ABSTRACT

Title of Thesis: EVALUATION OF PUBLIC HEALTH RISK FOR *ESCHERICHIA COLI* O157:H7 IN CILANTRO

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The supply chain of cilantro was modeled for growth and die-off of *Escherichia coli* (*E. coli*) O157:H7 from infield and harvesting, transportation and storage and ultimately consumption at home. Using Visual Basic for Applications (VBA) macros and @RISK software, a simulation model was developed for exposure and estimation of illnesses. Test scenarios were modeled to determine the relative importance of different factors on the risk of illness. The developed model was simulated using Monte Carlo technique and Latin Hypercube sampling for 100,000 iterations.

Results showed an increase in the mean *E. coli* O157:H7 concentration along the supply chain for cilantro grown in both winter and summer weather conditions. In the winter, the mean pathogen concentration increased from 5.6×10^{-5} CFU/g to 24.7 CFU/g from after harvest to after home storage, respectively. In summer conditions, the mean pathogen concentration increased from 3.2×10^{-4} CFU/g to 5.2×10^{-2} CFU/g. The inner quartile ranges (IQRs) for the same model conditions showed a decrease in *E. coli* O157:H7 concentration along the supply chain for cilantro grown in both winter and summer weather conditions. This indicates a majority of situations result in a decrease in *E. coli* O157:H7

concentration along the supply chain however rare situations can occur where the concentration will increase greatly. With a prevalence of 0.1% *E. coli* O157:H7 contamination for cilantro post-harvest used for illustration, the model predicted the mean number of illnesses per year due to the consumption of *E. coli* O157:H7 contaminated cilantro in the United States as 86 and 164 for cilantro grown during winter and summer conditions, respectively.

Sensitivity analysis results indicated that transportation temperatures and quality of irrigation water had the largest impact on the number of illnesses per year. Scenario testing results for different risk factors demonstrated the importance of limiting and reducing cross contamination along the production chain, especially at higher initial prevalence levels and preventing temperature abuse during transportation from farm to retail, when reducing overall risk of illness. The developed risk model can be used to estimate the microbiological risks associated with *E. coli* O157:H7 in cilantro and determine areas along the supply chain with the most effect on the final concentration per serving for future mitigation strategies.

EVALUATION OF PUBLIC HEALTH RISK FOR *ESCHERICHIA COLI* O157:H7 IN CILANTRO

By

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
CDF	Cumulative Density Functions
CFU	Colony Forming Unit
E. coli	*Escherichia coli*
FDA	U.S. Food and Drug Administration
FDOSS	Foodborne Disease Outbreak Surveillance System
FSMA	Food Safety Modernization Act
GAP	Good Agricultural Practices
HUS	Hemolytic Uremic Syndrome
IQR	Inner Quartile Range
MPD	Maximum Population Density
N/a	Not Available
QMRA	Quantitative Microbial Risk Assessment
RTE	Ready-to-Eat
U.S.	United States
WHO	World Health Organization

Chapter 1: Introduction

An increased number of foodborne disease outbreaks have been associated with fresh produce and herbs during the past decade as the consumption of these products has increased. From 1996 to 2015, the United States (U.S.) Food and Drug Administration (FDA) reported nine foodborne outbreaks linked to basil, parsley and cilantro, which resulted in 2,699 illnesses and 84 hospitalizations (FDA, 2018). One such herb outbreak was attributed to *Escherichia coli* O157:H7. Additionally, in 2016, cilantro was implicated in an *E. coli* O157:H7 outbreak resulting in 96 illnesses and 19 hospitalizations (IDPH, 2016).

Ready-to-eat (RTE) produce, particularly herbs like cilantro, are often added as one of many ingredients in dishes making it difficult to identify the cause of an illness, and typically requires no heat preparation or kill step before consumption. Quantitative microbial risk assessments (QMRA) using predictive microbiology and sensitivity analyses have proved useful to assist managing food safety risks along the supply chain. QMRA can help RTE manufacturers focus their attention and resources in the most effective ways toward reducing contamination and cross-contamination. The ultimate goal of this study was to develop a risk model for *E. coli* O157:H7 in fresh cilantro and evaluate the areas with the highest effect on microbiological contamination along the supply chain. This will allow industry and regulatory agencies to focus on specific areas along the supply chain where potential intervention strategies can be applied to reduce public health risks.

Chapter 2: Literature Review

2.1 *Escherichia coli* O157:H7

Escherichia coli belong to the Gram-negative Enterobacteriaceae family and are facultative anaerobic short rod shaped bacteria (ICMSF, 1996). Strains of *E. coli* differ serologically based on somatic, flagellar, and capsular antigens (ICMSF, 1996). *E. coli* was first identified as a pathogen in 1982 when it was associated with two foodborne outbreaks (Riley et al., 1982). These bacteria naturally occur in the intestinal track of some cattle and become inhabitants of the intestinal tract of other symptomatic and asymptomatic carriers, including humans (ICMSF, 1996). *E. coli* spread through fecal-oral transmission, meaning an infected animal will shed the bacteria in their stool and other animals may become infected through unintentional consumption of the feces.

While most *E. coli* are harmless, there are six categories of pathogenic *E. coli* (pathotypes) that cause illness and diarrhea, collectively referred to as diarrheagenic *E. coli* (CDC, 2014). These six pathotypes are (1) shiga toxin-producing, (2) entrotoxigenic, (3) enteropathogenic, (4) enteroaggregative, (5) enteroinvasives, and (6) diffusely adherent *E. coli*. The model created in this study will focus only on the pathogenic shiga toxin-producing *E. coli* O157:H7 which has been a significant food safety concern in the U.S. Infection symptoms vary but often include stomach cramps, diarrhea, and vomiting (CDC, 2014). Most people recover within 5–7 days after the first symptoms, however some people develop severe or life-threatening symptoms. Five to ten percent of diagnosed individuals develop hemolytic uremic syndrome (HUS), usually resulting in hospitalization and kidney damage or failure (CDC, 2014).

While there are multiple shiga toxin producing *E. coli* (non-O157, O157:H7 and more), *E. coli* O157:H7 is the most commonly identified shiga toxin–producing *E. coli* in North America. *E. coli* O157:H7 is estimated to cause 63,153 cases of foodborne illnesses, 2,138 hospitalizations, and 20 deaths in the U.S. per year (FDA, 2012a; Scallan et al., 2011a). Despite its animal origins and historical association with ground beef products, a large portion of *E. coli* O157:H7 multistate outbreaks in the last 13 years have implicated produce, including romaine lettuce, RTE salads, sprouts, spinach and varying or unknown food vectors from multiple Mexican style restaurants (CDC, 2018a).

2.2 Foodborne illness

2.2.1 General burden

In 2011, the Centers for Disease Control and Prevention (CDC) reported that, foodborne pathogens and/or unspecified agents cause an estimated 48 million foodborne illnesses, 128,000 hospitalizations, and 3,000 deaths each year in the U.S., leading to an estimated economic loss of $77.7 billion per year (Scallan et al., 2011a,b; Scharff, 2011). Of these illnesses, hospitalizations and deaths, 20%, 44% and 44%, respectively can be contributed to 31 known pathogens (Scallan et al., 2011a). Of the hospitalizations due to domestically acquired foodborne illnesses, 4% is attributed to *E. coli* O157:H7 (Scallan et al., 2011a).

The CDC began publishing annual summaries of foodborne disease outbreaks [two or more illnesses attributed to the same contaminated food or beverage] based on information provided by state and local health department in 2011 (CDC, 2017). The most recent Surveillance for Foodborne Disease Outbreaks annual report indicated that there were 839 foodborne disease outbreaks reported in 2016, resulting in 14,259 illnesses, 875 hospitalizations, and 17 deaths. Again shiga toxin-producing *E. coli* (including serogroup O157:H7) was one of the leading causes of hospitalizations (CDC, 2018b). It is important to remember that these are just the foodborne outbreaks reported, many more illnesses go unreported annually and isolated/sporadic illnesses are not included in this survey. This can explain the large disparity in numbers compared to the Scallan et al. (2011) annual estimates.

The CDC additionally publishes data estimates through the Foodborne Diseases Active Surveillance Network (FoodNet) which has conducted public health surveillance on *Campylobacter*, *Salmonella*, *Listeria*, shiga-toxin producing *E. coli* O157, *Vibrio*, and *Yersinia* since 1996; *Cryptosporidium* and *Cyclospora* since 1997 and shiga-toxin producing *E. coli* non-O157 since 2000 (CDC, 2015). Currently, this information is based on laboratory-confirmed cases and cases diagnosed using culture-independent methods collected by FoodNet personnel at each testing site then transmitted to the CDC (CDC, 2015). In 2015, FoodNet identified 20,098 laboratory confirmed infections, as well as 4,598 hospitalizations and 77 deaths related to those infections. The number of shiga-toxin producing *E. coli* O157 infections per 100,000 persons in 2015 was reported to be 465 (FoodNet, 2015).

The overall incidence of infections in 2015 caused by CDC monitored pathogens has reduced an estimated 30% since the 1996-1998 surveillance (FoodNet, 2015). However the reduction in illness rates between the 2015 survey and the 2011-2014 survey periods have not been statistically significant (FoodNet, 2015). A similar reduction pattern was observed in *E. coli* O157, also with a statistically insignificant reduction since 2012 (FoodNet, 2015).

The FDA Food Safety Modernization Act (FSMA), which aims to protect public health by establishing a new modern food safety system through the focus on prevention of food safety hazards rather than response to issues, was signed into law on January 4, 2011 (FDA, 2011). However, many of the rules were not published until 2015 and most were not effective until 2016 (FDA. 2018b). Some rules are still not in effect for small scale business and farms (FDA. 2018b). The effects of FSMA on food safety and foodborne illness and outbreak rates will not be fully understood for a least a few more years.

2.2.2 Foodborne illness and outbreaks associated with ethnic foods and herbs

A report published in 2012 by Mintel (a research company) indicated that as the U.S. population becomes more diverse, it encourages the growth of the ethnic restaurant industry. U.S. retail sales of ethnic foods totaled around $11 billion in 2013 and were estimated to generate more than $12.5 billion by 2018 (Hartman, 2016). A majority of these sales come from Mexican/Hispanic and Asia/Indian influenced foods (Hartman, 2016). However with this increased accessibility, a greater number of foodborne illness outbreaks have been linked to ethnic foods. An examination of CDC foodborne illness data showed an increase in the percentage of outbreaks associated with ethnic foods from 3% in 1990 to 11% in 2000 (Simmone et al., 2004).

A study of foodborne illness outbreaks associated with Mexican cuisine showed that fresh vegetables and eggs were most often identified as the cause. Red salsa, especially, was related to 70 foodborne outbreaks (2,280 illness cases) from 1990 to 2006 (Franco and Simonne 2009). Another report analyzing the foodborne illness outbreak data from the CDC's Foodborne Disease Outbreak Surveillance System (FDOSS) from 1973 to 2008 identified 136 outbreaks (four attributed to *E. coli* O157:H7) associated with salsa or guacamole, two foods commonly containing cilantro and receive no heat step (Kendall et al, 2013). Of those outbreaks 84% were attributed to restaurants. All *E. coli* O157:H7 related outbreaks in this group were attributed to restaurants (Kendall et al, 2013). Fresh herbs, like cilantro, are often added as one ingredient in many, making it difficult to identify the cause of an illness, if one occurs. Lee (2012) pointed out that regardless of the type of ethnic cuisines, those containing cooked ingredients have fewer microbial quality and safety issues than those containing raw ingredients, such as cilantro.

From 1996 to 2015, the FDA reported nine foodborne outbreaks linked to basil, parsley and cilantro, which resulted in 2,699 illnesses and 84 hospitalizations (FDA, 2018). One such herb outbreak was attributed to *E. coli* O157:H7. From 1996 to 2017, the CDC has linked cilantro or cilantro containing products to 12 outbreaks (Table 1; CDC, 2018c). The largest outbreak attributed to cilantro as the only food vehicle occurred in 2016, when cilantro was implicated in a Chicago Mexican restaurant outbreak of *E. coli* O157:H7 resulting in 96 illnesses and 19 hospitalizations (IDPH, 2016). Again it's important to note that many of the food vehicles listed by the CDC include multiple ingredients, making it difficult to say if cilantro was the contaminated ingredient. Table 1 only lists the outbreaks where cilantro was specially mentioned as the food vehicle or contaminated ingredient. There could be many more where cilantro was one of many ingredients but was not listed in this database due to its difficulty to isolate the contamination to one ingredient. For example, during that same time period the CDC specifically linked 127 outbreaks to salsa, which commonly contains cilantro (CDC, 2018c). Three of the salsa outbreaks were attributed to shiga toxin-producing *E. coli*.

Table 1: CDC reported cilantro related bacterial outbreaks; 1996-2017

Year	Etiology	Serotype or Genotype	Illnesses	Food Vehicle	Food Contaminated Ingredient
1999	*Salmonella enterica*	Thompson	35	cilantro, unspecified	N/a
2001	*Salmonella enterica*	Newport	8	cilantro, unspecified	spices
2002	*Salmonella enterica*	Newport	13	cilantro, unspecified	N/a
2003	*Bacillus cereus; Clostridium perfringens*	N/a	20	cilantro, unspecified	N/a
2005	*Salmonella enterica*	Manhattan	5	sandwich, pork; cilantro, unspecified	N/a
2007	*Salmonella enterica*	Newport	46	tomato, unspecified; avocado, unspecified; guacamole, unspecified; cilantro, unspecified	N/a
2008	*Salmonella enterica*	Montevideo	101	cheese, unspecified; cilantro, unspecified; chicken, raw	N/a
2013	*Salmonella enterica*	Typhimurium	64	quesadillas; chicken; cheese; tortilla, unspecified; cilantro, unspecified	N/a*
2014	*Salmonella enterica*	Braenderup	12	steak (beef taco), cilantro, onions	N/a
2016	*Escherichia coli*, shiga toxin-producing	O157:H7	96	cilantro	N/a
2016	*Salmonella enterica*	Enteritidis	16	onions; cilantro, unspecified	onion; cilantro
2017	Unidentified	N/a	5	salsa	cilantro; green onion/scallion; onion; tomato; spices

*N/a: Not available

2.3. Cilantro

2.3.1 Consumption

According to the most recent CDC FoodNet Population Survey Atlas of Exposures conducted in 2006 and 2007, only about 17% of the U.S. population consumes fresh cilantro with the highest percentages of citizens eating cilantro in California at 34% of those surveyed and the lowest at 6.6% in New York (CDC, 2007). However, more recent studies have shown the consumption of herbs in the U.S. has grown dramatically in the last few years. A Neilsen Company study in 2016 determined that the herb markets had grown 23% in the U.S. from $237 million in sales in 2012 to $299 million in 2016 (Nielson, 2016). This could likely be attributed to the growing diversity in the U.S. population as well as the increased interest in ethnic food, as described in the previous section. With cilantro being one of the most popular herbs and spices in the U.S. (Cedar Hills, 2011), growing attention needs to be paid to the safety of this increasingly consumed commodity.

2.3.2 E. coli O157:H7 prevalence in cilantro

A World Health Organization (WHO) review conducted in 1998, found that 19.5% (n = 41) of cilantro samples taken in Mexico in 1995 were contaminated with *E. coli* O157:H7 (WHO, 1998). In contrast, a 2018 FDA microbiological surveillance of cilantro, parsley or basil identified no *E. coli* O157:H7 in any of the 276 foreign or 407 domestic samples (FDA, 2018). While these FDA results are promising, the WHO data and recent outbreaks highlight the ability of *E. coli* to contaminate and survive on cilantro and cause foodborne illness.

2.3.3 Production

Cilantro *(Coriandrum sativum)* are the fresh leaves of the coriander herb, sometimes called “Chinese parsley” or “Mexican parsley.” This fragrant annual is grown in nearly every country across the globe, due in part to its ability to grown in a wide range of climatic conditions (Smith et al., 2011). The largest growing regions are Mexico and California where cilantro can be grown year round, with Mexico being the number one exporter (Produce Blue Book, 2019). In the U.S., California is the top producer followed by Arizona, Oregon, and Washington. In California, cilantro is grown primarily along the south and central coast in Ventura, Monterey, Santa Barbara and San Benito counties (Smith et al., 2011).

When planted in the summer season, cilantro will mature within 40 to 45 days, in winter, maturity can take up to 55 days[1]. Optimal growth temperatures range from 50°F to 85°F (10°C to 30°C), anything warmer will cause the cilantro to bolt, or flower, which reduces the development of the desired foliage (Smith et al., 2011). However, full sun exposure is optimal for growth (Produce Blue Book, 2019). To keep the soil moist, most farmers will use overhead sprinklers for long periods of time every two days until the emergence of seedlings, after which most growers will continue to use sprinklers but likely would not water more than every five to six days[1] (Smith et al., 2011). While some growers may supplement the soil with applications of nitrogen, because of the progression of good agricultural practices (GAPs) and the concern for microbiological contamination, most

[1] Personal communication with Richard Smith, a Farm advisor in Vegetable Crop Production and Weed Science at UC Davis (2018).

growers will not apply manure as a fertilizer[1]. Additionally, most California soils have adequate availability of micronutrients when sustainable farming practices are followed (Smith et al., 2011).

Cilantro is ready to be harvested as soon as the plant is about four to six inches tall (World Crops, 2019). Harvesting is most often done by hand with small, rounded knifes to slice the plant at the ground level (Produce Blue Book, 2019). Bunches are then tied in the field and the bottom is cut in a straight line. Sometimes cilantro is harvested by machines, usually when the herb is destined for food service or future processing like drying (Produce Blue Book, 2019). Cilantro can be bunched in the field and placed into boxes or packed in plastic bags for food service (Smith et al., 2011).

Fresh cilantro intended for retail is usually loaded into refrigerated trucks on ice without further processing at temperatures between 34 and 36°F (1 and 2.2°C) and transported directly to the retail/grocery stores[2]. Transportation from production areas in California can take several hours for local deliveries and up to seven days for deliveries to the east coast and Canada[2]. Cilantro intended for food service is sometimes processed further with chemical washes, cutting and modified atmosphere packaging[2]. A typical shelf life for cilantro is 14 to 21 days at frozen temperatures with 95-100% humidity (PPOD, 2019). Modified atmospheric packaging to reduce the exposure to ethylene can extend the shelf life at warmer temperatures between 40° to 50°F (4°-10°C). However, this would just

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[2] Personal communication with a representative of grower, shipper and distributor of fresh produce, including cilantro, based in southern California (2019).

delay yellowing and plant decay, but not prevent microbial growth as some *E. coli* have been shown to grow at temperatures around 47°F (ICMSF, 1996).

Due to the limited available data and the variability in cilantro processing depending on the manufacturing and packing companies and customer requirements, the focus of this study is on cilantro intended for retail. A flow diagram of the growth, harvesting and production of retail cilantro and potential sources of contamination used in the exposure assessment for this model is provided in Figure 1. A further explanation of the exposure assessment is provided in the following section.

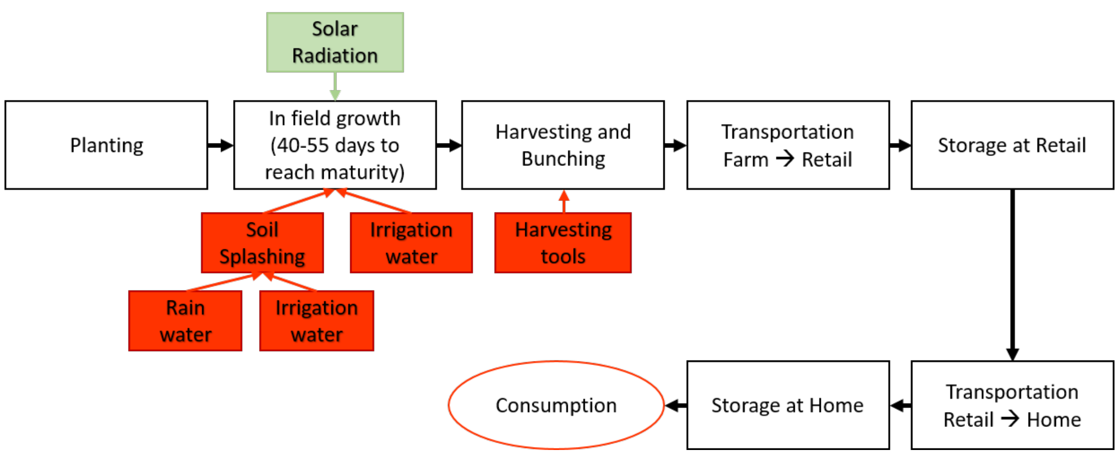

Figure 1: General production flow and framework of the QMRA model for E. coli O157:H7 in fresh cilantro. Red boxes indicate sources of E. coli O157:H7 contamination. Green boxes indicate sources of E. coli O157:H7 inactivation.

2.4 Potential sources of microbiological contamination and inactivation along cilantro supply chain

There are three main stages of retail cilantro production leading up to consumer consumption; infield production, harvesting and bunching, and transportation and storage. Each stage offers opportunities for contamination and growth of *E. coli* with very little chances for control or inactivation.

2.4.1 Infield

Cilantro and other fresh herbs are grown low to the ground year-round in moderate weather locations, like California, with multiple potential sources of microbial hazards. These sources include, but are not limited to, animal or human feces and cross contamination, irrigation water, and soil amendments (FAO/WHO, 2008). *E. coli* naturally occur in the intestinal track of some cattle and become inhabitants of the intestinal tract of other symptomatic and asymptomatic carriers, including humans and deer (ICMSF, 1996). This bacterium can spread through fecal-oral transmission. Infected deer or other grazing animals may contaminate cilantro through defecation if they gain access to the fields and asymptomatic infected humans may contaminate cilantro crops during harvest and handling if they have improperly washed their hands. The FDA recently indicated that the cilantro industry should pay particular attention to the quality of irrigation water; soil amendment and biosolids; animal management; worker health and hygiene; farm sanitation; transportation; and programs to monitor produce safety practices (FDA, 2016).

The relatively cool humid weather conditions in California is conducive for the growth and survival of *E. coli* (Takeuchi and Frank, 2000). If drier weather is to occur, as has been more common in California in the recent years, the increased requirement for additional water applications can introduce potential pathogens, either through possibly contaminated irrigation water or increased soil splashing caused by the popular use of sprinkler irrigation (FDA & WGA, 2013). Inadequate water quality has the potential to be a direct contaminate or spread contamination in the production of fresh produce crops (HHS, 1998). Although the identification of the point of contamination in a food is extremely difficult, studies have shown there is increasing evidence of contamination of produce by irrigation water in recent years (Uyttendaele et al., 2015; FDA, 2019).

Essential oils from both the seeds and leaves of the cilantro plant have antimicrobial properties as well as many other biological activities (Silva & Domingues, 2017). One study indicated that the minimum inhibitory effect of cilantro leaf and seed oil for *E. coli* O157:H7 was 0.125% and >2%, respectively (Kang and Song, 2018). Patel et al. (2018) observed a 0.5-1.5 log reduction of inoculated *E. coli* O157:H7 over two weeks after washing with distilled water; however, the focus of that study was the reduction of pathogens on cilantro using different plant based essential oils. The antimicrobial activity of this oil depends on the major constituents in the oil and their concentrations, which is affected by climatic conditions, growth region, vegetative state of the plant, and which part of the plant the oil is extracted *i.e.* seed versus leaf extracts (Msaada et al., 2009; Msaada et al, 2007; Telci et al., 2006; Silva & Domingues, 2017). Currently, there are no studies on the inactivation of small levels of *E. coli* O157:H7 on unharvested cilantro leaves due to the presence of its naturally occurring oil levels, rather than with concentrated oil extract.

2.4.2 Harvesting and bunching

Harvesting for retail is regularly performed by hand with small rounded knifes. Multiple studies have shown that *E. coli* O157:H7 can be transferred to fresh cut produce through blades and cutting during harvesting (Buchholz et al., 2014; Buhholz et al., 2012; Zilelidou et al., 2015). After harvesting, cilantro is bunched in the field by hand then packaged in boxes, increasing the risk of microbial cross contamination to uncontaminated bunches from workers hands and already contaminated bunches.

2.4.3. Transportation and storage

Time and temperature during transportation and storage (at both retail and at home) are factors that affect the microbiological safety of fresh cilantro. Fresh cilantro is stored on ice in refrigerated trucks for up to seven days during the transportation from farm to retail. Just like irrigation water, improperly or untreated ice water can spread existing contamination as well as be a source. During storage and transportation, the temperatures should be maintained at low levels (< 41°F or 5°C) to prevent the growth of human pathogens. However *E. coli* O157:H7 does survive at refrigerated temperatures, further emphasizing the importance of preventing contamination infield (Uyttendaele et al., 2001).

2.5 Quantitative microbial risk assessment

Although popular and used as a means of verification, the direct screening for pathogens by processors through ingredient and finished product testing can lead to a false sense of safety when no pathogens are detected. This is because pathogen contamination on leafy greens is considered to be rare (EFSA, 2014) and contamination is likely low in concentration and non-uniform in distribution, meaning although an ingredient or finished product tested negative for the presence of pathogens, low levels may be present in the un-sampled portion. Relying only on pathogen screening could likely lead to the release of contaminated product. This is why it is important for processors to look at their production as a whole from receiving ingredients to processing and packaging when trying to assess the potential for contamination in order to prevent it rather than find it after the fact.

With the introduction of FSMA and the increased focus on prevention, fresh culinary herb producers have begun to support the implementation of food safety programs that utilize risk assessment techniques (FDA & WGA, 2013). One such risk assessment technique is QMRA. A proper QMRA, according to the Codex Alimentarius Commission, consists of a hazard identification of the pathogenic microorganism, an exposure assessment to determine the amount of microorganism consumed or inhaled, a dose-response assessment determining if illness will occur based on dose and finally risk characterization that integrates all the information to estimate public harm (Codex Alimentarius, 1999). The goal of a QMRA is to provide adequate scientific evidence for industry and regulatory agencies to make risk management decisions to ultimately decrease the risk to public health. QMRA, which generates estimates of risk from consumption of a certain pathogen in a certain food or food category can also be used to evaluate where in the supply chain that risk is most attributed through sensitivity analyses. These evaluations provide highly valuable information to both regulatory agencies and industries in order to focus their time, attention and resources into the most effective areas for risk mitigation.

Several studies have developed QMRA for enteric pathogens, including *E. coli* O157:H7, in leafy greens at field level (Franz et al., 2008; McKellar et al., 2014, Allende et al., 2017) and for the entire farm-to-consumption supply chain (Danyluk and Schaffner, 2011; Pang et al., 2017). Limitations of these studies and most QMRA models for farm-to-fork consumption includes the difficulty associated with the low prevalence of foodborne pathogens making validation of these models using experimental data impossible

(McKellar et al., 2014; De Keuckelaere et al., 2015). Many assumptions are required to develop these models because data for specific parameters are lacking such as pathogen transfer from irrigation water to crops, pathogen penetration, and survival in or on food crops (De Keuckelaere et al., 2015). Because of this, many studies repeat unproven assumptions (De Keuckelaere et al., 2015).

A review of recently published scientific literature, found no QMRA models specifically targeting the contamination, growth and die-off of *E. coli* O157:H7 in cilantro or herbs from farm-to-fork in the U.S. However one Indian study by Kundo et al. (2018), evaluated the risks of diarrheal disease from consumption of cilantro and other herbs collected from local markets. While it determined that there was a 59% likelihood of exhibiting diarrheal symptoms from consuming cilantro from Indian markets, it focused on contaminated products taken directly from the market and reduction of the risk of illnesses by post-harvest washing and disinfection (Kundo et al., 2018). Likely due to a lack of data and information, the Kundo et al. (2018) QMRA model did not include contamination from environmental sources, such as irrigation water and soil or harvesting tools which could affect the level of contamination with *E. coli* O157:H7 prior to retail sale. Because cilantro in the U.S. is usually not further processed after harvest and bunching or prior to retail sale, more focus needs to be on infield contamination.

Currently, there is a need for a QMRA model incorporating both growth and die-off of *E. coli* O157:H7 from different environmental sources in the field and processing along the entirety of the fresh cilantro supply chain to: (1) provide estimates of contamination levels along different stages of the supply chain; (2) provide estimates of the expected risks of *E. coli* O157:H7 illnesses from consumption of a serving of fresh cilantro in the U.S.; and (3) identify the most important factors affecting the frequency of contamination and the growth of *E. coli* O157:H7 in fresh cilantro and the number of illness cases due to its consumption.

Chapter 3: Research Objectives

The aim of this study was to develop a QMRA model to evaluate the public health risks associated with consumption of a serving of cilantro contaminated with *E. coli* O157:H7 in the U.S., and to evaluate growth and post-harvest factors affecting illness numbers.

Specifically, the objectives of this study were to:

1. Develop a QMRA model to estimate the risks of *E. coli* O157:H7 illnesses in the U.S. population due to consumption of a serving of fresh cilantro.

2. Provide estimates of contamination levels of *E. coli* O157:H7 in fresh cilantro along different stages of the supply chain.

3. Perform sensitivity analyses to identify important factors affecting the number of illness cases from *E. coli* O157:H7 and gain insights into areas where future mitigation strategies should be applied in the production of fresh cilantro in order to protect consumers.

4. Perform scenario analyses for different risk situations to evaluate their effects on the probability of human illnesses per serving of cilantro and the number of illnesses per year from the consumption of cilantro.

Chapter 4: Methods

4.1 Overview

The QMRA model developed in this study is a description of *E. coli* O157:H7 contamination of fresh cilantro in field, the growth and inactivation of that contamination along the supply chain, and the risk of illness per serving upon consumption. The stochastic model considers that *E. coli* O157:H7 contamination can occur via sub quality irrigation water, soil splash from rain and sprinkler irrigation, and contamination via harvesting tools. Wildlife contamination is generally random and unpredictable, making quantification of it difficult (Liu et al., 2013). Contamination coming from wildlife and other routes including soil amendments and harvest workers were not considered in this QMRA model due to lack of available data. Cilantro's ability to grow well in direct sunlight was accounted for by including solar inactivation of *E. coli* O157:H7 during sunlight hours on days without rain. Because of the variety of parameters that affect the antimicrobial activity of cilantro oil and the limited information available on inactivation of small levels of *E. coli* on cilantro leaves due to the oil, inactivation caused by cilantro oil was not considered for this model.

Production stages included in this model are shown in Figure 1. Due to the limited information from industry and varying levels of additional processing by individual cilantro producers, this model only includes cilantro intended for retail and not food service which is sometimes sold with additional post-harvest processing.

During the storage and transportation stages, time and temperature were modeled to demonstrate the growth and inactivation of *E. coli* O157:H7 likely to occur prior to arriving at the consumer. Once at the consumer, this study used a dose-response model using the level of contamination at consumption estimated by the exposure assessment to determine probability of illness based on a serving of cilantro. From there the number of illnesses per the U.S. population was determined using the percent of the population known to consume cilantro. Table 2 provides a summary of the variables and parameters considered in this QMRA model.

Due to limitations in available information and situations unknown, not all potential risk and inactivation factors are included in this model therefore the quantitative outcome will not be able to predict the entire situation of the *E. coli* O157:H7 contamination in fresh cilantro. Potential contamination factors omitted from this model include introduction of animal and/or human feces, improperly treated biological soil amendments, improperly treated ice, cross contamination caused by bulk packaging, recirculated air in the refrigerated trucks, and retail and home handling. The potential inactivation due to the antimicrobial activity of coriander leaf oil was also not considered in this model.

Table 2: Overview of variables, point-estimate values, statistical distributions, and formulas used in the QMRA model for the summer season

Parameters	Symbol	Description, Value or Formula	units	Source
Growth				
Growth time	Day_{lim}	=ROUND(RiskUniform(40,45),0)	days	Personal Communication[a]
Number of days cilantro are watered via sprinkler irrigation	Irr_{lim}	=ROUND((Day_{lim})/5.5,0)	days	Personal Communication[a]
Number of rainy days	$Rain_{lim}$	=RiskPert(0,2.6,8)	days	NCDC, 2019
Average soil *E. coli* concentration	Cs	=10^RiskNormal(0.44,0.79,RiskTruncate(0,))	CFU/g	Lenehan et al., 2005
Prevalence of *E. coli* in soil	$Prev_{Soil}$	=RiskBinomial(1,0.37)	%	Holvoet et al., 2013
Likely *E. coli* concentration. If detected 1, if not 0	Eco_{Soil}	=IF($Prev_{Soil}$ =1, Cs, 0)	CFU/g	Calculated
E. coli O157:H7 ratio to *E. coli* in soil	Rs	=10^RiskNormal(-1.9,0.6,RiskTruncate(,0))	--	Ottoson et al., 2011
E. coli concentration in irrigation water	Cw	=RiskUniform(1,235)	CFU/100mL	LGMA, 2016
E. coli O157:H7 ratio to *E. coli* in irrigation water	Rw	=10^RiskNormal(-1.9, 0.6,RiskTruncate(,0))	--	Ottoson et al., 2011
Amount of water transferred to the plant during irrigation	Irr_{trans}	=RiskUniform(1.8,21.6)	mL/g produce	Allende et al., 2017
Splashing caused by irrigation	Irr_{Splash}	=RiskPert(0.02,0.04,0.06)	--	Allende et al., 2017
Splashing caused by rain	$P_{Rsplash}$	=1	--	Allende et al., 2017
Soil transfer from splashing	$Soil_{Trans}$	=RiskBetaGeneral(0.4,0.8,.05,16.4)	g /g prod	Allende et al., 2017
Bacteria transferred from soil to plant from irrigation	Bac_{irr}	=RiskUniform(0.35,0.9)	--	Allende et al., 2017
Bacteria transferred from soil to plant from rain	Bac_{rain}	=RiskUniform(0.35,0.9)	--	Allende et al., 2017
Sun hours per day	Sun_{hrs}	=RiskPert(8,12,13)	h	Holiday Weather, 2019
Daily *E. coli* O157:H7 concentration increase due to irrigation water	C_{Irr}	=(Cw/100*Rw*Irr_{trans})	CFU/g	Calculated
Daily *E. coli* O157:H7 concentration increase due to rain splashing	$C_{RSplash}$	=(Eco_{Soil} *Rs* $Soil_{Trans}$* Bac_{rain})* $P_{Rsplash}$	CFU/g	Calculated
Daily *E. coli* O157:H7 concentration increase due to irrigation splashing	C_{IrrS}	=(Eco_{Soil} *Rs* $Soil_{Trans}$* Bac_{irr})* Irr_{Splash}	CFU/g	Calculated
E. coli die-off on the plant	S_{Inact}	=10^(−0.52 ∗ (Sun_{hrs} / 24))	--	Ottoson et al., 2011

E. coli O157:H7 concentration on plant by harvest	C_{field}	Created VBA function	CFU/g	Calculated
Harvesting/Bunching/Packing				
Transfer rate from contaminated cilantro to sterile blade	$R_{c\text{-}b}$	=RiskUniform(0,0.13)	%	Zilelidou et al., 2015 & Pang et al., 2017
Transfer rate from harvesting blades to cilantro	$R_{c\text{-}b}$	= 0.13	%	Yang et al., 2012 & Pang et al., 2017
E coli O157:H7 transferred from contaminated blades to cilantro	$N_{h\text{-}c}$	= C_{field}*($R_{c\text{-}b}$/100) * ($R_{c\text{-}b}$/100)	CFU	Calculated
Concentration increase of *E coli* O157:H7 in cilantro from harvesting blades	$C_{harvest}$	=$N_{h\text{-}c}$/(32*4)	CFU/g	Zilelidou et al., 2015 & Pang et al., 2017
Initial concentration prior to bunching	$Conc_{FH}$	= C_{field} + $C_{harvest}$	CFU/g	Calculated
Transportation—Harvest to retail				
Transportation time	t_{FR}	=Riskuniform(0.5,168)	h	Personal Communication[b]
Temperature during transportation	$Temp_{FR}$	=RiskUniform(0,2.2)	°C	Smith et al., 2011 & Personal Communication[b]
Storage at				
Retail storage time	t_R	=RiskTriang(0.5,4,7)x24	h	Pang et al., 2017
Retail storage temperature	$Temp_R$	=RiskNormal(4.4441,2.9642,RiskTruncate(0, 20.56))	°C	EcoSure, 2008
Transportation—retail to home				
Transportation time	t_{RH}	=RiskLognorm(1.421,0.46478,RiskTruncate(0 .1833,3.8667),RiskShift(-0.24609))	h	Pang et al., 2017; EcoSure, 2008
Temperature before putting in home refrigerator	$Temp_{bH}$	=RiskNormal(8.386,3.831,RiskTruncate(0, 20))	°C	EcoSure, 2008
Temperature during transportation	$Temp_{RH}$	=1/2 x ($Temp_R$ + $Temp_{bH}$)	°C	Calculated
Home/retail Storage				
Time to first (home storage)	tf	=RiskWeibull(1.13,2.84)*24	h	Pouillot et al., 2010
Time to last (home storage)	tl	=RiskWeibull(1.7,7.96)*24	h	Pouillot et al., 2010
Time selected (home storage)	t_H	=1/2 x (tf +tl)	h	Calculated
Home storage temperature	$Temp_H$	=RiskNormal(3.4517,2.4442,RiskTruncate(-5,17.22))	°C	EcoSure, 2008

Growth/die-off parameter				
Growth model parameter	b	=0.023	--	McKellar and Delaquis, 2011
Growth model temperature minimum	T_{min}	=1.335-5.766 X b	°C	McKellar and Delaquis, 2011
Die-off rate	k	=RiskLognorm(0.013,0.001,RiskShift(0.001)) /2.303	log CFU/g/h	McKellar and Delaquis, 2011
Retail growth rate	μ_R	=(b x ($Temp_R$- T_{min}))^2/2.303	log CFU/g/h	Calculated
To retail transportation growth rate	μ_{RTran}	=(b x ($Temp_{FR}$ - T_{min}))^2/2.303	log CFU/g/h	Calculated
To home transportation growth rate	μ_{HTran}	=(b x ($Temp_{RH}$ - T_{min}))^2/2.303	log CFU/g/h	Calculated
Home growth rate	μ_H	=(b x ($Temp_H$ - T_{min}))^2/2.303	log CFU/g/h	Calculated
Growth/die-off calculation				
Growth or die-off during transportation from harvest	Q_{tran1}	=IF($Temp_{FR}$ >5,1,0)	--	Calculated
Change in concentration during transportation from harvest	G_{tran1}	=IF(Q_{tran1}=1,μ_R*t_R,-k*t_R)	log CFU/g	Calculated
Concentration of *E. coli* O157:H7 after transportation from harvest	C_{tran1}	=$LogConc_{FH}$+G_{Tran1}	log CFU/g	Calculated
Growth or die-off during retail storage	Q_R	=IF($Temp_R$ >5,1,0)	--	Calculated
Change in concentration during retail storage	G_R	=IF(Q_R=1,μ_R*t_R,-k*t_R)	log CFU/g	Calculated
Concentration of *E. coli* O157:H7 after retail storage	C_R	=C_{tran1}+G_R	log CFU/g	Calculated
Growth or die-off during transportation from retail	Q_{tran2}	=IF($Temp_{RH}$ >5,1,0)	--	Calculated
Change in concentration during transportation from retail	G_{tran2}	=IF(Q_{tran2}=1,μ_R*t_R,-k*t_R)	log CFU/g	Calculated
Concentration of *E. coli* O157:H7 after transportation from retail	C_{tran2}	=C_R+G_{Tran2}	log CFU/g	Calculated
Growth or die-off during home storage	Q_H	=IF($Temp_H$>5,1,0)	log CFU/g	Calculated
Change in concentration during home storage	G_H	=IF(Q_H=1,μ_R*t_R,-k*t_R)	log CFU/g	Calculated
Concentration of *E. coli* O157:H7 after home storage	C_H	=C_{Tran2}+G_H	log CFU/g	Calculated
Limit of contamination level	L	=IF(C_H <7, C_H,7)	log CFU/g	Calculated

Concentration after home storage	C_H	=Power(10,L)	CFU/g	Calculated
Serving				
Serving size	Ser	=RiskTriang(0.8,2,32)	g	Nutritionix, 2019
Dose per contaminated serving	D	$=C_H$*Ser	CFU/serving	Calculated
Dose Response				
Prevalence of contamination	$prev_0$	0.1	%	Pang et al., 2017 & Danyluk & Schaffner, 2011
Dose-response parameter - α	α	=0.267	--	Cassin et al., 1998
Dose-response parameter - β	β	=229.2928	--	Cassin et al., 1998
Probability of illness per serving	P	$=(1-(1+D/\beta\hat{}(-\alpha))*prev_0$	--	Cassin et al., 1998
Risk Characterization				
U.S. population	N_{pop}	=328,355,826	people	DOC-Census Bureau, 2019
% of U.S. population that consumes cilantro	N_{cil}	=RiskTriang(6.6,17,34.1)	%	CDC, 2007
Number of servings consumed per person per year	N_P	=52	--	CDC, 2007
Number of servings consumed per year in United States	N_{CS}	$=N_{pop}$*(Ncil/100)*N_P	--	Calculated
Number of illness cases per year	N_{cases}	$=N_{CS}$*P	Cases/yr	Calculated

[a] Personal communication with Richard Smith, a Farm advisor in Vegetable Crop Production and Weed Science at UC Davis (2018).

[b] Personal communication with a representative of grower, shipper and distributor of fresh produce, including cilantro, based in southern California (2019).

4.2 Parameters

4.2.1 Infield

Seasonality, solar radiation and rainfall have been shown to have important impacts on the overall *E. coli* contamination infield (Allende et al., 2017). This is especially important for cilantro that has very little, if any, post-harvest processing. The infield portion of this model is based on a previously created model by Allende et al. (2017) that describes the variable *E. coli* contamination of spinach during harvest based on the varying and daily contamination via irrigation water (C_{Irr}), soil transfer from splashing of irrigation (C_{IrrS}) or rain water ($C_{RSplash}$), and the daily inactivation of *E. coli* due to solar radiation (S_{Inact}).

The outcome of the first portion of this model (C_{field}) is the compilation of contamination of *E. coli* O157:H7 in cilantro (CFU/g) from each day of the growing season up to the final day of harvest based on random selection of days where rain, irrigation or sunshine occur. These modeling parameters are in (Table 2). The total number of days of rain ($Rain_{lim}$), although randomly selected when they would occur, is based on the average number of rain days per month in Santa Barbara, a primary cilantro growing region in California, in both winter and summer seasons (Table 3; NCDC, 2019). Conversations with Dr. Richard Smith at University of California Davis indicated that the California cilantro industry, irrigates only every five to six days, so the total number of days of irrigation (Irr_{lim}) is based on the number of days until harvest divide by 5.5. Any day it did not rain was considered a sunny day (P_{sun}). If irrigation occurred, it was assumed to be a sunny day and solar inactivation was still considered for that day. Table 3 lists the growth parameters for both winter and summer conditions.

Table 3: Growth parameters for summer and winter growth periods

Growth Parameter	Summer	Winter
Days from planting until harvest	= ROUND(RiskUniform(40,45),0)	= ROUND(RiskUniform(50,55),0)
Rain days	= ROUND(RiskPert(0,2.6,8),0)	= ROUND(RiskPert(0,6.1,9.3),0)
Irrigation days	= ROUND((Days until harvest)/5.5,0)	= ROUND((Days until harvest)/5.5,0)
Sun days	= Days until harvest – Rain days	= Days until harvest – Rain days

The VBA function created for this model which simulates the variability and randomness of weather by randomly generating scenarios of rain, irrigation and days of sun is shown in Figure 2. This function accounts for the unpredictability if rain may occur for multiple consecutive days or not at all for many days. Although irrigation needs are somewhat based on the number of rain days, the days of irrigation were given the same chance as rain days (one in three) to occur until the max number of irrigation days were met. The function is a loop of each day in the field adding the daily increase in *E. coli* loads through different contamination events and subtracting the number of bacteria inactivated due to daily solar radiation based on the random selection of rain, irrigation or sun days. The different sources of contamination are described further in this section.

```
Option Explicit

Function ConcField(daylim As Integer, rainlim As Integer, irrlim As Integer, Crain As Double, Sdecay As Double, Cirr As Double, CirrS As Double) As Double


Dim day As Integer, rain As Integer, irr As Integer
Dim number As Integer, Cfield As Double


day = 1
rain = 0
irr = 0

Do While day < daylim
    number = Application.WorksheetFunction.RandBetween(1, 3)
        If number = 1 Then
           If rain < rainlim Then
                Cfield = Cfield + Crain
                rain = rain + 1
                day = day + 1
            End If
        ElseIf number = 2 Then
            If irr < irrlim Then
                 Cfield = Cfield * Sdecay + Cirr + CirrS
                 irr = irr + 1
                 day = day + 1
            End If
        Else: number = 3
            If irr + rain < daylim Then
                Cfield = Cfield * Sdecay
                day = day + 1
            End If
        End If
Loop

ConcField = Cfield

End Function
```

Figure 2: VBA Excel function created to randomly select rain, irrigation or sun (blue row in Table 2)

Irrigation

While various types of irrigation exist throughout agriculture including furrow/flood irrigation, sprinkler/overhead irrigation and drip irrigation, the cilantro industry relies most heavily on sprinkler irrigation, so that is the system addressed in this model (Smith et al., 2011). In sprinkler irrigation, the water comes into direct contact with the edible portion of cilantro, posing a relatively high risk for contamination, in comparison to the other means of irrigation.

The California Leafy Green Products Handler Marketing Agreement (LGMA), a volunteer organization whose goal is to assure the safety of leafy greens, recommends that concentration of generic *E. coli* in irrigation water should not exceed 235 CFU/100 ml (LGMA, 2016). While the products covered in the LGMA do not include cilantro or herbs in general, many of the producers involved with LGMA may also grow cilantro and herbs in separate areas of their farms and it is likely that they share water sources. Because water testing results are maintained privately by most agricultural producers, very limited data are available publically on the overall quality of irrigation water. For that reason, a uniform distribution (min=1 CFU/100 ml, max=235 CFU/100 ml) was used for this QMRA model. The minimum distribution value represents the detection limit of generic *E. coli* in irrigation water and the maximum (235 CFU/100 ml) is LGMA's highest acceptance criteria for any single sample of irrigation water (LGMA, 2016). This model assumes that cilantro producers are in compliance with LMGA standards.

Currently, there is no information on an industry or regulatory accepted correlation between pathogenic and generic *E. coli* available. Thus, due to a lack of data, the number of *E. coli* O157:H7 in irrigation water was estimated from the amount of generic *E. coli* present using a lognormal distribution of the ratio of verotoxin-producing *E. coli* (VTEC) to generic *E. coli* identified in cattle manure (Ottoson et al., 2011). This ratio was used in this study to model the ratio of *E. coli* O157:H7 to generic *E. coli* for both soil contamination (R_s) and water contamination (R_w) as was done in previous QMRA models for leafy greens (Pang et al., 2017). These parameters are shown in Table 2. This model assumed that all *E. coli* O157:H7 in the irrigation water that was collected on the plant would attach to it. This conservative assumption has been used for previous risk assessments that investigated infield contamination (Hamilton et al., 2006, Pang et al., 2017; Allende et al., 2017).

Field experiments on spinach were used to determine the amount of irrigation water transferred to produce during each irrigation event (Allende et al., 2017). No studies were identified in literature that investigated the amount of water transferred to cilantro or herbs during overhead irrigation. Allende et al. (2017) field experiments took samples of 75 spinach leaves from five areas at 0 cm, 50 cm, 100 cm, 200 cm and 400 cm away from the irrigation sprinklers before and after 20 minutes of irrigation. The water was collected off the spinach leaves and weighed. The obtained data was fitted to a risk uniform distribution between 2.8 ml/g of produce to 21.6ml/g of produce (Allende et al., 2017). This distribution was used in this model to determine the amount of irrigation water transferred to the cilantro (Irr_{trans}, Table 2). Based on discussions with Dr. Smith, irrigation only occurred every five to six days (Irr_{lim}, Table 2). The calculation for daily increase of *E. coli* O157:H7 contamination attributed to irrigation water is detailed in Table 2 (C_{Irr}).

Soil transfer due to splashing

Estimations for the concentration *E. coli* in soil used in this model was based on a seven month experiment on the effect of proximity of cattle feeding sites on fecal bacteria in soil conducted by Lenehan et al. (2005). Since industry and regulatory guidance for production of fresh culinary herbs in the U.S. proposes that concentrated animal feeding operations be at least 400 ft (122 m) away from the edge of the crop (FDA & WGA, 2013) only the soil samples collected from the furthest distances in Lenehan et al. (2005) study were accounted for in this QMRA study. The *E. coli* concentration levels in the soil were described in this model by a normal distribution with μ= 0.44 log CFU/g and σ = 0.11 log CFU/g from the 30 meters distances in Lenehan et al. (2005) data (C_s). It was assumed for this model that this contamination level was the same for all months. The prevalence of *E. coli* in soil ($Prev_{Soil}$) was assumed based on a study by Holvoet et al. (2013) who identified a 37% (n=276) prevalence of enumerable (>10 CFU/g) *E. coli* in soil samples from an open air lettuce production. The same ratio of *E. coli* O157:H7 to generic *E. coli* used for the ratio in irrigation water as used for soil (R_s; Ottoson et al., 2011).

This model assumes that contamination of the cilantro from soil occurs due to splashing from rain and overhead (sprinkler) irrigation. The same Allende et al. (2017) spinach study used to determine water transfer due to irrigation conducted the same sampling scenarios for soil transferred due to sprinkler irrigation. Again five sampling areas located at 0 cm, 50 cm, 100 cm, 200 cm and 400 cm from the irrigation sprinklers were selected and irrigated for 20 min, then a total of 1,500 spinach leaves were randomly taken between the five selected zones and observed for presence or absence of soil on the surface (Allende et al., 2017). The probability of splashing from irrigation used in this model was estimated from Allende et al., (2017) data to be a risk pert distribution (Irr_{Splash})

with the minimum amount of splashing occurring on 2% of leaves, the max on 6% and most likely 4% of the leaves are splashed (Table 2). To evaluate the amount of soil transferred to the leaf surface, Allende et al. (2017) randomly selected 50 spinach leaf samples taken before and after the irrigation event in each of the five sampling zones then weighed soil remaining on the leaf surface after drying. Once statistical outliers were removed the amount of soil on produce (g soil/g produce) was described using a beta general distribution ($Soil_{Trans}$; Table 2).

This model assumes that if rain occurs there is a 100% chance that soil splashing will occur ($P_{Rsplash}$) and the amount of soil transfer is represented by the same beta general distribution as the irrigation water soil transfer ($Soil_{Trans}$). This conservative assumption was also utilized by the Allende et al. (2017) QRMA model. Allende et al. (2017) estimated that the amount of bacterial transfer caused by soil transfer was represented by a uniform distribution between 35 to 90% of the soil transferred (Bac_{irr}, Bac_{rain}, Table 2). Based on this and the ratio for pathogenic to generic *E. coli* described early, the distribution of *E. coli* O157:H7 concentration in cilantro due to soil splashing during an irrigation/ rain event was calculated (C_{IrrS}, $C_{RSplash}$, Table 2).

Decay due to UV radiation

As described in earlier sections, cilantro grows well in direct sunlight. Ultra violet (UV) radiation, like that produced from the sun can reduce pathogenic bacteria. Based on literature, *E. coli* is expected to reduce on the surface of leafy green produce and herbs when exposed to UV light (Islam et al., 2004; Ottoson et al., 2011). However, the inactivation rate is a function of specific physical factors including temperature, light intensity and solar radiation depending on the production area. Allende et al. (2017) adapted data from the Ottoson et al. (2011) climate chamber experiments using climatological data relevant to Spain. The inactivation of *E. coli* (S_{Inact}) follows a first-order kinetic equation (Allende et al., 2017; Ottoson et al., 2011; Table 2).

Because of the similarities in the Mediterranean climates of Spain and California, the inactivation rate generated by Allende et al. (2017) is a suitable rate for the decay of *E. coli* O157:H7 in the similar climate of California and will therefore be used in this model. Only solar radiation (intensity and duration) was considered to cause bacterial inactivation. The amount of sun per day used in this model was based on the average number of sun hours in Santa Barbara, CA represented in a risk pert distribution assuming the minimum amount of sun in a day is eight hours, the max is 13 hours and most days have 12 hours of sunlight (Sun_{hrs}; Table 2). It was assumed that the environmental temperature did not affect bacterial inactivation on the plant tissue.

4.2.2 Harvesting and bunching

Contamination from harvesting tools is one possible source of *E. coli* O157:H7 in the production of fresh cilantro. Pathogens could become attached to harvest blades and then contaminate subsequent bunches harvested afterward. Cilantro is usually cut at the base during harvesting rather than being dug from the ground. For that reason, only contamination from the harvesting blade was accounted for in this portion of the model, rather than transfer of soil to the plant from the harvesting tools.

A transfer rate of 0.13% ($R_{c\text{-}b}$) of *E. coli* O157:H7 passed from a contaminated harvest blade to uncontaminated produce was used for this model based on a calculated average of *E. coli* O157:H7 transference to lettuce from harvesting tools (Yang et al., 2012). This same transfer rate was used by Pang et al. (2017) for a QMRA of *E. coli* O157:H7 in heads of lettuce. Zilelidou et al. (2015) found in their study of bacterial transference during the cutting of lettuce, that the transfer of *E. coli* O157:H7 from contaminated lettuce to sterile knife is less than that from contaminated knife to uncontaminated lettuce. Zilelidou indicated this is likely due to the fact that the whole blade of the knife comes into contact with the contaminated lettuce, whereas only a small part of the lettuce contacts the knife. Unfortunately, they did not quantify how much less the transfer would be so due to a lack of available data, it was assumed in this model that a harvester's knife would become contaminated with some percentage uniformly distributed between 0 and 0.13% ($R_{c\text{-}b}$) of the *E. coli* O157:H7 on the plant at harvest ($N_{h\text{-}c}$).

Based on information from Yang et al. (2012) and Pang et al. (2017), it was assumed in this QMRA that each contaminated blade will evenly transfer the pathogenic microorganisms to multiple consecutive bunches. In their study on the transfer of pathogens during multiple cuts of lettuce, Zilelidou et al. (2015) determined that low levels of *E. coli* O157:H7 rapidly decreased after four cuts. With that information, this model assumes that *E. coli* is transferred evenly to the four subsequent bunches (32 grams each) after the harvesting blade had been initially contaminated ($C_{harvest}$). Prior to boxing, the concentration of *E. coli* O157:H7 ($Conc_H$) on the harvested cilantro is the sum of the concentration from the field prior to harvest (C_{field}) and the contamination caused by the harvesting tools ($C_{harvest}$).

4.2.3 Transportation and storage

Field to retail

Once packaged together, it is assumed in this model that the prevalence of contamination stays the same, however the concentration of *E. coli* O157:H7 increases or decreases during transportation and storage as a function of time and temperature. This model considers the transportation from field to retail (t_{FR}, $Temp_{FR}$) and retail to home (t_{RH}, $Temp_{RH}$). The data used to describe these distributions in our model were obtained from the literature, from on-line sources, and through personal communication with a representative of grower, shipper and distributor of fresh produce, including cilantro, based in southern California (to protect confidentiality, names of individuals and companies were not disclosed). Based on communications with a cilantro production representative, the trucks used for transportation of cilantro from harvest to retail stores are held between 34°F and 36°F (1.1°C and 2.2°C). Assuming some cilantro is in constant contact with the ice and others have reached equilibrium with the refrigerated truck, the transportation temperature between harvest and retail is assumed to be a uniform distribution between min = 0°C and max = 2.2°C (Table 2). Transportation time to retail can vary from local deliveries under an hour to cross country deliveries to the east coast taking up to seven days.

Retail storage

For refrigerated storage at retail, a normal distribution (μ=4.4441°C, σ=2.9642°C) of EcoSure Cold Temperature Report (2007) data for refrigerated products, generated by Pang et al. (2017), was used to represent the retail storage temperature for fresh cilantro ($Temp_{R;}$ Table 2). This distribution has previously been used to represent temperature for storage of fresh cut lettuce, which is held in similar if not the same areas as cilantro in grocery stores and has a comparable shelf life (Pang et al., 2017). This normal distribution was truncated at 0°C and 20.56°C (Pang et al., 2017). The time distribution for retail storage used in this model was previously expressed by Pang et al. (2017) for fresh cut lettuce as a triangular distribution of a minimum of 12 hours, a max of seven days and a the most likely storage time being four days (t_R).

Retail to home

Temperature during transportation from retail to home was described as the average of the temperature from the time of retail storage ($Temp_R$) and the temperature before putting the cilantro in to a home refrigerator ($Temp_{bH}$). This procedure was previously used for QMRA models for both unpasteurized milk and fresh-cut lettuce (Latorre et al., 2011; Pang et al., 2017). In the same manner as the retail storage temperatures, the data for temperature right before home refrigerator storage ($Temp_{bH}$) from the EcoSure report (2007) was fit to a normal distribution (μ=8.3858°C, σ=3.8314°C) and truncated at 0°C and 20°C (Pang et al., 2017). EcoSure data for transportation time (t_{RH}) for all refrigerated produces fit to a lognormal distribution (μ=1.421 h, σ=0.46478 h), truncated at 0.1833 h and 3.8667 h (Pang et al., 2017). In an effort to account for the differences in the refrigerated products represented in the 2007 EcoSure data and fresh cut lettuce, Pang et al. (2017) shifted the lognormal distribution -0.24609. This adaptation was used in this model to represent fresh cut cilantro which is stored in similar ways.

Home storage

In a study of RTE foods, Pouillot et al. (2010) fit data on the time food spent in home storage from first consumption (tf) and last consumption (tl) into two separate Weibull distributions. For this model, the Pouillot data on RTE bagged salads, as there were no data available for herbs, was averaged between the two values to represent the time cilantro was stored at home (t_H, Table 2). This same averaging approach was used by Pang et al. (2017) in their QMRA for fresh cut lettuce. A normal distribution (μ=3.4517°C, σ=2.4442°C) truncated at -5°C and 17.22°C was used to describe temperature during home storage ($Temp_H$) based on data from the EcoSure Cold Temperature Report (EcoSure, 2008; Pang et al., 2017).

Growth-death model

Due to a lack of data on the growth and inactivation of *E. coli* O157:H7 on cilantro due to temperature changes, the McKellar and Delaquis (2011) growth-death model developed for minimally processed leafy green vegetables (lettuce and spinach) was used to predict the potential fluctuation of *E. coli* O157:H7 concentration on cilantro during transit and storage for this QMRA model. The McKellar and Delaquis (2011) model has been referenced in about 50 scholarly articles since its publication, including being referenced for multiple growth models and risk assessments in produce. This combined pathogen growth and death model utilized by this study excludes both the lag and maximum population density (MPD), and calculates bacterial growth or die-off depending on if the temperature is above or below the minimum growth temperature for *E. coli* O157:H7 on cilantro (McKellar and Delaquis, 2011; Pang et al., 2017). At temperatures exceeding the minimum growth temperature, the increase in number of *E. coli* O157:H7 cells was determined by the growth model (simplified to growth rate multiplied by time; μ*t), while at temperatures below minimum growth temperature, the decline of pathogen cells was determined by a die-off model (simplified to negative die-off rate multiplied by time; -k*t). Assuming no lag phase or MPD is a conservative approach likely overestimating the growth of the pathogen. The growth (μ) and die-off equation parameters from McKellar and Delaquis (2011) used in this study are b = 0.023 and T_{min} = 1.2023 and death rate k is described by a lognormal distribution (μ=0.013 CFU/g/h, σ=0.0010

CFU/g/h. The growth and death equations for all stages of transportation and storage from farm to fork are described in Table 2 under the growth/die-off parameter and calculation sections.

This QMRA model uses 5°C as the minimum growth temperature of *E. coli* O157:H7 base on historical data (Nauta and Dufrenne, 1999; Palumbo et al., 1995; Rajkowski and Marmer, 1995; Tamplin et al., 2005). It should be noted, a study conducted by Khalil and Frank (2010) on the behavior of *E. coli* O157:H7 showed that the pathogen does not grow at 8°C on damaged cilantro. The study also indicated that the behavior of pathogens on damaged leafy greens differs than that on undamaged leaves (Khalil and Frank, 2010). Due to this finding and a lack of data on the growth of the pathogen on undamaged cilantro, it was determined this temperature was not the most representing of the situation modeled here. Additionally, in the study conducted by Koseki and Isobe (2005), no decline of *E. coli* O157:H7 cells on cut lettuce was found at 5°C temperature, this supports the determination by Khalil and Frank (2010) that pathogens on damaged leafy greens may behave differently.

4.3 Dose response model

To calculate the ingested dose or dose per serving (D) the concentration of *E. coli* O157:H7 after home storage (C_H) was multiplied by the distribution of serving sizes (Table 2). The probability of illness from ingested dose (P) was estimated using the Beta-Poisson dose response model ($P = 1 - (1 + D/\beta)\ \alpha$) created by Haas et al. (1983) and multiplied by the assumed prevalence rate that 0.1% of cilantro is contaminated with *E. coli* O157:H7 ($prev_0$; Table 2). This prevalence has been used to predict illnesses from consuming servings of lettuce (Pang et al., 2017). The dose response model parameters α and β for this

QMRA model were taken from Cassin et al. (1998). This model assumes that one cell of *E. coli* O157:H7 is capable of causing illness and all cells are equally capable (Cassin et al., 1998). This assumption is in line with published data indicating the infectious dose may be fewer than 100 cells and has been as low as 1 CFU (Meng et al., 2007). These parameters were used for other QMRA models including ones to estimate *E. coli* O157: H7 in leafy greens by Danyluk and Schaffner (2011) and Pang et al. (2017). Since no models or parameters were identified for cilantro or herbs, these parameters were most fitting.

The serving size for cilantro varies widely depending on its purpose in the dish. It is often added in small amounts as a garnish or whole bunches are utilized in sauces or chutneys. Due to the limited data on the consumption of cilantro and herbs in the U.S., the serving size (Ser, Table 2) selected in this model is a triangular distribution with the minimum being the weight of one sprig (0.8 g), the maximum being one bunch (32 g) and assuming a majority of the U.S. population is eating around 2 g in a serving.

Herb consumption data has not been studied in the recent years. The most recent U.S. consumption data for cilantro is from the 2007 Foodborne Active Surveillance Network (FoodNet) Population Survey Atlas of Exposures that estimated only about 17% of the U.S. population eats cilantro at least once a week (CDC, 2007). Although studies have shown herb consumption has increased in the last few years, due to lack of more up to date data, a triangular distribution was used to represent the amount of the U.S. population that consumes cilantro at least once a week (N_{cil}). Again due to limited information, the number of servings consumed per the U.S. population is based on the assumption that the percent of the U.S. population that eats cilantro eats exactly one serving per week (N_p). The U.S. population was 328,355,826 on January 29, 2019, according to the statistics on U.S. Department of Commerce - Census Bureau (DOC-Census Bureau, 2019). An estimate of the annual number of cases in the U.S. was calculated as the product of the probability of illness and the number of servings consumed by the U.S. population per year (N_{cs}; Table 2). Spearman's correlation coefficients were used for sensitivity analyses to identify important parameters along the cilantro supply chain affecting public health risk of *E. coli* O157:H7 illnesses.

4.4 Scenario analysis

In this QMRA model, a total of five different risk scenarios at varying parameter levels were analyzed to evaluate the effects of each parameter on the probability of illness per serving of cilantro and the number of illness cases per year due to consumption of cilantro against the baseline model of cilantro grown in summer conditions.

4.4.1 Elevated microbiological contamination in irrigation water

The model was run with five different generic *E. coli* levels in irrigation water to represent situations where irrigation water of inferior microbial quality are used during cilantro growth infield. In the baseline model, it was assumed that the microbial quality of irrigation water used was in compliance with LGMA recommendations (generic *E. coli* being no more than 235 CFU/100ml). The different point values describing irrigation water quality that exceeded the 235 CFU/100 ml limit used in this scenario analysis are provided below:

- 500 CFU/100 ml
- 1,000 CFU/100 ml
- 1,500 CFU/100 ml
- 2,000 CFU/100 ml

- 2,500 CFU/100 ml

4.4.2 Temperature abuse during transportation from farm to retail

The model was run with five different temperature values during transportation from farm to retail to represent situations where temperature abuse during transportation may occur. In the baseline model, it was assumed that the transportation temperature of cilantro between harvest and retail is a uniform distribution between the min = 0°C for cilantro in contact with ice and a max = 2.2°C for cilantro that has reached equilibrium with the refrigerated transportation trucks (Table 2). With the understanding that refrigerated trucks have the potential for malfunction, are regularly opened and closed exposing the inside to warmer temperatures and/or transportation of fresh produce may not always occur under refrigeration different point values describing different temperatures at or above the refrigerated temperatures described in the base model are used in this scenario. The temperatures used for analysis are provided below:

- 2°C
- 4°C
- 6°C
- 10°C
- 12°C

4.4.3 Increased popularity of cilantro in the U.S. population

Due to a lack of up to date consumption data and an understanding that the popularity of herbs in the U.S. has increased since the CDC's FoodNet Population Survey in 2006/2007, the model was run with five different varying levels of cilantro popularity in the U.S. in an attempt to better represent the actual situation. In the baseline model, it was assumed that, the U.S. popularity of cilantro was a triangular distribution between 6.6% (New York consumption) and 34.1% (California consumption) with the most likely percentage being about 17% representing the average percent of the U.S. population that consumes fresh cilantro (CDC, 2007; Table 2). The different point values describing increasing levels of popularity used in this scenario analysis are provided below:

- 20%
- 30%
- 40%
- 50%
- 60%

Because the popularity of cilantro is being used to represent the number of people in the U.S. that consume cilantro weekly, it has no effect on the probability of illness per serving, so this was not investigated for this scenario. Only the changes in the number of illnesses per year caused by the consumption of cilantro contaminated with *E. coli* O157:H7 were evaluated.

4.4.4 Varying levels of E. coli *O157:H7 prevalence post-harvest*

The model was run with five different initial prevalence levels to represent situations where contamination is more widespread than expected. In the baseline model, it was assumed that 0.1% of all cilantro is contaminated with *E. coli* O157:H7 (Table 2). The different point values describing increasing levels of prevalence used in this scenario analysis are provided below:

- 0.1% (baseline)
- 1%

- 3%
- 5%
- 10%

4.4.5 The effect of cross contamination on varying levels of E. coli O157:H7 prevalence

Due to a lack of applicable data, the potential for cross contamination during harvesting, bunching, packaging and home processing was not included the baseline model. The way untreated bunches of cilantro are packed into boxes in the field, already contaminated bunches could transfer *E. coli* O157:H7 through direct contact during storage and transportation. In an FDA expert panel to estimate risk associated with *E. coli* O157:H7 on lettuce, the agency determined that the prevalence of contamination increased by a factor of 1- to 2-fold (most likely 1.2-fold) due to cross contamination during washing (FDA, 2012b). Due to lack of available data on the effects of cross contamination of herbs in direct contact, this prevalence increase was assumed to be appropriate (FDA, 2012b). A pert distribution between 1- and 2- fold increase with a most likely increase of 1.2-fold was applied to each prevalence level described in the previous scenario.

4.5 Model

This risk model was simulated with the Monte Carlo simulation technique by using @Risk 7.5 risk modeling software (Palisade Corp., Ithaca, NY). The model was run with 100,000 iterations per simulation for each scenario based on previously published reports (Danyluk and Schaffner, 2011; Latorre et al., 2011; Pang et al., 2017) and to reflect the variability to be expected based on available data. The Latin Hypercube sampling method with a fixed initial seed was used to sample different values for input parameters and variables; however, due to the complexity of the model and the incorporation of the random

VBA macro function within the growth portion of the model, no matter if a fixed initial seed is used, the results will always be slightly different every time it is run. The results presented in the following section are representative examples of what the results are when the model and all the scenarios are run concurrently. Multiple runs of the model and scenarios revealed small changes in the means values of each output however the inner quartile ranges (IQRs) are all very similar. Additionally the outcomes of each scenario with varying parameters run in this model depict the same relative relationships despite how many times the models are run. Distributions describing the variability of model parameters were developed using Excel and the @Risk software.

Chapter 5: Results and Discussions

5.1 Estimated contamination

The growth, inactivation and contamination parameters were integrated to estimate the concentration of *E. coli* O157:H7 on cilantro grown during winter and summer sun and rain conditions right before harvest, after cutting and through home storage (Table 4). The mean concentration of *E. coli* O157:H7 increased along the supply chain for both winter and summer conditions. The mean pathogen concentration on cilantro grown during winter conditions increased from 5.6×10^{-5} CFU/g (IQR of 4.6×10^{-9} to 4.6×10^{-7} CFU/g) to 24.7 CFU/g (IQR of 6.7×10^{-10} to 2.3×10^{-7} CFU/g) right before harvest to after home storage, respectively. The maximum value for the concentration of *E. coli* O157:H7 on cilantro grown in winter conditions after home storage was 2,469,540.7 CFU/g for one iteration of this model, this outlier is significantly higher than the 99th percentile value of 7.0×10^{-4} CFU/g. Because this maximum value was so high, the mean value was larger than the 99th percentile range.

Although cilantro grown in summer conditions had IQRs with higher concentrations of the pathogen than that grown winter conditions, the mean increase along the supply chain was very similar. The mean pathogen concentration for cilantro grown in summer conditions increased from 3.2×10^{-4} CFU/g (IQR of 4.8×10^{-8} to 3.2×10^{-6} CFU/g) to 5.2×10^{-2} CFU/g (IQR of 6.6×10^{-9} to 1.6×10^{-6} CFU/g). The maximum value for the concentration of *E. coli* O157:H7 on cilantro grown in summer conditions after home storage was 4,277.07 CFU/g for one iteration of this model, this outlier is significantly higher than the 99th percentile value of 2.0×10^{-4} CFU/g. Because this maximum value was so high, the mean value was larger than the 99th percentile range.

Table 4: Estimated concentration levels of E. coli O157:H7 on cilantro at different stages along the supply chain

Stage along Supply Chain	**Concentration of *E. coli* O157:H7 (CFU/g)**						
	Median	Mean	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Winter right before harvest	4.5×10^{-8}	5.6×10^{-5}	2.2×10^{-11}	4.6×10^{-9}	4.6×10^{-7}	1.6×10^{-5}	2.0×10^{-4}
Winter after cutting	4.5×10^{-8}	5.6×10^{-5}	2.2×10^{-11}	4.6×10^{-9}	4.6×10^{-7}	1.6×10^{-5}	2.0×10^{-4}
Winter after home Storage	1.2×10^{-8}	24.7	1.1×10^{-12}	6.7×10^{-10}	2.3×10^{-7}	2.2×10^{-5}	7.0×10^{-4}
Summer right before harvest	3.8×10^{-7}	3.2×10^{-4}	3.7×10^{-10}	4.8×10^{-8}	3.2×10^{-6}	8.1×10^{-5}	9.8×10^{-4}
Summer after cutting	3.8×10^{-7}	3.2×10^{-4}	3.7×10^{-10}	4.8×10^{-8}	3.2×10^{-6}	8.1×10^{-5}	9.8×10^{-4}
Summer after home storage	9.6×10^{-8}	5.2×10^{-2}	1.7×10^{-11}	6.6×10^{-9}	1.6×10^{-6}	1.4×10^{-4}	3.7×10^{-3}

It is also important to note that the IQRs decrease along the supply chain from right before harvest to after home storage for both cilantro grown in winter and summer conditions despite the increase in the mean concentration in *E. coli* O157:H7 for both conditions. A decrease of contamination from before harvest to after home storage can be explained by extended storage and transportation times at cold storage below 5°C which this model calculates as an inactivation of *E. coli* O157:H7. Because this base model assumes the transportation temperatures are as the industry described them, below 2.2°C, it makes sense that a majority of situations would result in a decrease of *E. coli* O157:H7 along the supply chain. However, as described early, the increase seen in mean contamination levels is caused by the outlier maximum value cases that represent situations where the parameters are conducive to growth along the supply chain. This is reflected in real life where illnesses from cilantro are seen rarely but outbreaks have occurred.

The mean increase in microbiological load from contaminated knives during harvest ($C_{harvest}$) was 2.0×10^{-11} CFU/g and 9.0×10^{-11} CFU/g for winter and summer growing conditions, respectively, with 99% of results from both growth conditions being less than about 7.4×10^{-10} CFU/g (Figure 3). These low concentrations lead to the appearance in the results that no increase in contamination occurred as a result of harvesting.

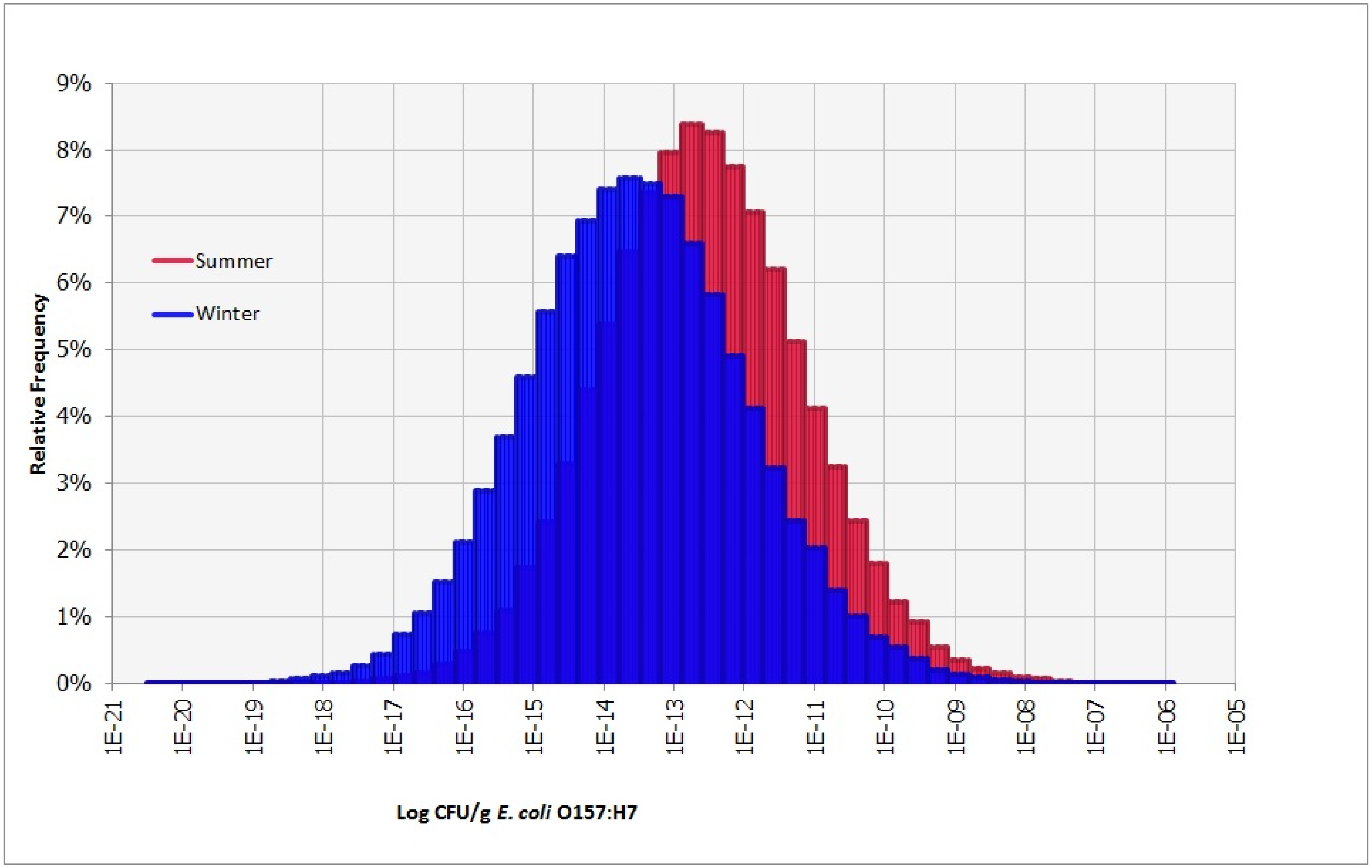

Figure 3: Distribution of the E. coli O157:H7 in (log CFU/g) transferred to cilantro during harvesting with a blade for cilantro grown in winter (blue) and summer (red) growth conditions

5.2 Probability of illness per serving

The ingested dose of *E. coli* O157:H7 was integrated using a Beta-Poisson dose-response model to calculate the probability of illness based on consuming a single serving of fresh cilantro grown during winter and summer growth conditions (Table 5). The average probability of illness per serving of fresh cilantro grown in winter conditions is 2.4×10^{-8} (IQR of 6.8×10^{-15} to 2.5×10^{-12} CFU/g). The probability of illness per serving of fresh cilantro grown during summer conditions is larger than that of winter conditions at an average of 5.1×10^{-8} (IQR of 6.8×10^{-14} to 1.8×10^{-11} CFU/g).

Table 5: Probability of illness from consuming one serving of fresh cilantro in the U.S. population for winter and summer growing conditions

Scenarios	**Probability of Illness per Serving**						
	Median	Mean	1^{st} Percentile	25^{th} Percentile	75^{th} Percentile	95^{th} Percentile	99^{th} Percentile
Winter Growth Conditions	1.2×10^{-13}	2.4×10^{-8}	9.3×10^{-18}	6.8×10^{-15}	2.5×10^{-12}	2.6×10^{-10}	8.4×10^{-9}
Summer Growth Conditions	1.0×10^{-12}	5.1×10^{-8}	1.5×10^{-16}	6.8×10^{-14}	1.8×10^{-11}	1.6×10^{-9}	4.5×10^{-8}

5.3 Number of illness cases per year

The number of cases per year was based on probability of illness per serving and cilantro consumption data for the U.S. population. If the outcome of an iteration of the model resulted in less than one illness per year, it was assumed that no illness occurred. The mean number of illnesses per year due to consumption of *E. coli* O157:H7 contaminated cilantro in the United States as 86 (IQR of 2.1×10^{-5} to 8.1×10^{-3}) and 164 (IQR of 2.1×10^{-4} to 5.8×10^{-2}) for cilantro grown during winter and summer conditions, respectively. The maximum number of possible illnesses per year for both growing conditions are extremely high (3,588,586 cases for winter and 2,767,109 cases for summer) causing the averages to be above the 99th percentile values. This reflects the complexity of the model and how extremely unlikely it is that an outbreak of that size would occur. While the results indicate that about 90%-95% of the time (for winter and summer conditions, respectively) no illness will occur from consuming cilantro grown in these conditions, the 95th and 99th percentile values indicate that given the right combination of parameters individual illnesses and outbreaks are possible.

Table 6: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated fresh cilantro in the U.S. population for winter and summer growing conditions

Scenarios*	Number of Cases per Year						
	Median	Mean	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Winter Growth Conditions	3.9×10^{-4}	86	2.9×10^{-8}	2.1×10^{-5}	8.1×10^{-3}	1	27
Summer Growth Conditions**	3.2×10^{-3}	164	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147

*Assume summer and winter growing conditions were applied to the entire year.

**Used as baseline values for scenario testing below

5.4 Sensitivity analysis

The spearman's rank order correlation was used to determine which input values had the highest effect on the total number of illnesses caused by the consumption of a serving of *E. coli* O157:H7 contaminated cilantro. The number of illnesses per year caused from the consumption of cilantro grown in the winter conditions were most sensitive to the following inputs (Figure 4): retail storage temperature (0.40), ratio of *E. coli* O157:H7 to generic *E. coli* in irrigation water (0.27), hours of sun per day (-0.26), number of rain days (0.24), home storage temperature (0.20), serving size (0.16), generic *E. coli* in irrigation water (0.16), transportation from harvest to retail (-0.15), amount of water transferred to the plant during irrigation (0.12) and prevalence of generic *E. coli* in the soil; (0.09).

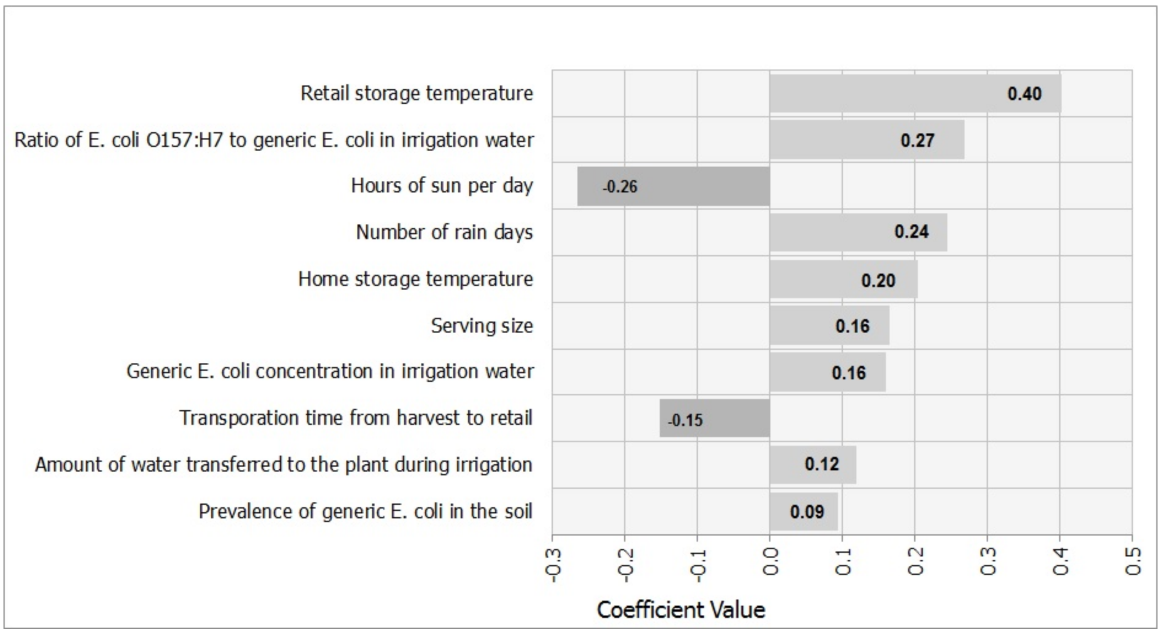

Figure 4: Tornado graph showing the most important variables affecting the estimated number of E. coli O157:H7 illness cases per year for cilantro grown under winter conditions. Spearman correlation coefficients were obtained from @Risk sensitivity analysis and were shown next to each bar.

The number of cases per year caused by the consumption of cilantro grown in the summer conditions were most sensitive to the following inputs (Figure 5): retail storage temperature (0.42), ratio of *E. coli* O157:H7 to generic *E. coli* in irrigation water (0.29), hours of sun per day (-0.24), home storage temperature (0.21), serving size (0.17), generic *E. coli* concentration in irrigation water (0.17), transportation time from harvest to retail (-0.16), amount of water transferred to the plant during irrigation (0.12), number of days cilantro is grown infield (-0.10) and temperature of cilantro before putting it in the home refrigerator (0.08)

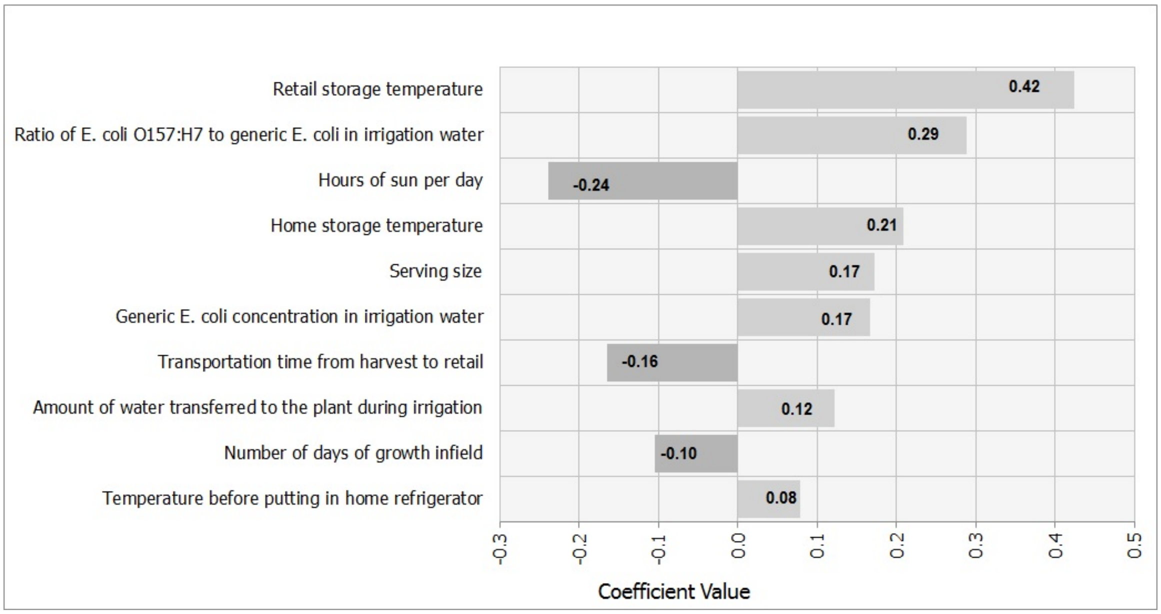

Figure 5: Tornado graph showing the most important variables affecting the estimated number of E. coli O157:H7 illness cases per year for cilantro grown under summer conditions. Spearman correlation coefficients were obtained from @Risk sensitivity analysis and were shown next to each bar.

5.5 Scenario analyses

The following five scenarios were evaluated for their influence on the relative risk of developing an illness after consuming a serving of retail cilantro in the U.S. as compared to the baseline model for cilantro grown in summer conditions;

(1) Varying levels of elevated microbiological contamination in irrigation water,

(2) Vary levels of temperature abuse during transportation from farm to retail,

(3) Varying levels of increased popularity of cilantro in the U.S. population,

(4) Varying levels of *E. coli* O157:H7 prevalence post-harvest, and

(5) The effect of cross contamination on varying *E. coli* O157:H7 prevalence levels.

To best demonstrate the influence of these parameters, the fold change in the mean number of illnesses caused by the consumption of cilantro is described in tables and the relative probabilities of illness are presented as cumulative density functions (CDF) for each varying input. The change in the probability of illness was not evaluated for the increased popularity of cilantro where the varying levels would have no effect. The results are discussed in the following sections.

5.5.1 Elevated contamination levels in irrigation water

Different levels of irrigation water quality that exceeded the LGMA 235 CFU/100 ml for generic *E. coli* guideline were evaluated. The predicted number of cases per year increased from 2.2-fold to 6.0-fold of the baseline number of illnesses when generic *E. coli* concentration in irrigation water increased from 1 - 235 CFU/100 ml to 2,500 CFU/100 ml (Table 7). As the amount of generic *E. coli* increased so did the probability of illness, as demonstrated in the CDF in Figure 6. The probability of illness is higher for all levels of water contamination than in the baseline model.

Table 7: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated cilantro in the U.S. population for the baseline model and different generic E. coli concentrations in irrigation water

Irrigation water quality (CFU/100 ml)	**Number of Cases per Year**							
	Median	Mean	Fold change*	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Baseline	3.2×10^{-3}	164	--	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147
500	1.6×10^{-2}	361	2.2	3.0×10^{-6}	1.1×10^{-3}	0.3	23	671
1,000	3.1×10^{-2}	569	3.5	6.0×10^{-6}	2.1×10^{-3}	0.5	41	1,322
1,500	4.6×10^{-2}	572	3.5	8.0×10^{-6}	3.2×10^{-3}	0.8	62	1,816
2,000	0.1	872	5.3	1.1×10^{-5}	4.2×10^{-3}	1.0	86	2,599
2,500	0.1	991	6.0	1.4×10^{-5}	5.1×10^{-3}	1	101	3,177

*Fold changes were calculated by comparing mean values of each of the irrigation water quality scenarios with the mean value of the baseline model.

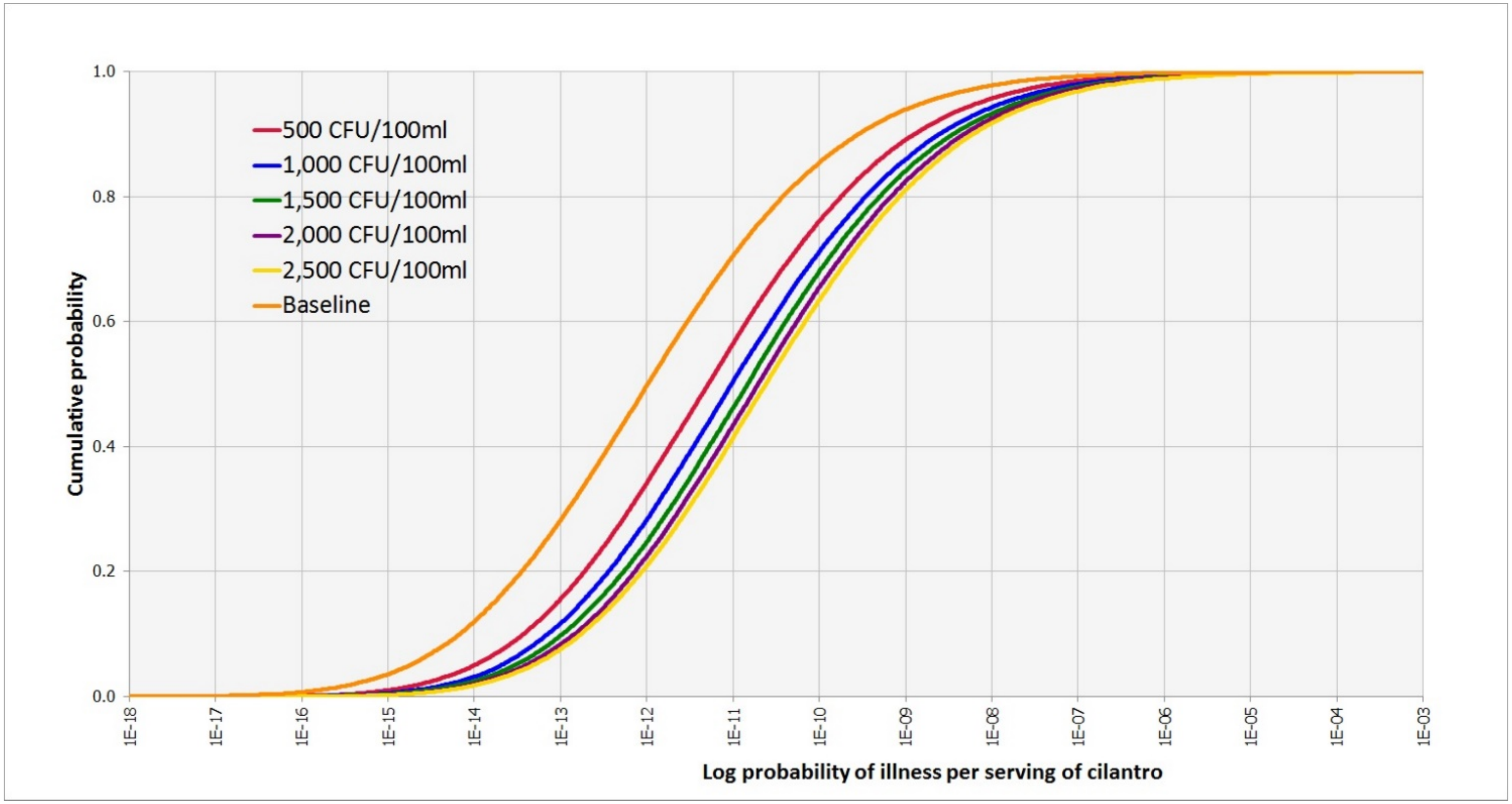

Figure 6: Cumulative density functions of the probability of illness per serving of cilantro for the baseline model (orange) and five increased levels of irrigation water contamination. Distributions are shown on a logarithmic scale.

5.5.2 Temperature abuse during transportation from farm to retail

Different temperatures that were either at the high end of the values described by the cilantro industry professional or exceed them were evaluated for the duration of transportation from farm to retail. The mean predicted number of cases per year increased from 106 cases per year for cilantro transported at a constant 2°C to 13,674 cases per year for cilantro transported at constant 12°C (Table 8). This is an 83.4-fold increase from the base model run at a uniform distribution between 0-2.2°C. The CDF representing the varying probabilities of illness in Figure 7, indicate that the risk of a single serving of cilantro remains about the same when transported at constant temperatures between 0 and 4°C.

Table 8: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated cilantro in the U.S. population for the baseline model and different transportation temperatures from farm to retail.

Transportation Temperature (°C)	Number of Cases per Year							
	Median	Mean	Fold change*	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Baseline	3.2×10^{-3}	164	--	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147
2	3.2×10^{-3}	106	0.6	4.0×10^{-7}	2.1×10^{-4}	0.1	5	154
4	3.2×10^{-3}	143	0.9	4.5×10^{-7}	2.1×10^{-4}	0.1	5	148
6	3.0×10^{-2}	613	3.7	4.3×10^{-6}	1.9×10^{-3}	0.5	44	1,279
10	0.3	3,810	23.2	1.7×10^{-5}	1.6×10^{-2}	8	961	32,657
12	2	13,674	83.4	3.1×10^{-5}	6.4×10^{-2}	68	11201	324,002

*Fold changes were calculated by comparing mean values of each of the temperature scenarios with the mean value of the baseline model.

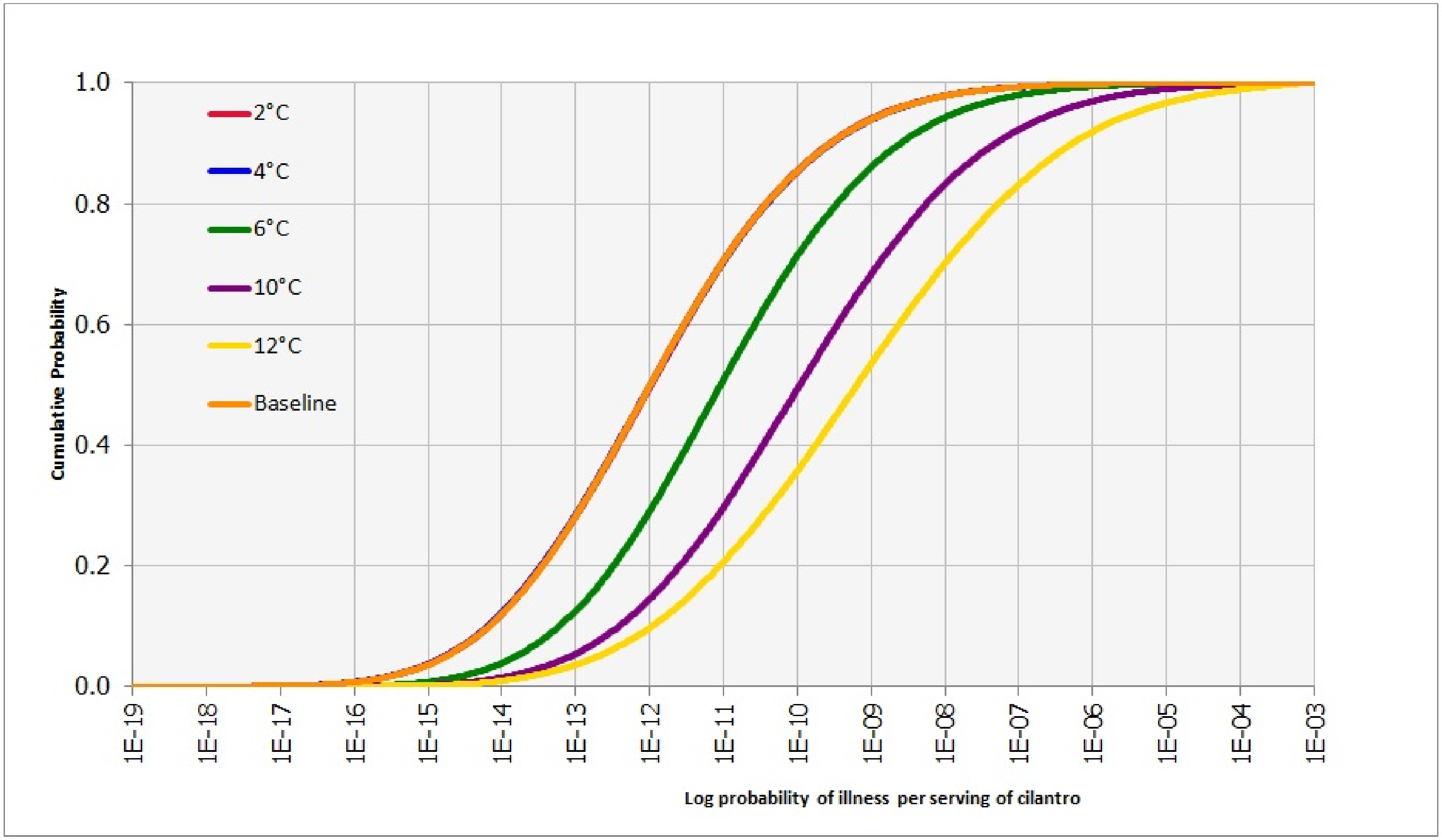

Figure 7: Cumulative density functions of the probability of illness per serving of cilantro for the baseline model (orange) and five temperatures to represent temperature abuse during transportation from farm to retail. Distributions are shown on a logarithmic scale.

5.5.3 Increased popularity of cilantro in the U.S. population

With the knowledge that the popularity of herbs has increased in U.S. since the 2007 CDC survey but having a lack of current data to reflect that increase, this QMRA model was run at different levels of popularity to evaluate the effect of this increase. The predicted number of cases per year increased from 0.8-fold to 3.3-fold of the baseline when popularity increased to 60% (Table 9). Although the increase in mean number of illnesses increases with popularity, illnesses were not observed (number of illnesses was less than one) for 90% of the model iterations in all scenarios.

Table 9: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated cilantro in the U.S. population for the baseline model and different cilantro popularity levels in the U.S.

U.S. Pop. That Consumes Cilantro (%)	**Number of Cases per Year**							
	Median	Mean	Fold change*	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Baseline	3.2×10^{-3}	164	--	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147
20	3.5×10^{-3}	127	0.8	4.9×10^{-7}	2.3×10^{-4}	0.1	6	164
30	5.2×10^{-3}	227	1.4	7.4×10^{-7}	3.5×10^{-4}	0.1	8	254
40	7.0×10^{-3}	277	1.7	9.8×10^{-7}	4.6×10^{-4}	0.1	11	326
50	8.7×10^{-3}	342	2.1	1.3×10^{-6}	5.8×10^{-4}	0.2	13	413
60	1.0×10^{-2}	547	3.3	1.5×10^{-6}	6.9×10^{-4}	0.2	16	540

*Fold changes were calculated by comparing mean values of each of the percent popularity scenarios with the mean value of the baseline model.

5.5.4 Varying levels of E. coli O157:H7 prevalence post-harvest

Due to a lack of available data on the prevalence levels of *E. coli* O157:H7 in cilantro grown in the U.S. the initial 0.1% prevalence was used for this study, adapted from other studies (Pang et al., 2017). To better understand how the prevalence may affect the relative number of illnesses per year caused by consuming cilantro, different levels of *E. coli* O157:H7 prevalence were evaluated against the baseline. The predicted number of cases per year increased from 13.4 fold to 89.1-fold when prevalence increased from baseline (0.1%) to 10% (Table 10). As the prevalence increased so did the probability of illness, as demonstrated in the CDFs in Figure 8.

Table 10: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated cilantro in the U.S. population for the baseline model and different initial E. coli O157:H7 prevalence levels

Prevalence of Contamination (%)	Number of Cases per Year							
	Median	Mean	Fold change*	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Baseline	3.2×10^{-3}	164	--	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147
1	3.1×10^{-2}	2,199	13.4	4.5×10^{-6}	2.1×10^{-3}	0.6	47	1,491
3	9.6×10^{-2}	5,640	34.4	1.3×10^{-5}	6.2×10^{-3}	2	145	4,489
5	0.2	10,104	61.6	2.3×10^{-5}	1.0×10^{-2}	3	244	7,877
10	0.3	14,601	89.1	4.4×10^{-5}	2.1×10^{-2}	6	510	15,325

*Fold changes were calculated by comparing mean values of each of the prevalence scenarios with the mean value of the baseline model.

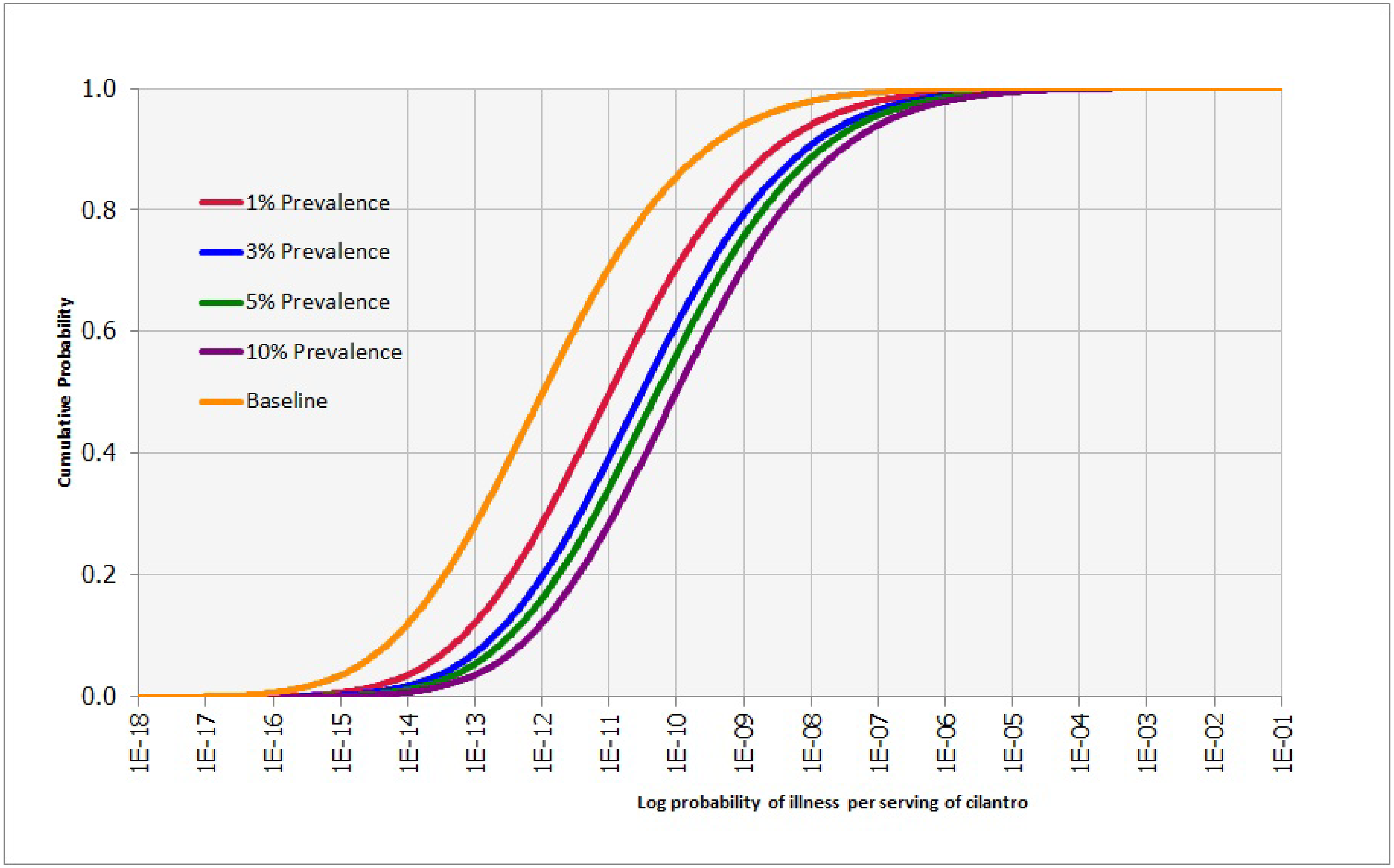

Figure 8: Cumulative density functions of the probability of illness per serving cilantro for the baseline model (orange) and four increased levels of E. coli O157:H7 prevalence. Distributions are shown on a logarithmic scale.

5.5.5 The effect of cross contamination on varying E. coli O157:H7 prevalence levels

To better understand how cross contamination may affect the relative number of illnesses per year caused by cilantro, a triangular distribution of min=1-fold, most likely=1.2-fold and max=2-fold increase was multiplied by the prevalence level to evaluate the effects of cross contamination (Table 11). This cross contamination factor was applied to the same varying levels of prevalence (0.1%-10%) as described in the previous scenario against the baseline. The predicted number of cases per year increased from 1.8-fold to 124.2-fold when considering the potential for cross contamination as prevalence levels increased from baseline (0.1%) to 10% (Table 11). The mean number of illness cases increased for all corresponding prevalence that did not incorporate cross contamination (Table 10). As the prevalence increased so did the probability of illness, as demonstrated in the CDFs in Figure 9.

Table 11: Number of illness cases per year due to consumption of E. coli O157:H7 contaminated cilantro in the U.S. population for the baseline model and different initial E. coli O157:H7 prevalence levels accounting for potential cross contamination

Prevalence of Contamination (%)	**Number of Cases per Year**							
	Median	Mean	Fold change*	1st Percentile	25th Percentile	75th Percentile	95th Percentile	99th Percentile
Baseline – No CC	3.2×10^{-3}	164	--	4.6×10^{-7}	2.1×10^{-4}	5.8×10^{-2}	5	147
0.1	4.1×10^{-3}	299	1.8	5.5×10^{-7}	2.6×10^{-4}	0.1	6	191
1	4.1×10^{-2}	3,255	19.9	5.7×10^{-6}	2.8×10^{-3}	0.7	66	1,958
3	0.1	7,953	48.5	1.6×10^{-5}	7.9×10^{-3}	2	207	6,102
5	0.2	11,574	70.6	2.6×10^{-5}	1.3×10^{-2}	4	336	10,424
10	0.4	20,363	124.2	5.3×10^{-5}	2.7×10^{-2}	7	643	21,536

*Fold changes were calculated by comparing mean values of each of the prevalence and cross contamination scenarios with the mean value of the baseline model.

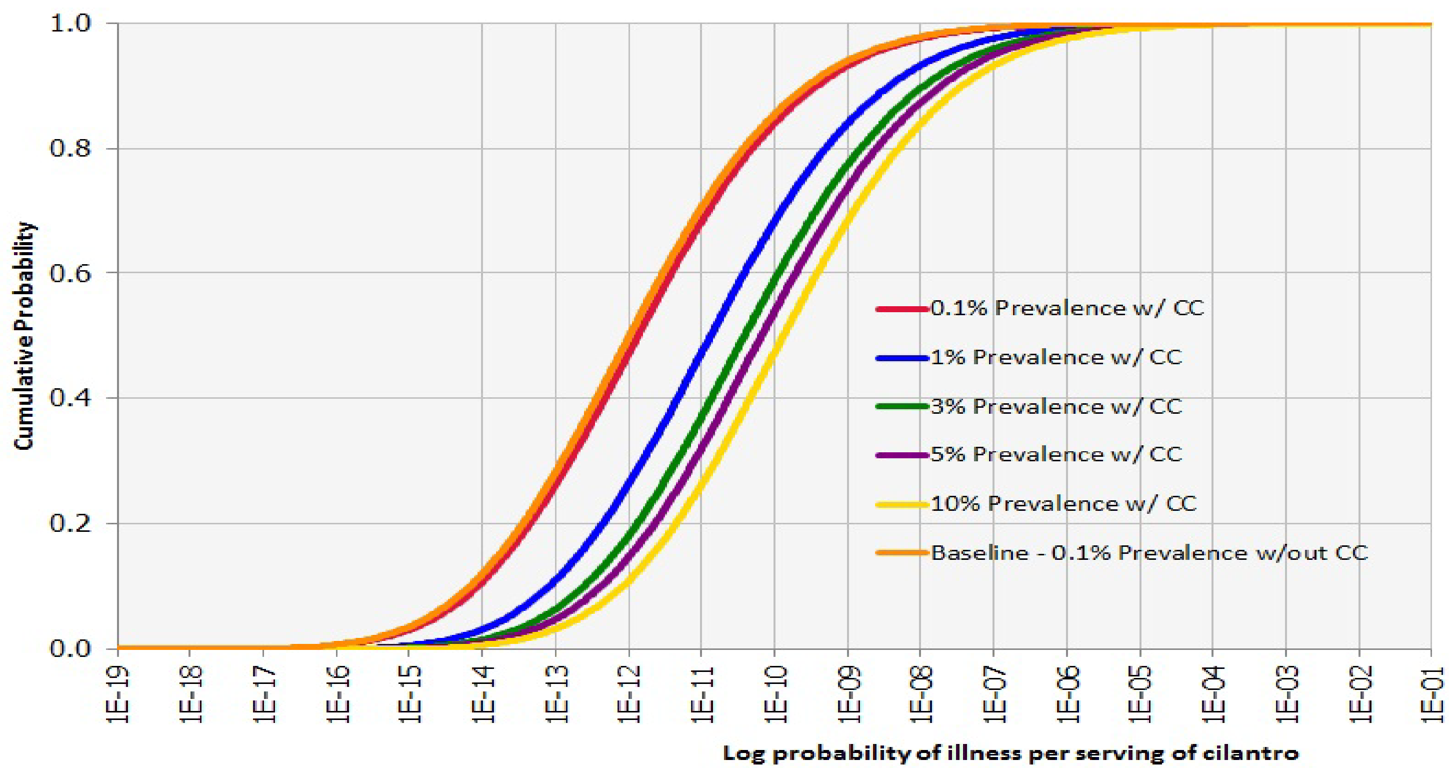

Figure 9: Cumulative density functions of the probability of illness per serving cilantro for the baseline model (orange) and five varying levels of E. coli O157:H7 prevalence incorporating the potential effects of cross contamination. Distributions are shown on a logarithmic scale.

5.6 Discussion

This QMRA model was generated to estimate the number of illnesses caused by the consumption of a serving of *E. coli* O157:H7 contaminated fresh cilantro in the U.S. and to identify contributing parameters from farm-to-fork. Although several studies in the last decade have created QMRA models for *E. coli* in fresh cut leafy greens infield (Franz et al., 2008; McKellar et al., 2014, Allende et al., 2017) and through the farm-to-fork supply chain (Danyluk and Schaffner, 2011; Pang et al., 2017), only one recent study focused on herbs likelihood to cause illness from *E. coli* O157:H7 contamination. The Kundo et al. (2018) QMRA model focused on determining the effects of post market mitigation techniques in reducing contamination of cilantro grown in India and did not include contamination from environmental sources, such as irrigation water and soil or harvesting tools which could affect the level of contamination with *E. coli* O157:H7 prior to retail sale. Because retail cilantro in U.S. is usually not further processed after harvest and bunching, a larger focus needs to be on infield contamination and growth.

Allende et al. (2017) determined that seasonality, solar radiation and rainfall were important impacts on the overall *E. coli* contamination of spinach infield, however their model used leafy green data from previous studies to investigate the effects. There is currently a lack of data specific to the infield growth of herbs, like cilantro. Although many of the growing conditions such as climate and location will be similar for herbs and leafy greens, things like transfer of rain and irrigation water, transfer of soil due to splashing and die-off of pathogens are going to be more plant specific due to leaf size, proximity to the ground and other factors including antimicrobial properties. In this study, QMRA modeling was used to study contamination from irrigation water, soil splash from rain and irrigation and harvesting tools to determine their effects on *E. coli* O157:H7 contamination of cilantro. The potential for solar die-off of the pathogen during infield growth was also considered. However most of the parameters used to run this model, including irrigation contamination, the ratio of pathogen to generic *E. coli* in soil and water and bacteria attachment rates were quantified using different distributions from limited data provided from leafy green studies (Table 2), which are larger and hardier crops.

This model provides a mathematical description of the cilantro grown infield and could be used to better understand the effect of direct sunlight, overhead sprinkler irrigation and time in the field on pathogen growth. In comparing the concentration of *E. coli* O157:H7 right before harvest for both cilantro grown in winter and summer conditions (Table 4) IQR concentration values were higher for cilantro grown in the summer conditions. This could suggest that the longer growth periods and exposure to more sun days in winter conditions (Table 3) could reduce the level of pathogen contamination. This is further supported by the amount of sun per day cilantro receives in field and the number of days cilantro is grown infield having -0.24 and -0.10 Spearman correlation coefficients, respectively, for their effect on the number of illness cases from consuming cilantro grown in winter conditions.

The day at which the last rain or irrigation event occurred would also have an effect on the concentration of *E. coli* O157:H7 at harvest, with cilantro receiving rain or irrigation the day of harvest having no time for solar inactivation of the last contamination event. The food safety guidelines for the harvest of fresh culinary herbs, developed together by government agencies and industry, advise that prior to harvest farmers should “schedule irrigation so as to avoid exposing the plants to excessive mud and soil.” (FDA & WGA,

2013) and the FDA specifically advises farmers to allow for a “die off period” of one or two days since the last watering event prior to harvest to allow for a reduction of microbiological contamination. Due to the random nature of the model and the limited number of rain and irrigation days incorporated into the model, a watering event occurring on the last day would not occur in most cases; however, the inclusion of the possibility in the model would likely overestimate the risk of illness.

This QMRA studied the effects of irrigation water contamination assuming that the water was in compliance with LMGA guidelines for having no more than 235 CFU/100 ml of generic *E. coli* in any given sample. While this is ideal, contamination events of irrigation water have occurred in the past and irrigation/reservoir water has been cited as the cause of recent romaine lettuce outbreaks (FDA, 2019) suggesting that irrigation water can have large effects on outbreaks. This was supported by the sensitivity analysis run in this study that indicated irrigation water quality and the ratio of pathogenic to generic *E. coli* in water had significant effects in the number of illness cases per year from consuming cilantro grown in both winter and summer conditions. To evaluate the effect of different irrigation water contamination levels on the number of illnesses per year, this QMRA model was run at five different contamination levels above LGMA guidelines, ranging from 500 to 2,500 CFU/100 ml. As expected based on the sensitivity analysis, the increase in the number of illnesses per year increased along with the level of contamination. Unfortunately because there is no known correlation between generic and pathogenic *E. coli* and the ratio used for this model was an estimation taken from manure amended soil, the parameters in this model may differ substantially from real situations. Ideally more applicable ratios from studies in irrigation water will be performed in the future and a better estimate of exactly how this increase in contamination of irrigation water can be evaluated.

The predictive model used in this study accounted for the change in temperature conditions through combined growth and die-off models applied above and below the threshold temperature of 5°C. Some of the longest storage conditions that cilantro is exposed to is during transportation from farm to retail establishments across the country. According to discussions with an industry professional this transportation time can take up to seven days. With the increase attention to sanitary transportation of food due to the implementation of FSMA, times and temperatures are being more closely monitored. However due to a lack of more specific information this model assumes that the temperature during transportation does not go higher than described by the industry expert (34-36°F/1.1-2.2°C). In reality the temperature can fluctuate especially if stops are made to unload deliveries to multiple locations. To study the effects of potential temperature abuse during this stage of the supply chain this model was run five times at five temperature levels, four above the values described in the baseline. As temperature increased above 2°C so did the probability of illness per serving and number of illnesses per year caused by consumption of cilantro. This change was very minimal for temperatures below 5°C as this is the threshold value for the determination of whether the growth or die-off equations are used. Anything below this temperature is calculated as a pathogen die-off. The results indicate that if temperature abuse exceeds refrigeration temperature and approaches ambient temperature the number of illnesses per year can significantly increase. At 12°C (53.6°F), a 83.4 fold increase was observed in the number of mean illness cases per year from the baseline study which assumed industry standards were being maintained. The estimates provided by this model are based on point estimates for the temperature values, assuming temperature does not fluctuate. Unless the transportation vehicle is not refrigerated at all, it is unlikely that the values closer to ambient temperature would be maintained for the entirety of the transportation. Again it is more likely that the temperature would fluctuate above and below the 5°C growth/die-off threshold.

As stated throughout this report, the consumption data for cilantro, and herbs in general, in the U.S. is out dated and likely an underestimate of the actual popularity of the herb today. Because better data does not exist, this model was run five times at varying point estimates of popularity, assuming a range of 20%-60% of the U.S. population consumes cilantro once a week. As the popularity increased so did the mean number of illnesses per year. As stated in section 5.3, if the outcome of the model resulted in less than one illness per year, it was assumed that no illness occurred. Although the mean number of illnesses increases with popularity (127 to 547 illnesses per year for 20% and 60% popularity respectively), illnesses were not observed for 90% of the model iterations. This indicates that although popularity may increase it does not have a strong effect on the overall likelihood of illness associated with *E. coli* O157:H7 and cilantro.

With the limited data available for *E. coli* O157:H7 prevalence in cilantro varying widely from <1% (FDA, 2018) to 19.5% (WHO, 1998), to better understand how the prevalence may affect the relative number of illnesses per year caused by cilantro, different levels of contamination prevalence were evaluated against the baseline. As prevalence increased so did the likelihood for illness per serving of cilantro. When better prevalence data becomes available in the future, this study will be able to reflect that change.

While previous QMRA studies described the cross contamination rates from contaminated produce to processing facilities back to uncontaminated produce, cilantro for retail receives no processing outside cutting for harvesting, bunching infield and packaging into boxes. However, this does not mean that cross contamination does not occur along the cilantro supply chain. The means by which cilantro are bunched and then bunches are further packaged together in boxes on ice lends itself to cross contamination. Cross contamination occurs when, during direct or indirect contact, a fraction of bacteria from one item is being lost to another, but no new bacteria are being generated from this contact. That is why in past studies the FDA represented cross contamination as an increase in prevalence, rather than an increase in microbiological load. This could have one of two effects, it could lower the level of concentration below the infectious dose or it could increase the likelihood that each cilantro serving is contaminated therefore increasing the probability of illness. Because *E. coli* O157:H7 has such a low infectious dose, this model and others assumed even one cell could cause illness (Meng et al., 2007; Cassin et al., 1998). For this reason the potential for cross contamination to have a diluting effect was not investigated in this study. Since no study that measured cross contamination from direct contact scenarios similar to bunching and packing of cilantro was available, to test the effects of cross contamination the range of multiplying factors 1- to 2-fold (most likely 1.2-fold) previously identified as the prevalence increase for washed lettuce, was applied to the five levels of *E. coli* O157:H7 prevalence tested in this study. When compared to the baseline, which did not incorporate cross contamination, and other scenarios excluding the cross contamination factor, the mean number of illness cases per year was larger when incorporating cross contamination. When larger prevalence values were evaluated the effect of cross contamination was even more evident. The mean number of illnesses for a 10% prevalence with and without incorporating cross contamination was 20,363 (124.2 fold increase from baseline) and 14,601 (89.1 fold increase from baseline), respectively. Although this prevalence is unlikely in a real world setting, unless extreme negligence is observed along the supply chain or an intentional contamination event occurred, these results indicate that cross contamination can have a substantial effect on public health and should be further studied and prevented.

This QMRA estimated the risk of contracting an illness from a serving of *E. coli* O157:H7 contaminated cilantro in the U.S. as being very low, with over 90% of iterations of the base model, using both winter and summer conditions, resulting in no illness. However in the 95 and 99 percentiles of these models the number of illnesses per year ranged from 1-27, for cilantro grown in winter conditions and 5-147 illness per year for cilantro grown in summer conditions. During a search of the literature, no other QMRA model for cilantro and *E. coli* O157:H7 has been identified so verification of the outcome is difficult. However the rarity of illnesses described in the model results is reflected in the historically low number of illness cases attributed to *E. coli* O157:H7 in cilantro. Additionally the range in illnesses reflected in the 95 to 99 percentile range suggest that outbreaks can occur and are similar to the number of cases associated with the *E. coli* O157:H7 contaminated cilantro outbreak in 2016 (96 cases) as well as the number of cases that have been related to outbreaks involving salsa products (2,280 cases/16 years = 142 cases/year) which usually contain cilantro (IDPH, 2016; Franco and Simonne, 2009). The large variances for both winter and summer growing condition for this study can be attributed to the complexity of the model and incorporating a random function during the growth and inactivation models infield.

Discerning between and identifying the variability and uncertainty are important aspects of any QMRA study. This risk assessment is a one-dimensional model combining variability represented by the random selections accounting for the inherent heterogeneous of a population and uncertainty represented by probability distributions of individual parameters. There are a number of data gaps, assumptions and limitations within this study that need to be identified for future studies to address. The soil concentration of generic *E. coli* was based on samples taken from areas in close proximity to cattle areas. Assuming the cilantro producers are following industry guidance, this soil concentration may overestimate the likelihood of contamination (Lenehan et al., 2005). The ratio of pathogenic to generic *E. coli* used in this study was taken from a study of cattle manure amended soil (Ottoson et al., 2011) and used for both irrigation water and soil. Currently, there is no information on industry or regulatory accepted correlation between pathogenic and generic *E. coli* available; thus, the estimate used in this model does not represent all situations. Additionally, the same distribution is unlikely to represent both water and soil, particularly those used in the field production of cilantro. Because the ratio comes from manure amended soil, the ratio may be higher than what is found in cilantro production, which does not usually utilize manure amendments.

The exclusion of the antimicrobial activity of the cilantro oil in this model would likely result in an overestimation of the concentration of *E. coli* O157:H7 along the supply chain. The effect of cilantro leaf oil on the inactivation of this pathogen along the supply chain could be incorporated into this model when such data becomes available.

The initial prevalence of contaminated cilantro prior to harvest is unknown, this model assumes a 0.1% prevalence of contamination, as multiple other QMRA models have for leafy greens prior to processing in the past (Pang et al., 2017; Danyluk & Schaffner, 2011). While this study tried to account for this unknown by running five scenarios at varying prevalence levels, if future studies identified a more specific prevalence related to *E. coli* O157:H7 in cilantro production the results would be able to better reflect reality.

The goal of any risk assessment is to provide highly valuable information about parameters that impact pathogen levels along the supply chain of a certain food to both regulatory agencies and industry in order to focus their time, attention and resources into the most effective areas of risk mitigation. This QMRA model was developed with available data from scientific literature and conversations with industry professionals and was used to provide an estimate of the risk of contracting an illness from consuming a serving of *E. coli* O157:H7 contaminated cilantro in the U.S. In addition, this model estimated the contamination, growth and inactivation of that pathogen along the supply chain to demonstrate the rate at which contamination grows from pre-harvest, post packaging and post home storage. Future studies can adapt this model to provide a more accurate representation of the contamination of cilantro with *E. coli* O157:H7 from farm to fork as more data becomes available and fill in the data gaps and limitations of this study.

Chapter 6: Conclusion & Suggestions for Future Research

The QMRA model developed for this study can provide risk managers and policy-makers a way to investigate and analyze key factors in the contamination of fresh cilantro with *E. coli* O157:H7 along the supply chain. Current practices for fresh cilantro intended for retail was estimated based on a thorough review of published scientific literature, industry and regulatory guidance and conversations with an industry professional. While no other past QMRA models have estimated the number of illnesses attributed to the consumption of a serving of *E. coli* O157:H7 contaminated cilantro in the past, the predicted number of cases per year based on the model developed in this study is comparable to the low number of illnesses and outbreaks contributed to *E. coli* O157:H7 contaminated cilantro per year. The model outcomes also reflect the ability of this combination to contribute to sporadic outbreaks such as the 2016 cilantro outbreak and those associated with red salsa, a commonly cilantro containing food. The QMRA model provides a method to assess how individual parameters, like travel times and temperatures or pathogen prevalence in the crop soil can impact the concentration of the pathogen at different stages of production as well as the illness incidence. With that knowledge risk managers can determine where to focus their time, resources and money to most efficiently address risk.

The results indicate that retail storage temperature and level of contamination and ratio of pathogenic to generic *E. coli* in irrigation water had the most impact on increased risk of illness from consumption of cilantro grown in both the summer and winter conditions, other than serving size. The number of sun hours and the transportation time from harvest to retail had the biggest impacts on the reduction of risk for both summer and winter growing conditions. The number of days of growth additionally had a reducing impact on the risk of consumption for cilantro grown during the summer. These results suggest that risk management could focus on temperature control at retail as well as quality of the irrigation water to reduce risk. The different risk scenario results found in this model indicated the importance of limiting and reducing cross contamination along the production chain, especially at higher initial prevalence levels, as well as the importance of preventing temperature abuse during transportation from farm to retail, when reducing overall risk of illness.

Additional research specific to the production of cilantro is needed. Some critical data gaps were identified in this QMRA study including, prevalence of generic *E. coli* and/or *E. coli* O157:H7 in the soil cilantro is grown in, initial prevalence of contaminated cilantro, the inactivation of *E. coli* O157:H7 on cilantro due to the presence of cilantro leaf oil, transfer rate of pathogens due to cross contamination during bunching and packaging, and up to date detail cilantro consumption data in the U.S. While this model was focused on retail cilantro, which does not receive any additional processing after harvest, the effects of processing and handling of cilantro at home and restaurants on the overall risk of illness have not been sufficiently studied and future studies incorporating these would be helpful.

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