CROSS-CONTAMINATION, MODELING, AND RISK ASSESSMENT FOR PATHOGENS ON FRESH-CUT PRODUCE

By

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Abstract of Dissertation

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The overall goal of this dissertation was to understand key factors that influence the microbial safety of fresh-cut leafy greens and tomatoes during processing. Laboratory experiments, computer modeling and risk assessment were used to achieve these objectives. In Chapter II, *E. aerogenes* and avirulent *E. coli* O157:H7 were found to be suitable surrogates for pathogenic *E. coli* O157:H7 when comparing mean log reduction when washing with water. These organisms were not suitable surrogates for studying transfer from inoculated to non-inoculated lettuce leaves during water washing. Post-inoculation drying time and water volume influenced transfer of pathogens during washing but the ratio of inoculated to non-inoculated leaves did not have a statistically significant effect. Chapter III demonstrates that while curli producing *E. coli* O157:H7 attach significantly more to lettuce than the non-producing curli strain, curli production has no significant effect on cross-contamination to non-inoculated lettuce during water washing. In Chapter IV, the growth of *Salmonella, E. coli* O157:H7 and *L. monocytogenes* on leafy greens during transportation was predicted using real-world

transport truck data using time series analysis to simulate transit temperatures. Most models showed relatively close agreement, but some models predicted less growth due to the use of modified atmosphere growth conditions or the use of limited published data. A Quantitative Microbial Risk Assessment (QMRA) was developed to calculate the probability and the total number of illness caused by *Salmonella* on cut tomatoes in Chapter V. QMRA results showed that using a mechanical slicer, and slicing at 4°C decreases the transfer of *Salmonella* on cut tomatoes. These findings from these studies will help improve the safety fresh cut leafy greens and tomatoes.

Dedication

I dedicate this dissertation to my parents, Emmanuel and Micheline Charles, for always pushing me, believing in me and praying for me although they don't understand what I am studying.

I also dedicate this work to Philip Vegdahl, my dearest husband for loving me unconditionally

Baby Vegdahl, I am doing this for you too, I am looking forward to meeting you

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I would like to thank Dr. Donald Schaffner for his guidance, patience, his mentorship, and financial supports. You have been more than advisor.

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Preface

This dissertation is a compilation of four studies aiming at understanding Microbial Safety of Fresh-Cut produce during processing. Chapter I is the literature review and provides in depth information that will supplement the following chapters. Chapters II and III include studies in which Dr. Donald Schaffner is the primary investigator. Chapter II aims to find a suitable surrogate for *E. coli* O157:H7 during fresh-cut lettuce washing. Chapter III investigates the influence of curli producing *E. coli* O157:H7 transfer on fresh-cut lettuce during washing. For the two studies, Ann Vegdahl performed the experiments and data analysis and wrote the first draft of the manuscript.

Chapter IV and V are the results of collaboration between multiple institutions. These works were funded by Michigan State University Fresh-Cut produce grant in which Dr. Elliot Ryser is the primary investigator. Chapter IV is a collaboration between Dr. Elliot Ryser (Michigan State University), Dr. Keith Vorst (California Polytechnic Station University), and Dr. Donald Schaffner and focused on predicting the growth of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* on fresh-cut leafy greens during transportation using time series analysis. Finally, Chapter V is also collaboration between Dr. Elliot Ryser, and Dr. Donald Schaffner and is a quantitative microbial risk assessment of *Salmonella* on sliced tomatoes. In the last two studies, Ann Vegdahl also performed all the data analysis; build the mathematical models using the data provided by the collaborators.

Chapter I: Literature Review

Fresh Produce outbreaks

For the last two decades, the number of food-borne outbreaks associated with fresh-cut produce has increased tremendously (187). From 1973 and 1997, lettuce, melon, seed sprouts and fruits juices were often associated with foodborne outbreaks (187). Additional outbreaks have been linked to tomato (54, 85), cilantro (39, 203), parsley (130), spinach, green onions (199), carrot and cabbage (183). Many pathogens have been associated fresh produce outbreaks including *Salmonella*, pathogenic *E. coli*, *Shigella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia* spp., and *Bacillus cereus*. *Salmonella* is the most frequent cause of fresh produce outbreaks, and has been associated with 48% of fresh produce outbreaks occurring between 1973 and 1997(187).

The exact cause of the apparent increase in fresh produce outbreaks is unclear; however, many factors might explain this development. First, the consumption of fresh produce increased significantly in recent years. This increase in consumption does not explain the overall increase in outbreaks. The rise in purchases of fresh-cut and bagged produce items may have introduced additional risk, since the cutting process liberates moisture and nutrients, which can facilitate pathogen growth. Growth in imports of fresh produce cultivated in regions with lower water quality may have raised risk as well. Enhanced epidemiological surveillance, and an increase in the fraction of the population that are at risk (e.g. the elderly and immuno-compromised) may also have contributed to the increase (198).

Source of microbial contamination

Understanding the routes of produce contamination is important in designing strategies to prevent future outbreaks (31). Analysis of produce-associated outbreaks revealed that ~80% of outbreaks originated at the farm, but contamination can arise at multiple points both pre- and post-harvest.

Pre-harvest contamination

Enteric pathogens bacteria like *E. coli* and *Salmonella* can be found in the gut of ruminants like cattle, sheep, and goats (64). These bacteria can also be found in poultry, pigs, fish, waterfowl, rodents and insects, as well as soil, and various aqueous environments. An average of 80 millions tons of manure is produced every year by the US animal industry (179). Manure is applied in the field to dispose of animal wastes and to fertilize soil, but if not properly composed, it can spread disease. The presence of flying insects from manure piles to leaf surface is an additional contamination risk factor (179).

Irrigation water may be another risk factor to plant contamination. Cattle in a nearby field were implicated as the source of *E. coli* O157: H7 in a multistate outbreak, and the authors of a study that examined the outbreak concluded that the soil was irrigated with contaminated water (191). In area where fresh water supply is scarce, low quality water sources are often used which increase the risk of microbial contamination (91, 106). Rainwater has been traced back to be the cause of several outbreaks (52). Since *E. coli* and *S. enterica* survive very well in soil and water, rainwater is a contributing factor to crop contamination (75, 86).

Post-Harvest contamination

Human and mechanical contact can also impact the microbiology safety of fresh produce during harvest. Farm workers were linked to a cholera outbreak in sliced watermelon (3), highlighting the importance of proper worker hygiene including hand washing (28). Once harvested, fresh produce may experience human contact, immersion in water, cutting and slicing, any of which have potential for either causing contamination or enhancing bacterial growth (113, 114). Fruits and vegetables may be exposed to warm temperatures during transport, which can also increase risk (28).

Pathogenic *E. coli*

E. coli is gram-negative bacilli within the family of *Enterobacteriacea* (19, 62), and while most strains are non-pathogenic, *Escherichia coli* O157: H7 was first recognized as a human pathogen after causing two outbreaks in 1982. *E. coli* can be isolated on either rich or selective media at 37°C under aerobic condition. MacConkey agar and eosin methylene-blue select for *E. coli* on the basis on morphology or metabolism. The pathogen can also be identified via biochemical test as well. Indole test is an efficient biochemical test to differentiate member of *Enterobacteriaceae* since 99% *E. coli* strains are indole positive (15). *E. coli* is serotyped based on O (somatic), H (flagella) and K (capsular) surface antigen profile. Other molecular methods have also been developed to quickly identify pathogenic *E. coli* using nucleic acid probes, polymerase chain reaction, and other molecular methods (131).

E. coli has been classified in 6 different categories based on virulence factors and symptoms: Enterohemorrhagic (EHEC), Enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC) and diffusely adherent (DAEC)

(34). They all share O (lipolysaccharide, LPS) and H (flagella) antigens (131). Most pathogenic *E coli* strains are extracellular pathogens, but EIEC can invade and replicate within epithelial cells. All pathogenic *E. coli* strains produce Shiga toxin, which binds to globotriaosylceramide (Gb), a receptor on the surface of eukaryotic cells. Shiga toxin can indirectly damage cells by causing the release of cytokines. The ability to make toxin alone does not make *E. coli* pathogenic, as other virulence factors including fimbrial adhesins, enterotoxins, cytotoxins, capsule and LPS are also required. Initial symptoms of *E. coli* infection generally appear 1-2 days after eating contaminated food, but longer incubation periods (3-5 days) have also been documented. Symptoms include diarrhea and abdominal cramps, and may last 4-10 days. Symptoms may also be more severe and potentially life threatening, including bloody diarrhea, intravascular destruction of red blood cells, and hemolytic uremic syndrome (197).

Survival and Growth

The survival and growth of *E. coli* depend on factors including temperature, pH, and water activity. As a natural inhabitant of the mammalian intestine, it is not surprising that *E. coli* grows very well at 37°C. Many isolates do not grow well at temperatures above 45°C and below 8-10°C. The optimal pH for *E. coli* growth is between 5.5 and 7.5, depending on the source of acid (organic vs. inorganic) as well other factors. The minimum pH for growth is 4.0 (36). There are several studies on mathematical models for the effect of temperature and pH and sodium nitrite on pathogenic *E. coli* growth kinetics (36, 59, 144, 192). Water activity also affects the survival and growth of *E. coli* O157: H7. Buchanan and Bagi developed a mathematical model on the effect of different

sugars and sodium chloride on the survival of *E. coli* and found that the pathogen can survive for days in low water activity for weeks even at low temperature (35).

Although *E. coli* will not grow below pH 4, there have been numerous outbreaks associated with acidic foods including sausages, apple cider and other juices, which clearly indicate that *E. coli* can survive well under acidic conditions. Pathogenic *E. coli* can survive for weeks or months in mayonnaise (224), fermented sausages (48), apple cider (225), and cheddar cheese (166). The acid tolerance of *E. coli* appears to be regulated, at least in part by the expression of sigma factor *rpoS* (47).

E. coli O157:H7 leafy greens outbreaks

There have been numerous pathogenic *E. coli* outbreaks linked to leafy greens across the US. Table 1 provides of such outbreaks occurring since 1993 (65).

Table 1: Selected *E. coli* O157:H7 lettuce outbreaks between 1993 and 2008

Produces	**Cases**	**Location**	**Month and Year**
Salad bar	53	WA & MT	August 1993
Lettuce	70	MT	July 1995
Lettuce	20	ME	September 1995
Lettuce	11	OH	October 1995
Lettuce	61	CT	May-June 1996
Salad	2	CA	May 1998
Lettuce	72	NE	February-March 1999
Lettuce	29	WA & ID	July-August 2002
Lettuce	57	CA	October 2003 and May 2004
Lettuce	71	NY, PA, & NJ	September 2006

Lettuce	81	IA, MN, & WI	November 2006
Lettuce	9	WA	May 2008

Curli expression

Curli are very thin, coiled extracellular structures expressed on the surface of both *E. coli* and *S. enterica*, and may affect cell adhesion onto surfaces, including those of fresh produce. Curli expression is dependent of several environmental factors. Some strains of *E. coli* produce curli at 26°C but not always at 37°C (141). *Csg* genes encode for curli in *E. coli* and are regulated by environmental conditions (32). The presence of curli on bacteria surfaces varies for each strain. Uhlich et al. surveyed a total of 49 *E. coli* O157 isolates and found that only 2 strains (ATCC 43894 and 43895) expressed curli, and most strains had a point mutation in the *csgD* promoter region leading to non-curli production (205). Curli are also involved in biofilm formation and binding to various cellular structures (32). When under stress, the expression of curli may help bacterial cells aggregate to each other and surfaces to increase the chance of survival. Several studies have found curli to play a role in attachment to abiotic surface. The attachment of *E. coli* O157 K-12 (a curli-overproducing strain) to polystyrene and thermonox plastic, glass coverslips, polystyrene, stainless steel and glass have been investigated (112, 148, 158). Ryu and Beuchat showed significant differences in attachment to stainless steel coupons between curli producing and non-producing *E. coli* strains (171, 172).

Salmonella

Salmonella is a known cause of foodborne disease since the late 1800s causing an estimated of 1.3 billion illnesses annually, worldwide. The US Center for Disease Control and Prevention (CDC) reports most of domestic *Salmonella* infections are foodborne.

Symptoms include vomiting, diarrhea, cramps and fever. Young children, pregnant women, elderly and immune-compromised individuals are at higher risk. *Salmonella* can be classified in three groups based on the host preferences. The first group includes *S.* Typhi and *S.* Paratyphi, which only infects humans not animals (136, 162). The second group includes the serotypes that are specific to one host but may also infect other hosts as well. *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg are part of the last group, which are commonly recovered from humans each year (26).

Salmonella can also be grouped into two species: *S. bongori* and *S. enterica*. The latter includes over 2500 distinct serovars. *S. enterica* serovar Enteritidis and *S. enterica* Typhimurium cause more than half of salmonellosis (180). *Salmonella* are often differentiated by flagella (H), somatic antigens (O), virulence (V), and capsular (K). The organism can be identified by agglutination test of specific antibodies. Those tests are not specific so cross-reactivity between O antigens of *Salmonella* and other *Enterobacteriacea* can occur. Further classification of serotypes had traditionally been based on the antigenicity for the flagella H antigens that are specific for *Salmonella* (82).

Salmonellae are gram-negative, facultative anaerobe, rod-shaped motile, pathogenic bacteria. Salmonellae are versatile, and can survive and grow under various environmental conditions, can survive for extended periods of time in the environment, and are transmitted by vectors like rats, flies, birds and reptiles. *Salmonella* grow at temperatures from 5 to 47°C with optimum growth at 35-37°C. They are subject to thermal destruction at temperatures above 54°C. The optimum pH for *Salmonella* growth ranges from 6.5 and 7.5 and some strains can grow at pH 4 or 9. *Salmonella* can grow in

foods down to water activity (Aw) 0.94 but can survive even at very low Aw <0.2 (83, 122).

Salmonella are widely distributed in nature. Salmonellosis has been linked to a variety of foods including eggs, poultry, milk, seafood, beef, pork, and fresh produce as well as dried foods like nuts (24). *Salmonella* is often associated with fresh fruits and vegetables such as apple, cantaloupe, alfalfa sprout, mango, lettuce, cilantro, unpasteurized orange juice, tomato, melon, celery and parsley (161).

Listeria monocytogenes

Listeria was first described in the published literature as a veterinary pathogen in 1926 and 1927 (126, 152). It is a small, Gram-positive, 1-2 μm long, non-spore forming and facultative anaerobic rod-shaped organism. It can growth at temperatures between -0.4 and 50°C, with an optimum between 30 to 37°C. *Listeria* can grow at pH values as high as 9.6, and it's growth is inhibited by pH values lower than 5.6. Organisms grown at 37°C show little or no motility, but *Listeria* is motile at room temperature (95, 149, 212). *L. monocytogenes* is catalase positive and oxidase negative and expresses a beta-hemolysin, which produces zones of clearing on blood agar.

The genus *Listeria* includes six species: *L. monocytogenes*, *L. innocua*, *L. welshimeri, L. seeligeri, L. ivanovii* and *L. grayi*. Listeriosis is a disease caused by the genus *Listeria* and *L. monocytogenes* is the major pathogenic species in both animals and humans (117). Listeriosis unfortunate effects are well documented and comprise two disease syndromes: invasive and non-invasive. In invasive listeriosis the organism infects sterile parts of the body, including the liver (218), spleen (9), cerebral spinal fluid and blood (23, 51). Non-invasive listeriosis causes gastroenteritis and this form typically

occurs in healthy individuals. Invasive listeriosis occurs in immune-compromised individuals including pregnant women, where is can lead to spontaneous abortion or stillbirth.

The principal route of infection by *L. monocytogenes* is the ingestion of contaminated foods, which pass through the stomach acid. The surviving cells of *L. monocytogenes* may infect intestinal cells, escape engulfment by phagosomes, and multiplying within the cytoplasm and spread between cells (10, 110). *L. monocytogenes* escapes intracellular killing within macrophages by lysis of the phagosomal membrane. Escape into the cytoplasm is mediated by secretion of listeriolysin O (LLO). Superoxide dismutase and catalase activities do not correlate with virulence (216). The uterine contents or the central nervous system may be invaded via the circulatory system (118). *L. monocytogenes* is able to survive and grow in the amniotic fluid, which often leads stillbirth or abortion in pregnant women (53).

L. monocytogenes can be found in a wide variety of animal species. It has been isolated in plant, soil, surface water, sewage, slaughterhouse waste, cow milk, cattle, goats and poultry, and occasionally from wild animals (81, 215). *Listeria* can also be found in raw sewage, abattoirs, cattle markets and poultry packing plants (20). It is can be found in processed, ready-to-eat (RTE) refrigerated meat and dairy products. *L. monocytogenes* can also be isolated from a variety of produce types, including cabbage (153), corn (13), carrots (96, 170, 193), lettuce (7, 56, 72, 200), cucumbers (92, 119), parsley (80), and salad vegetables (60, 188).

Time series analysis

Time series are data points measured over time. It can be continuous or discrete. In a continuous time series, data points are collected at every instance of time, whereas discrete time series data are collected at specific time (4). Time series analysis used a statistical procedure to fitting time series data to a mathematical model. The past observations are collected and analyzed; and the future events can theoretically be predicted using the model (4). Time series modeling typically uses a stochastic process, since the future cannot be predicted with certainty. Time series models can follow patterns including trend, cyclical, seasonal or irregular types. A trend time series is when the data increase, decrease or remain constant over long periods of time. A cyclic time series describes changes caused by circumstances, which repeats in cycle; seasonal times series are variation within year or with the seasons. An irregular time series is caused by unpredictable influences and cannot be repeated in a particular pattern (4).

The concept of stationarity of stochastic processes represents a form of statistical equilibrium. The mean and variance for stationary process are independent of time. Time series data that show trends or seasonal patterns are non-stationary. Since this makes modeling difficult, different power transformations (square root, cube root, log etc.) are often used to transform the data into stationary. To determine a proper model for a given times series data, autocorrelation must be assessed. Autocorrelation shows how one data point relates to prior and subsequent data points in the series.

Two common models used in times series analysis are the autoregressive conditional heteroskedasticity (ARCH) and generalized autoregressive conditional

heteroskedasticity (GARCH) models. ARCH/GARCH models provide a volatility measure (similar to a standard deviation), which can then be used in risk analysis (63).

Microbial Risk Analysis

Predictive Microbiology

Predictive microbiology is a scientific field that quantifies microbial behavior in the food environment using mathematical models. Predictive models are useful tools to improve food safety and quality (142). Models can predict the outcome of events that have not occurred or have not been observed. In a mathematical model, input variables are generally factors such as time, temperature, production volume that determine the type and magnitude of the response or output variable (142).

Microbe behavior in food environment can be studied as a function of the food's properties such as temperature, pH, acid type, salt concentration, etc. Bacterial responses can be predicted using mathematical models including growth rate and lag time (142). Predictive models can be divided in three groups: *primary models* which aim to describe the kinetics of a process defining growth and/or inactivation phases; *secondary models* which describe the effect of environmental condition on the values of the parameters in the primary models; and *tertiary models*, which are based on computer software programs, those allow model inputs to be entered and estimates to be observed through graphs (142).

The square root-type model proposed by Ratkowsky et al describes a linear relationship between the square root of the maximum growth rate and temperature: $\sqrt{\mu_{max}} = b^* (T-T_{min})$ where T_{min} is the minimum temperature below which the growth rate is equal to 0 (165). Many square-root type bacterial growth models have been published

for fresh produce including leafy greens (55, 167), asparagus (168), and cut tomatoes (145).

Quantitative Microbial Risk Assessment

Risk analysis is a tool that fosters problem solving and decision-making. It facilitates the gathering of information, risk analysis, drawing conclusions, and communicating information. Risk analysis has been applied to assess microbial threats to public health (93). Risk analysis is generally defined as consisting of three interconnected components: risk assessment (the scientific and systematic evaluation of a known or potential risk; risk management (the evaluation, selection and implementation of policies to address a risk) and risk communication (the exchange of information among all interested party affected by a risk) (50).

Microbial risk assessment can be described as consisting of four steps, hazard identification where the microorganisms capable of causing adverse health effect are identified; hazard characterization, where a qualitative and/or quantitative evaluation of adverse health effect associated with the organism is performed; exposure assessment where the frequency and levels of ingestion of a pathogen are determined; and finally risk characterization, which provides a complete picture of the risk to the risk manager for decision making. Quantitative microbial risk assessment (QMRA) uses the framework above and brings information and data together in a quantitative way, often using a mathematical model (50, 93).

QMRA as practiced today often uses Monte Carlo simulation (41). There are a variety of tools that can be used to implement a QMRA and one of the most common is an “add-in” for Microsoft Excel called @RISK (Palisade Corporation, 2004). The

@RISK software combines the ease of spreadsheet based formulae and calculations with the power of Monte Carlo simulation. The software allows the user to define the contents of spreadsheet cells to vary according to a particular distribution. Probability distributions in @RISK are then used to generate complicated risk analysis.

Examples of published QMRAs include those for pathogens in leafy greens (55, 73, 202), hand hygiene (57, 181), handwashing (125, 182), deli meat (154-156), beef (127, 134, 135), and poultry (132, 133).

Chapter II: In search of a suitable surrogate: Transfer of *E. aerogenes*, avirulent *E. coli* O157:H7 and pathogenic *E. coli* O157:H7 during lettuce washing

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Abstract

Cross-contamination during washing has been identified as an important factor in controlling risk in leafy greens. Research with foodborne pathogens at pilot plant scale or larger can be problematic, so validated non-pathogenic or avirulent surrogates are needed. This study quantifies cross-contamination by *Enterobacter aerogenes*, avirulent *E. coli* O157:H7, and pathogenic *E. coli* O157:H7 during laboratory scale lettuce washing. The experimental variables were post-inoculation drying time (10 min vs. 2 h), water volume (100 ml vs. 1L), and ratio of inoculated to non-inoculated lettuce pieces (1:5 vs. 1:20). Overnight cultures were centrifuged and re-suspended in peptone water prior to inoculation. A single piece of romaine lettuce was spot inoculated with ~ 6 log CFU of bacteria, dried, and washed with the non-inoculated pieces in a stainless-steel bowl for 30 seconds. Log reduction on the inoculated lettuce and log % transfer to the non-inoculated lettuce were determined. Data were analyzed using multiple linear regression analysis, t-test for mean comparison in Statplus. The log reduction on the inoculated piece ranged between 0.5 to 2.7 log CFU/piece; and the log % transfer to the non-inoculated lettuce pieces ranged between 0 and -1.8 log % CFU/piece. This study revealed that post-inoculation drying time and water volume are significant variables when studying the log reduction ($p<0.05$). Post-inoculation drying time, water volume and microbe strain are important variables to consider when quantifying transfer to non-inoculated lettuce piece ($p<0.05$). This study showed that *E. aerogenes* and avirulent *E. coli* are suitable surrogates for pathogenic *E. coli* O157:H7 when considering mean log reduction ($p>0.05$). When comparing mean log % transfer, neither *E. aerogenes* nor

avirulent *E. coli* were suitable surrogates ($p<0.05$). More research is needed to find suitable surrogates for *E. coli* O157:H7 in lettuce cross-contamination research.

Introduction

The consumption of fruits and vegetables has increased in recent years, likely due to increased emphasis on a diet containing more fresh fruits and vegetables. Many foodborne disease outbreaks have been linked to fresh produce including melons, sprouts, apples, berries, tomatoes and leafy greens (87, 187). Processing and handling of fresh leafy greens do not involve a kill step, and present methods include washing in water containing a sanitizer and cooling below 4°C to reduce pathogen transfer. Washing can remove soil, plant debris, pesticides, and microorganisms (6, 16, 22, 78, 105, 108, 220). Wash water that does not contain sanitizers or which is improperly sanitized can facilitate bacterial cross-contamination to non-contaminated produce during the washing process (6, 14).

A quantitative microbial risk assessment by Danyluk and Schaffner supports the hypothesis that cross-contamination during washing of leafy greens can increase risk (55). Many researchers have studied the effect of washing on reducing microorganisms in leafy greens (5, 79, 102, 190, 211) but there are limited data quantifying cross-contamination from inoculated to non-inoculated lettuce leaves (211, 221). One recent study quantified cross-contamination rates of *E. coli* O157:H7 from one inoculated lettuce leaf to non-inoculated leaves via un-chlorinated tap water (100 ml) while considering different wash time periods (10 and 30 s, 2 and 5 min) (94), and showed that the wash times studied had little effect on cross-contamination rates. These authors concluded that water volume and produce concentration might be key variables needing further investigation in cross-contamination studies.

Research with foodborne pathogens at pilot plant scale or larger are either very costly or unsafe; therefore, validated non-pathogen or avirulent surrogates for studying cross-contamination are needed. The present study was undertaken to investigate and quantify cross-contamination by three organisms. We used a non-foodborne pathogen (*Enterobacter aerogenes*), a three strain cocktail of avirulent *E. coli* O157:H7, and five strain cocktail of pathogenic *E. coli* O157:H7 with the goal of identifying a suitable surrogate for pathogenic *E. coli* O157:H7 for use in pilot plant scale research. Water volume, inoculated to non-inoculated lettuce ratio and post-inoculation drying time variables were also considered.

Materials and methods

Preparation of Stainless Steel bowls and pots

Round stainless steel bowls with a dimension 7.62Hx17.78Wx7.62D cm were used for 100 ml of water, and a 4-quart stainless steel pot was used for the larger volume (1000 ml). The surfaces were disinfected with 70% ethanol and air-dried prior to each experiment.

Produce

Bags of red and green romaine lettuce were purchased from a local supermarket (Somerset, NJ). The lettuce was stored at 4°C prior to use, and used within 5 days of purchase. Lettuce piece was cut into 3 x 3 cm pieces using sterile scissors. A total of 1:5 or 1:20 (inoculated: non-inoculated) lettuce pieces were used per experiment.

Bacterial Strains

Three different sets of microorganisms were used in separate experiments. *Enterobacter aerogenes* B199A is a nonpathogenic microorganism used to study cross-contamination in our lab and elsewhere (223). The avirulent cocktail consisted of three strains of *E. coli* O157:H7 CV2B67, 6982-2 and 6980-2 strains provided by Dr. Joshua Gurtler (US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Lab) (66, 219). The pathogenic cocktail contained five strains of *E. coli* O157:H7 H1730, 4042, 4045, EC4191, and EC4206 provided by Dr. Linda Harris (University of California, Davis) (100). *E. aerogenes* and the three avirulent *E. coli* O157:H7 strains were resistant to nalidixic acid; the five-strain *E. coli* O157:H7 cocktail was resistant to rifampicin. Prior to each experiment, one colony was inoculated in 10 ml of Tryptic Soy Broth (TSB) with 50 μg/ml of nalidixic acid/rifampicin and incubated overnight for 24h at 37°C. One ml of the culture was transferred to a micro-centrifuge tube, and was centrifuged at 0.6 x g for 10 min. Cells were washed twice, removing the supernatant and suspending the cell pellets in 1 ml of 0.1% peptone. The pellets were re-suspended in 1 ml of peptone to achieve a final concentration of ~6log CFU/ml. The final concentration was verified by enumeration on MacConkey or Tryptic Soy Agar with nalidixic acid or rifampicin.

Transfer between lettuce pieces.

One color leaf (red or green) was selected for inoculation and the other color was selected to represent non-inoculated leaves, depending on color availability. Ten μl of bacterial suspension (6 log CFU/ml) was spot inoculated on a lettuce piece and left to dry for either 10 min or 2 h. One hundred ml, or 1000 ml of sterile water was poured into the

designated stainless steel container. Inoculated and non-inoculated pieces were transferred into the containers and mixed using a sterile plastic spoon for 30 seconds. All lettuce pieces as well as the wash water were sampled as indicated below. All experiments were performed in triplicate.

Enumeration

Each lettuce piece was transferred to a 3oz sterile Whirl-Pak bag containing 10 or 40 ml of 0.1% peptone buffer. Lower volumes of buffer were used for *E. coli* testing to lower the detection limit. Leaf samples were homogenized for 2 min, serially diluted, and surface plated on MacConkey and Tryptic Soy agar with 50 μg/ml of nalidixic acid or rifampicin as appropriate. The wash water was also sampled. Samples were incubated for 24h at 37°C. Colonies were enumerated, and counts were expressed as log CFU/lettuce piece, and log CFU/ml for the water.

Calculation and Data analysis

Data were analyzed using multiple linear regression analysis and t-test using Statplus (AnalystSoft, 2016). Log reduction of bacteria on the inoculated leaves, and the log % transfer to the non-inoculated lettuce pieces were calculated as shown below:

Total CFU in the system= [CFU on the initially inoculated lettuce piece+ Σ (CFU on the non-inoculated lettuce pieces)+ CFU in the water]

Log Reduction on the initially inoculated piece = Log (CFU previously non-inoculated lettuce pieces + CFU wash water) - Log (CFU initially inoculated piece post wash)

Transfer Rate to non-inoculated pieces (%) = (CFU on non-inoculated lettuce piece/ Total CFU in the system)*100

A surrogate was considered failsafe for log reduction if the surrogate's log reduction was lower than the log reduction of pathogenic *E. coli* O157:H7. A surrogate was considered failsafe for cross-contamination if the log % transfer of the surrogate is greater than the pathogen. Conversely, a surrogate is fail dangerous if its log reduction is greater than the pathogen, of if the log % transfer is lower than the pathogen.

Results

Reduction on inoculated lettuce pieces

The log reduction of *E. aerogenes*, avirulent *E. coli* O157:H7, and pathogenic *E. coli* O157:H7 on the initially inoculated lettuce leaf is shown for all the variables in Table 1, and the multiple linear regression analysis on this log reduction data is summarized in Table 2. Results showed that post-inoculation drying time ($p<0.001$), and water volume ($p=0.021$) had a statistically significant effect on the calculated log reduction. Inoculated to non-inoculated lettuce leaf ratio and organism type (e.g. *E. aerogenes* vs. avirulent *E. coli* vs. pathogenic *E. coli*) did not have a statistically significant influence on log reduction ($p>0.05$). The difference in log reduction between organisms was also compared at the same volume, drying time, and lettuce ratio. The log reduction between the three types of bacteria was barely significant ($p=0.04$) under only one condition (1000 ml of water, 10 min post-inoculation drying time and 1:20 lettuce ratio (data not shown).

The mean log reductions were compared pairwise between organisms within a single condition using t-test, and the results are shown in Table 3. There were no

significant differences when comparing *E. aerogenes* vs. pathogenic *E. coli* O157:H7 and pathogenic vs. avirulent *E. coli* O157:H7 log reductions across any of the conditions studied. The mean log reductions were either not statistically significant ($p>0.05$) or were failsafe. There were two conditions where significant differences were observed between *E. aerogenes* and avirulent *E. coli* log reductions (Table 3). First, for 100 ml of water, 10 min post-inoculation drying time, 1:5 lettuce ratio, the mean log reductions were 2.06, and 2.54 for *E. aerogenes*, avirulent *E. coli* O157:H7 respectively ($p=0.023$). Second, for 1000 ml water, 10 min post-inoculation drying time, 1:20 lettuce ratio, the mean log reductions were 0.75 and 2.67 for *E. aerogenes* and avirulent *E. coli* respectively ($p=0.033$).

Transfer to non-inoculated lettuce pieces

The log % transfer of the three different bacterial inocula to the non-inoculated lettuce pieces is summarized in Table 4, and the multiple linear regression analysis comparing the log % transfer data is shown in Table 5. The results of the multiple linear regression analysis (Table 5) revealed that water volume and the choice of bacterial type (*E. aerogenes*, avirulent *E. coli* or pathogenic *E. coli*) have a highly significant effect ($p<0.001$) on transfer of bacteria from inoculated to non-inoculated leaves during washing. The post-inoculation drying time also has an effect that just meets the conventional criterion for significance ($p=0.047$). The ratio of inoculated to uninoculated lettuce pieces ($p=0.11$) did not have a statistically significant effect on log percent transfer. The log % transfer of each bacterial type was also compared at the same water volume, drying time, and lettuce piece ratio. There was a significant difference ($p<0.001$) between bacterial types at the lower water volume (100 ml) independent of the post-

inoculation drying time and lettuce piece ratio (data not shown). When higher water volumes were used (1000 ml) no significance difference ($p>0.05$) between bacterial types was observed regardless of post-inoculation drying time and lettuce piece ratio (data not shown).

The means log percent transfer were also compared pairwise between bacterial types (e.g *E. aerogenes* vs. avirulent *E. coli*; *E. aerogenes* vs. pathogenic *E. coli*; avirulent *E. coli* vs. pathogenic *E. coli*) within single conditions (Table 6). The log % transfer was significantly different when comparing *E. aerogenes* and avirulent *E. coli*, for all the conditions except two. Potential surrogates were fail dangerous 11 times, failsafe seven times, and there was no difference six times out of the 24 conditions studied.

Discussion

Prior research has shown that wash water may spread pathogenic bacteria from contaminated pieces of produce to previously uncontaminated pieces of produce during washing (5, 94, 102, 222). However, none of these articles studied the effect of lettuce ratio, water volume and post-inoculation drying time of bacterial transfer to the contaminated and non-contaminated lettuce pieces.

Our study showed that log reductions on inoculated pieces were highly variable and ranged between 0.5 to 2.7 log CFU overall. Jensen *et al.* studied cross-contamination of *E. coli* O157:H7 from inoculated to non-inoculated lettuce leaves during 30s, 1, 2 or 5 min of washing and the water volume was 100 ml. Regardless of the wash times, the log reduction averaged 2-2.5 log CFU (94). These results are consistent with our study where the log reduction of *Enterobacter aerogenes,* avirulent and pathogenic *E. coli* O157:H7

on the inoculated piece for 100 ml of water was ~2.5 log CFU as well. Zhang *et al.* also investigated the transfer of *E. coli* O157:H7 from an inoculated lettuce piece to non-inoculated pieces during washing. Similarly to our study, the log reduction on the inoculated lettuce piece was 2.1 log CFU and 2.5 log CFU transferred to the non-inoculated lettuce pieces.

Patel *et. al* investigated 5 *Salmonella* serovars attachment properties to lettuce and cabbage surface. Theirs showed that the organisms irreversibly attached to the lettuce as soon as 5 min (146). Those results suggest that longer drying times may lead to stronger attachment of the organism to the lettuce, and thus less transfer to non-contaminated leaves. This is consistent with our results since the log reduction on the inoculated lettuce piece after 2 h drying time was lower than the log reduction after 10 min for all the conditions tested. Similarly, the log % transfer to non-contaminated lettuce pieces at 10 min is higher than at 2 h for all experimental scenarios.

Another objective of our study was to find a suitable surrogate of *E. coli* O157:H7 in experiments involving cross-contamination on lettuce during washing. There are very few peer-reviewed articles exploring the suitability of non-pathogenic surrogates for fresh produce washing studies. Peri et al proposed *E. coli* K-12 LMM 1010 to be a suitable surrogate for *E. coli* O157 under acidic and alkaline conditions and reduced water activities (150). Sapers et al proposed *E. coli* ATCC25922 to exhibit similar behavior as the pathogens after washing apples with hydrogen peroxide (178). Another study confirmed that *E. coli* ATCC25922 could be a useful candidate in attachment studies involving romaine lettuce (97). Our study found *E. aerogenes* and avirulent *E. coli* to be suitable surrogates for pathogenic *E. coli* O157:H7 when comparing mean log reduction

during water washing as the differences were generally not statistically significant ($p>0.05$) or where different, were failsafe. Unfortunately, neither *E. aerogenes* nor avirulent *E. coli* were suitable surrogates for pathogenic *E. coli* O157:H7 when comparing the log % transfer. In most cases, differences were statistically significant ($p<0.05$), and showed no clear pattern of failsafe or fail dangerous results. We should point out that although the analysis above concludes that that suitable surrogate may be available for comparing mean log reduction, the number of replicates for these experiments ($n=3$) are far fewer than for the log % transfer experiments ($n=15$ and 60) and that experiments with more replicates might yield different results. Although the results of our study are generally disappointing with respect to log % transfer, clearly more research is needed to select suitable surrogates for *E. coli* O157:H7 to study transfer during lettuce washing. We are encouraged to note that, for the ratios studied here (1:5 vs. 1:20 lettuce pieces), inoculated to non-inoculated leaf ratio does not appear to influence log reduction and the log % transfer.

Tables

Table 1: Log reduction of *E. aerogenes*, avirulent *E. coli* O157:H7 and pathogenic *E. coli* O157:H7 on inoculated romaine lettuce leaves

Organism	Lettuce Ratio[1]	Water Volume	Drying time	Mean	SD[2]	Min	Max	Range[3]
E. aerogenes	1:5	100 ml	10 min	2.06	0.04	2.03	2.11	0.08
			2 hrs	1.68	0.73	0.86	2.25	1.39
		1000 ml	10 min	2.59	0.30	2.40	2.94	0.54
			2 hrs	1.06	0.77	0.58	1.95	1.37
	1:20	100 ml	10 min	2.22	0.44	1.71	2.51	0.80
			2 hrs	1.77	0.34	1.46	2.14	0.68
		1000 ml	10 min	0.75	0.34	0.42	1.09	0.67
			2 hrs	0.67	0.86	0.07	1.65	1.58
Avirulent *E. coli* O157:H7	1:5	100 ml	10 min	2.54	0.07	2.46	2.58	0.12
			2 hrs	1.91	0.85	0.93	2.50	1.57
		1000 ml	10 min	2.67	0.45	2.16	3.04	0.87
			2 hrs	0.82	0.50	0.27	1.26	0.99
	1:20	100 ml	10 min	2.54	0.07	2.46	2.58	0.12
			2 hrs	1.91	0.85	0.93	2.50	1.57
		1000 ml	10 min	2.67	0.45	2.16	3.04	0.87
			2 hrs	0.82	0.50	0.27	1.26	0.99
Pathogenic *E. coli* O157:H7	1:5	100 ml	10 min	1.53	1.17	0.31	2.65	2.34
			2 hrs	1.68	0.14	1.52	1.78	0.26
		1000 ml	10 min	2.66	0.17	2.49	2.83	0.34
			2 hrs	0.82	0.26	0.52	0.99	0.47
	1:20	100 ml	10 min	2.52	0.23	2.38	2.79	0.41
			2 hrs	1.52	0.36	1.12	1.82	0.70
		1000 ml	10 min	2.56	0.64	1.82	2.93	1.11
			2 hrs	1.24	1.03	0.06	1.97	1.91

[1]Inoculated leaves to uninoculated leaves, [2]Standard deviation, and [3]Maximum minus minimu

Table 2: Multiple linear regression summary for log reduction of *E. aerogenes*, avirulent *E. coli* O157:H7, pathogenic *E. coli* O157:H7 and their interactions with the water volume, drying time and lettuce ratio

Variables	Coefficient	Standard Error	LCL[1]	UCL[2]	t Statistic	p-level[3]
Intercept	2.440	0.285	1.871	3.009	8.566	0.001
Lettuce Ratio	-0.004	0.010	-0.025	0.017	-0.407	0.685
Drying Time	-0.009	0.001	-0.011	-0.006	-6.009	0.001
Water Volume	0.001	0.001	-0.001	0.001	-2.366	0.021
Organism	0.103	0.096	-0.089	0.295	1.071	0.288

[1]Lower confidence limit, [2]Upper confidence limit and [3]Variables significant at $p<0.05$ are shaded

Table 3: Comparison of the mean log reduction of *E. aerogenes*, avirulent *E. coli* and pathogenic *E. coli* O157:H7 on the inoculated lettuce piece

Organisms	Water Volume	Lettuce Ratio	Drying Time	First[1] mean log reduction	Second[2] mean log reduction	p-value[3]	Fail Safe[4]
E. aerogenes vs. avirulent *E. coli O157:H7*	100 ml	1:5	10 min	2.06	2.54	0.023	safe
			2 hrs	1.68	1.91	0.826	-
		1:20	10 min	2.22	2.54	0.352	-
			2 hrs	1.77	1.91	0.976	-
	1000 ml	1:5	10 min	2.59	2.67	0.664	-
			2 hrs	1.06	0.82	0.583	-
		1:20	10 min	0.75	2.67	0.033	safe
			2 hrs	0.67	0.82	0.743	-
E. aerogenes vs. pathogenic *E. coli*	100 ml	1:5	10 min	2.06	1.53	0.239	-
			2 hrs	1.68	1.68	0.713	-
		1:20	10 min	2.22	2.52	0.482	-
			2 hrs	1.77	1.52	0.508	-
	1000 ml	1:5	10 min	1.77	1.52	0.508	-
			2 hrs	1.06	0.82	0.664	-
		1:20	10 min	0.75	2.56	0.089	-
			2 hrs	0.67	1.24	0.749	-
Avirulent *E. coli* vs. pathogenic *E. coli*	100 ml	1:5	10 min	2.54	1.53	0.146	-
			2 hrs	1.91	1.68	0.985	-
		1:20	10 min	2.54	2.52	0.162	-
			2 hrs	1.91	1.52	0.764	-
	1000 ml	1:5	10 min	2.67	2.66	0.676	-
			2 hrs	0.82	0.82	0.723	-
		1:20	10 min	2.67	2.56	0.440	-
			2 hrs	0.82	1.24	0.920	-

[1]Mean log reduction for first organism in first column, [2]Mean log reduction for second organism in first column, [3]p-value is from a t-test where the means log reduction were compared, [4]Safe means Fail safe (.i.e. using the surrogate over-estimates transfer), Dangerous means Fail Dangerous (.i.e. using the surrogate under-estimates transfer), "-" means no difference. Failsafe rows are shaded lightly, and no difference rows are unshaded.

Table 4: Log % transfer of *E. aerogenes*, avirulent *E. coli* O157:H7 and pathogenic *E. coli* O157:H7 from inoculated romaine lettuce pieces to non-inoculated lettuce pieces

Organism	Lettuce Ratio[1]	Water Volume	Drying time	Mean	SD[2]	Min	Max	Range[3]
E. aerogenes	1:5	100 ml	10 min	-1.07	0.3	-1.68	-0.47	1.21
			2 hrs	-1.2	0.72	-1.98	-0.23	1.74
		1000 ml	10 min	-1.46	0.52	-2.17	-0.54	1.63
			2 hrs	-1.41	0.34	-1.81	-0.62	1.19
	1:20	100 ml	10 min	-0.71	0.28	-1.09	-0.07	1.01
			2 hrs	-0.08	0.82	-1.51	1.11	2.61
		1000 ml	10 min	-1.43	0.44	-1.97	-0.28	1.69
			2 hrs	-1.59	0.30	-1.36	-0.25	1.12
Avirulent *E. coli* O157:H7	1:5	100 ml	10 min	-0.74	0.30	-1.36	-0.25	1.12
			2 hrs	-1.34	0.49	-1.99	-0.33	1.66
		1000 ml	10 min	-1.2	0.60	-2.93	-0.33	2.60
			2 hrs	-0.66	0.29	-1.05	-0.19	0.86
	1:20	100 ml	10 min	-0.60	0.27	-1.27	-0.04	1.23
			2 hrs	-1.55	0.97	-3.58	-0.31	3.27
		1000 ml	10 min	-1.03	0.46	-2.47	-0.10	2.37
			2 hrs	-1.91	0.53	-0.32	-0.61	2.63
Pathogenic *E. coli* O157:H7	1:5	100 ml	10 min	-0.49	0.62	-1.22	0.35	1.58
			2 hrs	-0.51	0.54	-1.02	0.32	1.34
		1000 ml	10 min	-1.79	0.62	-2.55	-0.78	1.78
			2 hrs	-1.43	0.49	-1.84	-0.33	1.52
	1:20	100 ml	10 min	-1.52	0.55	-2.67	-0.5	2.17
			2 hrs	-0.67	0.39	-1.46	0.15	1.61
		1000 ml	10 min	-1.52	0.55	-2.67	-0.50	2.17
			2 hrs	-1.7	0.36	-2.22	-0.63	1.59

[1]Inoculated leaves to uninoculated leaves, [2]Standard deviation, [3]Maximum minus minimum

Table 6: Multiple linear regression summary for log % transfer of *E. aerogenes*, avirulent *E. coli* O157:H7, pathogenic *E. coli* O157:H7 and their interactions with the water volume, drying time, lettuce ratio

Variables	Coefficient	Standard Error	LCL[1]	UCL[2]	t Stat	p value[3]
Intercept	-0.359	0.095	-0.546	-0.172	-3.764	0.001
Lettuce Ratio	-0.006	0.004	-0.013	0.001	-1.590	0.112
Drying Time	-0.001	0.001	-0.002	0.001	-1.989	0.047
Water Volume	-0.001	0.001	-0.001	-0.001	-14.397	0.001
Organism	-0.138	0.027	-0.190	-0.085	-5.136	0.001

[1]Lower confidence limit, [2]Upper confidence limit, [3]Variables significant at $p<0.05$ are shaded

Table 7: Comparison of the mean log % transfer of *E. aerogenes*, avirulent *E. coli* O157:H7 and pathogenic *E. coli* O157:H7 to non-inoculated lettuce pieces

Organisms	Water Volume	Lettuce Ratio	Drying Time	First[1] mean log transfer	Second[2] mean log transfer	p-value[3]	Fail Safe?[4]
E. aerogenes vs. avirulent *E. coli O157:H7*	100 ml	1:5	10 min	-1.07	-0.74	0.016	dangerous
			2 hrs	-1.2	-1.34	0.531	-
		1:20	10 min	-0.71	-0.6	0.034	dangerous
			2 hrs	-0.08	-1.55	0.000	safe
	1000 ml	1:5	10 min	-1.46	-1.2	0.137	-
			2 hrs	-1.41	-0.66	0.000	dangerous
		1:20	10 min	-1.43	-1.03	0.000	dangerous
			2 hrs	-1.59	-1.91	0.000	safe
E. aerogenes vs. pathogenic *E. coli*	100 ml	1:5	10 min	-1.07	-0.49	0.003	dangerous
			2 hrs	-1.2	-0.51	0.016	dangerous
		1:20	10 min	-0.71	-1.52	0.000	safe
			2 hrs	-0.08	-0.67	0.000	safe
	1000 ml	1:5	10 min	-1.46	-1.79	0.272	-
			2 hrs	-1.41	-1.43	0.924	-
		1:20	10 min	-1.43	-1.52	0.376	-
			2 hrs	-1.59	-1.7	0.107	-
Avirulent *E. coli* vs. pathogenic *E. coli*	100 ml	1:5	10 min	-0.74	-0.49	0.084	dangerous
			2 hrs	-1.34	-0.51	0.001	safe
		1:20	10 min	-0.6	-1.52	<0.001	dangerous
			2 hrs	-1.55	-0.67	<0.001	safe
	1000 ml	1:5	10 min	-1.2	-1.79	0.023	dangerous
		1:20	2 hrs	-0.66	-1.43	<0.001	dangerous

	10 min	-1.03	-1.52	<0.001	dangerous
	2 hrs	-1.91	-1.7	0.012	safe

[1]Mean log reduction for first organism in first column, [2]Mean log reduction for second organism in first column, [3]p-value is from a t-test where the means log reduction were compared, [4]Safe means Fail safe (.i.e. using the surrogate over-estimates transfer), Dangerous means Fail Dangerous (.i.e. using the surrogate under-estimates transfer), "-" means no difference. Fail dangerous rows are shared darkly, failsafe rows are shaded lightly, and no difference rows are unshaded.

Chapter III: Curli production and cross-contamination by *E. coli* O157:H7 E0018- and E0018+ during fresh-cut lettuce washing mediated by different inoculation locations

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Abstract

E. coli O157:H7 express extracellular proteins called curli that are essential for surface colonization and which interact with mammalian host immune systems. Curli production has been shown influence attachment to food surfaces including alfalfa sprouts, lettuce, and spinach. This study quantified the transfer rates of curli producing *E. coli* O157:H7 vs. non-curli producing *E. coli* O157:H7 from inoculated to non-inoculated lettuce pieces during washing. Lettuce pieces were inoculated with ~ 6 log CFU of *E. coli* O157:H7 E0018+ (curli positive) or E0018- (curli negative) on the surface, the cut-edges, and both surface/cut-edges. The inoculated lettuce piece was washed with ten (10) non-inoculated lettuce pieces in 500 ml of water. The log reduction on the inoculated lettuce piece and the log % transfer to the non-inoculated pieces were determined. Results showed that the log reduction of E0018- on the inoculated lettuce was higher than E0018+ regardless on the inoculation location. The log reduction was the lowest when the cut-edges were inoculated regardless of the strain (0.41 and 0.70 log CFU/piece) confirming that *E. coli* O157:H7 is more difficult to remove from cut-edges than from leaf surfaces. The log % transfer of the curli producing strain to the non-inoculated lettuce pieces was significantly higher than for the non-curli producing strain ($p<0.05$). Neither strain preferred the non-inoculated lettuce surface nor the cut-edges after washing. These results point to the need to further understand and develop new intervention methods that could reduce cross-contamination of pathogens during washing.

Introduction

E. coli O157:H7 is the most common enterohemorrhagic *Escherichia coli* (EHEC) and contributes significantly to human infections and foodborne disease outbreaks. More than twenty percent of *E. coli* outbreaks have been linked to fresh produce (164), and lettuce is the most common type of produce implicated. Lettuce is widely consumed in the United States with a farm value of over $1.5 billion/year Calvin 2006}. Researches to find strategies to essentially eliminate pathogens on fresh produce without unduly affecting product quality have been ongoing for some time (21). Lettuce producers use one or more washing steps to reduce food safety risk and prolonging shelf life. Research has shown that the efficacy of washing is reduced when microorganisms are associated with stomata, cracks, or cut surfaces in plant tissues (217).

E. coli expresses extracellular proteins called curli, which are important for surface colonization and interacting with the host immune system. Curli are thin highly stable coiled fibers of varying lengths (6-12 nm wide), that self-assemble outside the cell (32, 45, 158). Curli are a major component protein of *E. coli* biofilm (115), and are resistant to degradation by proteases and denaturation by detergents (45). Curli are depolymerized by strong denaturants such as formic acid or hexafluoroisopropanol (45). The ability to produce curli fimbriae is encoded on two divergently transcribed operons *csgDEFG* and *csgBAC*; and is highly regulated and controlled by several environmental and chemical signals including temperature, osmolarity and oxygen (76, 140, 159). Curli are primarily expressed during stationary phase and temperatures below 30°C) although some clinical isolates can express curli at 37°C (11, 141, 226). Studies have suggested

that curli play an important role in mediating attachment to surfaces such as alfalfa sprouts (201), lettuce (69), and spinach (109).

Curli producing *E. coli* O157:H7 have been shown to have a stronger association with leaf surface of produce; and strains isolated from plants appear to produce significantly more curli compared to the ones isolated from animals and humans (69, 109, 121, 147). Interestingly, curli deficient strains survive better under acidic conditions, persist longer in soil and resist protozoan predation compare to curli producing strains (42). Boyer et al. showed that curli producing *E. coli* O157:H7 E0018+ attached in significantly greater number to both the cut-edges and whole lettuce pieces compare to non-producing curli E0018- (27). Our study builds on that by (27), and here we seek to quantify the cross-contamination rates of curli producing *E. coli* O157:H7 from inoculated surfaces to the non-inoculated lettuce during water washing depending on the inoculation location.

Methods and Materials

Preparation of stainless steel bowls and produce

A stainless-steel bowl with a dimension 7.62Hx17.78Wx7.62D cm was used for 500 ml of water. It was disinfected with 70% ethanol and air-dried prior to each experiment. Bags of red and green baby romaine lettuce were purchased from a local supermarket and stored at 4°C prior to experiment. Each lettuce leaf was cut into 3 x 3 cm^2 pieces with sterile scalpel.

Selection of strains

E. coli O157:H7 strain deficient in curli production (E0018-) and its corresponding curli producing version (0018+) were used for all experiments. Strain E0018- is a calf isolate, and strain E0018+ was obtained from E0018- (172). The both strains were made nalidixic acid resistant, and kindly provided by Dr. Renee Boyer at Virginia Tech. Curli phenotype was confirmed by plating strains on Tryptic Soy Agar (TSA) supplemented with 40 μg/ml Congo red dye and 20 μg/ml Commassie brilliant blue dye. Curli producing strains bind Congo red dye producing red colonies while curli negative colonies are unable to bind the dye producing white or colorless colonies.

Inoculum preparation

Frozen cultures (-80 °C) of each *E. coli* strain were streaked on TSA plate prior to each experiment. A single colony from TSA was inoculated in 10 ml of Tryptic Soy Broth (TSB) and incubated overnight for 24 h at 37°C. One ml of the culture was transferred to a micro-centrifuge tube and centrifuged at 0.6 x g for 10 min. Cells were washed twice by removing the supernatant and re-suspending in 1 ml of 0.1% peptone to achieve a final concentration of ~7 log CFU/ml. The final concentration was verified by enumeration on TSA plus nalidixic acid.

Leaf surface and cut-edge inoculation

Eleven lettuce pieces were used in each experiment (1 inoculated and 10 non-inoculated pieces). The designated leaf was inoculated with 10 μl of either curli producing (E0018+) or non-producing (E0018-) *E. coli* O157:H7 on the leaf surface, the cut-edges, or both the surface and the cut-edges (Figure 1). The inoculated piece was

dried for 2 hours in a biosafety cabinet, and the final concentration of ~6 log CFU/piece was confirmed.

Transfer between lettuce pieces and enumeration

Dry inoculated lettuce pieces were transferred to the stainless steel bowl and washed with 500 ml of water for 30 seconds. The outermost edges (~2 mm) of all lettuce leaves were excised on all four sides using a sterile scalpel, resulting in cut-edge and whole leaf samples. Each sample (whole leaf and cut-edge) was transferred to a separate 3 oz. sterile Whirl-Pak bag containing 10 ml of buffer and homogenized for 2 min. Each bag and the used wash water were sampled, serially diluted and surface plated on TSA plus nalidixic acid. The plates were incubated for 24 h at 37°C, and colonies were counted the following day. Data were analyzed using Microsoft excel and Statplus. All experiments were performed in triplicate.

Data analysis and Calculation

The equations below were used to calculate the log reduction on the inoculated lettuce piece and the log percent transfer to the non-inoculated lettuce pieces.

Total CFU in the system = CFU on the water + Σ CFU on inoculated leaf (surface and edge) + Σ CFU on the non-inoculated lettuce pieces (surface and edge)

Log reduction on the inoculated leaf = Log total CFU in the system - Log CFU on the inoculated leaf part

Log % transfer to the non-inoculated lettuce surface = Log [(CFU on one non-inoculated lettuce surface/CFU total in the system) * 100]

Log % transfer to the non-inoculated lettuce cut-edges = Log [(CFU on one non-inoculated lettuce cut-edges/CFU total in the system) * 100]

ANOVA, and t-tests were performed to determine statistical significance using Statplus.

Results

Log reduction on inoculated lettuce

The log reduction of *E. coli* O157:H7 0018- and 0018+ on the inoculated lettuce is shown in Figure 2. The uppercase XY and Z show statistically significant differences for curli + *E. coli* O157:H7 between treatments. The lower case xy and z show statistically significant differences for curli - *E. coli* O157:H7 between treatments. The uppercase A and B show significance between curli + and curli – strains, within an inoculation location.

Washing with water produced greater log reductions in the curli negative strain E0018- compared to the curli positive strain E0018+ regardless of the inoculation location. The differences in log reduction achieved between the curli positive and negative strains were significantly different when either the surface or the cut-edges were inoculated ($p<0.05$). Although differences in the same direction were observed for the experiments where the surface and cut-edges were co-inoculated, those differences were not statistically significant.

The mean log reduction of the curli negative strain E0018- on the surface and the cut-edges were 3.19 and 0.70 log CFU/lettuce piece respectively, and this difference was significantly different ($p<0.05$). When the surface/cut-edges were inoculated, the log reduction on the surface and the cut-edges respectively, both equal to 1.42 log CFU, with no significant difference.

The mean log reduction of the curli positive strain E0018+ on the surface and the cut-edges were 1.06 and 0.41 log CFU respectively, although the difference was not statistically significant. When surface/cut-edges were inoculated; the log reduction on the surface and the cut-edges were 1.08 and 0.54 log CFU/lettuce piece respectively, although also not statistically significantly different.

Log % transfer to non-inoculated surfaces and water

The log percent transfer of *E. coli* O157:H7 0018- and 0018+ to the non-inoculated leaves is shown in Figure 3. When the leaf surface was inoculated (Figure 3A), the curli positive strain more readily transferred to both uninoculated surfaces and uninoculated cut edges, and this difference was statistically significant ($p<0.05$). The transfer of the curli positive strain was about 10-fold greater (-1 log percent or 0.1% vs. -2 log percent or 0.01%). The same trend was evident when the cut edges were inoculated (Figure 3B): the curli positive strain more readily transferred to both non-inoculated surfaces and cut edges. This difference was also statistically significant ($p<0.05$). As might be expected from the prior results, when the surface and cut-edges were co-inoculated (Figure 3C), the trend was also the same and the curli positive strain more readily transferred both uninoculated surfaces and uninoculated cut edges. This difference was also statistically significant ($p<0.05$).

Figure 3D shows the log % transfer of the curli positive and negative strains from inoculated surfaces, cut-edges, and co-inoculated surface/cut-edges to the wash water. When only the leaf surface was inoculated, the mean log % transfer to the wash water was almost 100% (or 2 log %) either the curli positive or negative strains, and the difference was not statistically significant. The transfer from inoculated cut-edges to the wash water was slightly lower but the difference was not statistically significant. The results when both the surface/cut-edges were inoculated were similar to when the cut edges were inoculated. Although the curli positive strain showed greater mean transfer to the wash water than the curli negative strain, the difference was not statistically significant.

Log % transfer within an inoculated leaf from surface to cut-edge or vice versa

Since the inoculated leaves were a different color than the uninoculated leaves we could also quantify transfer within a given leaf from surface to cut-edge or vice versa. These results are shown in Figure 4. The log % transfer of the curli negative strain from surface to cut-edge or vice versa was roughly equivalent and ~ -0.5 log percent transfer (or 0.3 %). The log % transfer of the curli positive strain from inoculated surface showed a greater mean log % transfer to the cut edges than vice versa, however these differences were not statistically significant.

Discussion

The overall range of log reductions on the inoculated leaf with water washing we observed ranged from 0.41 (curli-positive, cut edge inoculated) to 3.19 log CFU/lettuce piece (curli-negative, surface inoculated) and is generally consistent with other studies using a similar design. Jensen et al. found that the log reduction on surface inoculated

lettuce ranged between 2 and 2.5 log CFU when washed with 100 ml of water (94). Vegdahl and Schaffner (Chapter 2) reported log reduction ranging from 0.5 to 2.5 log CFU for water washing studies using virulent and avirulent *E. coli* O157:H7 strains surface-inoculated on to lettuce.

Our results show the least log reduction with water washing when the cut-edges were inoculated regardless of curli production (0.41 and 0. 70 log CFU), which is consistent with finding in many other studies (27, 84, 194-196).

Water washing removed significantly ($p<0.05$) fewer curli-producing *E. coli* O157:H7 from lettuce leaves compared to non-curli producing *E. coli* O157:H7 which suggests that the curli producing strain attached more tightly to lettuce leaves. The published literature indicates a complex relationship between curli expression and surface attachment. It has been shown that curli expression facilitates *E. coli* attachment to polystyrene surfaces (159), but not attachment to stainless steel surfaces (173). Research with *Salmonella* and showed the presence of curli is important for strengthening adhesion to parsley (103).

Reports of reductions on the surface of fresh produce during are quite common, but studies quantifying cross-contamination during washing are quite scarce. Jensen et al. showed that the percent transfer from *E. coli* O157:H7 to non-inoculated lettuce pieces was approximately 1% (~0 log percent transfer) when washed with 100 ml of water, and using 1:10 (inoculated: non-inoculated) lettuce piece ratio (94) and Schaffner (Chapter 2) showed that the log % transfer of surface inoculated *E. aerogenes*, avirulent *E. coli* O157:H7, and pathogenic *E. coli* ranged between 1% (~0 log percent transfer) and 0.02% (-1.8 log percent transfer) % when washed with 100 and 1000 ml and the lettuce ratio

was 1:5 and 1:20. These findings (94) (Chapter 2) are generally consistent with those reported here, where transfer ranged from a high of 0.14% (-0.85 log percent transfer) to a low of 0.005% (-2.27 log percent transfer).

While our results clearly shown *E. coli* are less readily removed from cut edges, and that curli production impedes removal from both surfaces and edges, once the cells are in the wash water, they show no tendency to preferentially attach to either surfaces or cut edges, since transfer rates were not statistically significantly different. This is in contrast to Takeuchi et al. {Takeuchi 2000} who compared the attachment of *E. coli* O157:H7, *Salmonella typhimurium*, *L. monocytogenes* and *P. fluorescens* to intact and damaged lettuce tissues by plate count and confocal scanning laser microscopy, and showed *E. coli* O157:H7 attached preferentially to the cut-edges of the lettuce. Our findings are also in contrast to those of Seo and Frank (184) who showed that *E. coli* O157:H7 preferentially attached to cut lettuce leaf edges vs. the intact leaf surface.

While curli clearly influence attachment, other factors are also influencing the associate of *E. coli* O157:H7 with the surface of fresh produce. Cell surface hydrophobicity, presence of fimbriae and flagella, and production of extracellular polymeric substances (EPS) have all been shown to influence attachment (58). Our study was unique, as it quantified not only the removal of *E. coli* from cut edges and intact surfaces, but also subsequent re-association with those same surfaces of in previously uncontaminated lettuce. While curli have clearly influenced the attachment patterns on inoculated lettuce, the ability to produce curli did not significantly influence the ability of detached *E. coli* cells in the wash water to re-attach. Further research in needed to

understand and control bacterial cross-contamination during the washing of fresh produce.

Figures

Figure 1: *E. coli* O157:H7 inoculation on the leaf surface, cut-edge and surface and edges diagram.

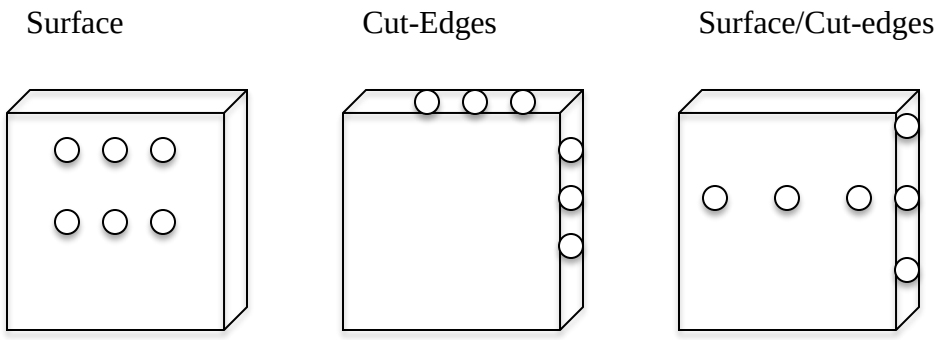
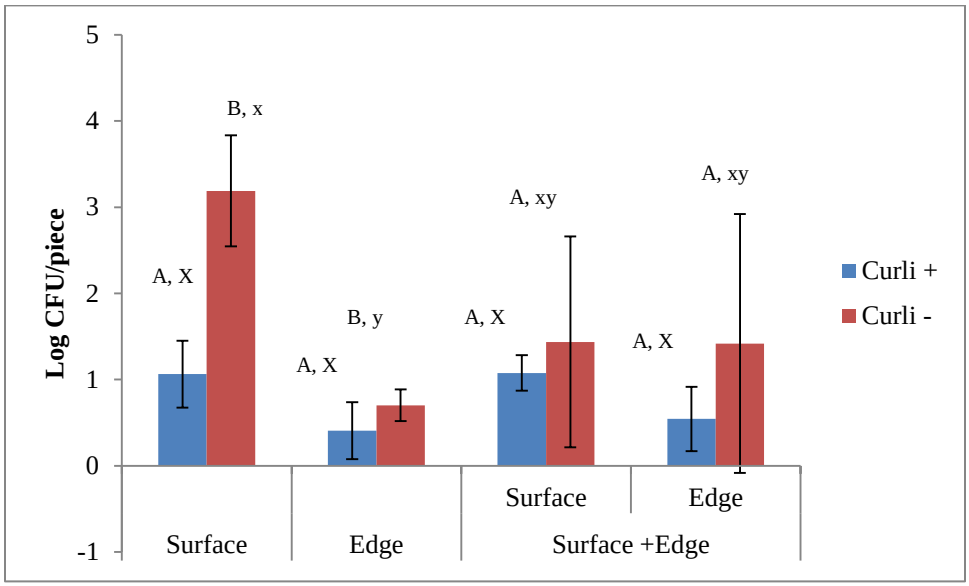
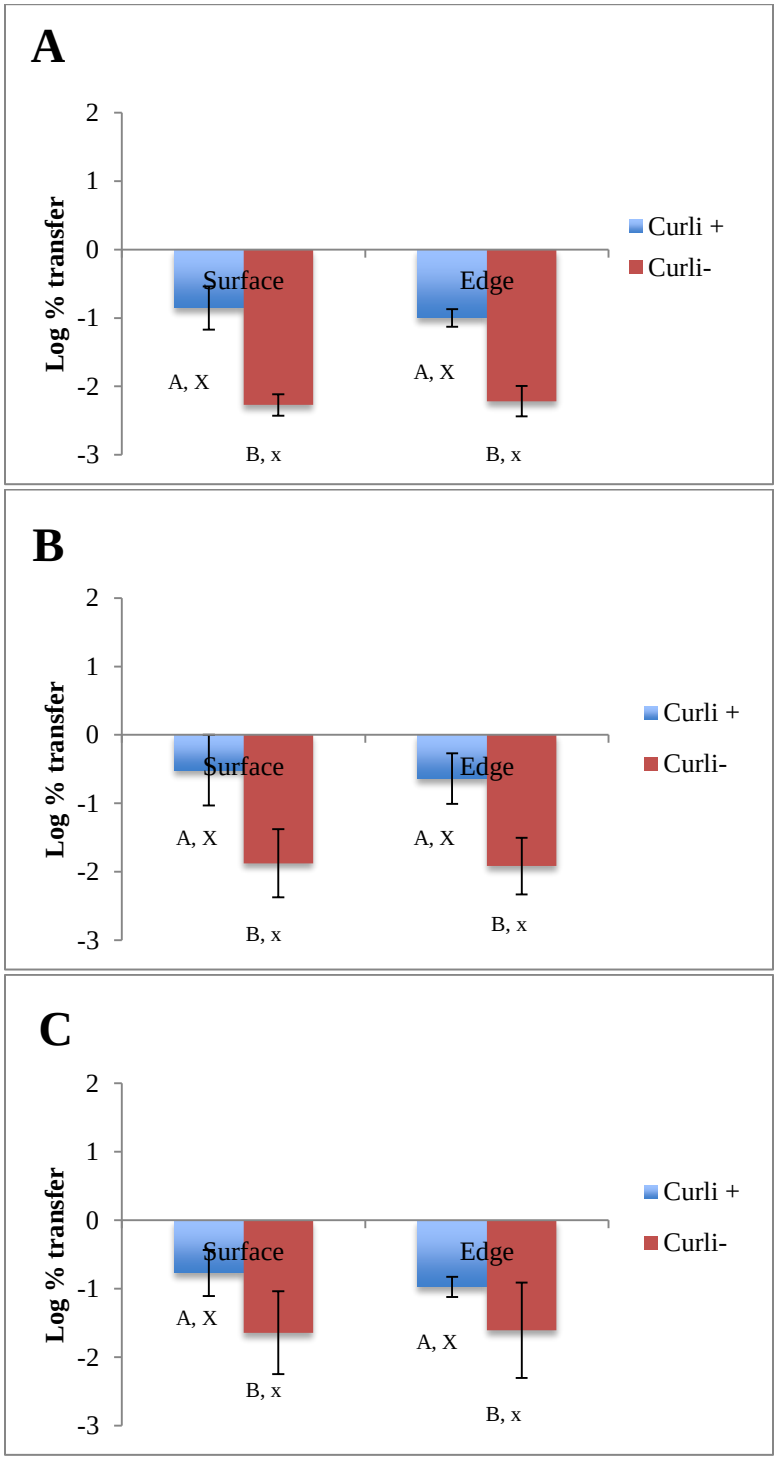


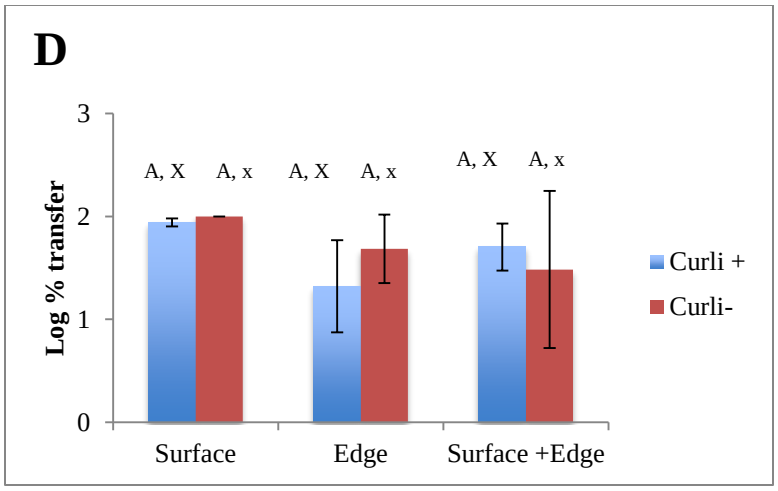
Figure 2: Log reduction of *E. coli* O157:H7 E0018+ and E0018- on inoculated lettuce by inoculation location.



XYZ shows statistically significant differences for curli + *E. coli* O157:H7 between treatments
xyz statistically significant differences for curli - *E. coli* O157:H7 between treatments
AB shows significance between curli + and curli – strains, within an inoculation location
Errors bars represent the standard deviation around the mean (n=3)

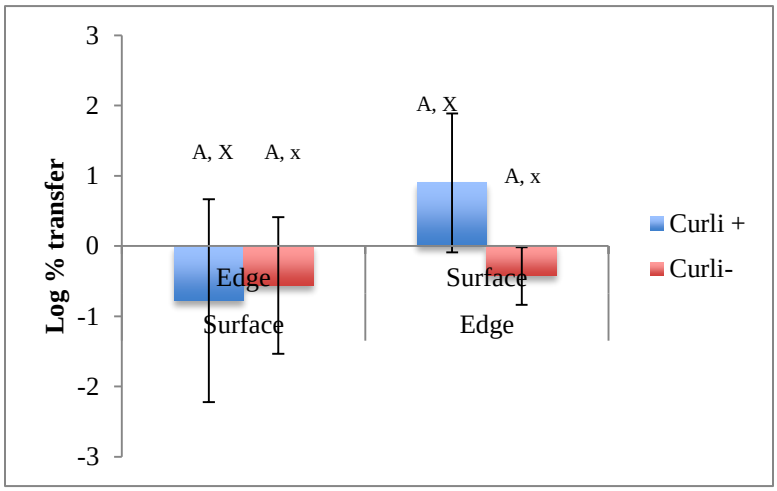
Figure 3: Log % transfer of *E. coli* O157:H7 0018- and 0018+ to non-inoculated lettuce pieces depending on inoculation of the lettuce (A) surface, (B) cut-edges, (C) both surface/cut-edges, and to the wash water (D).





XYZ shows statistically significant differences for curli + *E. coli* O157:H7 between treatments
xyz statistically significant differences for curli - *E. coli* O157:H7 between treatments
AB shows significance between curli + and curli – strains, within an inoculation location
Errors bars represent the standard deviation around the mean (n=30 for the non-inoculated pieces and n=3 for the water)

Figure 4: Log % transfer of *E. coli* O157:H7 0018+ and 0018- to the non-inoculated part of the inoculated lettuce.



XYZ shows statistically significant differences for curli + *E. coli* O157:H7 between treatments
xyz statistically significant differences for curli - *E. coli* O157:H7 between treatments
AB shows significance between curli + and curli – strains, within an inoculation location
Errors bars represent the standard deviation around the mean (n=3)

Chapter IV: Modeling growth of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* on cut leafy greens during transportation using time series analysis

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Abstract

Leafy greens are frequently implicated in foodborne disease outbreaks, and are believed to be the most common cause of foodborne illness in the United States. Refrigeration at 5°C or less will prevent the growth some pathogens that may be present on leafy greens. The research presented here combines real time dynamic transit temperature with many previously published growth models to estimate the growth of *Salmonella* (6 models), *E. coli* O157:H7 (6 models), and *L. monocytogenes* (4 models) during leafy greens transportation. Temperatures extracted from 18 trucks an extensive dataset described in previously published paper (33) were fitted into a time series distributions using @Risk 7.0 software and the generalized autoregressive conditional heteroscedasticity (GARCH) model with parameters (1, 1) gave a satisfactory fit for temperature profiles. Each of the 18 trucks was simulated 10000 times to be in transit for 60 hours where temperature could change every 5 minutes. Mean log increase of each pathogen was predicted by each of the model assuming a cut leafy green substrate. The predicted mean log increases ranged from 0 to 1.97 log CFU/g (*Salmonella*), 0 to 2.19 Log CFU/g (*E. coli* O157:H7) and 0 and 4.15 log CFU/g (*L. monocytogenes*). Most simulated trucks showed almost no growth (less than 0.5 log CFU/g). This research highlights the value of time series analysis in simulating temperature profiles during transport and the value in using multiple predictive models for pathogen growth.

Introduction

Leafy greens are frequently implicated in foodborne disease outbreaks. Confirmed single etiology outbreaks linked to leafy greens reported that Norovirus was most commonly implicated followed by *E. coli* O157:H7 and *Salmonella* (87). *Listeria monocytogenes* has also been linked to leafy greens recalls and at least one recent outbreak (207). *L. monocytogenes* is particularly concerning because it can grow even at acceptable, low refrigerated temperatures (213). Leafy greens can become contaminated with pathogens by a variety of means including contaminated irrigation water, improperly composted manure used for fertilizer, and by the feces of feral animals. Contamination can also occur during processing, including washing, cutting and storage (87).

Refrigeration at 5°C or less will prevent the growth of *Salmonella* and pathogenic *E. coli* that may be present on leafy greens (2, 99, 204), and will also severely restrict the growth of *L. monocytogenes*. Storage of leafy greens at 5°C or above may allow pathogens to multiply, increasing the risk of foodborne disease (107). The FDA Model Food Code identifies cut-leafy greens as a food that requires time and temperature control for safety (68). The need for temperature control includes control during the shipment of fresh-cut leafy greens by tractor trailers or other means. If the tractor trailer loads are subjected to temperature above 5°C, they may be rejected by distribution centers or wholesale markets on arrival (40, 44, 73, 98, 176). Temperature must be controlled carefully, however as temperatures below ~0°C can lead to damage of plant tissues by freezing. This will lead to quality loss and a potential for greater subsequent microbial growth in the damaged tissue after the temperature rises again.

Time series analysis techniques are used to analyze data collected over time. Time series are mathematically defined as a set of vectors $x(t)$, $t = 0, 1, 2\ldots$ where t represents the time elapsed and the variable x(t) is treated as random variable (49, 88, 163). The variable x can be anything that changes over time (e.g temperature, stock price, concentration of a chemical, etc). The procedure of modeling time series data is termed Time Series analysis (88). Past observations are collected and analyzed and used for future forecasting and simulation. One key attribute of time series data is that they are not independent, such that the temperature at one time is not truly random; it depends on the temperature in the previous time interval. There are many time series models that can be used to represent different stochastic processes (25, 49, 88, 104, 151). The generalized autoregressive conditional heteroscedasticity (GARCH) model is one such time series model that has been extensively used in the published literature (63).

Predictive microbiology used mathematical models predict the growth of bacteria based environmental conditions (169). Several growth models have been developed to predict the growth of *Salmonella spp*. (99, 123, 160, 175, 209), *E. coli* O157:H7 (55, 99, 116, 160, 209), and *L. monocytogenes* (99, 123, 175) in leafy greens. The objectives of our study were to model fresh-cut leafy greens transport truck temperatures using time series analysis; and subsequently to model the growth of *E. coli* O157:H7, *Salmonella* spp, *L. monocytogenes* during transportation of fresh-cut leafy greens while using the temperatures forecast by the time series models.

Material and Methods

Temperature data

The truck transport temperature data used in our study were kindly provided by Brown et al. (33) and are summarized in Table 1. A total of sixteen shipments were monitored over one year period; and sensors recorded temperatures at intervals that did not exceed 5 min. Over 300,000 data points were collected and analyzed. Data from truck 2 and 14 were split in half (Truck 2.1, 2.2 and Truck 14.1, 14.2 respectively) to facilitate handling of the large data sets.

Time Series Analysis

Temperature data were fitted to time series distributions using @risk 7.0 Software (Palisade Corporation, Ithaca, NY). Data were transformed to be stationary; and GARCH (1, 1) model determined to provide the best fit for temperature profiles using the Akaike information criterion (AIC) (174). The GARCH (1,1) model parameters for the trucks and their respective stationarity transformation are shown in Table 2. Truck 14.2 was not included in our analysis due to difficulty in suitably transforming the data.

Simulation modeling

The GARCH (1,1) parameters were used to predict future truck temperature during transportation. Shipments times varied between 31 hours to 84 hours with an average of 64 hours (33). Therefore, each of the eighteen trucks was simulated to be in transit for 60 hours where temperature could change every 5 minutes. Published leafy greens growth rate models for *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* were used to estimate the log increase by each pathogen during transportation. Pathogen

model parameters are shown in Table 3. Models were generally of the form of the standard square root model: $\sqrt{\mu_{max}} = b(\mathrm{T} - \mathrm{Tmin})$ and the parameters (b and T_{min}) were as provided by the authors. In some case the transformation was different (116, 123, 160) or the time units were different (55). The parameters values indicated as being from Koseki and Isobe (99) for broth were from the Pathogen Modeling Program, but are as reported by Koseki and Isobe (99).

These growth rate models were integrated into the time series models and Monte Carlo simulation of 10,000 iterations for each of 16 trucks were performed using @Risk 7.0. The simulation output was the predicted log increase of pathogen concentration after 60 h of transit. The log increase of pathogen concentration was capped at the biologically plausible level of 9 log CFU in the simulation.

Results

Modeling truck temperature during transit

Descriptive statistics for the truck temperature data are shown in Table 1. The minimum temperature for the trucks ranged from -14.39 to 1.56°C and the maximum ranged from 4.83 to 9.67°C. The mean truck temperature ranged from 0.90 to 3.70°C. Skewness is a measure of the asymmetry of data around its mean, where a skewness of 0 means the data are not skewed. The skewness for most truck temperatures was slightly positive (right tailed, 0.0-0.5). Truck 3 had a high negative skew (-4.76) while trucks 5 and 15 had higher positive skews (1.95 and 0.94 respectively). Kurtosis is another attribute of distributions, where greater kurtosis values indicate more and/or a greater number of outliers than a normal distribution. Most trucks had low kurtosis values with the exception of Trucks 3, 5, 12 and 15 where the kurtosis values were equal to 43.97,

4.32, 4.26 and 2.99 respectively. Most trucks had over 10,000 time and temperature data points.

The parameters estimates (μ, ω, α, β, r) for the GARCH (1, 1) model are shown in Table 2. The definitions of the parameters are μ: mean, ω: volatility parameter, α: error coefficient, β: autoregressive coefficient, and r: assumed temperature at time zero. Table 2 also shows the corresponding transformation applied to the temperature data to achieve stability. Most trucks required a first order difference with some of additive seasonality. However, truck 9 and 13 required a seasonal difference and log difference specifically to achieve stationarity.

Modeling the growth of Salmonella spp., E. coli O157:H7*, and L. monocytogenes*

The log increase of *Salmonella* on fresh-cut leafy greens during transportation is summarized in Table 4 and shown in Figure 1A. The minimum log predicted increase of *Salmonella* was always 0 for the all trucks and models. The mean log increase ranged from 0 to 4.34 log CFU/g. The mode and median were generally 0; however they were as high as 9 log CFU/g for some trucks. Overall, Mishra et al (123) model had the highest mean log increase, and the Sant'Ana et al model (177) predicted the lowest *Salmonella* growth. Trucks 2.1, 2.2, 10, 11 and 12 estimated negligible growth of *Salmonella* (less than 0.5 log CFU/g) regardless of the model used.

The log increase of *E. coli* O157:H7 on fresh-cut leafy greens during transportation is summarized in Table 5 and shown in Figure 1B. The minimum predicted *E. coli* O157:H7 log increase was always 0 log CFU/g (just as with *Salmonella*). The mean log increase ranged from 0 to 2.19 Log CFU/g. The mode was 0 for all the trucks and except when the Puerta-Gomez model (160) was used for Trucks 13 to 15. The

median log increases were equal to zero or less than 1 log CFU/g with few exceptions. In Truck 15, the median log increases were 1.29 and 1.51 respectively for PMP (99) and Veys (209) models. Most trucks had a predicted increase of less than 1 log CFU after 60 h in transit with the exception of Trucks 1, 14.1, and 15. The growth model developed by Veys et al (209) predicted the greatest increase in *E. coli* O157:H7 populations relative to all other models (Figure 1B). Danyluk and Schaffner model (55) predicted the least increase in *E. coli* O157:H7 concentration during transportation.

The log increase of *L. monocytogenes* on fresh-cut leafy greens during transportation is summarized in Table 6 and shown in Figure 1C. The minimum log increase of *L. monocytogenes* for all trucks was 0 except for Trucks 10, 11 and 13 where the minimum increase was 0.31 log CFU as predicted by the Koseki and Isobe model (99). The maximum log increase varied greatly ranging from 0.68 to 9 log CFU/g. The mean log increase of *L. monocytogenes* ranged between 0 and 4.15 log CFU. The mode log increase of *L. monocytogenes* was less than or approximately 1 for most trucks, except for Truck 13 and Truck 15 predicted by Koseki and Isobe model (99) and PMP model {Koseki} respectively. The median log increase ranged between 0 and 2.57 log CFU/g. Overall, the PMP model (99) predicted the highest mean log increase of *L. monocytogenes,* followed by Koseki and Isobe (99) then Mishra (123) and finally Sant'Ana (175), which tended to estimated the lowest log increase (Figure 1C).

Discussion

Modeling the temperature data using time series analysis enabled the generation of a very large number of realistic simulated temperature profiles. This is in contrast to other studies that used normal or uniform distributions to model temperatures in

microbial risk assessment (55, 202). In those studies, a specific time and temperature is randomly chosen via Monte Carlo, and simulations were run with that specific temperature.

The sixteen growth models from 16 published reports used in our simulation gave different predictions from one another. The log increase of *Salmonella* during transit is shown in Table 3 and Figure 1. The mean log increase for *Salmonella* during transit predicted by Mishra et al., Koseki and Isobe, PMP and Puerta-Gomez et al. were very similar *(77, 99, 124, 160)*. Mishra et al used growth data from 8 published studies for *S. enterica* on romaine and iceberg lettuce, fresh-cut celery, baby spinach, and cilantro at various temperatures ranging from 7 to 37°C. Koseki and Isobe performed laboratory experiments where they inoculated iceberg lettuce (3X3 cm) with *S.* Enteritidis ATCC BAA-708, ATCC 4933, *S.* Typhimurium ATCC 29057, 29629 and 29630 (99) at 5 temperatures from 5 to 25°C. The PMP model is based on data from Gibson et al. (77) using *S.* Thompson, *S.* Stanley, and *S. Infantis* in Tryptone Soy Broth at 5 storage temperatures between 10 and 30°C (77). Puerta-Gomez et al. studied a single strain, *S.* Typhimurium LT2 on spinach at 4 temperatures between 10 and 37°C (160). The similarity of all four models is encouraging, as it shows that models based on literature data (124), experimental data in leafy greens with multiple strains (99), a single strain (160) or growth in nutrient broth (77) all result in similar predictions. The similarity of the broth data is particularly surprising as bacteria growing on the surface of foods experience multiple additional stress factors including limitations in nutrient diffusion, and competition with natural flora compared to bacteria grown in liquid media (189). It appears that damaged or fresh-cut leafy greens promote pathogen growth under

conditions (120, 137) that approximate those found in nutrient broth (77). Our understanding of the factors that control pathogen growth in leafy green is still developing as earlier studies concluded that limited *Salmonella* growth at temperature below 10°C (138), while a recent publication reports the startling observation that *Salmonella* can apparently grow at 4°C in liquid from fresh-cut leafy greens (101).

The Sant'Ana model predicted the lowest increase of *Salmonella* during transit (176). Sant'Ana et al group determined growth parameters by inoculating *S. enterica* 277 and 386 and *S. typhimurium* 13076 on 2x2 cm iceberg and crisp lettuce stored at 6 temperatures between 7 and 30°C. Most importantly, the lettuce pieces were packaged under modified atmosphere 5% O_2, 15% CO_2 and 80% N_2. Modified atmosphere packaging (MAP) is commonly believed to slow the growth of pathogens, in addition to extending the shelf life of minimally processed fruits and vegetables (8). Published studies examining the fate of pathogens on packaged lettuce have led to different outcomes. Horev et al (89) studied on the effects of MAP on *S. enterica* on the surface of lettuce at 8 and 20°C, and found no effect of MAP (10% O_2, 10% CO_2 and 80% N_2) on *Salmonella* concentration although MAP did inhibit growth of indigenous microflora. Oliveira et al investigated the growth of *E. coli* O157:H7, *Salmonella spp*. and *L. monocytogenes* on shredded Romaine lettuce at 5 and 25 °C. They concluded that the MAP conditions they used (20% O2, 2% CO2) had no significant effect on pathogen survival or growth at refrigerated temperature (139). Previous studies have shown that a low concentration of O_2 combined with a high concentration of CO_2 (10% or more) will reduce microbial growth (29, 90). Since Sant'Ana et el used a CO_2 concentration of 15%, this might explain the difference in the *Salmonella* log increase prediction.

The Veys and PMP models estimated the highest mean log increases for *E. coli* O157:H7. Veys et al. inoculated *E. coli* O157:H7 on 4x4 cm lettuce pieces (type not specified) and stored it at 5, 10, 25 and 37°C for different time periods (209). They used four different *E. coli* O157 strains isolated in Brazil, 2 from cows, 1 from manure and one from lettuce wash water. The PMP model used 3 strains of *E. coli* O157:H7 (933, 45753 and A9218-C1) grown in Brain Heart Infusion broth at 7 temperatures from 5 to 42°C (38).

The models developed by MacKellar and Delaquis, and Koseki and Isobe predicted slightly lower growth of *E. coli* O157:H7 during transit compared to the models above, while the model by Puerta-Gomez et al. showed slightly less growth than that of MacKellar and Delaquis, and Koseki and Isobe. McKellar and Delaquis used data from 13 published reports containing data for *E. coli*'s growth on lettuce and spinach at various temperatures. The leafy greens data used by MacKellar and Delaquis included treatments such as modified atmosphere packaging, heat treatments, and chlorine dips. Koseki and Isobe used 6 *E. coli* O157:H7 strains (ATCC 35150, 43889, 43895, 51657, 700378, ATCC-BAA-460) on 3x3cm iceberg lettuce incubated at 5 temperatures between 5 and 25 °C. Puerta-Gomez et al. studied a three-strain cocktail of *E. coli* (BAA-1427, BAA-1428, and BAA-1430) on spinach at 4 temperatures between 10 and 37°C (160).

The model developed by Danyluk and Schaffner provides the most striking contrast to those of all the other authors, as this model predicted almost no *E. coli* growth during transit. Danyluk and Schaffner extracted growth data on fresh-cut lettuce from 7 published studies (55). These authors noted a substantial difference between their predictions and those of the PMP and Koseki and Isobe, specifically because of their

decision to exclude data from cored iceberg lettuce at 30°C which were significantly higher than other data. These authors did foreshadow the eventual modification of their model noting that "future work focusing on the growth of *E. coli* O157:H7 on cut leafy greens at temperatures above 23°C… may alter the predictive growth model…"Clearly use of the Danyluk and Schaffner model should be undertaken very carefully given the very different nature of it's predictions from the other 5 models presented here.

The PMP model estimated the highest growth for *L. monocytogenes*. The PMP model was developed using a single strain of *L. monocytogenes* (Scott A) in Tryptose Phosphate Broth at 5 temperatures between 5 and 37°C. This is not surprising given the earlier comments fewer stress factors for bacteria grown in liquid media (189). The predictions of Koseki and Isobe and Mishra et al. were similar for *L. monocytogenes*. Koseki and Isobe used 6 strains of *L. monocytogenes* (ATCC 19111, 19117, 19118, 13932, 15313, and 35152) inoculated on 3x3 cm of iceberg lettuce and stored 5 temperatures between 5 and 25°C. Mishra et al. used data on *L. monocytogenes* growth on leafy greens collected from 17 articled in the peer reviewed literature.

As was the case with the *Salmonella* model noted above, predictions from Sant'Ana et al model estimated the lowest growth of *L. monocytogenes* during transit. Sant'Ana et al used three strains of *L. monocytogenes* 4b (413, 494 and 581) inoculated onto 2x2 cm pieces of iceberg and crisped lettuce stored at 6 temperatures between 7 and 30°C. As noted above these authors package the lettuce in 5% O_2, 15% CO_2 and 80% N_2 and the MAP may have slowed down the growth of *L. monocytogenes*.

Conclusions

This project was undertaken to explore times series analysis as a method to compare the performance of predictive growth models for three important pathogens on leafy greens, simulating real dynamic transit temperature. Despite the fact that the various models used were from different in experimental designs, used different bacterial strains, growth media and incubation temperatures, the model to model differences were generally small. In a few cases, models based on experiments using MAP shown less growth than models than used non-MAP data. In one case a model based on a small data-set that chose to specifically exclude some published data did result in very low predicted growth. Finally, the results show that simulated truck-to-truck variation is probably a bigger contributor to prediction differences than most model to model variation.

Tables

Table 1: Summary statistics of truck temperature for 16 leafy green transporting trucks as described by Brown et al (33).

Truck number	Temperature °C								Number of Observations
	Minimum	Maximum	Mean	Standard Deviation	Mode	Median	Skewness	Kurtosis	
Truck 1	1.56	7.94	3.70	1.29	2.94	3.72	0.42	-0.43	10,760
Truck 2.1	0.94	4.83	2.71	0.51	3.06	2.67	0.14	0.12	15,100
Truck 2.2	0.56	5.44	2.71	0.69	3.22	2.67	0.06	-0.29	15,101
Truck 3	-14.39	7.50	2.34	1.47	2.11	2.33	-4.76	43.97	21,920
Truck 4	-0.28	7.61	2.06	0.96	2.00	2.06	0.28	0.11	19,824
Truck 5	0.78	9.67	3.03	1.43	2.56	2.72	1.95	4.32	18,631
Truck 6	0.61	6.17	2.32	0.54	2.39	2.33	-0.06	0.85	15,646
Truck 7	-0.22	7.33	1.83	0.88	2.00	1.83	0.17	-0.25	23,236
Truck 8	-0.72	5.17	1.66	0.95	1.72	1.61	0.30	-0.41	26,882
Truck 9	-1.22	7.06	0.90	1.16	-0.28	0.61	0.47	-0.78	27,559
Truck 10	0.50	5.67	2.61	0.95	2.72	2.61	0.20	-0.78	17,242
Truck 11	0.56	6.50	2.62	0.48	2.67	2.61	0.25	0.45	24,840
Truck 12	0.83	7.17	2.50	0.58	2.56	2.50	0.46	4.26	14,112
Truck 13	0.50	6.50	2.22	0.60	2.00	2.17	0.11	0.26	14,254
Truck 14.1	-2.39	5.89	2.05	1.03	1.28	2.00	0.26	-0.08	12,105
Truck 15	-0.78	8.56	1.63	1.00	1.39	1.50	0.94	2.99	9,504
Truck 16	-0.83	5.17	1.53	1.01	1.94	1.72	-0.15	-0.77	7,480

Table 2: GARCH (1,1) temperature model coefficients for 16 leafy green transporting trucks.

	Model parameters[1]					
Trucks	**μ**	**ω**	**α**	**β**	**r**	**Transformation[1]**
Truck 1	0.00016	0.00496	0.00297	0.00362	-0.07934	Difference (1)
Truck 2.1	-0.00012	0.00293	0.00171	0.00213	-0.05556	Difference (1)
Truck 2.2	-0.00014	0.00636	0.00389	0.00467	-0.00049	Difference (1), Additive seasonality (4)
Truck 3	0.00009	0.01535	0.00907	0.01079	0.00000	Difference (1)
Truck 4	0.00001	0.00585	0.89412	0.01383	0.28334	Difference (1), Additive seasonality (12)
Truck 5	0.00002	0.00195	0.87154	0.00556	0.11224	Difference (1), Additive seasonality (6)
Truck 6	0.00002	0.00503	0.00301	0.00367	0.22558	Difference (1), Additive seasonality (15)
Truck 7	0.00005	0.02182	0.02461	0.02781	0.05919	Difference (1), Additive seasonality (5)
Truck 8	-0.00010	0.01194	0.00708	0.00840	-0.33362	Difference (1), Additive seasonality (74)
Truck 9	0.00016	0.00282	0.92289	0.02804	0.00000	Seasonal difference (1)
Truck 10	-0.00005	0.00104	0.00061	0.00076	-0.15516	Log difference (1), Additive seasonality (26)
Truck 11	0.00001	0.01075	0.00683	0.00832	-0.00096	Difference (1), Additive seasonality (37)
Truck 12	-0.00003	0.00657	0.00401	0.00483	0.00999	Difference (1), Additive seasonality (74)
Truck 13	0.00000	0.00142	0.00083	0.00103	0.00000	Log difference (1)
Truck 14.1	0.00045	0.00636	0.97111	0.02177	-0.16667	Difference (1)
Truck 15	0.00919	0.00714	0.87629	0.24982	-3.16670	Seasonal difference (1)
Truck 16	-0.00035	0.01579	0.06735	0.02195	-0.01314	Difference (1), Additive seasonality (41)

[1]Model parameter definitions are μ: mean, ω: volatility parameter, α: error coefficient, β: autoregressive coefficient, r: assumed temperature at time zero.

[2]Transformation required to stabilize the time series data.

Table 3: *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* growth rate model parameters for growth on leafy greens, where the model is: $\sqrt{\mu_{max}} = b(T - Tmin)$

Organism	Growth Substrate	*b*	Growth Rate Units	T_{min} (°C)	Source
Salmonella spp.	Lettuce	0.033	√Log CFU/h/°C	4.96	(99)
Salmonella	Broth	0.037	√Log CFU/h/°C	6.27	(77) via (99)
S. enterica	Lettuce	0.0178	√Log CFU/h/°C	6.65	(175)
S. *typhymurium*	Spinach	0.000296	Log CFU/h/°C	5.88	(160)
Salmonella spp.	Lettuce	0.027	√Log CFU/h/°C	5.42	(209)
S. enterica	Leafy greens	0.02	√Ln CFU/h/°C	-0.571	(123)
E. coli O157:H7	Lettuce	0.033	√Log CFU/h/°C	4.45	(99)
E. coli O157:H7	Broth	0.032	√Log CFU/h/°C	2.67	(38) via (99)
E. coli O157:H7	Leafy greens	0.0616	√Log CFU/day/°C	2.628	(55)
E. coli O157:H7	Lettuce	0.023	Log CFU/h/°C	1.20	(116)
E. coli O157:H7	Spinach	0.000605	Log CFU/h/°C	4.76	(160)
E. coli O157:H7	Lettuce	0.028	√Log CFU/h/°C	1.58	(209)
L. monocytogenes	Lettuce	0.016	√Log CFU/h/°C	-4.26	(99)
L. monocytogenes	Broth	0.027	√Log CFU/h/°C	-0.44	(37) via (99)
L. monocytogenes	Lettuce	0.0144	√Log CFU/h/°C	1.96	(175)
L. monocytogenes	Leafy greens	0.023	√Ln CFU/h/°C	0.0599	(123)

Table 4: Descriptive statistics for the log increase of *Salmonella* during transportation as predicted by six published models using time series simulated data from 16 trucks.

Truck Number	Model	Minimum	Maximum	Mean	Mode	Median	Standard deviation
Truck 1	Koseki and Isobe 2005	0.00	4.07	0.31	0.00	0.10	0.46
	PMP	0.00	3.93	0.17	0.00	0.00	0.36
	Sant'Ana 2012	0.00	0.74	0.02	0.00	0.00	0.06
	Gomez 2013	0.00	1.08	0.08	0.00	0.03	0.10
	Veys 2016	0.00	3.20	0.16	0.00	0.04	0.27
	Mishra 2017	0.00	4.73	1.27	1.25	1.20	0.66
Truck 2.1	Koseki and Isobe 2005	0.00	0.33	0.00	0.00	0.00	0.01
	PMP	0.00	0.02	0.00	0.00	0.00	0.00
	Sant'Ana 2012	0.00	0.01	0.00	0.00	0.00	0.00
	Gomez 2013	0.00	1.62	0.34	0.21	0.31	0.22
	Veys 2016	0.00	0.20	0.00	0.00	0.00	0.00
	Mishra 2017	0.00	1.82	0.18	0.00	0.13	0.18
Truck 2.2	Koseki and Isobe 2005	0.00	1.44	0.00	0.00	0.00	0.03
	PMP	0.00	0.00	0.00	0.00	0.00	0.00
	Sant'Ana 2012	0.00	0.13	0.00	0.00	0.00	0.00
	Gomez 2013	0.00	3.11	0.50	0.00	0.41	0.39
	Veys 2016	0.00	1.57	0.00	0.00	0.00	0.02
	Mishra 2017	0.00	2.56	0.16	0.00	0.06	0.24
Truck 3	Koseki and Isobe 2005	0.00	8.25	0.19	0.00	0.00	0.53
	PMP	0.00	9.00	0.12	0.00	0.00	0.48
	Sant'Ana 2012	0.00	1.78	0.02	0.00	0.00	0.09
	Gomez 2013	0.00	4.37	0.30	0.00	0.15	0.40
	Veys 2016	0.00	4.53	0.10	0.00	0.00	0.32

	Mishra 2017	0.00	6.33	0.69	0.00	0.43	0.80
Truck 4	Koseki and Isobe 2005	0.00	9.00	0.45	0.00	0.00	1.36
	PMP	0.00	9.00	0.38	0.00	0.00	1.34
	Sant'Ana 2012	0.00	9.00	0.10	0.00	0.00	0.52
	Gomez 2013	0.00	9.00	0.76	9.00	0.33	1.22
	Veys 2016	0.00	9.00	0.30	0.00	0.00	1.05
	Mishra 2017	0.00	9.00	0.81	0.00	0.23	1.39
Truck 5	Koseki and Isobe 2005	0.00	9.00	0.14	0.00	0.00	0.61
	PMP	0.00	9.00	0.09	0.00	0.00	0.55
	Sant'Ana 2012	0.00	9.00	0.02	0.00	0.00	0.19
	Gomez 2013	0.00	9.00	0.27	0.00	0.13	0.46
	Veys 2016	0.00	9.00	0.08	0.00	0.00	0.46
	Mishra 2017	0.00	9.00	0.61	0.00	0.40	0.75
Truck 6	Koseki and Isobe 2005	0.00	2.04	0.03	0.00	0.00	0.13
	PMP	0.00	2.66	0.01	0.00	0.00	0.07
	Sant'Ana 2012	0.00	0.35	0.00	0.00	0.00	0.01
	Gomez 2013	0.00	1.34	0.16	0.00	0.10	0.18
	Veys 2016	0.00	1.87	0.01	0.00	0.00	0.06
	Mishra 2017	0.00	4.45	0.52	0.00	0.43	0.42
Truck 7	Koseki and Isobe 2005	0.00	9.00	0.28	0.00	0.00	0.78
	PMP	0.00	9.00	1.00	0.00	0.00	2.34
	Sant'Ana 2012	0.00	2.24	0.03	0.00	0.00	0.13
	Gomez 2013	0.00	6.99	0.41	0.00	0.19	0.57
	Veys 2016	0.00	7.25	0.15	0.00	0.00	0.47
	Mishra 2017	0.00	8.45	0.73	0.00	0.36	0.96
Truck 8	Koseki and Isobe 2005	0.00	2.68	0.02	0.00	0.00	0.11

	PMP	0.00	2.30	0.01	0.00	0.00	0.07
	Sant'Ana 2012	0.00	0.86	0.00	0.00	0.00	0.02
	Gomez 2013	0.00	4.75	0.61	0.00	0.45	0.57
	Veys 2016	0.00	1.45	0.01	0.00	0.00	0.06
	Mishra 2017	0.00	3.79	0.22	0.00	0.05	0.35
Truck 9	Koseki and Isobe 2005	0.00	9.00	0.03	0.00	0.00	0.37
	PMP	0.00	9.00	0.03	0.00	0.00	0.40
	Sant'Ana 2012	0.00	9.00	0.01	0.00	0.00	0.24
	Gomez 2013	0.00	9.00	0.41	0.00	0.31	0.49
	Veys 2016	0.00	9.00	0.02	0.00	0.00	0.24
	Mishra 2017	0.00	9.00	0.24	0.00	0.13	0.41
Truck 10	Koseki and Isobe 2005	0.00	9.00	0.02	0.00	0.00	0.26
	PMP	0.00	9.00	0.02	0.00	0.00	0.31
	Sant'Ana 2012	0.00	9.00	0.01	0.00	0.00	0.19
	Gomez 2013	0.00	9.00	0.48	0.60	0.53	0.19
	Veys 2016	0.00	9.00	0.01	0.00	0.00	0.18
	Mishra 2017	0.00	9.00	0.10	0.00	0.03	0.32
Truck 11	Koseki and Isobe 2005	0.00	2.94	0.00	0.00	0.00	0.04
	PMP	0.00	5.75	0.05	0.00	0.00	0.22
	Sant'Ana 2012	0.00	0.45	0.00	0.00	0.00	0.01
	Gomez 2013	0.00	3.30	0.25	0.00	0.13	0.33
	Veys 2016	0.00	3.19	0.05	0.00	0.00	0.17
	Mishra 2017	0.00	9.00	0.07	0.01	0.03	0.17
Truck 12	Koseki and Isobe 2005	0.00	2.80	0.04	0.00	0.00	0.15
	PMP	0.00	2.58	0.01	0.00	0.00	0.08
	Sant'Ana 2012	0.00	0.37	0.00	0.00	0.00	0.01
	Gomez 2013	0.00	2.06	0.22	0.00	0.14	0.25

	Veys 2016	0.00	1.58	0.01	0.00	0.00	0.06
	Mishra 2017	0.00	3.70	0.44	0.00	0.32	0.43
Truck 13	Koseki and Isobe 2005	0.00	9.00	0.54	0.00	0.00	1.81
	PMP	0.00	9.00	0.48	0.00	0.00	1.74
	Sant'Ana 2012	0.00	9.00	0.22	0.00	0.00	1.11
	Gomez 2013	0.00	9.00	0.70	9.00	0.37	1.54
	Veys 2016	0.00	9.00	0.42	0.00	0.00	1.60
	Mishra 2017	0.00	9.00	0.84	9.00	0.17	1.78
Truck 14.1	Koseki and Isobe 2005	0.00	9.00	1.49	0.00	0.04	2.60
	PMP	0.00	9.00	1.29	0.00	0.00	2.53
	Sant'Ana 2012	0.00	9.00	0.40	0.00	0.00	1.17
	Gomez 2013	0.00	9.00	0.89	9.00	0.32	1.53
	Veys 2016	0.00	9.00	1.04	0.00	0.00	2.10
	Mishra 2017	0.00	9.00	1.92	0.00	0.97	2.38
Truck 15	Koseki and Isobe 2005	0.00	9.00	1.26	0.00	0.34	2.17
	PMP	0.00	9.00	1.02	0.00	0.08	2.14
	Sant'Ana 2012	0.00	9.00	0.36	0.00	0.01	1.24
	Gomez 2013	0.00	9.00	0.63	9.00	0.12	1.58
	Veys 2016	0.00	9.00	0.85	0.00	0.14	1.82
	Mishra 2017	0.00	9.00	1.97	0.00	1.47	1.94
Truck 16	Koseki and Isobe 2005	0.00	9.00	0.07	0.00	0.00	0.33
	PMP	0.00	7.72	0.04	0.00	0.00	0.25
	Sant'Ana 2012	0.00	1.74	0.01	0.00	0.00	0.05
	Gomez 2013	0.00	5.40	0.56	0.00	0.34	0.63
	Veys 2016	0.00	3.39	0.03	0.00	0.00	0.17
	Mishra 2017	0.00	7.14	0.36	0.00	0.11	0.56

Table 5: Descriptive statistics for the log increase of *E. coli* O157:H7 during transportation as predicted by six published models using time series simulated data from 16 trucks

Truck Number	Models	Minimum	Maximum	Mean	Mode	Median	Standard Deviation
Truck 1	Koseki and Isobe 2005	0.00	5.96	0.57	0.00	0.33	0.69
	PMP	0.00	8.02	1.20	0.00	0.97	1.02
	Danyluk and Schaffner 2011	0.00	0.04	0.01	0.00	0.01	0.01
	McKellar and Delaquis 2011	0.00	5.46	0.83	0.00	0.71	0.61
	Gomez 2013	0.00	1.12	0.07	0.00	0.03	0.10
	Veys 2016	0.00	7.23	1.34	0.00	1.17	0.93
Truck 2.1	Koseki and Isobe 2005	0.00	0.60	0.00	0.00	0.00	0.01
	PMP	0.00	1.40	0.02	0.00	0.00	0.07
	Danyluk and Schaffner 2011	0.00	0.01	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	1.27	0.06	0.00	0.01	0.11
	Gomez 2013	0.00	1.66	0.34	0.25	0.31	0.22
	Veys 2016	0.00	1.62	0.06	0.00	0.00	0.13
Truck 2.2	Koseki and Isobe 2005	0.00	1.52	0.01	0.00	0.00	0.05
	PMP	0.00	1.06	0.01	0.00	0.00	0.04
	Danyluk and Schaffner 2011	0.00	0.02	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	1.78	0.07	0.00	0.00	0.16
	Gomez 2013	0.00	2.85	0.50	0.00	0.42	0.39
	Veys 2016	0.00	4.63	0.07	0.00	0.00	0.19
Truck 3	Koseki and Isobe 2005	0.00	8.93	0.25	0.00	0.00	0.66
	PMP	0.00	9.00	0.53	0.00	0.06	0.98
	Danyluk and Schaffner 2011	0.00	0.00	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	7.33	0.50	0.00	0.18	0.75
	Gomez 2013	0.00	4.40	0.30	0.00	0.14	0.40
	Veys 2016	0.00	9.00	0.64	0.00	0.20	1.00
Truck 4	Koseki and Isobe 2005	0.00	9.00	0.52	0.00	0.00	1.51
	PMP	0.00	9.00	0.82	0.00	0.00	1.79

	Danyluk and Schaffner 2011	0.00	1.73	0.01	0.00	0.00	0.03
	McKellar and Delaquis 2011	0.00	9.00	0.69	0.00	0.06	1.42
	Gomez 2013	0.00	9.00	0.77	9.00	0.32	1.25
	Veys 2016	0.00	9.00	0.88	0.00	0.05	1.74
Truck 5	Koseki and Isobe 2005	0.00	9.00	0.17	0.00	0.00	0.63
	PMP	0.00	9.00	0.41	0.00	0.05	0.91
	Danyluk and Schaffner 2011	0.00	0.38	0.00	0.00	0.00	0.01
	McKellar and Delaquis 2011	0.00	9.00	0.42	0.00	0.17	0.76
	Gomez 2013	0.00	9.00	0.28	0.00	0.13	0.50
	Veys 2016	0.00	9.00	0.53	0.00	0.17	0.93
Truck 6	Koseki and Isobe 2005	0.00	2.81	0.06	0.00	0.00	0.18
	PMP	0.00	5.52	0.24	0.00	0.06	0.39
	Danyluk and Schaffner 2011	0.00	0.03	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	2.93	0.30	0.00	0.19	0.35
	Gomez 2013	0.00	2.08	0.16	0.00	0.09	0.18
	Veys 2016	0.00	4.83	0.36	0.00	0.20	0.46
Truck 7	Koseki and Isobe 2005	0.00	9.00	0.35	0.00	0.00	0.92
	PMP	0.00	9.00	0.35	0.00	0.00	0.92
	Danyluk and Schaffner 2011	0.00	0.09	0.00	0.00	0.00	0.01
	McKellar and Delaquis 2011	0.00	9.00	0.57	0.00	0.14	0.93
	Gomez 2013	0.00	7.74	0.42	0.00	0.20	0.58
	Veys 2016	0.00	9.00	0.74	0.00	0.14	1.28
Truck 8	Koseki and Isobe 2005	0.00	4.22	0.03	0.00	0.00	0.15
	PMP	0.00	4.92	0.09	0.00	0.00	0.31
	Danyluk and Schaffner 2011	0.00	0.03	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	5.35	0.12	0.00	0.00	0.29
	Gomez 2013	0.00	4.99	0.61	0.00	0.45	0.58
	Veys 2016	0.00	5.30	0.13	0.00	0.00	0.35
Truck 9	Koseki and Isobe 2005	0.00	9.00	0.03	0.00	0.00	0.39
	PMP	0.00	9.00	0.09	0.00	0.00	0.47
	Danyluk and Schaffner 2011	0.00	1.20	0.00	0.00	0.00	0.01

	McKellar and Delaquis 2011	0.00	9.00	0.12	0.00	0.01	0.38
	Gomez 2013	0.00	9.00	0.41	0.00	0.31	0.51
	Veys 2016	0.00	9.00	0.15	0.00	0.00	0.50
Truck 10	Koseki and Isobe 2005	0.00	9.00	0.02	0.00	0.00	0.29
	PMP	0.00	9.00	0.04	0.00	0.00	0.37
	Danyluk and Schaffner 2011	0.00	0.11	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	9.00	0.05	0.00	0.00	0.29
	Gomez 2013	0.00	9.00	0.48	0.60	0.53	0.20
	Veys 2016	0.00	9.00	0.06	0.00	0.00	0.41
Truck 11	Koseki and Isobe 2005	0.00	9.00	0.02	0.00	0.00	0.27
	PMP	0.00	8.37	0.37	0.00	0.03	0.69
	Danyluk and Schaffner 2011	0.00	0.05	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	5.22	0.38	0.00	0.16	0.54
	Gomez 2013	0.00	2.88	0.25	0.00	0.13	0.32
	Veys 2016	0.00	6.14	0.02	0.00	0.00	0.15
Truck 12	Koseki and Isobe 2005	0.00	5.93	0.06	0.00	0.00	0.19
	PMP	0.00	5.49	0.21	0.00	0.01	0.41
	Danyluk and Schaffner 2011	0.00	0.03	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	3.53	0.26	0.00	0.12	0.36
	Gomez 2013	0.00	2.04	0.22	0.00	0.14	0.25
	Veys 2016	0.00	4.31	0.31	0.00	0.11	0.46
Truck 13	Koseki and Isobe 2005	0.00	9.00	0.60	0.00	0.00	1.90
	PMP	0.00	9.00	0.77	0.00	0.00	2.05
	Danyluk and Schaffner 2011	0.00	2.60	0.01	0.00	0.00	0.08
	McKellar and Delaquis 2011	0.00	9.00	0.70	0.00	0.02	1.77
	Gomez 2013	0.00	9.00	0.72	9.00	0.38	1.57
	Veys 2016	0.00	9.00	0.85	0.00	0.01	2.06
Truck 14.1	Koseki and Isobe 2005	0.00	9.00	1.64	0.00	0.10	2.71
	PMP	0.00	9.00	2.18	0.00	0.61	2.98
	Danyluk and Schaffner 2011	0.00	9.00	0.02	0.00	0.00	0.12
	McKellar and Delaquis 2011	0.00	9.00	1.75	0.00	0.66	2.42

	Gomez 2013	0.00	9.00	0.91	9.00	0.32	1.57
	Veys 2016	0.00	9.00	2.19	0.00	0.86	2.84
Truck 15	Koseki and Isobe 2005	0.00	9.00	1.44	0.00	0.48	2.31
	PMP	0.00	9.00	2.13	0.00	1.29	2.47
	Danyluk and Schaffner 2011	0.00	9.00	0.03	0.00	0.01	0.28
	McKellar and Delaquis 2011	0.00	9.00	1.76	0.00	1.16	2.01
	Gomez 2013	0.00	9.00	0.64	9.00	0.12	1.60
	Veys 2016	0.00	9.00	2.23	0.00	1.51	2.37
Truck 16	Koseki and Isobe 2005	0.00	6.37	0.09	0.00	0.00	0.37
	PMP	0.00	8.79	0.23	0.00	0.00	0.64
	Danyluk and Schaffner 2011	0.00	0.06	0.00	0.00	0.00	0.00
	McKellar and Delaquis 2011	0.00	6.17	0.24	0.00	0.00	0.50
	Gomez 2013	0.00	5.22	0.56	0.00	0.35	0.62
	Veys 2016	0.00	7.83	0.29	0.00	0.00	0.66

Table 6: Descriptive statistics for the log increase of *L. monocytogenes* during transportation as predicted by six published models using time series simulated data from 16 trucks

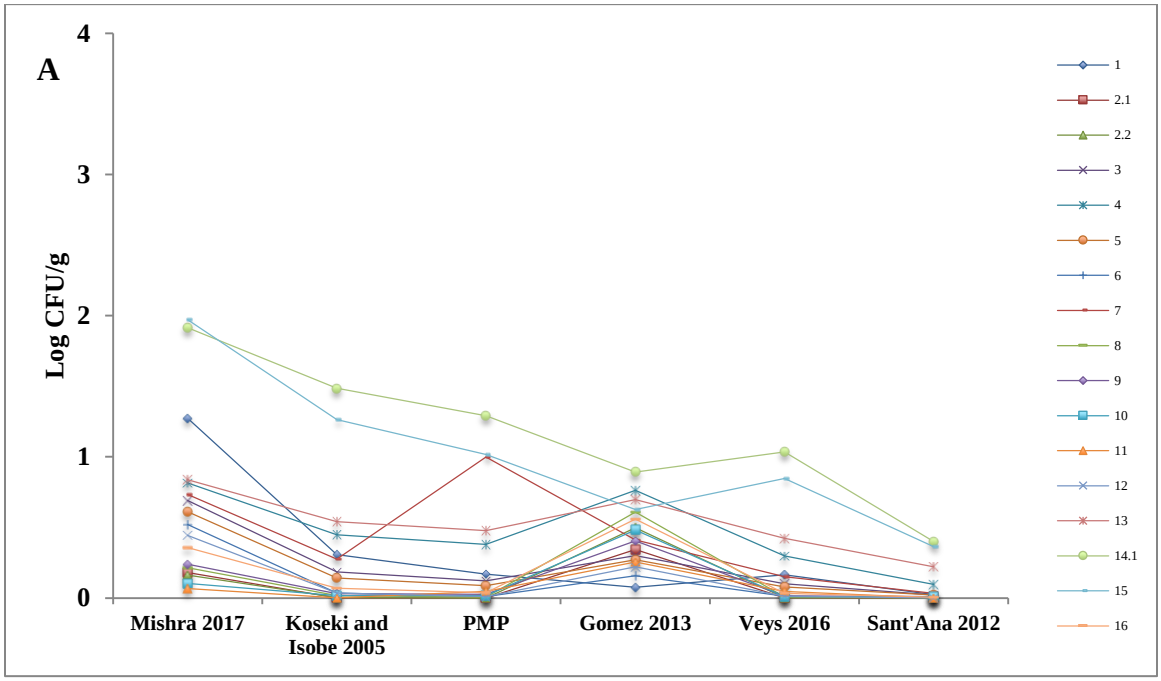
Trucks	Model	Minimum	Maximum	Mean	Mode	Median	Standard Deviation
Truck 1	Koseki and Isobe 2005	0.00	0.00	0.00	1.42	1.61	0.60
	PMP	0.00	8.35	2.07	1.55	1.90	1.14
	Sant'Ana 2012	0.37	0.06	0.71	0.00	0.20	0.20
	Mishra 2017	0.00	5.65	1.22	0.86	1.11	0.74
Truck 2.1	Koseki and Isobe 2005	0.00	2.24	0.59	0.49	0.56	0.28
	PMP	0.00	2.66	0.30	0.00	0.21	0.31
	Sant'Ana 2012	0.00	1.62	0.16	0.00	0.09	0.19
	Mishra 2017	0.00	0.68	0.00	0.00	0.00	0.02
Truck 2.2	Koseki and Isobe 2005	0.00	0.82	0.01	0.00	0.00	0.04
	PMP	0.00	1.99	0.18	0.00	0.10	0.22
	Sant'Ana 2012	0.00	0.82	0.01	0.00	0.00	0.04
	Mishra 2017	0.00	2.85	0.15	0.00	0.03	0.25
Truck 3	Koseki and Isobe 2005	0.00	7.16	1.13	0.00	0.96	0.85
	PMP	0.00	9.00	1.20	0.00	0.73	1.41
	Sant'Ana 2012	0.00	2.71	0.15	0.00	0.03	0.25
	Mishra 2017	0.00	8.97	0.74	0.00	0.41	0.93
Truck 4	Koseki and Isobe 2005	0.00	9.00	1.17	0.00	0.72	1.42
	PMP	0.00	9.00	1.34	0.00	0.40	2.07
	Sant'Ana 2012	0.00	9.00	0.24	0.00	0.01	0.66
	Mishra 2017	0.00	9.00	0.92	0.00	0.19	1.59
Truck 5	Koseki and Isobe 2005	0.00	9.00	1.06	0.00	0.92	0.78
	PMP	0.00	9.00	1.04	0.00	0.67	1.21
	Sant'Ana 2012	0.00	9.00	0.12	0.00	0.03	0.28
	Mishra 2017	0.00	9.00	0.65	0.00	0.38	0.87

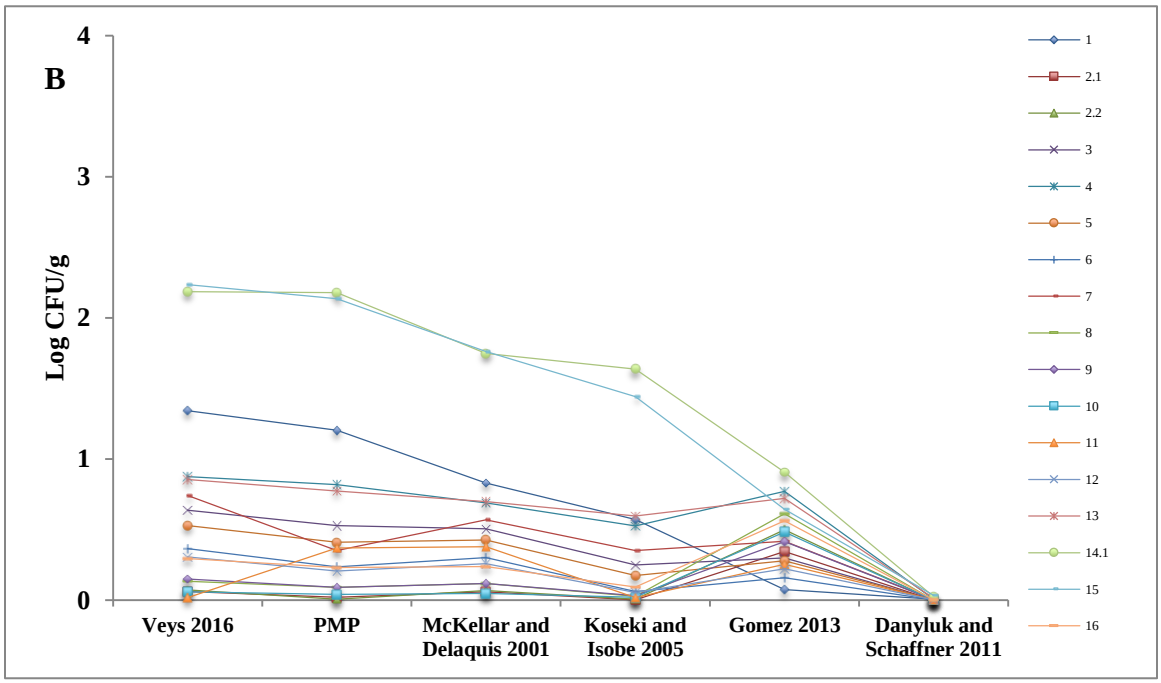
Truck 6	Koseki and Isobe 2005	0.01	3.88	1.02	0.99	0.97	0.47
	PMP	0.00	5.38	0.89	0.00	0.73	0.73
	Sant'Ana 2012	0.00	0.91	0.08	0.00	0.03	0.10
	Mishra 2017	0.00	3.45	0.52	0.00	0.41	0.47
Truck 7	Koseki and Isobe 2005	0.00	7.96	1.15	0.00	0.89	1.02
	PMP	0.00	9.00	2.14	0.00	0.60	2.92
	Sant'Ana 2012	0.00	3.10	0.18	0.00	0.02	0.33
	Mishra 2017	0.00	9.00	0.81	0.00	0.34	1.14
Truck 8	Koseki and Isobe 2005	0.00	4.42	0.54	0.00	0.41	0.51
	PMP	0.00	8.76	0.37	0.00	0.07	0.64
	Sant'Ana 2012	0.00	1.84	0.03	0.00	0.00	0.09
	Mishra 2017	0.00	4.98	0.21	0.00	0.02	0.41
Truck 9	Koseki and Isobe 2005	0.00	9.00	0.64	0.00	0.56	0.49
	PMP	0.00	9.00	0.41	0.00	0.21	0.68
	Sant'Ana 2012	0.00	9.00	0.04	0.00	0.00	0.26
	Mishra 2017	0.00	9.00	0.23	0.00	0.09	0.49
Truck 10	Koseki and Isobe 2005	0.31	9.00	0.57	0.38	0.46	0.45
	PMP	0.00	9.00	0.17	0.00	0.04	0.56
	Sant'Ana 2012	0.00	5.87	0.01	0.00	0.00	0.14
	Mishra 2017	0.00	9.00	0.09	0.00	0.01	0.34
Truck 11	Koseki and Isobe 2005	0.22	9.00	0.45	0.27	0.34	0.37
	PMP	0.00	8.55	0.99	0.00	0.65	1.09
	Sant'Ana 2012	0.00	8.92	0.01	0.00	0.00	0.15
	Mishra 2017	0.00	9.00	0.09	0.00	0.01	0.41
Truck 12	Koseki and Isobe 2005	0.00	3.53	0.92	0.69	0.84	0.52
	PMP	0.00	5.30	0.77	0.00	0.57	0.75
	Sant'Ana 2012	0.00	1.44	0.07	0.00	0.02	0.11
	Mishra 2017	0.00	4.29	0.45	0.00	0.30	0.48
Truck 13	Koseki and Isobe 2005	0.23	9.00	1.22	9.00	0.62	1.67

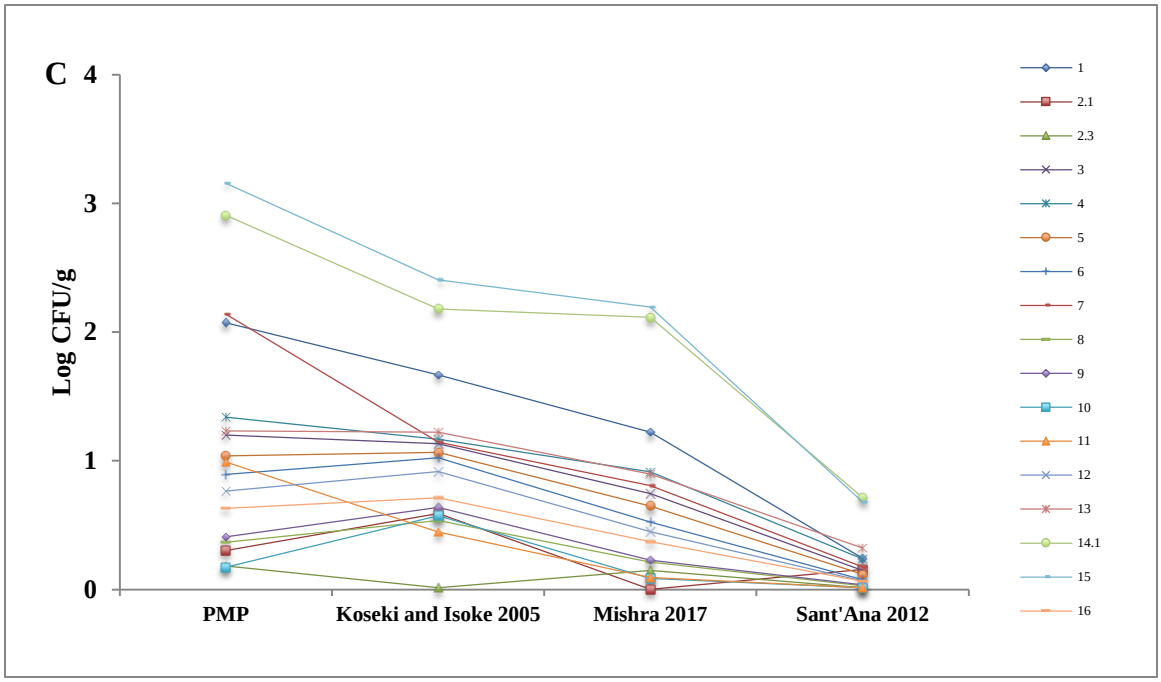
	PMP	0.00	9.00	1.23	9.00	0.28	2.23
	Sant'Ana 2012	0.00	9.00	0.32	0.00	0.00	1.17
	Mishra 2017	0.00	9.00	0.90	0.00	0.13	1.94
Truck 14.1	Koseki and Isobe 2005	0.00	9.00	2.18	0.00	1.53	2.18
	PMP	0.00	9.00	2.91	0.00	1.70	3.11
	Sant'Ana 2012	0.00	9.00	0.72	0.00	0.18	1.35
	Mishra 2017	0.00	9.00	2.12	0.00	1.04	2.61
Truck 15	Koseki and Isobe 2005	0.00	9.00	2.41	0.00	2.06	1.81
	PMP	0.00	9.00	3.16	9.00	2.57	2.54
	Sant'Ana 2012	0.00	9.00	0.68	0.00	0.35	1.23
	Mishra 2017	0.00	9.00	2.20	0.00	1.64	2.13
Truck 16	Koseki and Isobe 2005	0.00	5.78	0.71	0.00	0.53	0.69
	PMP	0.00	9.00	0.63	0.00	0.18	1.01
	Sant'Ana 2012	0.00	2.67	0.06	0.00	0.00	0.16
	Mishra 2017	0.00	5.86	0.37	0.00	0.08	0.64

Figures

Figure 1: Log increase of (A) *Salmonella spp*., (B) *E. coli* O157:H7, and (C) *Listeria monocytogenes* during transportation



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Chapter V: Quantitative Microbial Risk Assessment for *Salmonella* on Sliced Tomatoes

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Abstract

Quantitative microbial risk assessment (QMRA) has been used to evaluate the risk of foodborne illness associated with a particular pathogen and a particular food product. A QMRA approach was applied to estimate the risk of illness from *Salmonella* associated with the consumption of sliced tomatoes. A cross-contamination model was developed from laboratory data *Salmonella* for transfer by a mechanical slicer under different slicing conditions. Data, models, and user inputs were used to create a simulation model in Excel using the software add-in @Risk perform Monte Carlo simulation. Scenarios included different prevalence levels for *Salmonella* on fresh, whole tomatoes (1/30, 1/300 and 1/3000 tomatoes) and different initial concentrations of *Salmonella* (0, 1, 3 and 6 log CFU/tomato). The QMRA simulated production runs corresponded to a total of one million servings per run. Not surprisingly, the QMRA predicts that higher concentration and/r high prevalence of *Salmonella* resulted in a higher total number of illness. Using an electrical slicer and slicing at a temperature of 4°C resulted in a lower number of illnesses. Sanitizing the blade halfway through a production run did not significantly reduce the number of illnesses compare to a production run with no cleaning. Most illnesses were as result of consuming an initially uncontaminated tomato, which was subsequently cross-contaminated by the slicer blade. This QMRA provides a preliminary framework for determining the risk of illness from *Salmonella* arising from sliced tomatoes. The results of the QMRA depend strongly on assumptions regarding the prevalence and concentration of *Salmonella* on whole tomatoes, as well as storage time and temperature throughout the distribution chain, which currently remained limited.

Introduction

Salmonella cause an estimated one million foodborne illnesses in the United States, and 19,000 hospitalizations and 380 deaths (180). Outbreaks of salmonellosis have been linked to a wide variety of fresh fruits and vegetables including apple, cantaloupe, alfalfa sprout, mango, lettuce, cilantro, tomato, melon, celery and parsley (161). There were 15 multistate *S. enterica* outbreaks associated with whole tomatoes between 1973 to 2010, resulting in 1952 reported illnesses, 284 hospitalizations and 3 deaths (18). *Salmonella* outbreaks have also been associated with pre-sliced or diced tomatoes. At least 65 people were sickened after consuming diced tomatoes served at Chipotle in Minnesota in 2015(111, 208). The ultimate source of *Salmonella* on whole tomatoes is typically assumed to be environmental, e.g. animal contaminating arising in the field, use of contaminated irrigation water, etc (30). Trace back investigations from outbreaks linked to tomatoes are challenging as ill individuals may have difficulty recalling package labeling or even tomato type especially if tomatoes have already been cut or sliced (18). Microbiological tests of any implicated tomatoes are also difficult because of their perishability. Finally, any link back to a given growing location is complicated by the speed with which fields are turned over after harvest. These factors mean that data on prevalence and concentration of *Salmonella* on implicated tomatoes are virtually non-existent.

QMRA is a tool increasingly used to evaluate the risk of foodborne illnesses. In the last decade, several QMRA have been developed to assess the risk of pathogens in fresh produce, including the risk of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on leafy green vegetables on salad bars (73); risk assessment of leafy greens from farm to

fork (55); QMRA on *E. coli* O157:H7 on lettuce based on survival from controlled studies in a climate chamber (143). QMRAs addressing meat safety have included models *L. monocytogenes* in Retail delicatessens (74, 154), *Salmonella* in Danish meatballs (127), risk of *Salmonella* and *L. monocytogenes* during pork and beef grinding (128).

Our study uses a quantitative microbial risk assessment (QMRA) approach to estimate the risk of illness arising from *Salmonella* associated sliced tomatoes, starting from the point of whole tomato receiving and ending with consumption. We developed a model for tomato cross-contamination by *Salmonella* during slicing based on published data (214).

Materials and Methods

Tomato Slicing Model

Data from Wang and Ryser (2016) on the transfer of *S. typhimurium* during tomatoes slicing were extracted and analyzed. Those authors assessed the spread of *Salmonella* from one inoculated tomato to 20 non-inoculated tomatoes subsequently sliced on the same slicer. The baseline conditions for the Wang and Ryser (2016) study used a post-inoculation hold time of 0 min, dry tomato surface, manual slicer at 23ºC, with a blade thickness of 0.95 cm (1/4”) using Torero tomatoes. Additional slicing variables used by Wang and Ryser (2016) included electric slicing, a 30 min post-inoculation wait time, wet tomato surface, slicing temperatures of 4 and 10 ºC), blade thicknesses of 0.48, 0.64 cm (3/16 and 3/8 in.), and the Rebelski and Bigdena tomato varieties. Wang and Ryser (2016) measured log CFU of *Salmonella* transferred to each tomato slice, and all their experiments were performed in triplicate.

We calculated the transfer rate from the blade to the tomato slice for each processing variable from those data as follows. The CFU on each tomato slice (S) was determined using equation 1. The total CFU coming out of the system (CFU_{Total}) was calculated by summing the total CFU on all tomato slices (Equation 2). The CFU remaining on the blade (B) after each tomato was calculated in two steps. First, the CFU on the blade after slicing tomato 1 was obtained by subtracting the CFU on the tomato slice 1 from the total CFU (equation 3a). Second, the CFU remaining on the blade subsequently was calculated by subtracting the CFU on the relevant tomato slice from the CFU remaining on the precedent blade (Equation 3b). The transfer rate from the blade to the tomato slice was calculated in two steps (Equation 4). The transfer rate (*t*) to the first tomato slice was obtained by dividing the CFU on the first tomato slice by the total CFU remaining on the blade after slicing the first tomato. The subsequent transfer rates were calculated dividing the CFU on the relevant tomato slice by the CFU remaining on the blade in the previous step. All the calculated transfer rates were log transformed and fit to a normal distribution.

1. $S = 10^{(\log S)}$
2. $CFU_{Total} = \sum (S_1 + S_{n+1} \ldots + S_{20})$
3. $B_1 = CFU_{Total} - S_1$ (a); $B_2 = B_1 - S_2$; $B_n = B_{n-1} + S_n$ (b)
4. $t_1 = S_1 / CFU_{Total}$ (a); $t_2 = S_2 / B_1$; $t_n = S_n - B_{n-1}$ (b)

The means and standard deviations of the log transformed transfer rates were compared using Statplus (AnalystSoft, Walnut, CA).

Tomato slicing model

A schematic diagram showing the implementation of the tomato-slicing cross-contamination model is illustrated in Figure 1 where T_x represent the number of CFU on tomato x entering the system, B_x represents the CFU on the blade used to slice tomato x, and S_x represents the CFU ending up on the tomato slices arising from tomato x and *t* the percent transferred from blade to tomato.

Overview of simulations variables and parameters

A literature search was conducted to obtain relevant published data on the behavior of *Salmonella* on cut tomatoes. The risk assessment is comprised of two sub-models: an exposure assessment model and a dose-response model. The exposure assessment starts with contaminated tomatoes arriving at the fresh-cut operation and ends at the consumer's home. The dose response model estimates the probability of illness from each contaminated sliced tomato, and calculates the total number of illnesses. Table 1 provides an overview of the simulation variables and distributions that were used in the risk model, which are discussed further in the results section. The table contains eight modules: (i) on arrival at the fresh-cut operation, (ii) transfer during slicing at the fresh-cut operation, (iii) sanitizing at the fresh-cut operation step, (iv) transportation to retail, (v) retail storage, (vi) home storage, (vii) serving size and dose-response and (viii) illnesses from the consumption of contaminated tomatoes. The first column indicates the spreadsheet cell reference of the variable. The second column describes the variable. The third and fourth columns show the source of the information based on user input, literature citation, and calculation, and the unit variable, respectively. The fifth column represents the value of the cell as a number, a formula or @Risk function. The sixth to

the 100th columns (not shown) represent individual tomatoes being sliced subsequently. Data models and user inputs were constructed in an Excel (Microsoft, Redmond WA) spreadsheet and were simulated using @Risk (version 7, Palisade, Newfield, NY) an excel spreadsheet add-in program. Ten thousand iterations were performed for each scenario using Monte Carlo Simulation. One production day where 100 tomatoes are sliced represented one iteration. Although 100 tomatoes are far less than the typical volumes processed in a fresh-cut plant typically 4000 to 88000 tomatoes per line per day), preliminary simulation results (not shown) indicated that simulating more realistic numbers of tomatoes produced essentially the same results. Given the very limited published data on *Salmonella* prevalence and concentration on whole tomatoes (17, 206), *Salmonella* prevalence was assumed to be either 1/30, 1/300 or 1/3000 tomatoes, and *Salmonella* concentration on positive tomatoes was assumed to be 0, 1, 3 or 6 log CFU/tomato. We simulated the effect of no cleaning vs. cleaning half-way through production (after 50 tomatoes) and all of the different slicing conditions from Wang and Ryser (2016).

Results

Comparison of mean log % transfer rates and p-values for S. typhimurium transfer from blade to tomatoes for different slicing parameters are provided in Table 2. Results showed that the mean log transfer rate of the baseline (post-inoculation hold time of 0 min, dry tomato surface, manual slicer at 23ºC, with a blade thickness of 0.95 cm (1/4") using Torero tomatoes) was significantly different compared to electrical slicing, slicing after a 30 min post-inoculation time, slicing a wet tomato, slicing at T=4°C, and slicing with a 0.48 cm blade ($p<0.05$). The baseline transfer rate was not significantly

different than slicing at T=10°C (p=0.0868), slicing with a 0.64 cm blade (p=0.3307), or for slicing Bigdena (p=0.7991) or Rebelski (p=0.8952) tomatoes. When the tomato surface was wet, the transfer rates were significantly different than at 4°C, slicing with a 0.64 cm or 0.48 cm blade or for slicing Bigdena or Rebelski varieties. Slicing at 4°C was significantly different ($p<0.05$) than slicing at 10°C, slicing with a 0.64 cm or 0.48 cm blade or for slicing Bigdena or Rebelski varieties.

Figure 2 shows a comparison between the actual transfer from one highly contaminated tomato (after 30 min hold-time post-inoculation) to 20 non-contaminated tomatoes and the simulated data from 10,000 iterations. The mean reductions from the simulation closely match the actual data. Although the actual data appear to show some tailing, it is important to realize that this is likely an artefact of the experimental detection limit, such that only counts above the detection limit would be observed. Figure 2 also shows the extreme variability that can occur in the simulation, such that very high counts and very low counts (below the experimental detection limit) can occasionally occur.

Risk Assessment Results

All the cells in the Excel spreadsheet used for subsequent risk calculations are summarized in Table 1. The prevalence and concentration of *Salmonella* on tomatoes arriving at the fresh-cut facility are described in the first section of Table 2 (in field). The QMRA model assumes that all contamination arises prior to arrival at the fresh cut facility. Prevalence assumption were informed by data from Bell *et al*. and USDA Microbiological Data Program (17, 206) which indicated prevalence rates of 1/279 that was around up to 1/300. A binomial distribution was used to calculate the percentage of positive tomatoes. Since no data were available to estimate the concentration of

Salmonella on positive tomatoes, mean and standard deviation were assumed to be concentration of *Salmonella* on positive tomatoes for exploratory purposes.

The transfer of *Salmonella* from contaminated tomato to blade, and from blade to tomatoes is calculated in the slicing at fresh-cut section of Table 1. The percent transfer *Salmonella* during slicing from different conditions were included as shown at the top of Table 2, where a normal (Gaussian) distribution was used to describe the log percent transfer of *Salmonella* from blade to tomato. The Risktruncate function was used to insure percent transfer retained between 0 and 100%. The log CFU remaining on the blade and log CFU transferred to the tomatoes were calculated. The sanitizer section of the QMRA evaluated the effect of a cleaning session occurring a slice 50 (halfway through processing). The sanitizer log reduction was simulated as normal distribution (μ=5, σ=1). The log CFU remaining on the blade and log CFU transferred to the tomatoes were determined after cleaning. Log CFU of any blade or tomato containing 0 CFU was set to "-1"

The simulated change of pathogen concentration during transportation was represented in the transportation section. Transportation temperatures and times included in the study were based on expert opinion (fresh-cut plant manager). A Pert distribution (2, 4, 7^{o}C) described temperature; and the transportation time was expressed by a uniform distribution (1 to 4 days). Growth model parameters *b* and T_0 from Pan and Schaffner were used to calculate the growth of *Salmonella* during transportation (145). The Retail section simulated *Salmonella* concentration change during retail storage. Since no retail sliced tomato temperature data were available in the published literature, we used potato salad temperature data was extracted from Ecosure Audit International cold temperature

database, and fitted these data to a normal distribution (61). The storage time was expressed by uniform distribution between 2-10 days based on expert opinion (fresh-cut plant manager) The Pan and Schaffner (2008) growth model was used to calculate the change in *Salmonella* concentration during retail storage.

The Consumer home section of Table 2 calculated the change in *Salmonella* concentration during home storage, and we assumed that each tomato slice would go to a different home. The storage model was largely based on data from Pouillot et al (157), where mean storage temperature was 4.06°C and the difference from the mean was expressed by Riskexpon (2.31), and the chance of being above or below the mean was determined by RiskBinomial (1, 0.5). Exponential and Weibull distributions described the time until first consumption and the time until last consumption respectively (157), and the actual storage time was a uniform distribution between these two values. In the case where the last consumption time was less than the first consumption time, the first consumption time was set as the actual consumption time. The Pan and Schaffner growth model was used to calculate the growth during home storage. The level of *Salmonella* growth was limited to 7 log CFU/tomato, as this was the highest concentration ever observed by(145).

The Serving Size and dose-response section assumes that one tomato slice is one serving, and uses the concentration of *Salmonella* per serving, and parameters of the dose response model from FAO to estimate the probability of illness (67). In the Illness section of Table 2, the total number of illnesses was calculated. The probability of illness was combined with the number of servings per iteration in a binomial distribution to predict

the number of illness arising per serving. The number of illness per slice is summed to calculate the total number of illness from the 100 tomato slices consumed.

Table 3 summarizes the total number of illness resulting from the simulations for different slicing variables when *Salmonella* was present on 1/30 tomatoes. The total number of illness was close to zero for all slicing parameters when the initial concentration of *Salmonella* was low (0 log CFU/tomato, or 1 CFU per tomato). As expected, the predicted number of illness increased with increasing initial concentration of *Salmonella*. When the starting concentration of *Salmonella* of 6 log CFU/tomato, Rebelski tomatoes or baseline slicing parameters caused the highest simulated number of illness 48.76 (± 19.88) and 45.44 (±19.33) per 100 tomatoes, respectively. Slicing tomatoes when wet or using thinner blades (0.64 cm and 0.48 cm) did not reduce the number of illnesses substantially. There were fewer simulated illnesses when the virtual tomatoes were processed with an electrical slicer or at 4°C. Independent of the slicing conditions, the fraction of illnesses cause by cross-contamination from blade increased with the initial concentration of the pathogen. This is not surprising, as higher starting concentrations on the tomato allow more *Salmonella* to transfer to the blade and then subsequently transfer to other tomatoes.

Table 4 and 5 summarizes the total number of illness resulting from the simulations for different slicing variables when Salmonella was present on 1/300 and 1/3000 tomatoes respectively. As can be seen in comparison with Table 3, the average number of illnesses drops as prevalence of *Salmonella* in incoming tomatoes decreases, and it rises as the concentration increases. As seen in Table 3, the average number of illnesses was the highest for the baseline slicing condition and for wet tomatoes and

thinner blades. Slicing at lower temperatures and using electrical slicer resulted in the lowest number of illnesses. It is clear that from Tables 3, 4 and 5 that most simulated illnesses (for any scenario) arise from cross-contamination from the blade during slicing.

There were no significant differences in the total number of illness when cleaning was simulated halfway through slicing (data not shown). Since the greatest *Salmonella* prevalence simulated was 1/30 tomatoes, and even when a highly contaminated tomato was sliced, the contamination would typically spread to only about 20 tomatoes.

Discussion

Many studies have quantified and/or modeled the transfer of pathogens during slicing including, *Listeria* during the slicing of deli meats(43, 46, 185, 210) or salmon fillets(1) norovirus during tomato slicing(186). Similar processes such as meat grinding have been amply investigated (70, 128, 129) . Those processes have been described through various models. Generally, the transfer of pathogens during slicing has a descending pattern. Many of those studies used a non-linear approach to develop slicing model. While those mathematical models were significant, further analyses are necessary to be included in a quantitative microbial risk assessment. In the current study, a model was developed to predict the transfer of *Salmonella* during tomato slicing under various slicing conditions. *Salmonella* transfer rates varied among the slicing conditions. The transfer rate was assumed to be constant regardless of time of contamination and the concentration of bacteria in the system. The results showed that important difference exist among the slicing methods. Mechanical slicer and 4°C slicing temperature transferred significantly less pathogen than the other slicing parameters (Table 2). Additional factors may also affect the transfer of pathogens during slicing such as blade

material and sharpness, the back pressure from meat loft/tomato, slicing speed (slices per minute), contact angle, area, texture and surface of the food (12, 185, 210).

The second part of this study simulated the cross-contamination of *Salmonella* during tomato slicing plant from the field to the consumer's home. The risk assessment estimated the probability of illness of *Salmonella* on one sliced tomato; and calculated the total number of illness within a specific slicing condition. Several assumptions were made to build this QMRA. It was assumed that first no reduction in the pathogen level occurs due to cutting, washing and partitioning. Second, potato salad temperature was similar to sliced tomatoes in retail refrigerators. Finally, the physical characteristic of the tomato (cooked or raw) in which the pathogen was transmitted did not affect the dose response relations. Critical data such as the prevalence and concentration of *Salmonella* in the field, storage time and temperature (transportation, retail and home) remained limited. Since our analysis integrated data from a single study, additional data for validation are needed. In conclusion, this risk analysis simulates a tomato slicing facility and estimates the total number of illnesses by *Salmonella*. This work is unique because it links quantitative cross-contamination of *Salmonella* during tomato slicing to predict public health outcomes. This article provides a robust tomato-slicing model and evaluates the risk of salmonellosis in cut-tomatoes. Although it is clear that more data may be needed, this analysis could become a valuable tool providing managers with information needed regarding practices and mitigation strategies in fresh-cut facilities.

Tables

Table 1. Overview of simulations variables and parameters

	Variable	Source	Units	Tomato 0	Tomato 1
	EXPOSURE ASSESSMENT				
F1	**Arriving at fresh-cut**				
F2	Every xth tomato is positive for *Salmonella*	(17)	1/percent	-	
F3	Is this tomato positive?	Calculated	Percent	=RiskBinomial(1,1/F2)	=RiskBinomial(1, 1/$F2)
F4	Mean Concentration of *Salmonella* on positive tomatoes	User input		-	
F5	SD concentration of Salmonella on positive tomatoes	User input		0.5	
F6	**Slicing at fresh-cut**				
F7	Log CFU of *Salmonella* on positive tomatoes	Calculated	Log CFU	=RiskNormal($F4, $F5)	=RiskNormal($F4, $F5)
F8	CFU of *Salmonella* on positive tomatoes	Calculated	CFU	=TRUNC(10^F7)	=TRUNC(10^G7)
F9	CFU of *Salmonella* added to the system	Calculated	CFU	=F8*F3	=G8*G3
F10	Mean of log % transfer from blade to tomato	(214)		-	
F11	SD of log % transfer from blade to tomato	(214)		-	
F12	Log % transfer from blade to tomato	Calculated	Log %	=RiskNormal($F10,$F11, RiskTruncate(-5,0))	=RiskNormal($F10,$F11,RiskTruncate(-5,0))
F13	Percent transfer from blade to tomato	Calculated	%	=10^F12	=10^G12
F14	Percent remaining on blade or % from tomato to blade, if tomato is positive	Calculated	%	=1-F13	=IF(1-G13<0,0,1-G13)
F15	CFU of *Salmonella* on tomato post slicing	Calculated	CFU	=TRUNC(F9*F13)	=TRUNC((F16+G9)*G13)

F16	CFU of *Salmonella* remaining on blade or going to blade, if tomato is positive	Calculated	CFU	=TRUNC(F9*F14)	=TRUNC((G9+F16)*G14)
F17	Log CFU of *Salmonella* on tomato post slicing	Calculated	Log CFU/slice	=IF(F15=0,-1,LOG(F15))	=IF(G15=0,-1,LOG(G15))
F18	Log CFU of *Salmonella* remaining on blade or going to blade, if tomato is positive	Calculated	Log CFU	=IF(F16=0,-1,LOG(F16))	=IF(G16=0,-1,LOG(G16))
F19	**Sanitizer**				
F20	Sanitizer every x slices	User input	Slice number	-	
F21	Sanitizer Remainder	Calculated			=MOD(G1,$F20)
F22	Sanitizer yes or no			1	=IF(G22=0,1,0)
F23	Sanitizer log reduction	User input	Log CFU	5	
F24	Sanitizer log reduction SD	User input	Log CFU	1	
F25	Sanitizer log red with SD	Calculated	Log CFU	=RiskNormal($F23,$F24)	=RiskNormal($F23,$F24)
F26	Sanitizer efficiency	Calculated	%	=(1-10^-F25)*F22	=(1-10^-G25)*G22
F27	CFU removed by sanitizer on blade	Calculated	CFU	=TRUNC(F16*F26)	=TRUNC(G16*G26)
F28	CFU remaining on the blade	Calculated	CFU/slice	=IF(TRUNC(F16-F27)<0,0, TRUNC(F16-F27))	=IF(TRUNC(((F28+G9)*G14)-G27)<0,0,TRUNC(((F28+G9)*G14)-G27))
F29	CFU transferred from blade to tomato	Calculated	CFU	=IF(TRUNC(F9*F14)<0,0,TRUNC(F9*F14))	=IF(TRUNC((F28+G9)*G13-G27)<0,0,TRUNC((F28+G9)*G13-G27))
F30	Log CFU transferred to tomato post sanitizer	Calculated	Log CFU	=IF(F29=0,-1,LOG(F29))	=IF(G29=0,-1,LOG(G29))
F31	Log CFU remaining on the blade	Calculated	Log CFU	=IF(F28=0,-1,LOG(F28))	=IF(G28=0,-1,LOG(G28))
F32	**Transportation**				
F33	Temperature Storage in the truck	User input	°C	=RiskPert(2,4,7)	
F34	Time storage in the truck	User input	Days	=RiskUniform(1,4)	
F35	Growth model b parameter	(145)		0.026	
F36	Growth model To parameter	(145)		-0.107	
F37	√Growth Rate per hour	Calculated	√Log CFU/hour	=F35*(F33-F36)	

F38	Growth Rate per hour	Calculated	Log CFU/hour	=F37^2	
F39	Growth per day	Calculated	Log CFU/day	=F38*24	
F40	Calculated log CFU increase of *Salmonella* during transportation	Calculated	Log CFU/day	=F39*F34	
F41	Log CFU of *Salmonella* on subsequent slices after transport	Calculated	Log CFU/slice	=IF(F30=-1,-1,$F$40+F30)	=IF (G30=-1,-1,$F$40+G30)
F42	**Retail**				
F43	Temperature Storage in retail	(61)	°C	=RiskNormal(5.72, 3.08)	
F44	Time storage in retail	(61)	Days	=RiskUniform(2,10)	
F45	Growth model *b* parameter	(145)	Log CFU/hour	0.026	
F46	Growth model T_o parameter	(145)		-0.107	
F47	√Growth Rate per hour	Calculated	√LogCFU/hour	=F45*(F43-F46)	
F48	Growth Rate per hour	Calculated	Log CFU/hour	=F47^2	
F49	Growth per day	Calculated	Log CFU/day	=F48*24	
F50	Calculated increase in Log CFU *Salmonella* during retail storage	Calculated	Log CFU/time	=F49*F44	
F51	Log Concentration of *Salmonella* on each slice	Calculated	Log CFU/slice	=IF(F43=-1,-1,$F$53+F43)	=IF (G41=-1,-1,$F$50+G41)
F52	**Consumer home**				
F53	Temperature, mean	(157)	°C	4.06	
F54	Temperature, difference from mean	(157)	°C	=RiskExpon(2.31)	=RiskExpon(2.31)
F55	Temperature, above or below the mean	Calculated	°C	=RiskBinomial(1,0.5)	=RiskBinomial(1,0.5)
F56	Home temperature used	Calculated	°C	=IF(F55=1, F53+F54, F53-F54)	=IF(G55=1, G53+G54, G53-G54)
F57	Time until first consumption	(157)	Days	=RiskExpon(0.255)	=RiskExpon(0.255)
F58	Time until last consumption	(157)	Days	=RiskWeibull(1.19,14)	=RiskWeibull(1.19,14)
F59	Time used if the last time is shorter than the first time	Calculated	Days	=IF(F57>F58,F57,0)	=IF(G57>G58,G57,0)
F60	Time from uniform distribution	Calculated	Days	=RiskUniform(F57,F58)	=RiskUniform(G57,G58)
F61	Time selected	Calculated		=IF(F59=0,F60,F59)	=IF(G57>G58,G57,0)
F62	Is the product past 15 day shelf	No units		=IF(F61+F44>15,1,0)	=IF(G61+G44>15,1,0)

	life				
F63	Growth *b* parameter	(145)	√LogCFU/hour	0.026	
F64	Growth model T_o parameter	(145)	C	-0.107	
F65	√Log CFU/hour	Calculated		=F63*(F56-F64)	=G63*(G56-G64)
F66	Growth Rate per hour	Calculated	Log CFU/hour	=F65^2	=G65^2
F67	Growth rate per day	Calculated	Log CFU/day	=F66*24	=G66*24
F68	*Salmonella* increase during home storage	Calculated	Log CFU	=F67*F61	=G67*G61
F69	Log CFU of *Salmonella* after home storage	Calculated	Log CFU	=IF (F51=-1,-1,F51+F68)	=IF(G51=-1,-1,G51+G68)
F70	Limit of level if 10^7	Calculated	Log CFU	=IF(F69>7,7,F69)	=IF(G69>7,7,G69)
	DOSE-RESPONSE ASSESSMENT				
F71	**Serving size and dose response**				
F72	Serving Size	User input	Slice	1	
F73	Concentration of *Salmonella* per serving	Calculated	CFU/slice	=IF (F70=-1,0, TRUNC(10^F70))	=IF(G70=-1,0, TRUNC(10^G70))
F74	Alpha	(71)	No units	0.1324	
F75	Beta	(71)	No units	51.45	
F76	Probability of illness	Calculated	Percent	=1-(1+F73/$F$75)^-$F$74	=1-(1+G73/$F$75)^-$F$74
F77					
F78	**Illnesses**				
F79	One iteration for			1	
F80	Was there illness?	Calculated	Illness	=RiskBinomial($F$78,F76)	=RiskBinomial($F$78,G76)
F81	Was the slice contaminated at consumption?	Calculated	No units	=IF(F73>0,1,0)	=IF(G73>0,1,0)
F82	Was the blade contaminated?	Calculated	No units	=IF(F28=0,0,1)	=IF(G28=0,0,1)
F83	Was there illness due to contaminated blade?	Calculated	Illnesses	=IF(F79=1, IF(F3=0,1,0),0)	=IF(G79=1, IF(G3=0,1,0),0)
F84	Was the tomato originally contaminated?	Calculated	No units	=IF(F3=1,1,0)	=IF(G3=1,1,0)
F85	Was there illness due to originally	Calculated	Illnesses	=IF(F79+F83=2,1,0)	=IF(G79+G83=2,1,0)

contaminated tomato?

Table 2: Comparison of mean log % transfer rates and p values for *S. typhimurium* transfer from blade to tomatoes for different slicing conditions.

					Temperature		Blade thickness		Tomato variety	
	Baseline[1]	Electrical	30 min hold	Wet surface	4°C	10°C	0.64 cm (3/8")	0.48 cm (3/16")	Bigdena	Rebelski
N	53	13	43	57	15	24	23	55	33	42
μ	-0.85	-0.29	-0.56	-0.53	-0.25	-0.60	-0.70	-0.60	-0.81	-0.87
σ	0.67	0.24	0.27	0.15	0.16	0.36	0.44	0.21	0.62	0.44
Electrical	0.0088									
30 min	0.0111	0.0192								
Wet	0.0006	0.0002	0.3375							
4°C	0.0016	0.6160	0.0001	<0.0001						
10°C	0.0868	0.0099	0.7086	0.2351	0.0010					
0.64 cm	0.3307	0.0038	0.1327	0.0104	0.0056	0.3775				
0.48 cm	0.0110	<0.0001	0.4549	0.0318	<0.0001	0.9102	0.1905			
Bigdena	0.7991	0.0514	0.0276	0.0029	0.0013	0.1295	0.4002	0.0429		
Rebelski	0.8952	<0.0001	0.0030	<0.0001	<0.0001	0.0131	0.1512	0.0002	0.6694	

[1]The baseline conditions (Wang and Ryser, 2016) used a post-inoculation hold time of 0 min, dry tomato surface, manual slicer at 23ºC, with a blade thickness of 0.95 cm (1/4") using Torero tomatoes.

Table 3: Simulated number of illnesses and fraction due to cross-contamination, arising from 100 sliced tomatoes by slicing condition when 1/30 tomatoes are contaminated.

Slicing condition	Initial *Salmonella* concentration per tomato (log CFU)	Illnesses from 100 tomatoes		Fraction of illness from	
		Mean	SD	Blade cross-contaminated tomato	Originally contaminated tomato
Baseline	0	0.42	0.79	0.35	0.65
	1	4.30	4.34	0.66	0.34
	3	22.46	14.66	0.87	0.13
	6	45.44	19.33	0.92	0.08
Electrical slicing	0	0.58	0.96	0.25	0.75
	1	4.01	3.48	0.50	0.50
	3	13.73	8.86	0.78	0.22
	6	28.37	14.09	0.88	0.12
30 min drying	0	0.37	0.86	0.46	0.53
	1	4.24	4.43	0.70	0.30
	3	19.62	12.88	0.88	0.12
	6	38.90	18.08	0.93	0.07
Wet tomato	0	0.53	4.00	0.32	0.68
	1	5.06	1.00	0.62	0.38
	3	21.78	13.01	0.86	0.14
	6	43.57	18.89	0.92	0.08
Slice at 4°C	0	0.58	0.95	0.21	0.79
	1	3.84	3.35	0.46	0.54
	3	12.73	8.10	0.76	0.24
	6	26.47	13.07	0.87	0.13
Slice at 10°C	0	0.50	0.91	0.33	0.67
	1	4.68	4.45	0.62	0.38
	3	19.92	12.83	0.85	0.15
	6	40.60	18.33	0.92	0.08
Blade thickness 0.64 cm (3/8")	0	0.64	0.89	0.25	0.47
	1	4.59	4.44	0.64	0.36
	3	21.61	13.96	0.86	0.14
	6	43.06	18.84	0.92	0.08
Blade thickness 0.48 cm (3/16")	0	0.49	0.92	0.34	0.66
	1	5.20	4.97	0.64	0.36
	3	22.82	14.40	0.87	0.13
	6	45.11	19.16	0.92	0.08
Bigdena Variety	0	0.42	0.80	0.34	0.66
	1	4.33	2.82	0.65	0.35
	3	22.29	19.37	0.87	0.13
	6	44.81	41.40	0.92	0.08
Rebelski Variety	0	0.42	0.82	0.35	0.65
	1	4.76	4.82	0.68	0.32
	3	0.42	0.82	0.94	0.13
	6	48.76	19.88	0.93	0.07

Table 4: Simulated number of illnesses and fraction due to cross-contamination, arising from 100 sliced tomatoes by slicing condition when 1/300 tomatoes are contaminated.

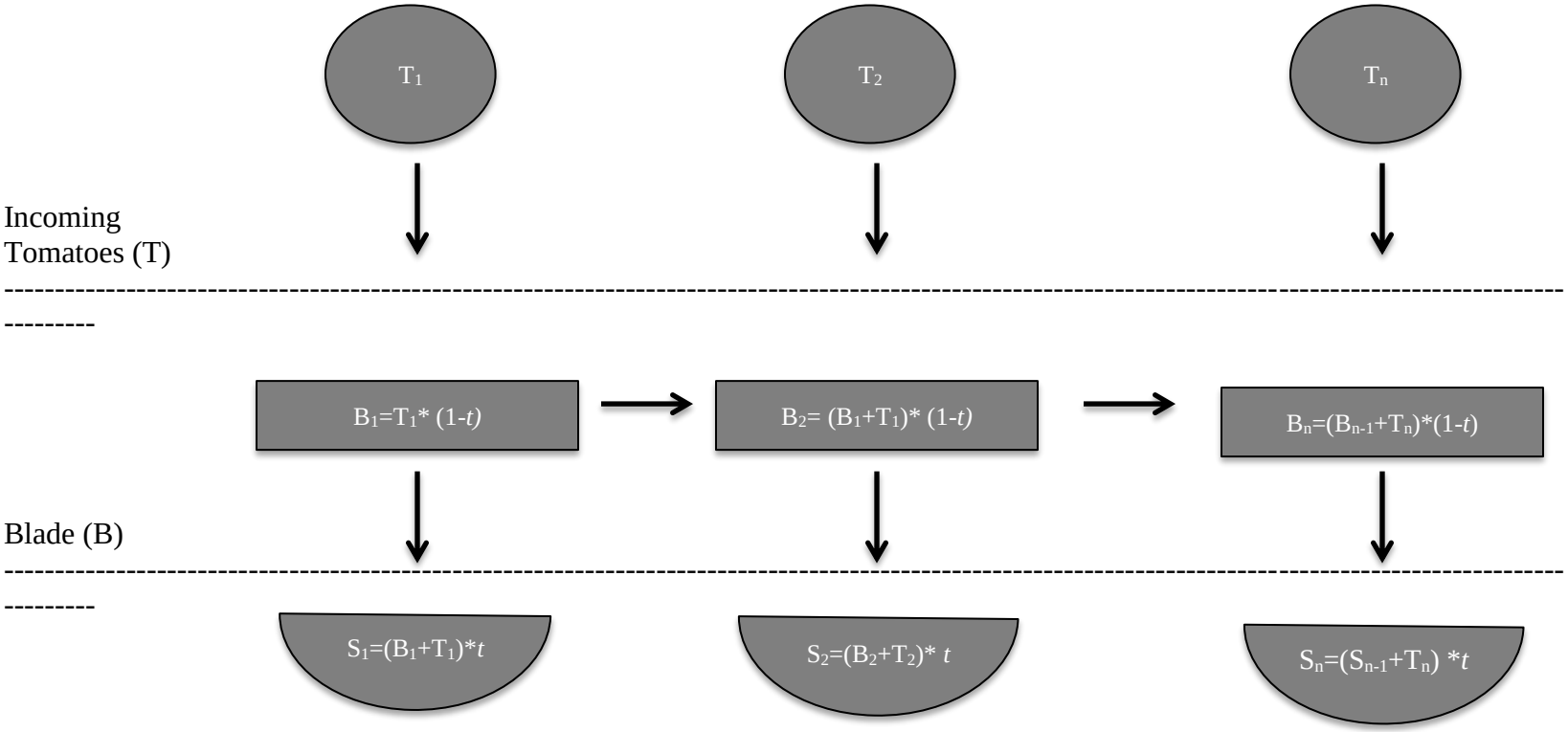
Slicing condition	**Initial *Salmonella* concentration per tomato (log CFU)**	**Illnesses from 100 tomatoes**		**Fraction of illness from**	
		Mean	**SD**	**Blade cross-contaminated tomato**	**Originally contaminated Tomato**
Baseline	0	0.18	0.42	0.07	0.93
	1	0.96	1.33	0.30	0.70
	3	3.42	5.71	0.72	0.28
	6	9.18	12.67	0.88	0.12
Electrical slicing	0	0.17	0.17	0.08	0.92
	1	0.87	1.07	0.23	0.77
	3	2.17	2.92	0.55	0.45
	6	5.12	6.26	0.79	0.21
30 min drying	0	0.19	0.44	0.08	0.89
	1	1.04	1.39	0.30	0.68
	3	3.04	4.62	0.68	0.32
	6	7.92	10.32	0.87	0.13
Wet tomato	0	0.20	0.46	0.09	0.91
	1	1.04	1.41	0.32	0.68
	3	3.10	4.84	0.72	0.29
	6	8.52	11.10	0.88	0.12
Slice at 4°C	0	0.16	0.41	0.08	0.92
	1	0.86	1.02	0.21	0.79
	3	2.06	2.66	0.53	0.47
	6	4.67	5.01	0.77	0.23
Slice at 10°C	0	0.19	0.45	0.10	0.90
	1	0.99	1.33	0.30	0.70
	3	3.01	4.60	0.68	0.32
	6	7.73	10.06	0.86	0.14
Blade thickness 0.64 cm (3/8")	0	0.19	0.45	0.10	0.90
	1	0.99	1.35	0.31	0.69
	3	3.22	5.11	0.70	0.30
	6	8.47	11.47	0.87	0.13
Blade thickness 0.48 cm (3/16")	0	0.19	0.46	0.09	0.91
	1	1.05	1.50	0.32	0.68
	3	3.41	5.31	0.71	0.29
	6	9.01	11.98	0.88	0.12
Bigdena Variety	0	0.18	0.42	0.09	0.92
	1	0.98	1.38	0.31	0.69
	3	3.36	5.43	0.71	0.29
	6	9.07	12.46	0.88	0.12
Rebelski Variety	0	0.18	0.42	0.07	0.93
	1	1.01	1.46	0.33	0.67
	3	3.83	6.33	0.75	0.25
	6	10.36	14.50	0.90	0.10

Table 5: Simulated number of illnesses and fraction due to cross-contamination, arising from 100 sliced tomatoes by slicing condition when 1/3000 tomatoes are contaminated.

Slicing condition	Initial *Salmonella* concentration per tomato (log CFU)	Illnesses from 100 tomatoes		Fraction of illness from	
		Mean	SD	Blade cross-contaminated tomato	Originally contaminated tomato
Baseline	0	0.16	0.37	0.01	0.99
	1	0.62	0.65	0.05	0.95
	3	1.03	1.86	0.26	0.74
	6	3.33	5.38	0.77	0.25
Electrical slicing	0	0.13	0.34	0.01	0.99
	1	0.57	0.59	0.04	0.96
	3	0.89	1.01	0.14	0.86
	6	2.18	2.57	0.62	0.38
30 min drying	0	0.16	0.38	0.02	0.98
	1	0.63	0.63	0.05	0.95
	3	1.00	1.57	0.22	0.78
	6	3.05	4.29	0.73	0.27
Wet tomato	0	0.16	0.38	0.01	0.99
	1	0.63	0.64	0.05	0.95
	3	1.02	1.73	0.24	0.76
	6	3.23	4.58	0.76	0.26
Slice at 4°C	0	0.12	0.33	0.01	0.99
	1	0.56	0.58	0.03	0.97
	3	0.88	0.95	0.13	0.87
	6	2.05	2.32	0.60	0.40
Slice at 10°C	0	0.16	0.37	0.02	0.98
	1	0.62	0.64	0.05	0.95
	3	0.99	1.56	0.22	0.78
	6	3.04	4.35	0.73	0.27
Blade thickness 0.64 cm (3/8")	0	0.16	0.38	0.01	0.99
	1	0.62	0.62	0.05	0.95
	3	1.03	0.62	0.24	0.75
	6	3.26	5.01	0.75	0.25
Blade thickness 0.48 cm (3/16")	0	0.16	0.37	0.01	0.99
	1	0.64	0.66	0.05	0.95
	3	1.04	1.79	0.25	0.75
	6	3.43	4.98	0.76	0.24
Bigdena Variety	0	0.16	0.37	0.02	0.98
	1	0.62	0.62	0.05	0.95
	3	1.04	1.90	0.26	0.74
	6	3.33	5.16	0.75	0.25
Rebelski Variety	0	0.16	0.37	0.01	0.99
	1	0.62	0.62	0.05	0.95
	3	1.09	2.15	0.28	0.71
	6	3.76	6.06	0.78	0.22

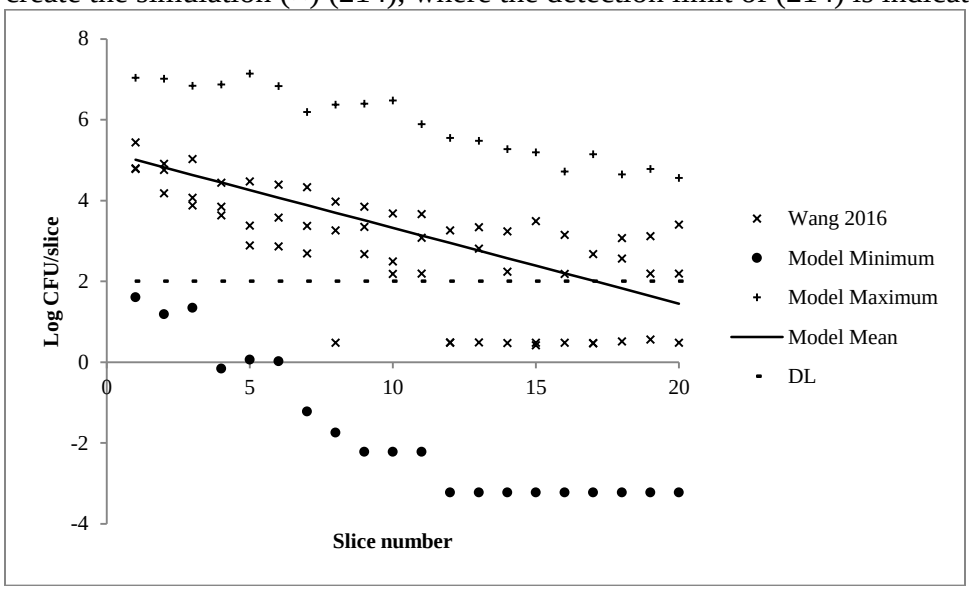
Figures

Figure 1. Illustration of the slicing model



t= % transfer from blade to tomato
1-*t* =% transfer from tomato to blade

Figure 2: Simulated transfer of *S. typhimurium* to tomatoes during slicing from highly contaminated tomato to 20 non-contaminated tomatoes (after 30 min hold-time post-inoculation) showing minimum (•), maximum (---) and mean transfers predicted for each slice number (——) from 10,000 iterations of the simulation compared to the data used to create the simulation (×) (214), where the detection limit of (214) is indicated by ---.



Chapter VI: Conclusions

This dissertation investigated the microbial safety of fresh-cut produce during processing in order to estimate risk. *E. aerogenes* and avirulent *E. coli* O157:H7 were suitable surrogates for pathogenic *E. coli* O157:H7 during washing of fresh-cut lettuce. These organisms were not suitable surrogates for pathogenic *E. coli* O157:H7 when comparing transfer from inoculated lettuce to non-inoculated lettuce during water washing. While curli production by pathogenic *E. coli* did reduce the ability of water washing to remove cells from inoculated lettuce, it did not have a significant effect on cross-contaminate during water washing. These conclusions point to the need to find suitable surrogates for *E. coli* O157:H7 cross-contamination during washing, and for further investigation into factors influence cross-contamination by pathogens during washing of fresh produce.

Published microbial growth models were combined with times series models of real dynamic transit temperatures to predict growth of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* during leafy greens transportation. The results show that most models agreed relatively well, but some models developed using modified atmosphere conditions or from limited published literature data shows lower growth rates. This work demonstrates the value of using time series analysis for simulation of pathogen growth and also shows that model-to-model differences appear to be generally less important that truck to truck differences encountered during transport. The probability and the total number of illness of *Salmonella* on sliced tomatoes were estimated using quantitative microbial risk assessment. The models indicate that using a mechanical slicer and slicing at a temperature of 4°C transferred significantly fewer pathogens compared to other

slicing parameters. The results from this dissertation should provide valuable guidance for future researchers and provide fresh cut food industry risk managers with useful information for mitigating risk in fresh-cut facilities.

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