (0.84) | 14 | 0.33 (0.24) | 7 | 0.15 (0.74) |
| Tomato | 23 | 0.13 (0.55) | 23 | -0.21 (0.33) | 21 | -0.23 (0.31) | 10 | -0.09 (0.81) |
| Melon | 37 | -0.37* (0.02) | 37 | -0.21 (0.20) | 37 | -0.20 (0.24) | 29 | -0.09 (0.64) |
| **Source water (All)** | 82 | -0.21* (0.05) | 79 | -0.34* (>0.01) | 72 | 0.009 (0.94) | 40 | -0.17 (0.29) |
| Jalapeño | 27 | -0.06 (0.76) | 26 | -0.11 (0.58) | 24 | 0.17 (0.41) | 11 | -0.12 (0.72) |
| Tomato | 25 | 0.31 (0.13) | 23 | -0.12 (0.57) | 21 | -0.42 (0.06) | 13 | 0.27 (0.37) |
| Melon | 30 | -0.29 (0.03) | 30 | 0.03 (0.87) | 30 | 0.32 (0.09) | 16 | -0.07 (0.80) |

** = Significant at the 0.05 level*

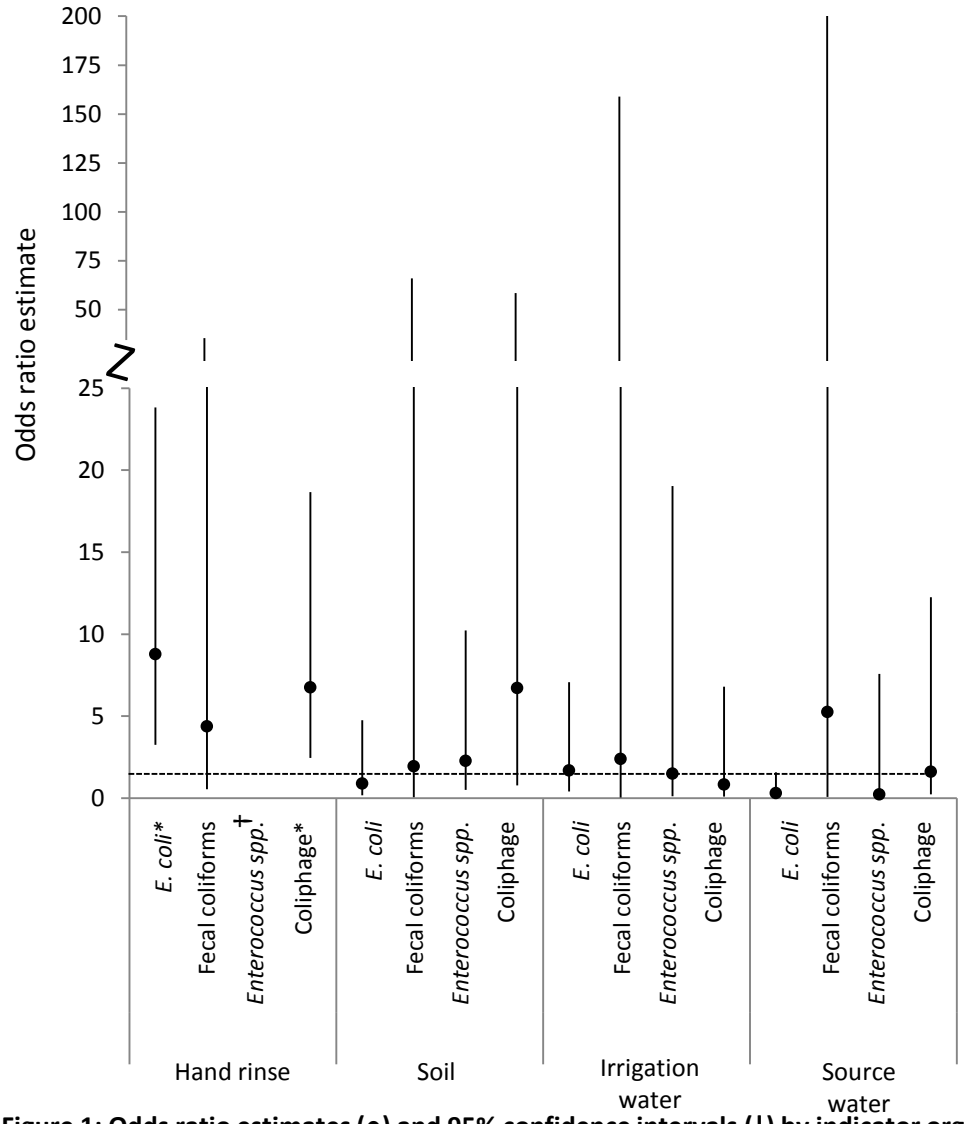


**Figure 1: Odds ratio estimates (●) and 95% confidence intervals (|) by indicator organism and sample type.** The dots represent odds ratio estimates of the frequency of association between indicator concentrations on produce and matched environmental samples controlling for produce type, year of collection, and step in the production process.

†Model estimates for hand contamination for *Enterococcus spp.* are unavailable as there were no hand samples that were free of *Enterococcus spp.*

Z= break in y-axis

*significant at the 0.05 level

--- = expected value of linear model estimate under the null hypothesis of zero association

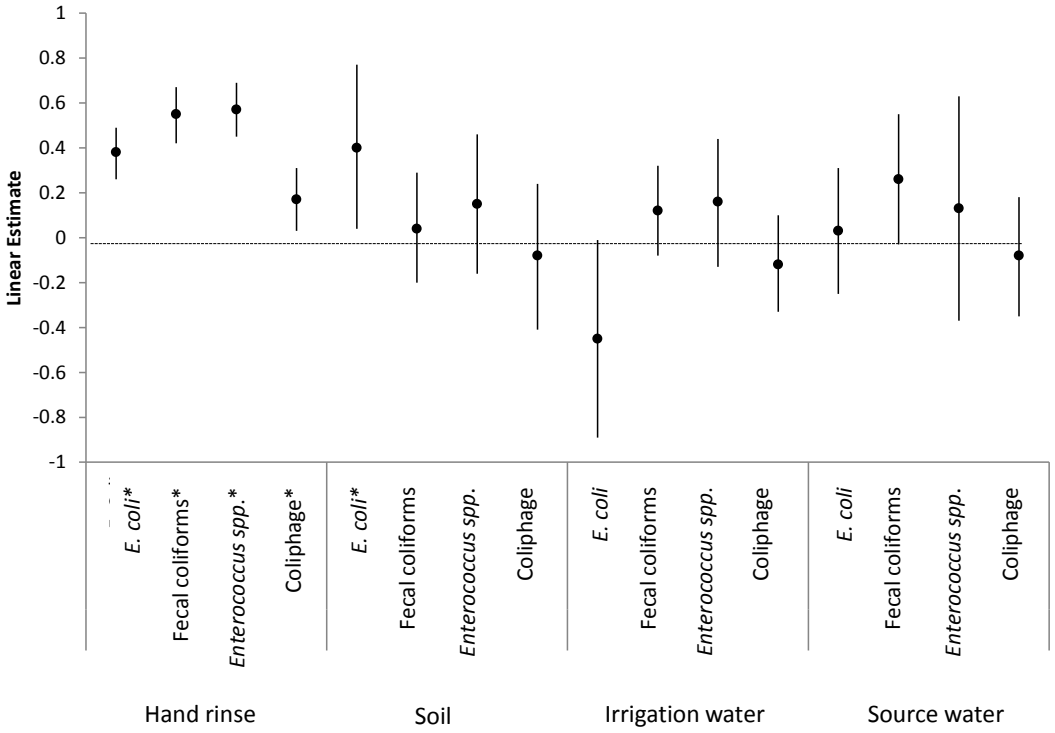


**Figure 2: Linear model estimates (●) and 95% confidence intervals (|) by indicator organism and sample type.** The dots represent linear model estimates of the magnitude of association between indicator concentrations on produce and matched environmental samples controlling for produce type, year of collection, and step in the production process.
*Significant at 0.05 level
--- = expected value of linear model estimate under the null hypothesis of zero association

# Chapter 3: Conclusion and Recommendations

## Conclusion

Identifying routes of microbiological contamination in the produce production process is imperative in order to improve the quality and safety of raw produce and ultimately reduce foodborne illnesses. Overall, our results showed a strong association between hand contamination and produce contamination. When adjusting for crop type, year of data collection, and step in the production process, produce is more likely to be contaminated if hands are contaminated for *E. coli* and somatic coliphage. Similarly, we found concentrations of *E. coli* , fecal coliforms, and *Enterococcus spp.* in hands were significantly, positively associated with concentrations of the same indicator organisms in produce. The presence of a relationship between produce and farmworkers hands may be explained by a number of transfer mechanisms. Factors such as microbial load and frequency of contact may provide evidence for these mechanisms.

Additionally, our results also showed that there was no association between soil and water contamination and produce contamination, except for *E. coli* in soil. The lack of association of soil and produce is likely due to plastic mulch used on farms that prevented soil from aerosolizing and subsequently adhering to the surface of produce. Water and produce contamination had no relationship, reinforcing findings of past studies that show drip irrigation is an effective technique to limit exposure of produce surfaces to water and providing evidence that

well water is a low-risk alternative as a water source. The results of this study helped to advance our understanding of mechanisms of fecal-associated microbial contamination on farms and will now allow us to explore appropriate interventions on the farm that will reduce levels of fecal contamination.

## Recommendations

- We found an association between hand contamination and produce contamination. The spread of fecal matter and enteric pathogens can be prevented if good hygiene practices are followed. It is recommend that farms follow the FDA's Good Agricultural Practices for hand hygiene and sanitation including:
    - Clean and properly maintained sanitation facilities within close walking distance of farmworkers
    - Hand washing stations close and accessible to farmworkers
    - Hygiene and sanitation training for farmworkers because, generally, farmworker knowledge of best hygiene practices is low [36, 37].
- Hands may become contaminated for a variety of reasons including when the farmworker is infected with an illness and hands become host to the pathogen. This is especially important in the agricultural production environment due to high rates of intestinal infection [95, 96] and non-enteric diseases (e.g. TB and HIV) in migrant workers compared to the general American population [97-99]. Ill farmworkers that serve as the reservoir of infectious microbes and pathogens may play a role in transmission to produce when their hands become host to microorganisms [96].
    - Farm managers should recognize the importance of the health of farmworkers.

- Despite high rates of enteric and other diseases, migrant farm workers may have inadequate healthcare due to language barriers, insufficient transportation, lack of health insurance, and immigration status [72, 100-104]. It is important that workers are not penalized (loss of pay) for staying home to recover from illness since doing so encourages workers to attend to harvests when they may be infectious. Other alternatives to improve the health of farmworkers should be considered in order to reduce the rate of illness in this population, especially because their health has an effect on the spread of foodborne illnesses.
- There were low levels of contamination in water samples and there was no association of water samples and produce rinses. This means that the techniques used on the farms enrolled in this study were effective. As a result, it is recommended that farms that use well water to irrigate their crops continue to do so since concentrations of microbial indicators were below EPA limits. Furthermore, it is recommended that farms continue to use drip irrigation methods to prevent produce surface exposure to potentially contaminated water.
- There were low levels of soil contamination and there was a lack of association between soil samples and produce rinses. The farms enrolled in this study used synthetic fertilizer, free of organic and manure-based soil amendments, so we encourage farms to consider the use of synthetic fertilizers to reduce microbial loads in soil. The lack of association may be explained by the use of plastic mulch placed over the soil that prevented it from aerosolizing and adhering to produce.

## References


[1] M. B. Batz, S. Hoffmann, and J. G. Morris, Jr., "Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation," *J Food Prot,* vol. 75, pp. 1278-91, Jul 2012.

[2] C. N. Berger, S. V. Sodha, R. K. Shaw, P. M. Griffin, D. Pink, P. Hand*, et al.*, "Fresh fruit and vegetables as vehicles for the transmission of human pathogens," *Environmental Microbiology,* vol. 12, pp. 2385-2397, Sep 2010.

[3] N. H. Bean, J. S. Goulding, C. L. Frederick, and F. J. Angulo, "Surveillance for Foodborne-Disease Outbreaks -- United States, 1988-1992," *MMWR CDC Surveill Summ,* vol. 45, pp. 1-55, 1996.

[4] L. R. Beuchat, "Pathogenic microorganisms associated with fresh produce," *J Food Prot,* vol. 59, pp. 203-216, 1996.

[5] S. Sivapalasingam, C. R. Friedman, L. Cohen, and R. V. Tauxe, "Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997," *J Food Prot,* vol. 67, pp. 2342-53, Oct 2004.

[6] J. A. Painter, R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo*, et al.*, "Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008," *Emerg Infect Dis,* vol. 19, pp. 407-15, Mar 2013.

[7] C. S. DeWaal, D. Banda, and k. Barlow. (2004, Outbreak Alert! Closing the Gaps in our food-safety net. *Center for Science in Public Interest*.

[8] M. F. Lynch, R. V. Tauxe, and C. W. Hedberg, "The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities," *Epidemiology and Infection,* vol. 137, pp. 307-315, Mar 2009.

[9] C. S. Jacobsen and T. B. Bech, "Soil survival of Salmonella and transfer to freshwater and fresh produce," *Food Research International,* vol. 45, pp. 557-566, 2012.

[10] S. L. Pollack. Consumer Demand for Fruit and Vegetables: The U.S. Example. *Economi Research Service, USDA*.

[11] K. Hoelzer, R. Pouillot, K. Egan, and S. Dennis, "Produce Consumption in the United States: An Analysis of Consumption Frequencies, Serving Sizes, Processing Forms, and High-Consuming Population Subgroups for Microbial Risk Assessments," *J Food Prot,* vol. 75, pp. 328-340, Feb 2012.

[12] "Surveillance for Foodborne Disease Outbreaks-United States, 2006," Centers for Disease Control and Prevention, Atlanta, GA2009.

[13] S. Sivapalasingam, E. Barrett, A. Kimura, S. Van Duyne, W. De Witt, M. Ying*, et al.*, "A multistate outbreak of Salmonella enterica serotype Newport infection linked to mango consumption: Impact of water-dip disinfestation technology," *Clinical Infectious Diseases,* vol. 37, pp. 1585-1590, Dec 2003.

[14] "Ongoing Multistate Outbreak of Escherichia coli serotype O157:H7 Infections Associated with Consumption of Fresh Spinach --- United States, September 2006," Centers for Disease Control and Prevention2006.

[15] W. G. Yuan, Donghai; Huh, Eui-Nam; Lee, Sungyoung, "Harness Human Sensor Networks for Situational Awareness in Disaster Reliefs: A Survey," *IETE Technical Review,* vol. 30, pp. 240-247, 2013

[16] J. B. Gregory, L. F. Webster, J. F. Griffith, and J. R. Stewart, "Improved detection and quantitation of norovirus from water," *Journal of Virological Methods,* vol. 172, pp. 38-45, Mar 2011.

[17] E. Franz and A. H. van Bruggen, "Ecology of E. coli O157:H7 and Salmonella enterica in the primary vegetable production chain," *Crit Rev Microbiol,* vol. 34, pp. 143-61, 2008.

[18] C. f. D. C. a. P. (CDC). (2011). *2011 Estimates of Foodbornes Illness in the United States*. Available: http://www.cdc.gov/features/dsfoodborneestimates/

[19] E. Scallan, R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy*, et al.*, "Foodborne Illness Acquired in the United States-Major Pathogens," *Emerg Infect Dis,* vol. 17, pp. 7-15, Jan 2011.

[20] A. M. Fabiszewski de Aceituno, Rocks, J. L., Jaykus, L. A., Leon, J. S., "Foodborne Viruses," in *Guide to Foodborne Pathogens*, 2nde ed: Oxford, 2013.

[21] C. S. DeWaal, X. A. Tian, and D. Plunkett, "Outbreak Alert!," *Center for Science in the Public Interest,* vol. 11, pp. 5-9, 2009.

[22] A. S. Sant'Ana, M. Landgraf, M. T. Destro, and B. D. G. M. Franco, "Prevalence and counts of Salmonella spp. in minimally processed vegetables in São Paulo, Brazil," *Food Microbiology,* vol. 28, pp. 1235-1237, 2011.

[23] M. Peleg, M. D. Normand, and M. G. Corradini, "A Study of the Randomly Fluctuating Microbial Counts in Foods and Water Using the Expanded Fermi Solution as a Model," *Journal of Food Science,* vol. 77, pp. R63-R71, Jan 2012.

[24] K. G. Field and M. Samadpour, "Fecal source tracking, the indicator paradigm, and managing water quality," *Water Research,* vol. 41, pp. 3517-3538, Aug 2007.

[25] F. F. Busta, T. V. Suslow, M. E. Parish, L. R. Beuchat, J. N. Farber, E. H. Garrett*, et al.*, "The Use of Indicators and Surrogate Microorganisms for the Evaluation of Pathogens in Fresh and Fresh-Cut Produce," *Comprehensive Reviews in Food Science and Food Safety,* vol. 2, pp. 179-185, 2003.

[26] J. M. Jay, "Indicators in food microbial quality and safety," in *Modern food microbiology*, 5th ed New York: Chapman & Hall, 1996.

[27] P. Hartman, Deibel, R., Sieverding, L., "Enterococci," in *Compendium of Methods for the Microbiological Examination of Foods*, ed, 1992, pp. 83-87.

[28] H. Aycicek, U. Oguz, and K. Karci, "Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey," *Int J Hyg Environ Health,* vol. 209, pp. 197-201, Mar 2006.

[29] P. B. Allwood, Y. S. Malik, S. Maherchandani, K. Vought, L. A. Johnson, C. Braymen*, et al.*, "Occurrence of Escherichia coli, noroviruses, and F-specific coliphages in fresh market-ready produce," *J Food Prot,* vol. 67, pp. 2387-90, Nov 2004.

[30] S. Endley, L. Lu, E. Vega, M. E. Hume, and S. D. Pillai, "Male-specific coliphages as an additional fecal contamination indicator for screening fresh carrots," *J Food Prot,* vol. 66, pp. 88-93, Jan 2003.

[31] C. Franz, W. H. Holzapfel, and M. E. Stiles, "Enterococci at the crossroads of food safety?," *Int J Food Microbiol,* vol. 47, pp. 1-24, Mar 1999.

[32] A. H. Paul, H. D. Robert, and M. S. Linda, "Enterococci," in *Compendium of methods for the microbiological examination of foods*, ed: American Public Health Association, 2001.
[33] Anonymous, "NFPA White Paper-Significance of coliforms and generic Echerichia coli on fresh produce and minimally processed produce " 2000.
[34] "Fact Sheets on the Subparts of the FSMA Proposed Rule for Produce Safety," F. a. D. Administration, Ed., ed. Maryland: U.S. Department of Health and Human Services, 2013.
[35] L. R. Beuchat and J. H. Ryu, "Produce handling and processing practices," *Emerging Infectious Diseases,* vol. 3, pp. 459-65, Oct-Dec 1997.
[36] F. a. USDA. (1998, Guide to minimize microbial food safety hazards for fresh fruits and vegetables. *Center for Food Safety and Applied Nutrition -- USDA*.
[37] J. M. Soon and R. N. Baines, "Food safety training and evaluation of handwashing intention among fresh produce farm workers," *Food Control,* vol. 23, pp. 437-448, Feb 2012.
[38] J. D. Barak and B. K. Schroeder, "Interrelationships of Food Safety and Plant Pathology: The Life Cycle of Human Pathogens on Plants," *Annu Rev Phytopathol,* May 29 2012.
[39] M. T. Jay, M. Cooley, D. Carychao, G. W. Wiscomb, R. A. Sweitzer, L. Crawford-Miksza*, et al.*, "Escherichia coli O157 : H7 in feral swine near spinach fields and cattle, central California coast," *Emerg Infect Dis,* vol. 13, pp. 1908-1911, Dec 2007.
[40] M. Iwasa, S. Makino, H. Asakura, H. Kobori, and Y. Morimoto, "Detection of Escherichia coli O157:H7 from Musca domestica (Diptera: Muscidae) at a cattle farm in Japan," *J Med Entomol,* vol. 36, pp. 108-12, Jan 1999.
[41] X. Guo, J. Chen, R. E. Brackett, and L. R. Beuchat, "Survival of Salmonella on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil," *J Food Prot,* vol. 65, pp. 274-9, Feb 2002.
[42] K. Ibenyassine, R. AitMhand, Y. Karamoko, N. Cohen, and M. M. Ennaji, "Use of repetitive DNA sequences to determine the persistence of enteropathogenic Escherichia coli in vegetables and in soil grown in fields treated with contaminated irrigation water," *Lett Appl Microbiol,* vol. 43, pp. 528-33, Nov 2006.
[43] M. Islam, J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang, "Fate of Salmonella enterica serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water," *Appl Environ Microbiol,* vol. 70, pp. 2497-502, Apr 2004.
[44] W. J. Reilly, G. I. Forbes, G. M. Paterson, and J. C. Sharp, "Human and animal salmonellosis in Scotland associated with environmental contamination, 1973-79," *Vet Rec,* vol. 108, pp. 553-5, Jun 27 1981.
[45] A. Forslund, J. H. Ensink, B. Markussen, A. Battilani, G. Psarras, S. Gola*, et al.*, "Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater," *Water Res,* vol. 46, pp. 5917-34, Nov 15 2012.
[46] E. E. Natvig, S. C. Ingham, B. H. Ingham, L. R. Cooperband, and T. R. Roper, "Salmonella enterica serovar Typhimurium and Escherichia coli contamination of root and leaf vegetables grown in soils with incorporated bovine manure," *Appl Environ Microbiol,* vol. 68, pp. 2737-44, Jun 2002.

[47] O. A. Ijabadeniyi, L. K. Debusho, M. Vanderlinde, and E. M. Buys, "Irrigation Water as a Potential Preharvest Source of Bacterial Contamination of Vegetables," *Journal of Food Safety,* vol. 31, pp. 452-461, 2011.
[48] E. D. Akins, M. A. Harrison, and W. Hurst, "Washing practices on the microflora on Georgia-grown cantaloupes," *J Food Prot,* vol. 71, pp. 46-51, Jan 2008.
[49] A. Tomas-Callejas, G. Lopez-Velasco, A. B. Camacho, F. Artes, F. Artes-Hernandez, and T. V. Suslow, "Survival and distribution of Escherichia coli on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions," *Int J Food Microbiol,* vol. 151, pp. 216-22, Dec 2 2011.
[50] E. C. Ailes, J. S. Leon, L. A. Jaykus, L. M. Johnston, H. A. Clayton, S. Blanding*, et al.*, "Microbial Concentrations on Fresh Produce Are Affected by Postharvest Processing, Importation, and Season," *J Food Prot,* vol. 71, pp. 2389-2397, Dec 2008.
[51] L. M. Johnston, L. A. Jaykus, D. Moll, M. C. Martinez, J. Anciso, B. Mora*, et al.*, "A field study of the microbiological quality of fresh produce," *J Food Prot,* vol. 68, pp. 1840-7, Sep 2005.
[52] F. National Advisory Committee on Microbiological Criteria For, "Response to questions posed by the food safety and inspection service regarding determination of the most appropriate technologies for the food safety and inspection service to adopt in performing routine and baseline microbiological analyses," *J Food Prot,* vol. 73, pp. 1160-200, Jun 2010.
[53] "Bacteriological Analytical Manual (BAM)," U.S. Department of Health and Human Services Maryland1998.
[54] S. L. Burnett and L. R. Beuchat, "Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination (Reprinted from Journal of Industrial Microbiology & Biotechnology, vol 25, pg 281-287, 2000)," *Journal of Industrial Microbiology & Biotechnology,* vol. 27, pp. 104-110, Aug 2001.
[55] A. Bowen, A. Fry, G. Richards, and L. Beauchat, "Infections associated with cantaloupe consumption: a public health concern," *Epidemiol Infect,* pp. 1-11, Dec 1 2005.
[56] CSPL. (2009). *Outbreak Alert! Database*. Available: http://www.cspinet.org/foodsafety/outbreak/outbreaks.php?column=subgroup&colval=Fruits&column1=Produce
[57] L. R. Beuchat, "Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables," *Microbes Infect,* vol. 4, pp. 413-23, Apr 2002.
[58] M. R. Wachtel, L. C. Whitehand, and R. E. Mandrell, "Association of Escherichia coli O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water," *J Food Prot,* vol. 65, pp. 18-25, Jan 2002.
[59] P. Luber, S. Brynestad, D. Topsch, K. Scherer, and E. Bartelt, "Quantification of campylobacter species cross-contamination during handling of contaminated fresh chicken parts in kitchens," *Appl Environ Microbiol,* vol. 72, pp. 66-70, Jan 2006.
[60] S. Bidawid, J. M. Farber, and S. A. Sattar, "Contamination of foods by food handlers: experiments on hepatitis A virus transfer to food and its interruption," *Appl Environ Microbiol,* vol. 66, pp. 2759-63, Jul 2000.
[61] M. Islam, J. Morgan, M. P. Doyle, and X. Jiang, "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,* vol. 67, pp. 574-8, Mar 2004.

[62] C. Barton Behravesh, R. K. Mody, J. Jungk, L. Gaul, J. T. Redd, S. Chen*, et al.*, "2008 Outbreak of Salmonella Saintpaul Infections Associated with Raw Produce," *New England Journal of Medicine,* vol. 364, pp. 918-927, 2011.

[63] K. K. Bradley, J. M. Williams, L. J. Burnsed, M. B. Lytle, M. D. McDermott, R. K. Mody*, et al.*, "Epidemiology of a large restaurant-associated outbreak of Shiga toxin-producing Escherichia coli O111:NM," *Epidemiol Infect,* vol. 140, pp. 1644-54, Sep 2012.

[64] E. C. Todd, J. D. Greig, C. A. Bartleson, and B. S. Michaels, "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 5. Sources of contamination and pathogen excretion from infected persons," *J Food Prot,* vol. 71, pp. 2582-95, Dec 2008.

[65] M. T. Jay-Russell, A. Bates, L. Harden, W. G. Miller, and R. E. Mandrell, "Isolation of Campylobacter from feral swine (Sus scrofa) on the ranch associated with the 2006 Escherichia coli O157:H7 spinach outbreak investigation in California," *Zoonoses Public Health,* vol. 59, pp. 314-9, Aug 2012.

[66] A. Mukherjee, S. Cho, J. Scheftel, S. Jawahir, K. Smith, and F. Diez-Gonzalez, "Soil survival of Escherichia coli O157:H7 acquired by a child from garden soil recently fertilized with cattle manure," *J Appl Microbiol,* vol. 101, pp. 429-36, Aug 2006.

[67] R. H. Shumway, R. S. Azari, and M. Kayhanian, "Statistical approaches to estimating mean water quality concentrations with detection limits," *Environ Sci Technol,* vol. 36, pp. 3345-53, Aug 1 2002.

[68] "Criteria Used in Model Selection Methods," in *Support SAS: The GLM Select Procedure*, SAS, Ed., ed, 2014.

[69] D. Beal, "SAS\ Code to Select the Best Multiple Linear Regression Model for Multivariate Data Using Information Criteria," NCSU, Ed., ed. Tennessee.

[70] G. Kampf and A. Kramer, "Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs," *Clin Microbiol Rev,* vol. 17, pp. 863-93, table of contents, Oct 2004.

[71] J. D. van Elsas, A. V. Semenov, R. Costa, and J. T. Trevors, "Survival of Escherichia coli in the environment: fundamental and public health aspects," *ISME J,* vol. 5, pp. 173-83, Feb 2011.

[72] K. Mehta, S. M. Gabbard, V. Barratt, M. Lewis, D. Carroll, R. Mines, "Findings from the National Agricultural Workers Survey (NAWS) 1997–1998: A demographic and employment profile of United States farmworkers.," O. o. t. A. S. f. P. U.S. Department of Labor, Office of Program Economics, Ed., ed. Washington D.C, 2000.

[73] J. C. de Wit and E. H. Kampelmacher, "Some aspects of microbial contamination of hands of workers in food industries," *Zentralbl Bakteriol Mikrobiol Hyg [B],* vol. 172, pp. 390-400, Jan 1981.

[74] J. C. de Wit and F. M. Rombouts, "Faecal micro-organisms on the hands of carriers: Escherichia coli as model for Salmonella," *Zentralbl Hyg Umweltmed,* vol. 193, pp. 230-6, Oct 1992.

[75] M. Abbaszadegan, M. S. Huber, C. P. Gerba, and I. L. Pepper, "Detection of enteroviruses in groundwater with the polymerase chain reaction," *Appl Environ Microbiol,* vol. 59, pp. 1318-24, May 1993.

[76] M. A. Borchardt, P. D. Bertz, S. K. Spencer, and D. A. Battigelli, "Incidence of enteric viruses in groundwater from household wells in Wisconsin," *Appl Environ Microbiol,* vol. 69, pp. 1172-80, Feb 2003.

[77] A. P. MacGowan, K. Bowker, J. McLauchlin, P. M. Bennett, and D. S. Reeves, "The occurrence and seasonal changes in the isolation of Listeria spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources," *Int J Food Microbiol,* vol. 21, pp. 325-34, Mar 1994.

[78] M. Estrada-Acosta, M. Jimenez, C. Chaidez, J. Leon-Felix, and N. Castro-Del Campo, "Irrigation water quality and the benefits of implementing good agricultural practices during tomato (Lycopersicum esculentum) production," *Environ Monit Assess,* Mar 31 2014.

[79] R. C. Almeida, A. Y. Kuaye, A. M. Serrano, and P. F. de Almeida, "[Evaluation and control of the microbiological quality of hands in foodhandlers]," *Rev Saude Publica,* vol. 29, pp. 290-4, Aug 1995.

[80] S. A. Ansari, V. S. Springthorpe, S. A. Sattar, S. Rivard, and M. Rahman, "Potential role of hands in the spread of respiratory viral infections: studies with human parainfluenza virus 3 and rhinovirus 14," *J Clin Microbiol,* vol. 29, pp. 2115-9, Oct 1991.

[81] G. M. Rayan and D. J. Flournoy, "Microbiologic flora of human fingernails," *J Hand Surg [Am],* vol. 12, pp. 605-7, Jul 1987.

[82] Y. Chen, K. M. Jackson, F. P. Chea, and D. W. Schaffner, "Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks," *J Food Prot,* vol. 64, pp. 72-80, Jan 2001.

[83] E. Tuladhar, W. C. Hazeleger, M. Koopmans, M. H. Zwietering, E. Duizer, and R. R. Beumer, "Transfer of noroviruses between fingers and fomites and food products," *Int J Food Microbiol,* vol. 167, pp. 346-52, Nov 1 2013.

[84] D. O. Cliver and K. D. Kostenbader, "Disinfection of Virus on Hands for Prevention of Foodborne Disease," *International Journal of Food Microbiology,* vol. 1, pp. 75-87, 1984.

[85] D. V. Mukherjee, B. Cohen, M. E. Bovino, S. Desai, S. Whittier, and E. L. Larson, "Survival of influenza virus on hands and fomites in community and laboratory settings," *Am J Infect Control,* vol. 40, pp. 590-4, Sep 2012.

[86] P. Rusin, S. Maxwell, and C. Gerba, "Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage," *J Appl Microbiol,* vol. 93, pp. 585-92, 2002.

[87] J. N. Mbithi, V. S. Springthorpe, J. R. Boulet, and S. A. Sattar, "Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces," *J Clin Microbiol,* vol. 30, pp. 757-63, Apr 1992.

[88] National Agricultural Statistics Service and U.S. Department of Agriculture. (2001, Agricultural chemical usage - fruit and vegetable agricultural practices. Available: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1568

[89] M. L. Signorini and L. S. Frizzo, "[Quantitative risk model for verocytotoxigenic Escherichia coli cross-contamination during homemade hamburger preparation]," *Rev Argent Microbiol,* vol. 41, pp. 237-44, Oct-Dec 2009.

[90] L. Verhoeff-Bakkenes, R. R. Beumer, R. de Jonge, F. M. van Leusden, and A. E. de Jong, "Quantification of Campylobacter jejuni cross-contamination via hands, cutlery, and cutting board during preparation of a chicken fruit salad," *J Food Prot,* vol. 71, pp. 1018-22, May 2008.
[91] Y. Rekah, D. Shtienberg, and J. Katan, "Disease Development Following Infection of Tomato and Basil Foliage by Airborne Conidia of the Soilborne Pathogens Fusarium oxysporum f. sp. radicis-lycopersici and F. oxysporum f. sp. basilici," *Phytopathology,* vol. 90, pp. 1322-9, Dec 2000.
[92] M. B. Pescod, "Wastewater Treatment and Use in Agriculture," 1992.
[93] D. Ongeng, G. A. Vasquez, C. Muyanja, J. Ryckeboer, A. H. Geeraerd, and D. Springael, "Transfer and internalisation of Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium in cabbage cultivated on contaminated manure-amended soil under tropical field conditions in Sub-Saharan Africa," *Int J Food Microbiol,* vol. 145, pp. 301-10, Jan 31 2011.
[94] K. Holvoet, I. Sampers, M. Seynnaeve, and M. Uyttendaele, "Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production," *Int J Food Microbiol,* vol. 171, pp. 21-31, Feb 3 2014.
[95] B. L. Ungar, E. Iscoe, J. Cutler, and J. G. Bartlett, "Intestinal parasites in a migrant farmworker population," *Arch Intern Med,* vol. 146, pp. 513-5, Mar 1986.
[96] M. D. Russell, M. T. Correa, C. E. Stauber, and J. A. Kase, "North Carolina Hispanic farmworkers and intestinal parasitism: a pilot study of prevalence and health-related practices, and potential means of foodborne transmission," *J Food Prot,* vol. 73, pp. 985-8, May 2010.
[97] J. S. Ortiz, "The prevalence of intestinal parasites in Puerto Rican farm workers in western Massachusetts," *American Journal of Public Health,* vol. 70, pp. 1103-5, Oct 1980.
[98] J. L. Jones, P. Rion, S. Hollis, S. Longshore, W. B. Leverette, and L. Ziff, "Hiv-Related Characteristics of Migrant Workers in Rural South-Carolina," *Southern Medical Journal,* vol. 84, pp. 1088-1090, Sep 1991.
[99] CDC, "Prevention and Control of Tuberculosis in Migrant Farm Workers Recommendations of the Advisory Council for the Elimination of Tuberculosis " *Morbidity Mortalality Weekly Report,* vol. 41, 1992.
[100] V. D. o. Health, "Final Report: Assessing the Health Status, Health Care Needs, and Barriers to Care For Migrant Farm Labor in Franklin, Addison, and Grand Isle Counties," February 2007.
[101] M. D. Russell, M. T. Correa, C. E. Stauber, and J. A. Kase, "North Carolina Hispanic Farmworkers and Intestinal Parasitism: A Pilot Study of Prevalence and Health-Related Practices, and Potential Means of Foodborne Transmission," *Journal of Food Protection,* vol. 73, pp. 985-988, May 2010.
[102] K. E. Emmi, J. M. Jurkowski, N. Codru, E. M. Bell, M. A. Kacica, and T. P. Carter, "Assessing the Health of Migrant and Seasonal Farmworkers in New York State: Statewide Data 2003-2005," *Journal of Health Care for the Poor and Underserved,* vol. 21, pp. 448-463, May 2010.
[103] E. Hansen and M. Donohoe, "Health issues of migrant and seasonal farmworkers," *Journal of Health Care for the Poor and Underserved,* vol. 14, pp. 153-164, May 2003.

[104] D. Villarejo, D. Lighthall, D. Williams III, A. Souter, R. Mines, B. Bade*, et al.*, "Suffering in Silence: A report on the health of California's agricultural workers," The California Endowement2000.

## Appendices

**Table 4. Linear and Logistic regression models describing *E. coli* concentrations on produce (N=196)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | -1.16* | -1.67 | -0.65 | | | |
| **Hands** | 0.38* | 0.26 | 0.49 | 8.79* | 3.24 | 23.82 |
| **Jalapeño** | 0.03 | -0.50 | 0.57 | 0.61* | 0.14 | 2.68 |
| **Melon** | 2.74* | 2.27 | 3.22 | | | |
| **Tomato** | | | | Referent | | |
| **Packing Shed** | 2.03* | 1.48 | 2.58 | 90.17* | 17.99 | 451.87 |
| **Distribution** | 0.79* | 0.21 | 0.37 | 13.26 | 3.36 | 52.33 |
| **After Harvest** | | | | Referent | | |
| **Year 1** | 0.16 | -0.23 | 0.55 | 31.31* | 6.60 | 148.43 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 5. Linear and Logistic regression models describing fecal coliforms concentrations on produce (N=194)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 2.45* | 1.50 | 3.40 | | | |
| **Hands** | 0.55* | 0.42 | 0.67 | 4.37 | 0.54 | 35.52 |
| **Jalapeño** | -0.69* | -1.20 | -0.17 | 0.32 | 0.05 | 1.97 |
| **Melon** | 1.11* | 0.65 | 1.57 | 3.12 | 0.20 | 48.54 |
| **Tomato** | | | | Referent | | |
| **Packing Shed** | 0.23 | -0.28 | 0.74 | 0.79 | 0.04 | 16.35 |
| **Distribution** | -0.05 | -0.45 | 0.36 | 0.45 | 0.08 | 2.63 |
| **After Harvest** | | | | Referent | | |
| **Year 1** | -1.14* | -1.60 | -0.68 | 0.78 | 0.13 | 4.59 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 6. Linear and Logistic regression models describing *Enterococcus spp.* concentrations on produce (N=196)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 0.93* | 0.05 | 1.81 | | | |
| **Hands** | 0.57* | 0.45 | 0.69 | - | - | - |
| **Jalapeño** | 0.19 | -0.27 | 0.64 | 0.64* | 0.24 | 1.71 |
| **Melon** | 2.97* | 2.57 | 3.36 | 13.21* | 2.42 | 72.17 |
| **Tomato** | | | | Referent | | |
| | | | | 0.77* | 0.20 | 3.05 |
| **Packing Shed** | 0.11 | -0.34 | 0.56 | 0.56 | 0.21 | 1.50 |
| **Distribution** | -0.08 | -0.44 | 0.29 | | | |
| **After Harvest** | | | | Referent | | |
| | | | | 0.09 | 0.02 | 0.46 |
| **Year 1** | -1.21* | -1.55 | -0.86 | | | |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 7. Linear and Logistic regression models describing somatic coliphage concentrations on hands and produce (N=143)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 0.57 | -0.08 | 1.22 | | | |
| **Hands** | 0.17* | 0.03 | 0.31 | 6.75* | 2.44 | 18.67 |
| **Jalapeño** | 0.01 | -0.57 | 0.59 | 0.50 | 0.16 | 1.59 |
| **Melon** | 2.39* | 1.86 | 2.92 | 1.42 | 0.45 | 4.53 |
| **Tomato** | | | | Referent | | |
| | | | | 5.25 | 0.80 | 34.27 |
| **Packing Shed** | 0.79* | 0.20 | 1.39 | 1.20 | 0.45 | 3.21 |
| **Distribution** | 0.17 | -0.30 | 0.63 | | | |
| **After Harvest** | | | | Referent | | |
| | | | | 0.93 | 0.29 | 3.01 |
| **Year 1** | -0.02 | -0.58 | 0.55 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 8. Linear and Logistic regression models describing *E. coli* concentrations on produce (N=84)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | -0.04 | -0.79 | 0.71 | | | |
| **Soil** | 0.40* | 0.04 | 0.77 | 0.90 | 0.17 | 4.74 |
| **Jalapeño** | 0.25 | -0.65 | 1.15 | 0.91 | 0.18 | 4.71 |
| **Melon** | 2.66* | 1.81 | 3.51 | 5.56* | 1.15 | 26.90 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -0.09 | -0.87 | 0.69 | 58.14* | 3.21 | >999.9 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 9. Linear and Logistic regression models describing fecal coliforms concentrations on produce (N=82)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 5.87* | 5.11 | 6.64 | | | |
| **Soil** | 0.04 | -0.20 | 0.29 | 1.95 | 0.06 | 66.08 |
| **Jalapeño** | -1.00* | -1.75 | -0.26 | 0.47 | 0.06 | 3.72 |
| **Melon** | 1.73* | 1.12 | 2.34 | 4.87 | 0.21 | 114.30 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -1.94* | -2.52 | -1.36 | 0.26 | 0.02 | 3.48 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 10. Linear and Logistic regression models describing *Enterrococcus spp.* concentrations on produce (N=84)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 3.73* | 2.86 | 4.60 | | | |
| **Soil** | 0.15 | -0.16 | 0.46 | 2.28 | 0.51 | 10.23 |
| **Jalapeño** | -0.06 | -0.87 | 0.76 | 0.75* | 0.19 | 3.06 |
| **Melon** | 3.99* | 3.30 | 4.67 | 24.51* | 1.32 | 454.49 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -1.19* | -1.84 | -0.54 | 0.07 | 0.003 | 1.25 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 11. Linear and Logistic regression models describing somatic coliphage concentrations on produce (N=62)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 1.05* | 0.17 | 1.94 | | | |
| **Soil** | -0.08 | -0.41 | 0.24 | 6.71 | 0.77 | 58.64 |
| **Jalapeño** | 1.34* | 0.53 | 2.16 | 13.83 | 0.63 | 303.08 |
| **Melon** | 3.23* | 2.58 | 3.88 | 9.81 | 1.47 | 65.52 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -0.62 | -1.48 | 0.24 | 0.16 | 0.006 | 4.04 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 12. Linear and Logistic regression models describing *E. coli* concentrations on produce (N=75)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 0.31 | -1.02 | 0.41 | | | |
| **Irrigation** | -0.45* | -0.89 | -0.01 | 1.69 | 0.40 | 7.07 |
| **Jalapeño** | -0.18 | -1.17 | 0.82 | 1.74 | 0.21 | 14.71 |
| **Melon** | 2.19* | 1.42 | 2.97 | 7.48* | 1.41 | 39.74 |
| **Tomato** | | | | Referent | | |
| **Year 1** | 0.61 | -0.14 | 1.35 | 52.43* | 3.08 | 891.42 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 13. Linear and Logistic regression models describing fecal coliforms concentrations on produce (N=72)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 6.09* | 5.46 | 6.72 | | | |
| **Irrigation** | 0.12 | -0.08 | 0.32 | 2.39 | 0.04 | 159.0 |
| **Jalapeño** | -0.50 | -1.25 | 0.25 | 0.20 | 0.009 | 4.42 |
| **Melon** | 1.50* | 0.91 | 2.10 | 1.70 | 0.05 | 60.85 |
| **Tomato** | | | | Referent | | |
| **Year 1** | -2.09* | -2.61 | -1.56 | 0.46 | 0.03 | 8.19 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 14. Linear and Logistic regression models describing *Enterococcus spp.* concentrations on produce (N=74)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 4.24* | 3.55 | 4.93 | | | |
| **Irrigation** | 0.16 | -0.13 | 0.44 | 1.49 | 0.12 | 19.04 |
| **Jalapeño** | -0.15 | -1.08 | 0.77 | 1.02 | 0.18 | 5.81 |
| **Melon** | 3.93* | 3.21 | 4.65 | 32.36* | 1.89 | 554.90 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -1.77* | -2.47 | -1.07 | 0.05* | 0.003 | 0.76 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 15. Linear and Logistic regression models describing somatic coliphage concentrations on produce (N=46)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 1.29* | 0.32 | 2.26 | | | |
| **Irrigation** | -0.12 | -0.33 | 0.10 | 0.83 | 0.10 | 6.80 |
| **Jalapeño** | 1.42* | 0.35 | 2.49 | 5.52 | 0.20 | 152.60 |
| **Melon** | 3.13* | 2.32 | 3.93 | 5.26 | 0.58 | 47.77 |
| **Tomato** | | | Referent | | | |
| **Year 1** | -0.65 | -1.41 | 0.11 | 0.20 | 0.01 | 3.34 |
| **Year 2** | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 16. Linear and Logistic regression models describing *E. coli* concentrations on produce (N=82)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | -0.28 | -0.91 | 0.35 | | | |
| **Source** | 0.03 | -0.25 | 0.31 | 0.31 | 0.06 | 1.57 |
| **Jalapeño** | 0.09 | -0.63 | 0.82 | 0.63 | 0.09 | 4.54 |
| **Melon** | 2.68* | 1.97 | 3.39 | 2.59 | 0.41 | 16.38 |
| **Tomato** | | | | Referent | | |
| **Year 1** | 0.47 | -0.11 | 1.05 | 60.81* | 3.35 | >999.99 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 17. Linear and Logistic regression models describing fecal coliforms concentrations on produce (N=75)**

| | Linear model | | | Logistic model | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | **OR** | **95% CL** | |
| **Intercept** | 6.36* | 5.57 | 7.15 | | | |
| **Source** | 0.26 | -0.03 | 0.55 | 5.26 | 0.08 | 330.95 |
| **Jalapeño** | -0.76* | -1.56 | 0.03 | 0.52 | 0.06 | 4.37 |
| **Melon** | 1.20* | 0.43 | 2.0 | 2.91 | 0.15 | 58.25 |
| **Tomato** | | | | Referent | | |
| **Year 1** | -2.62* | -3.24 | -2.00 | 0.15 | 0.01 | 2.36 |
| **Year 2** | | | | Referent | | |

*** = Significant at the 0.05 level**

**Table 18. Linear and Logistic regression models describing *Enterococcus spp.* concentrations on produce (N=79)**

| | Linear model | | | | Logistic model | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | | **OR** | **95% CL** | |
| **Intercept** | 4.81* | 4.05 | 5.57 | | | | |
| **Source** | 0.13 | -0.37 | 0.63 | | 0.23 | 0.007 | 7.58 |
| **Jalapeño** | -0.28 | -1.18 | 0.62 | | 0.87 | 0.20 | 3.83 |
| **Melon** | 3.34* | 2.47 | 4.21 | | 13.92 | 0.73 | 264.60 |
| **Tomato** | | | | Referent | | | |
| **Year 1** | -1.82* | -2.57 | -1.08 | | 0.03* | 0.002 | 0.43 |
| **Year 2** | | | | Referent | | | |

*** = Significant at the 0.05 level**

**Table 19. Linear and Logistic regression models describing somatic coliphage concentrations on produce (N=40)**

| | Linear model | | | | Logistic model | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Beta** | **95% CL** | | | **OR** | **95% CL** | |
| **Intercept** | 1.79* | 0.55 | 3.03 | | | | |
| **Source** | -0.08 | -0.35 | 0.18 | | 1.62 | 0.22 | 12.26 |
| **Jalapeño** | 0.96 | -0.11 | 2.03 | | 9.45 | 0.33 | 270.18 |
| **Melon** | 2.35* | 1.34 | 3.35 | | 2.64 | 0.34 | 20.28 |
| **Tomato** | | | | Referent | | | |
| **Year 1** | -0.71 | -1.70 | 0.28 | | 0.11 | 0.006 | 1.92 |
| **Year 2** | | | | Referent | | | |

*** = Significant at the 0.05 level**