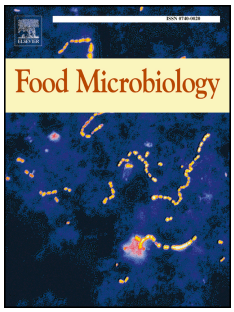

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Assessment of microbial risk during Australian industrial practices for *Escherichia coli* O157:H7 in fresh cut-cos lettuce: A stochastic quantitative approach

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Declaration of Interest

None

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Abstract

Escherichia coli O157:H7 risk associated with the consumption of fresh cut- cos lettuce during Australian industrial practices was assessed. A probabilistic risk assessment model was developed and implemented in the @Risk software by using the Monte Carlo simulation technique with 1,000,000 iterations. Australian preharvest practices yielded predicted annual

mean *E. coli* O157:H7 levels from 0.2 to -3.4 log CFU/g and prevalence values ranged from 2 to 6.4%. While exclusion of solar radiation from the baseline model yielded a significant increase in concentration of *E. coli* O157:H7 (-5.2 -log fold), drip irrigation usage, exclusion of manure amended soil and rainfall reduced *E. coli* O157:H7 levels by 7.4, 6.5, and 4.3-log fold, respectively. The microbial quality of irrigation water and irrigation type both had a significant effect on *E. coli* O157:H7 concentrations at harvest ($p<0.05$). The probability of illness due to consumption of *E. coli* O157:H7 contaminated fresh cut- cos lettuce when water washing interventions were introduced into the processing module, was reduced by 1.4-2.7-log fold ($p<0.05$). This study provides a robust basis for assessment of risk associated with *E. coli* O157:H7 contamination on fresh cut-cos lettuce for industrial practices and will assist the leafy green industry and food safety authorities in Australia to identify potential risk management strategies.

1. Introduction

Changes in consumers' behaviors and attitudes towards healthy diets over the last two decades have resulted in increased consumption of fresh produce (Rekhy and McConchie, 2014). Concomitantly, increased number of foodborne outbreaks linked to consumption of fresh produce have also been reported. Fresh produce is often eaten raw, receiving no or minimal processing, resulting in significant potential for pathogenic contamination across the value chain and thus, illness upon consumption. According to peer-reviewed literature collated from 1980 to 2016, fresh produce was involved in 571 outbreaks, which resulted in 72,855 cases of illness around the world (Machado-Moreira et al., 2019). While most of these outbreaks and cases of illness were recorded in North America and Europe (Machado-Moreira et al., 2019), the proportion of produce-linked outbreaks and cases of illness in Australia were less than 5% and

11%, respectively (Li et al., 2018). The most common type of fresh produce implicated in these outbreaks was leafy greens, which accounted for more than 50% of reported outbreaks (Machado-Moreira et al., 2019). A risk assessment conducted by the Food and Agriculture Organization (FAO) of the United Nations, ranked leafy greens as the highest priority in terms of the safety of fresh fruit and vegetables (WHO, 2008).

The etiological agents identified in outbreaks linked to leafy greens ranged from bacteria (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Bacillus* spp., *Clostridium* spp.) to viruses (hepatitis A virus, human norovirus) and protozoa (*Cyclospora* spp., *Cryptosporidium* spp.) (Herman et al., 2015). *E. coli* O157:H7 was the most commonly implicated contaminant, while cos lettuce was the most commonly implicated vehicle (Turner et al., 2019). Frequent involvement of *E. coli* O157:H7 in outbreaks linked with leafy greens makes this pathogen-commodity combination the highest risk concern for government agencies, industries, and the public in terms of produce safety (Anderson et al., 2011).

Australia has had a moderate rate of reported outbreaks and recalls related to leafy greens compared to the European Union and the United States (Li et al., 2018), and a very low prevalence of pathogenic bacteria in leafy greens (NSW/FA, 2007). However, cognizance of fresh produce-linked outbreaks in other nations, not only highlighted the existing potential for foodborne pathogens to be present on leafy greens in Australian production conditions, but also the urgent need for a rigorous risk assessment (Forum, 2018). To this end, Australian government officials have adopted a pro-active approach, and requested Food Standards Australia New Zealand (FSANZ) to identify appropriate regulatory and non-regulatory measures for Australia to manage food safety risks in leafy greens (Forum, 2018).

To minimize the risk associated with microbial hazards of leafy greens, effective food safety intervention strategies need to be implemented throughout production, processing, distribution and storage (Gil et al., 2015). As with other fresh produce, foodborne pathogen contamination of lettuce can occur anywhere along the supply chain. It is known that warm-blooded animals such as cattle are a natural reservoir for *E. coli* O157:H7 (Berry and Wells, 2010), and the use of cattle manure as a soil amendment could induce migration of *E. coli* O157:H7 not only into fresh produce but also into water supplies through soil (Russell and Jarvis, 2001). Even though Australian cattle manure has a very low prevalence of *E. coli* O157:H7 (1.7%) (Barlow and Mellor, 2010) compare to those of Florida (9%),an intense agricultural production region in the United States (Baker et al., 2019), it poses a significant health risk to consumers when used as a soil amendment in the production of leafy greens. While contaminated irrigation water and manure-amended soil are the main sources of preharvest contamination (Gil et al., 2015), interactions between produce and infected food handlers, contaminated surfaces or water, and imperfect postharvest conditions (i.e., retail and home storage temperature), are critical factors in postharvest contamination (Pang et al., 2017). Therefore, appropriate risk management strategies rely upon the proper understanding of each of these factors.

An enhanced understanding of food safety can be achieved through the usage of a risk assessment and risk management approach that comprises hazard identification and produce consumption scenarios in probabilistic modeling known as quantitative microbial risk assessment (QMRA). To address the complexity of food safety issues in the application of QMRA, the World Health Organization (WHO), and the FAO of the United Nations, both have a crucial role in the deliberations of international standard-setting bodies (Dennis et al., 2002). QMRA has

been widely applied in leafy greens across the value chain for *E. coli* or *E. coli* O157:H7 in many countries such as Japan (Koseki and Isobe, 2005), Netherlands (Franz et al., 2008), Canada (Ottoson et al., 2011), United States (Danyluk and Schaffner, 2011; Pang et al., 2017), and Spain (Allende et al., 2018; Castro-Ibanez et al., 2015; Franz et al., 2010; Rodriguez et al., 2011; Tromp et al., 2010), India (Kundu et al., 2018). To date, there is no published QMRA model of preharvest and postharvest practices in Australia for *E. coli* O157:H7 in cos lettuce. The unique geographical location of Australia offers a wide range of weather conditions and agricultural practices for leafy greens to be grown and harvested all year around. Concomitantly, daily exposure to environmental and climatological factors have been known to induce changes in microbial community structure in the phyllosphere during primary production (Vorholt, 2012). For example, there is greater emission of ultraviolet radiation in the Southern hemisphere than at an equivalent locations in the Northern hemisphere because the former receives 7% more solar radiation intensity and has cleaner air. (Gies, 2003). In a recent meta-analysis, Dao et al. (2020) found that environmental conditions have an important effect on the die-off rate of *E. coli* in manure-amended soils. Considering the higher levels of solar radiation and hours of sunlight in Australia than at an equivalent location in Northern hemisphere where the majority of the risk models have been developed, the generation of risk models from local data is essential so that the effects of agricultural and processing practices on the microbial safety of leafy greens can be assessed appropriately. The outputs of this model for lettuce may assist in the design of preventive measures and intervention strategies to reduce microbial risks for the Australian leafy green industry. Thus, the objective of this research was to develop a quantitative microbial contamination model to evaluate the impact of different preharvest practices and conditions (i.e. seasonality, solar radiation, rainfall, manure usage, irrigation water quality, and irrigation type)

and postharvest practices (washing, retail, postharvest and home storage) on the levels of *E. coli* O157:H7 associated with fresh cut-cos lettuce. The model simulation focused on conditions in Sydney, Australia as a representative example of an intensive agricultural region for leafy greens production in Australia.

2. Material and Methods

2.1. Overview of the model

Exemplar risk models that were developed in the Spain (Allende et al., 2017), and the United States (Pang et al., 2017) were used with modifications in distributions to mirror the Australian industrial leafy green practice to estimate the likelihood of *E. coli* contamination of leafy green. To this end, the QMRA model developed by Allende et al. (2017), was considered as a solid baseline to assess the effects of different agricultural practices and weather conditions on contamination of lettuce by *E. coli* O157:H7 (CFU/g) at preharvest level, whereas the QMRA model generated Pang et al. (2017) was used as a baseline to evaluate postharvest practices for fresh-cut cos lettuce in Australia. The probabilistic model that comprised industrial preharvest and postharvest activities for cos lettuce grown in Australia was built in Excel and executed in @Risk software (V 7.3.1. Palisade Corporation, US).

2.2. Baseline scenario

The baseline model was of cos lettuce planted in an open field in Sydney during summer (December-February) and assumed constant environmental conditions (Table 1). The summer plantation period (number of days between sowing and harvest) was provided by a commercial lettuce producer (Table 1). The average days of rain for each season over the last two decades were obtained from The Australian Bureau of Meteorology database (BOM, 2020). Due to the availability of vast amounts of meteorological data, a PERT distribution (min, most likely, max) was used for rainfall and solar radiation (Table 1). Based on the results from previous studies (Allende et al., 2017), the prevalence of *E. coli* on leafy greens during crop cultivation was assumed to be constant, and contamination through manure-amended soil and irrigation water were assumed to be steady throughout the growing period.

Recorded levels of *E. coli* in irrigation water and manure-amended soil in Australia were included as an input of the model (Table 2). The number of *E. coli* O157:H7 in irrigation water and manure-amended soil was predicted using the estimated ratio of *E. coli* O157:H7 to *E. coli* from Ottoson et al. (2011). Since overhead sprinkler irrigation is the most common irrigation method used by producers in the Australian leafy green industry, it was selected for the baseline scenario of cos lettuce production.

2.3. Risk factors for the preharvest module

In the preharvest module of the QMRA model, the level of *E. coli* O157:H7 was calculated on a daily basis considering the effect of growing time, rainfall, solar radiation, and microbial quality of irrigation water and manure-amended soil during the whole season (Table 1). Daily contamination arising from irrigation or rainwater splashing was calculated from the

occurrence of irrigation or rain. The total load for each consecutive day was calculated by running a loop summing the daily amount of contamination of *E. coli* O157:H7 from manure-amended soil, irrigation water, rain, and irrigation water splashing, and harvest tools, and subtracting the number of bacteria inactivated due to daily solar radiation. The output of this part of the model was the daily concentration of *E. coli* O157:H7 associated with cos lettuce during a growing season up to the final day of harvest as described by Allende et al. (2017).

2.3.1. Manure-amended soil

The distribution of the *E. coli* in the manure-amended soil was assessed from 72 samples collected during cultivation in Australia (Table 1) (data not shown). Aseptically collected samples were transported to National Association of Testing Authorities (NATA) accredited laboratory in Sydney within 24 hours and maintained at <10°C. Analysis of samples for *E. coli* followed ISO 16649-2 pour plate method modified by using ChromID medium (bioMerieux, Australia) incubated at 37°C for enumeration with a detection limit of 100 CFU/g. A RANDBETWEEN function between 0 and 1 (or 0 and 100% of the samples) was described and applied to the manure-amended soil data. The usage of a RANDBETWEEN function allowed a random number to be generated for each simulation within given intervals (from 0 to 1). A certain fraction (30/72) was above the detection limit (2.0 log CFU/g). Based on the value generated being above or below (30/72), an IF function was used to determine the concentration of *E. coli* O157:H7 in manure- amended soil (Table 1). In this model, it was assumed that *E. coli* O157:H7 contamination of cos lettuce from manure-amended soil occurred through splashing from rain and irrigation water and from harvest tools (Table 1). Data related to the quantitative transfer of bacteria from manure-amended soil to plant tissue through splashing was not

available for Australian conditions and was therefore taken from Allende et al. (2017). The probability of rain splashing was assumed to be 100%, indicating that there was always splashing from manure-amended soil during rainfall. Similarly, in the absence of quantitative data describing the transfer rate of *E. coli* O157:H7 from harvest tools to cos lettuce under Australian conditions, data was taken from Yang et al. (2012) (Table 1).

2.3.2. Irrigation water quality and irrigation type

The distribution of the *E. coli* levels in the irrigation water (Table 1) was based on 2062 samples taken in Australia (data not shown). Water samples were tested for indicator bacteria by Australian Standard/New Zealand Standard (AS/NZS) 4276.7 2007 for thermotolerant coliforms and *E. coli* using membrane filtration with a detection limit of 1 CFU/100 mL. A RANDBETWEEN function between 0 and 1 (or 0 and 100% of the samples) was again described and applied to irrigation water data with a detection limit of 1.0 log CFU/ml (Table 1).

In addition, using overhead irrigation as the baseline, the impact of drip and furrow irrigation were also assessed by using data given in Stine et al. (2005) (Table 5).

2.4. Risk factors for postharvest module

2.4.1. Washing

Washing of cos lettuce in Australia typically involves water and a sanitizer such as chlorine, peroxyacetic acid (PAA), or both in combination. The efficacy of water washing, chlorine, and PAA to decontaminate *E. coli* reduction of O157:H7 from cos lettuce leaves was

modeled using uniform, normal, and PERT distributions, respectively, based on the experimental data generated in this study (Table 1).

2.4.2. Cross-contamination during processing

Cross-contamination among individual lettuce heads during processing was modeled by using data from Perez-Rodriguez et al. (2011) to calculate the number of *E. coli* O157:H7 transferred during washing. Calculation details are presented in Table 1.

2.4.3. Conditions during storage and transportation

Nine cases containing nine 300 g of ready to eat fresh-cut lettuce were instrumented with a Hygrochron iButton™ (DS1923; Thermodata® Pty. Ltd.) temperature and RH sensor to measure conditions surrounding the produce; a thermistor probe (Tinytag Plus 2 TGP-4020; Hastings Data Loggers Ltd.) to measure produce temperature; and a GPS tracker (SU-6800 Mini; Simply Unified Pty. Ltd.) to track, locate, and measure distance travelled by the produce in real-time. Each instrumented case were placed in three key pallet levels (top, middle, and bottom) as described previously McKellar (2014b) across three different pallets. Time and conditions (temperature and RH), and GPS readings were recorded at 15-, and 5-min intervals, respectively. Four separate trials were carried out for retailers' supply chain operations for every season over a year, although some data was lost due to tracker loss or malfunction. Consequently, reliable temperature profiles were obtained for 32-casesof lettuce. The collected data were fitted to a PERT distribution to describe retail storage temperature for cos lettuce (Table 1), with an

assumed maximum duration of 10 days, an average of 7 days, and a minimum of half a day. Home storage time was modeled on US data from Pouillot et al. (2010). The temperature and duration of transportation from retail to home was estimated using calculations in a risk assessment study by Pang et al. (2017).

2.5. Microbial kinetics of E. coli O157:H7 on cos lettuce

For the preharvest module, the effect of solar radiation level (W/m^2) on the inactivation of *E. coli* O157:H7 in the field during cultivation in Australia was defined by the following die-off model;

$$log\ N_t/N_0 = -k * t \quad (1)$$

where *t* represents time (days), k is the die-off rate (log CFU/g/day), and where N_0 is the initial number of survivors, and N_t represents the number of survivors at a certain time (*t*) (Ottoson et al., 2011). Inactivation of *E. coli* O157:H7 on cos lettuce was calculated for Australian field conditions based on the relationship between die-off rate and solar radiation levels determined in previous studies (Allende et al., 2017; Ottoson et al., 2011) (Table 1 and 5).

To consider the dynamic nature of environmental conditions on survival of *E. coli* O157:H7 on fresh cut-cos lettuce in the postharvest module, a threshold temperature of 5°C was used as the cut-off between growth and inactivation (McKellar et al., 2014; Pang et al., 2017). While inactivation of *E. coli* O157:H7 was defined with a die-off model at temperatures below 5°C (Eq. 1), a growth model was used to determine the increase in *E. coli* O157:H7 numbers at temperatures above 5°C (Eq. 2) as proposed by Ratkowsky et al. (1982).

$$\mu = b(T - T_{min}) \quad (2)$$

In Eq. 2, μ is the growth rate (log CFU/g/h), b is the temperature coefficient, T and T_{min} are temperature (°C), and theoretical minimum growth temperature, respectively (Table 3).

2.6. Dose-response relationship and risk characterization

The daily dose of *E. coli* O157:H7 ($D, log\ CFU/day$) that the consumer is exposed to was defined as described by Hamilton et al. (2006);

$$D\ (CFU/day) = C_H \times M_{body} \times M_i \quad (3)$$

where C_H is the *E. coli* O157:H7 concentration in cos lettuce at home (log CFU/g), M_i is the daily consumption amount for the fresh cut-cos lettuce per person (g.person^{-1}.day^{-1}), and M_{body} is the human body mass (kg).

The Beta-Poisson dose-response model used in this study was based on animal studies, and first reported by Haas (1983). The risk predicted by the Beta-Poisson dose-response model was concordant with illness rates evidenced in foodborne outbreaks (Haas et. al 2000), thus it was found adequate to describe the human morbidity risk of a highly virulent pathogen like *E. coli* O157:H7 (Teunis et al. 2008). Concomitantly, the selected dose-response model was used in many risk assessments, including a QMRA for *E. coli* O157: H7 in leafy greens by Danyluk and Schaffner (2011) and Pang et al. (2017).

$$P_I(\lambda) = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \times \mathrm{Prev}_{AP} \quad (4)$$

In Eq. 4, $P_I(\lambda)$ is the probability of illness per day, D is the number of organisms ingested per day (i.e,. a dose), and α (0.267) and β (229.2928) are model parameters reported by Cassin et al. (Cassin et al., 1998) (Table 4).

An estimate of the annual risk of infection in Australia was calculated based on the assumption that irrigation of fresh produce and consumption of those fresh products is a daily event which occurs year-around (n=365) (Haas, 1983) (Table 4);

$$P_I(A)(\lambda) = 1 - [1 - P_I(\lambda)]^n \quad (5)$$

An estimate of the annual number of cases of illness in Australia was calculated as the product of the annual risk of infection and the population of Australia.

2.7. Scenario analysis

To assess the effect of different preharvest and postharvest practices on levels of *E. coli* O157:H7 on cos lettuce in Australia, 17 scenarios were evaluated (Table 5). Summer harvest was considered as the baseline scenario (Table 1) and modification in distributions were used to compare the outcome of each scenario (Table 5). The effects of different growing times, harvest seasons, rain events, and solar radiation were evaluated using six scenarios (Scenario 1 to 6). Another set of scenarios was used to evaluate the impact of agricultural practices with variable irrigation water quality and type (Scenario 7 to 12) and manure quality (Scenario 13). A final set of scenarios (Scenario 14 to 17) were used to evaluate the efficacy of water washing and the use of chlorine and PAA.

Preliminary analysis was used to determine the number of iterations required to provide an acceptable degree of stability in percentile risk estimates where repeated simulation runs with different numbers of iterations were performed and the output reproducibility as considered for the mean, median, and high-end (90th, 95th and 99th percentiles) forecasts. Each simulation was repeated five times to examine the output stability. While the minimum number of iterations

required to constantly stabilize high-end forecasts at < 0.5% variance was ~100,000 for preharvest module, it was ~200,000 for postharvest module. For each preharvest and postharvest scenario, to assure precision in high-end forecasts, the developed model was simulated five times by using @Risk, Version 7.3 with the Monte Carlo simulation technique using 1,000,000 iterations for each run by Latin Hypercube sampling.

3. Results and Discussion

Under Australian industrial preharvest practices, the developed probabilistic QMRA model predicted annual mean *E. coli* O157:H7 levels from 0.3 to -3.4 log CFU/g, and prevalence values ranged from 2 to 6.3 %. Based on the meta-analysis performed by Elias et al. (2019) on *E. coli* O157:H7 prevalence and concentration in lettuce, using available worldwide data, average preharvest practices yielded a higher prevalence (5-7.8%) and concentration values (from 0.48 to 3.04 log MPN/g) than Australian industrial preharvest practices. The results of this study highlights that a large variation of the prevalence and concentration of *E. coli* including O157:H7, VTEC, STEC in lettuce between developed countries such as Sweden (Soderstrom et al., 2005), Spain (Castro-Ibanez et al., 2015), and Germany (Fiedler et al., 2017), and developing countries including Iran (Khandaghi et al., 2010), Malaysia (Kuan et al., 2017), and Pakistan (Shah et al., 2015) and Australia might be attributed to different agricultural production environments, in particular meteorological differences. Even though it's been known that meteorological and environmental conditions have an important impact on the amount of *E. coli* O157:H7 on leafy greens at harvest, (Allende et al., 2017; Castro-Ibanez et al., 2015; Oliveira et al., 2012; Park et al., 2015), employment of a universal risk management strategy for in-field food safety management systems, may not be ideal under certain environmental conditions, and may either underestimate or overestimate microbial risk. This indicates the importance of

conducting further studies focusing on specific agricultural production environments over different seasons for reliable risk model development.

For Australia, the developed model predicted that the season in which cos lettuce was harvested had a significant influence on risk of *E. coli* O157:H7 contamination in the field (Figure 1a). For example summer harvest of cos lettuce occurs under a relatively high mean ambient temperature (23±0.9°C), a mid number of rainy days (6±2 days), and after the shortest growing cycle (35-45 days). These harvesting conditions yielded a mean predicted *E. coli* O157:H7 level of -1.1 log CFU/g with a prevalence value of 6.3% (Table 6). Even though summer harvest of cos lettuce had the highest solar radiation intensity ((370±13 W/m^2) and longer sun hours (10.4±1.7 h), spring harvest of cos lettuce yielded the lowest mean predicted level of *E. coli* O157:H7 with a value of -3.4 log CFU/g at harvest (Table 6). The lowest predicted levels of *E. coli* O157:H7 in spring harvest of cos lettuce might be attributed to individual or a combination of meteorological and environmental factors including the second highest solar radiation intensity (312.5±20.4 W/m^2) and sun hours (9.3±1.1 h) and most importantly, longer growing cycles (55 to 65 days) than the summer season. On the other hand, a winter harvest scenario with longest growth cycles (70-80 days) only reduced the mean number of *E. coli* O157:H7 by 0.6-log fold compared to summer season ($p < 0.05$). Even though in winter, increased bacterial decay would have been expected due to longer growing cycles season, the low temperature (13.7 ± 0.7°C) and lowest solar radiation intensity (69.4±15.7 W/m^2) might be the factors that have been associated with enhanced microbial survival (Oliveira et al., 2012). Interestingly, an autumn scenario yielded the highest mean levels of *E. coli* O157:H7 compared to other seasons ($p < 0.05$) and showed an increase of -0.2-log fold relative to summer

harvest ($p>0.05$) (Figure 1a). The combination of highest number of rainy days (10.3±2.3 days) and second lowest solar radiation (104.2±23.2 W/m^2) might be factors associated with the highest mean levels of *E. coli* O157:H7 at autumn harvest. This finding is in agreement with others (Allende et al., 2017; Castro-Ibanez et al., 2015; Machado-Moreira et al., 2019) in which rain events and solar radiation intensity were identified as the most significant weather related factors that lead to an increase in level of pathogens in fresh produce at autumn harvest. Overall, the large variation in the mean predicted *E. coli* O157:H7 levels on cos lettuce over different seasons might be influenced by a range of individual and interacting meteorological and environmental effects, such as solar radiation intensity and rainfall, and highlights the importance of further studies that explore the impact of each individual factors.

Further investigations of the effect of key weather-related factors in Australia on the survival of *E. coli* O157:H7 at preharvest level were performed by excluding solar radiation and rainfall from summer harvest as the baseline model (Figure 1a). The results revealed that both rainfall and solar radiation intensity have been identified as critical factors that affected the mean predicted *E. coli* O157:H7 levels on cos lettuce ($p<0.05$) in comparison to baseline model. While summer harvest with no solar radiation yielded a significant increase in mean predicted numbers of *E. coli* O157:H7 (5.8 log CFU/g with a -5.3 -log fold change), exclusion of rainfall reduced the mean number of *E. coli* O157:H7 on cos lettuce by 6.5-log fold relative to summer harvest (baseline model) (Table 6). High solar radiation intensity is linked with greater rates of die-off of enteric bacteria under both shady and sunny conditions (Sidhu et al., 2008). However, the limited data on the contribution of a rain event on the frequency and magnitude of pathogen transfer rate in field, highlights the importance of future studies focusing on these themes for precise characterization and management of microbial risk. To this end, the majority of fresh

produce safety management guidelines rely upon the microbial die-off rate between the last water application and harvest at a rate of 0.5 log CFU/day, for up to four days (FDA, 2015) with no consideration for any location based weather-related factors such as solar radiation intensity, number of rain events, etc. Moreover, the scarcity of the data on the quantification of microbial behavior in the field at specific growing locations other than the United States (where the die-off rate of 0.5 log CFU per day is based on), highlights the importance of risk model development based on local data and reflects the diversity of prevailing meteorological and environmental conditions.

In comparison with other vegetable production regions in Australia, Tasmania, an important producer of fresh produce in the country**,** is characterized with cool temperate climate with four distinct seasons, along with highly differentiated rainfall and available sun hours (Holtz et al. 2010). Thus, it is of great interest to assess the *E. coli* O157:H7 risk associated with the cultivation of cos lettuce in Tasmania. To this end, the impact of rainfall, solar radiation, and available sun hours on the *E. coli* O157:H7 levels at harvest in Launceston, Tasmania over summer season was evaluated using cos lettuce cultivated in Sydney (summer) as a baseline. In general, the predicted mean *E. coli* O157:H7 levels in cos lettuce that was cultivated in Tasmania were higher (95%CI=-1.91,6.34 Log CFU/g) with a -2.2%- log fold change than those of Sydney as baseline (Supplementary Figure 3).

The variation of the concentration of *E. coli* O157:H7 in Tasmania might not only be attributed to meteorological differences between sites but also spatiotemporal heterogeneity of farm and freshwater environments in the region. It further underscores the importance of risk

management strategies considering meteorological factors and spatiotemporal heterogeneity of the region for a reliable risk assessment.

Among agricultural practices, manure-amended soil and irrigation water are both major reservoirs for foodborne pathogen transfer to fresh produce and thereby, have an important effect on the microbial load on fresh produce at harvest (Cevallos-Cevallos et al., 2012; Girardin et al., 2005; Keraita et al., 2007; Monaghan and Hutchison, 2012). To this end, the removal of manure amendment from the QMRA model resulted in the lowest mean predicted level of *E. coli* O157:H7 with a value of -7.1 log CFU/g (95^{th}, 99^{th} and 99.9^{th} percentiles; -3.5,-2.4, and -1.4 log CFU/g, respectively), and reduced the level of *E. coli* O157:H7 on cos lettuce 6.5-log fold relative to that of summer cultivation levels (baseline model) (Table 6 and Figure 1b). The significant reduction in the mean level of *E. coli* O157:H7 levels at harvest when manure amended soil was excluded from baseline model (6.5-log fold) highlighted the fact that manure amended soil has an overarching effect on risk of *E. coli* O157:H7 in lettuce at harvest. This indicates that organic amendment quality should be monitored to eliminate future foodborne incidences, recalls, or outbreaks linked to leafy greens.

The impact on the irrigation water quality on the level of *E. coli* O157:H7 in cos lettuce was also assessed by using different sources of water in the QMRA model. Irrigation using tap water, surface water, or reclaimed water reduced the mean number of *E. coli* O157:H7 on cos lettuce by 3.5, -0.7, and -0.4-log fold relative to summer cultivation (baseline model), respectively (Table 6). As expected, there was significant difference among the irrigation water types used in this QMRA at selected conditions ($p<0.05$) (Figure 1b). The usage of tap water resulted in the lowest mean predicted *E. coli* O157:H7 levels on cos lettuce at summer harvest (-3.9 log CFU/g), followed by surface (0.8 log CFU/g), and reclaimed water (0.4 log CFU/g).

Considering that presence and persistence of foodborne pathogens in irrigation water sources are still major issues for public health (Liu et al., 2018), compliance with local guidelines is an important risk management practice. Australian Freshcare guidelines require irrigation water used in high risk produce (i.e., in which the edible parts are grown in direct contact with the soil or are consumed uncooked), including leafy greens, to have no more than 100 CFU generic *E. coli* per 100 ml of water (FPSC, 2019). Given the fact that the *E. coli* levels recommended by Freshcare standards (<100 CFU/100 ml) are lower than those of California Leafy Green Marketing Association in the United States (235 CFU/100 ml) (LGMA, 2013), compliance with Freshcare guidelines is good practice for the Australian leafy green industry in order to avoid potential contamination of fresh produce via irrigation water, and highlights the importance of risk characterization using locally acquired data.

In addition to the risks posed by manure-amended soil and irrigation water, the type of irrigation system also has an impact on the mean level of *E. coli* O157:H7 in cos lettuce at harvest. While summer harvest with overhead irrigation was used as a baseline, the impact of furrow and drip irrigation on mean level of *E. coli* O157:H7 in cos lettuce at harvest was also evaluated (Figure 1c). Drip and furrow irrigation resulted -8.2, and -6.1 log CFU/g of *E. coli* O157:H7 in cos lettuce at harvest (Table 6), and compared to overhead irrigation they both reduced the mean level of *E. coli* O157:H7 in cos lettuce at harvest by 7.5, and 5.6-log fold, respectively (Figure 1c). In addition to being a reservoir for foodborne pathogens, irrigation water is also considered to be potential vehicles for pathogen transfer from manure-amended soil to fresh produce (Allende et al. 2017). The usage of drip irrigation reduces the potential for contamination from manure amended soil via irrigation splashing (Allende et al., 2017; Stine et al., 2005).While overhead irrigation is more likely to introduce pathogens than furrow or drip

irrigation, it has been used not only provide water for lettuce leaves (Moyne et al. 2009) but also to cool down them (Smith et al. 2011). The latter became particularly important for lettuce as a cool season crop, with an optimal growing temperature of 23.8°C during the day and 7°C during the night (Coloumbe et al. 2020), especially in exceptionally productive agriculture regions such as California, Arizona in the US, and Victoria, Queensland in Australia. Being a cool-season crop, lettuce is particularly vulnerable to heat stress, therefore at the high end of temperature range, lettuce may bolt, causing bitterness and loose, fluffy heads. For regions where, daytime temperature is above the optimal growing temperature, it's recommended to use overhead irrigation only if the water quality is well controlled and potable (Uyttendaele et al. 2015). In response to recent *E. coli* O157:H7 outbreak linked to romaine lettuce from the Yuma area, new water safety requirements under the LGMA were put in place, in which the use of untreated surface water for overhead irrigation during the 21 days prior to harvest has been banned (LGMA, 2019). Even though the type of irrigation method chosen by a grower depends on several issues, codes of practices are required to stress the importance of the quality of the irrigation water source for ensuring safety of fresh produce (Uyttendaele et al. 2015).

The most important parameters and variables affecting *E. coli* O157:H7 levels on lettuce during preharvest practices using summer harvest as a baseline model were determined by Spearman's rank order correlation (Figure 2). Based on the results obtained from sensitivity analysis, sun hours (-0.44) and growing time (-0.21) were the most important input factors that were negatively correlated with contamination (Figure 2) In contrast, the amount soil transferred to produce by irrigation (0.53 and numbers of rainy days (0.32) were identified as the most important input factors that were positively correlated with predicted mean levels of *E. coli* O157:H7 on cos lettuce at harvest (Figure 2). Results revealed that among weather related

factors, sun hours, thereby, solar radiation was identified the most significant factor affecting the elimination of microbial risk, whereas rain event had the highest contribution to the increase of overall risk at preharvest level, and underlying the importance of locally acquired data usage. Since the amount soil transferred to produce by irrigation is dependent on the type of irrigation, selection of drip or furrow irrigation rather than overhead irrigation avoids the contamination from manure amended soil via irrigation water, and identified as an important risk management practices for growers to apply at preharvest level. Other influential factors that were positively correlated with predicted mean numbers of *E. coli* O157:H7 at harvest were *E. coli* concentration in manure-amended soil (0.11), amount of water transferred (0.11), bacterial transfer from soil to produce (0.09), and *E. coli* O157:H7 concentration after irrigation (0.06)(Figure 2). The corollary of these correlations is for implementation of good agricultural practices (GAP) to avoid contamination via manure-amended soil and is one of few factors within grower's control (Islam et al., 2004; Natvig et al., 2002). To this end, the Fresh Salad Producers Group - PMA Australia-New Zealand (Group, 2019) prohibits the use of untreated manure. and if untreated manure is applied, requires a 365 days exclusion period prior to production of leafy greens. For weather-related factors such as sunshine hours, growing time, and number of rainy days that growers have no control over and GAP cannot be applied to, growers are encouraged to assess their own risk, and take necessary actions to manage, eliminate, or reduce risk to an acceptable level.

The baseline model of this QMRA predicted that the mean number of cases of illness per year in Australia due to consumption of fresh cut-cos lettuce contaminated with *E. coli* O157:H7 was 43. Due to the lack of epidemiological data for leafy greens linked *E. coli* O157:H7 illness in Australia, no formal model validation was performed. Thus, the total number of *E. coli*

O157:H7 illness associated with the consumption of other food commodities were used to make a comparison. The incidence of *E. coli* O157:H7 infections reported in Australia was 17 cases per year, which includes only foodborne cases (OzNet, 2020). Taking into account an underreporting factor of 26.1% used by Danyluk and Schaffner (2011), the predicted number of cases of illness per year in Australia came to 65, clearly surpassing the reported number of cases of illness per year in Australia. The mean probability of illness per year for the baseline study was 4.9×10^{-7} (90^{th}, 95^{th}, and 99^{th} percentiles; 1.7×10^{-6}, 3.9×10^{-6}, and 7.0×10^{-6}, respectively). The mean probability of illnesses associated with the consumption of the *E. coli* O157:H7 contaminated lettuce reported by this study were similar with those of other developed countries including Canada (water rinsed lettuce; 1.0×10^{-7} per year), (Ottoson et al., 2011) and the United States (chlorine washed fresh-cut lettuce; 6.6×10^{-7} per year) (Pang et al., 2017). Even though a different dose-response relationship was used by Ottoson et al. (2011) and some important preharvest risk factors such as rainfall, solar radiation intensity along with contamination from manure amended soil through rain and irrigation water were excluded by Pang et al. (2017), the similarities between these findings could be linked to effective usage of food safety standards among these developed countries. Intervention strategies such as washing, differed significantly ($p < 0.05$) in their predicted capacity to reduce the number of cases of illness per year and probability of illness per serving due to consumption of *E. coli* O157:H7. According to the baseline model and relative to washing in water only, reduction in mean number of *E.coli* O157:H7 was greatest for the sequence of washing in water followed by washing in PAA (2.7-log fold reduction) or washing in water followed by washing in chlorine (2.2-log fold reduction). The reduction was lowest for washing in chlorine only (1.4-log fold reduction) or washing in PAA only (1.6-log fold reduction) (Table 7). The use of chlorine and PAA as sanitizers in wash

water have been studied extensively, and similar findings regarding the effectiveness of these sanitizers against *E. coli* O157:H7 on leafy greens have been reported by others (Lopez-Galvez et al., 2009; Petri et al., 2015; Veschetti et al., 2003). The enhanced efficacy of water washing, when followed by washing in PAA or chlorine, suggests that the use of sequential treatment would be a worthwhile focus of further research to optimize industrial cos lettuce washing practices.

A sensitivity analysis showed that the predicted number of cases of illness per year was most sensitive to retail storage temperature (0.14), home storage temperature (0.11), and washing (-0.10) among postharvest practices. Once a product is contaminated, exposure to abusive storage conditions during the postharvest stage may lead to microbial growth and a higher number of pathogens at consumption. Thus, postharvest temperature control was considered as one of the most important control measures affecting the predicted number of cases of illness cases per year and the probability of illness per serving due to consumption of *E. coli* O157:H7. Similar to others (Danyluk and Schaffner, 2011; Ottoson et al., 2011; Pang et al., 2017), this study highlighted the importance of environmental monitoring and maintenance of adequate postprocessing conditions to eliminate risk associated with presence of *E. coli* O157:H7.

The development of a QMRA model using local data that reflected Australian preharvest and postharvest practices for *E. coli* O157:H7 in cos (romaine) lettuce was a resource-intensive but invaluable activity as it enabled the generation of realistic estimates of risk. However, it was necessary to use several credible assumptions in the model due to a lack of specific data or knowledge. These assumptions included: steady contamination through manure-amended soil and irrigation water throughout the growing period (Allende et al., 2017); constant ratio between generic and pathogenic *E. coli* in irrigation water and manure-amended soil (Pang et al., 2017);

and constant environmental conditions during each season (Allende et al., 2017; Allende et al., 2018; Castro-Ibanez et al., 2015). Despite these limitations, the developed QMRA model remains useful because it provides, not only estimates of the risk, but also a baseline to compare different scenarios. This could aid Australian risk managers to mitigate and/or control risk involving *E. coli* O157:H7 in fresh cut- cos lettuce production.

Conclusion

Generation of QMRA models to assess Australian preharvest factors including: seasonality (growing time, temperature), weather (rainfall, solar radiation), agricultural practices (manure amended soil and irrigation water quality), and irrigation types (overhead, drip, furrow) and postharvest practices (washing, distribution, and storage) for *E. coli* O157:H7 in fresh cut-cos lettuce, will assist the leafy green industry and food safety authorities in Australia to identify potential risk reduction strategies. It was found that the level of *E. coli* O157:H7 in cos lettuce at harvest was significantly affected by a range of individual and interacting environmental effects, including solar radiation intensity, rainfall, growing time, manure usage, irrigation water quality, and irrigation types.While exclusion of manure and usage of drip irrigation reduced the level of *E. coli* O157:H7 in cos lettuce at harvest in comparison to the baseline model, the exclusion of solar radiation yielded the highest *E. coli* O157:H7 concentrations in cos lettuce at harvest.
In order to develop reliable microbial risk assesment models, these findings also emphasized the usage of local data that take into consideration the unique environmental conditions, and agricultural practices. Our data suggests that the use of a sequential washing treatment, as part of Australian industrial practices, could reduce the public health risk associated with *E. coli*

O157:H7 compared to water washing only. Meanwhile, temperature control was the most important postharvest control to reduce the predicted number of illness cases per year and probability of illness per serving due to consumption of *E. coli* O157:H7. Even though the QMRA presented here has a number of assumptions and limitations, it provides a valuable insight into the risk associated with leafy greens and provides a means for prioritizing risk management actions.

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4. Declaration of Interest

None

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Figure captions

Figure 1. Cumulative density functions for *E. coli* O157:H7 levels at harvest for a)seasonality (baseline(summer): (95%CI=-5.97,3.19); autumn:(95%CI=-4.46,4.44); winter:(95%CI=-5.28,3.50); spring:(95%CI=-8.42,1.13), and meterological factors (no solar (95%CI=1.74, 9.19); no rain:(95%CI=-8.64,-1.14) and b) agricultural practices (baseline(summer): (95%CI=-5.97,3.19); no manure (95%CI=-11.90, -2.96); tap water:(95%CI=-8.21,-0.27) surface water:(95%CI=-5.19,2.81); reclaimed water:(95%CI=-3.98,4.05), c)irrigation types (baseline(summer-overhead irrigation (95%CI=-5.97,3.19); drip irrigation:(95%CI=-13.06,-3.98) furrow irrigation:(95%CI=-10.98,-1.89).

Figure 2. Tornado graph indicating the most important parameters and variables affecting *E. coli* O157:H7 levels on lettuce during preharvest practices. Spearman's correlation coefficients obtained from @Risk sensitivity analyses are shown next to each bar.

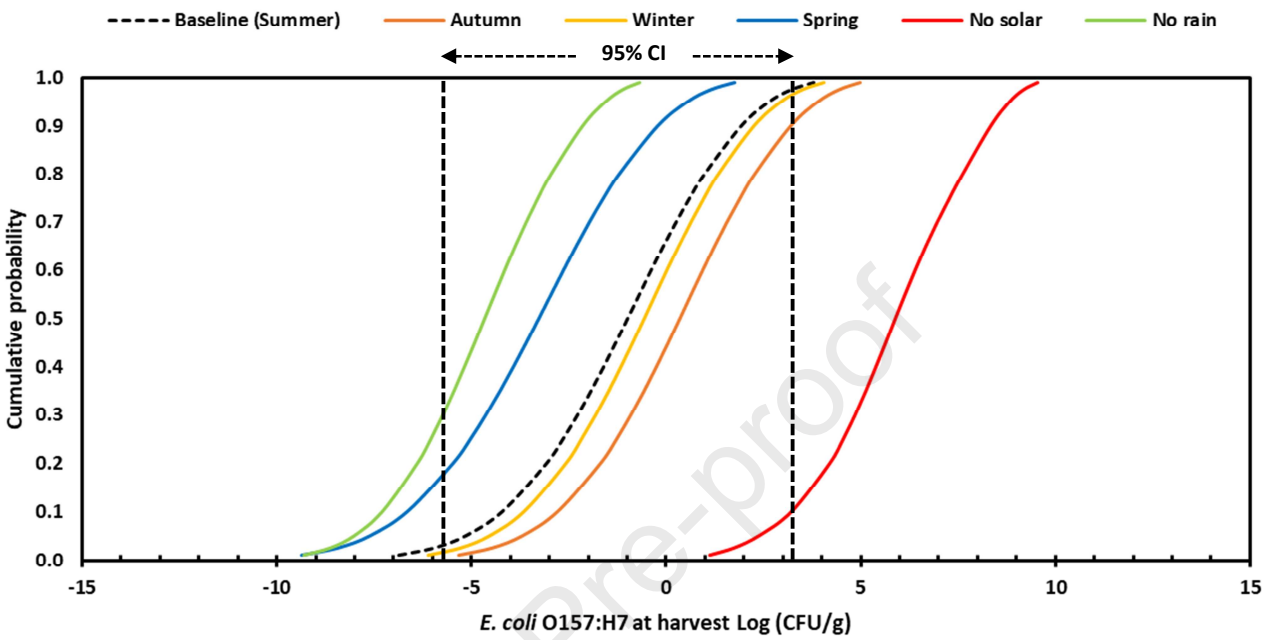

Figure 1a.

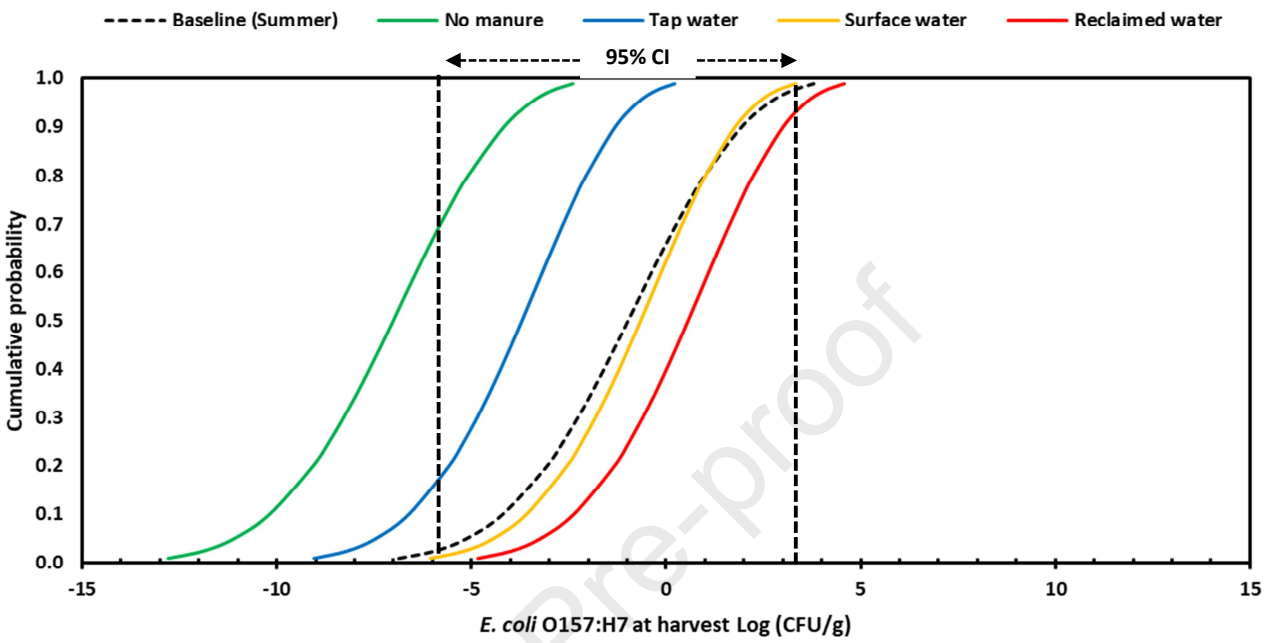

Figure 1b.

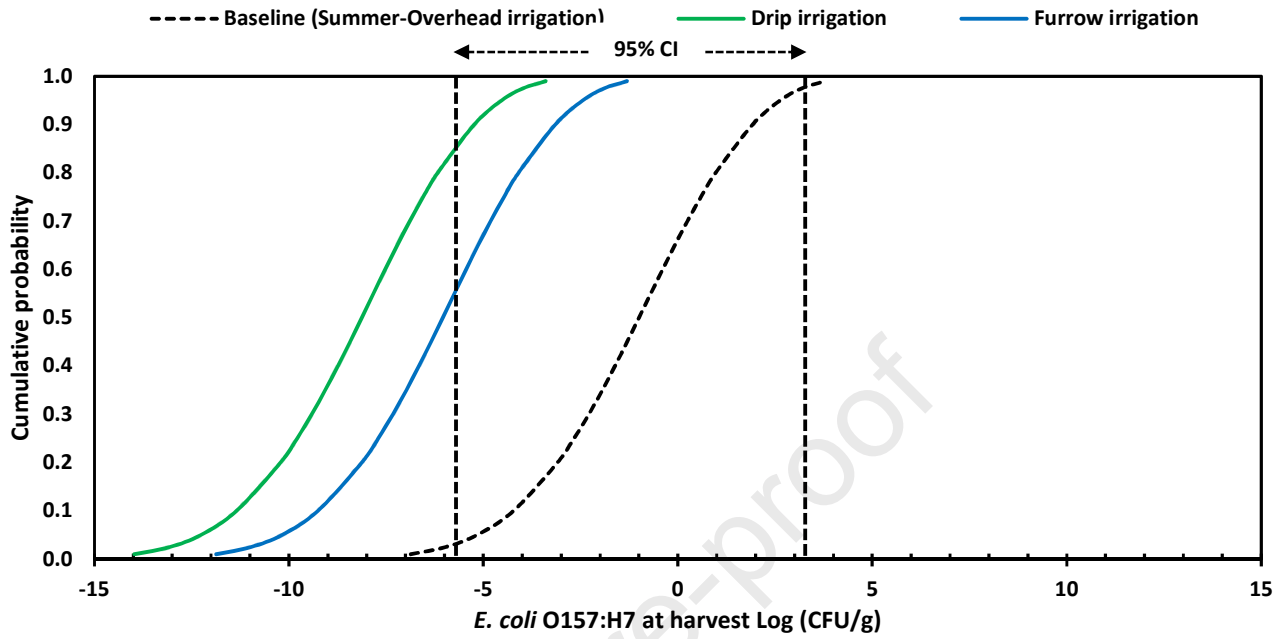

Figure 1c.

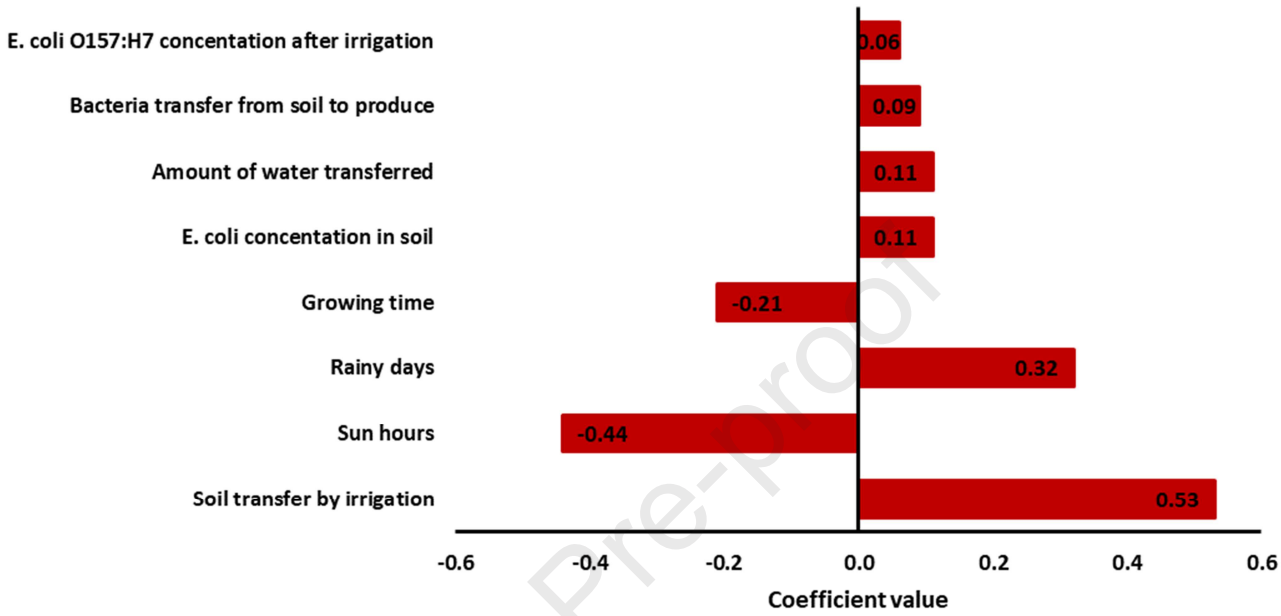

Figure 2.

Table 1. Overview of inputs and distributions for baseline model for levels of *Escherichia coli* on cos lettuce during preharvest conditions and practices in Australia. CFU – colony forming unit.

Symbol	Description	Distribution or value	Unit	Source
		Field conditions		
$D_{Growing}$	Growing time in summer	=Round(RiskPert(35,40,45),0)	Days	Local data
D_{Rain}	Rainy days in summer	=Round(RiskPert(1,6,11),0)	Days	Local data
		Contamination from manure-amended soil (MAS)		
$RANDBETWEEN_{MAS}$	Distribution randbetween (0,1)	=Randbetween(0,1)	-	Calculated
C_{MAS+}	*E. coli* concentration in positive MAS	=RiskCumul(0,229,{0,5.29,44.61,229},{0.11,0.22,0.58,1})	CFU/g manure	Local data
C_{MAS-}	*E. coli* concentration in negative MAS	=RiskUniform(0,100)	CFU/g manure	Local data
$Prev_{MAS}$	Prevalence in MAS	=30/72	-	Local data
R_{MAS}	*E. coli* O157:H7 ratio	=10^RiskNormal(1.9,0.6,RiskTruncate(,0))		Ottoson et al. (2011)
C_{MAS}	*E. coli* O157:H7 concentration in MAS	=If($Randbetween_{MAS}$ >$Prev_{MAS}$, $Log_{10}C_{MAS,}$ 0,C_{MAS+})*R	Log CFU/g manure	Local data
		Contamination from irrigation water (IW)		
$D_{Irrigation}$	Irrigation days in summer	= $D_{Growing}$ -D_{Rain}	Days	Calculated
C_{IW+}	IW quality	=RiskCumul(0,829.19,{0,4,31.6,236.38,829.19},{0.8,0.87,0.95,0.99,1})	CFU/100 ml water	Local data
$RANDBETWEEN_{IW}$	Distribution randbetween (0,1)	=Randbetween(0,1)	-	Calculated
$Prev_{IW}$	Prevalence of *E. coli* O157:H7 in IW	=101/2062	-	Local data
R_{IW}	*E. coli* O157:H7 ratio	=10^RiskNormal(1.9,0.6,RiskTruncate(,0))		Ottoson et al. (2011)
Tr_{OI}	Amount of IW transferred to produce during irrigation via overhead irrigation	=Riskuniform(1.8,21.6)	ml/g produce	Allende et al. (2017)
C_{IW}	Concentration of *E. coli* O157:H7 in IW	=If($Randbetween_{IW}$ $Prev_{IW}$,0, C_{IW+})*R	CFU/100 ml water	Calculated
ΔC_{IW}	Increase in *E. coli* O157:H7 concentration in IW	= $log_{10}(C_{IW})$* Tr_{OI} * $D_{Irrigation}$/100	Log CFU/g produce	Calculated
		Contamination from MAS via irrigation water splashing (IWS)		
P_{IWS}	Probability of IWS	=RiskPert(0.02,0.04,0.06)	-	Franz et al. (2008)
m_{S-L}	Amount of soil transferred to produce by IWS	=RiskBetaGeneral(0.4,0.8,0.05,16.4)	g soil/g produce	Allende et al. (2017)

t_{S-L}	Bacteria transfer from soil to produce	=RiskUniform(0.35,0.9)	%	Girardin et al. (2005)
ΔC_{IWS}	Increase in *E. coli* O157:H7 concentration in produce after IWS	$=C_{MAS} * D_{Irrigation} * m_{S-L} * t_{S-L} * P_{IWS}$	Log CFU/g produce	Calculated
	Contamination from MAS via rain splashing (RS)			
P_{RS}	Probability of RS	1		Allende et al. (2017)
ΔC_{RS}	Increase in *E. coli* O157:H7 concentration in produce after RS	$=C_{MAS} * D_{Rain} * m_{S-L} * t_{S-L} * P_{RS}$	Log CFU/g produce	Calculated
	Contamination from MAS via harvesting tools (HT)			
m_{soil_HT}	Attached soil on HT	10.22	g/blade	Yang et al. (2012)
n_{HT-L}	Number of *E. coli* O157:H7 cells per blade	$= C_{MAS} * m_{soil\_HT}$	Log CFU/blade	Calculated
t_{HT-L}	Transfer rate from HT to produce	0.0013	-	Yang et al. (2012)
ΔC_{HT}	Increase in *E. coli* O157:H7 concentration in produce after contact with HT	$= n_{HT-L} * t_{HT-L} /1500$	Log CFU/g produce	Calculated
	Reduction through solar radiation			
h_{Sun}	Sun hours per day	=RiskPert(8,10,14.4)	h	Calculated
P_{Sun}	Probability of sun	$=1 -(D_{Rain}/ D_{Growing})$	%	Calculated
k_{Summer}	Solar decay rate	0.65	Log CFU/g/day	Calculated, Ottoson et al. (2011)
ΔC_{SR}	Inactivation of *E. coli* O157:H7 by solar radiation	$= P_{Sun} * D_{Growing} *(k_{Summer} *(h_{Sun} /24)$	Log CFU/g produce	Calculated
	E. coli at harvest			
$C_{Harvest}$	Concentration of *E. coli* O157:H7 in produce at harvest	$= \Delta C_{IW} + \Delta C_{RS} + \Delta C_{IWS} + \Delta C_{HT} - \Delta CSR$	Log CFU/g produce	Calculated

Table 2. Concentration of *Escherichia coli* in irrigation water (IW) and manure-amended soil (MAS) used for the baseline model.

Concentration (CFU/ml IW)	**Number of positive samples**	**Cumulative probability F(x)**	**Concentration (CFU/g MAS)**	**Number of positive samples**	**Cumulative probability F(x)***
<1	1647	0.80	<1	8	0.11
1 to 10	142	0.87	1 to 10	8	0.22
10 to 100	172	0.95	10 to 100	26	0.58
100 to 500	80	0.99	100 to 500	-	0.58
>500	21	1.00	>500	30	1.00

* F(x) refers to the cumulative probability F(x) =i/(n+1), here i is the rank of the observed data point and n is the number of data used.

Table 3. Overview of inputs and distributions for baseline model for *E. coli* O157:H7 levels on lettuce during postharvest practices in Australia. CFU – colony forming unit.

Symbol	Description	Distribution or value	Unit	Reference
$Prev_{PP}$	Prevalence when entering processing plant	0.27	%	Local data
	Processing – washing and cross contamination			
ΔC_{WW}	Log reduction when washing with water	=RiskUniform(0.29,0.67)	Log CFU/g	This study
C_{WW}	*E. coli* O157:H7 level after washing	$=C_{Harvest}- \Delta C_{WW}$	Log CFU/g	Calculated
N_{AW}	CFU in a unit batch after washing	$= C_{WW}* Prev_{PP}$	Log CFU/g	Calculated
T_1	Transfer (%) from contaminated lettuce to flume	=RiskTriang(0,0.01,0.02)	%	Franz et al. (2011)
T_2	Transfer (%) from contaminated lettuce to shredder	=RiskTriang(0,0.02,0.02)	%	Franz et al. (2011)
T_3	Transfer (%) from contaminated lettuce to shaker	=RiskTriang(0,0.01,0.02)	%	Franz et al. (2011)
T_4	Transfer (%) from contaminated lettuce to centrifuge	=RiskTriang(0.01,0.04,0.08)	%	Franz et al. (2011)
T_5	Transfer (%) from contaminated lettuce to conveyor	RiskTriang(0,0.1,0.24)	%	Franz et al. (2011)
T_O	Overall transfer coefficient (%) from facilities to uncontaminated lettuce	= RiskTriang(9.9,15.33,18.83)	%	Franz et al. (2011)
N_{FS-L}	Number of cells transferred from lettuce to facility surfaces in a unit batch	$=N_{AW}*(T_1+T_2+T_3+T_4+T_5)$	Log CFU/g	Calculated
N_{L-FS}	Number of cells transferred from facility surfaces to lettuce in a unit batch	$= N_{AW}* T_O$	Log CFU/g	Calculated
N_{Final}	Number of cells in lettuce after processing in a unit batch	$=N_{AW}-N_{FS-L}+N_{L-FS}$	Log CFU/g	Calculated
S	Spread of contamination due to cross contamination	=Riskpert(1,1.2,2)		Pang et al. (2017)
$Prev_{AP}$	Prevalence after cross-contamination	$=Prev_{PP}*S$	%	Calculated
C_{AP}	Concentration of *E. coli* on cos lettuce after processing	$= N_{Final}/Prev_{AP}$	Log CFU/g	Calculated
	Transportation from processing to retail			
t_{P-R}	Transportation time	=RiskTriang(6,12,24)	h	This study
T_{P-R}	Processing to retail temperature	=RiskBetaGeneral(1.5217,1.3470,2.8376,4.9987)	°C	This study

		Retail storage condition		
t_R	Retail storage time	=RiskTriang(0.5,4,10)* 24	h	This study
T_R	Retail storage temperature	=RiskNormal(3.8,1.4,RiskTruncate(0,13.56))	°C	This study
		Transportation from retail to home		
t_{R-H}	Transportation time	=RiskLognorm(1.421,0.46478,RiskTruncate(0.1833,3.8667),RiskShift(-0.24609))	h	(Pang et al., 2017)
T_{R-H1}	Temperature before putting in home refrigerator	=RiskNormal(8.386,3.831,RiskTruncate(0,20))	°C	(Pang et al., 2017)
T_{R-H}	Transportation temperature, T_T (retail-home)	$=½*(T_R+T_{RH-1})$	°C	Calculated
		Home storage condition		
t_f	Time to first	=RiskWeibull(1.13,2.84)*24	h	(Pouillot et al., 2010)
t_L	Time to last	=RiskWeibull(1.7,7.96)*24	h	(Pouillot et al., 2010)
t_H	Time selected	$=½*(t_f+t_l)$	h	Calculated
T_H	Home storage temperature	=RiskNormal(3.4517,2.4442,RiskTruncate(-5,17.22))	°C	(Pang et al., 2017)
		Parameters for microbial kinetics		
b	Growth parameter	0.023		(McKellar, and Delaquis 2011; Pang et al., 2017)
T_{min}	Growth parameter	$=1.335*5.766×b$	°C	
k	Die-off rate	=RiskLognorm(0.013,0.001,Riskshift(0.001))/2.303	Log CFU/gh	
μ_{P-R}	Processing to retail growth rate	$=\left(b \times (T_{P-R} - T_{min})\right)^{2/2.303}$	Log CFU/gh	Calculated
μ_R	Retail growth rate	$=\left(b \times (T_R - T_{min})\right)^{2/2.303}$		
μ_{R-H}	Retail to home growth rate	$=\left(b \times (T_{R-H} - T_{min})\right)^{2/2.303}$		
μ_H	Home growth rate	$=\left(b \times (T_H - T_{min})\right)^{2/2.303}$		
		Microbial kinetics		
Q_{P-R}	Growth or die off?	$=IF(T_{P-R} > 5,1,0)$		
ΔC_{P-R}	Change in *E. coli* O157:H7 level	$IF(Q_{P-R} = 1, \mu_{P-R} \times t_{P-R}, -k \times t_{P-R})$	Log CFU/g	Calculated
C_{P-R}	Concentration of *E. coli* O157:H7 after processing to retail	$C_{WW}+ \Delta C_{P-R}$	Log CFU/g	
Q_R	Growth or die off?	$=IF(T_R > 5,1,0)$		
ΔC_R	Change in *E. coli* O157:H7 level	$IF(Q_R = 1, \mu_R \times t_R, -k \times t_R)$	Log	Calculated

			CFU/g	
C_R	Concentration of *E. coli* O157:H7 after retail	$C_{P\text{-}R} + \Delta C_R$	Log CFU/g	
$Q_{R\text{-}H}$	Growth or die off?	$=IF(T_{R-H} > 5,1,0)$		
$\Delta C_{R\text{-}H}$	Change in *E. coli* O157:H7 level	$IF(Q_{R-H} = 1, \mu_{R-H} \times t_{R-H}, -k \times t_{R-H})$	Log CFU/g	Calculated
$C_{R\text{-}H}$	Concentration of *E. coli* O157:H7 after retail to home	$CR + \Delta C_{R\text{-}H}$	Log CFU/g	
Q_H	Growth or die off?	$=IF(T_H > 5,1,0)$		
ΔC_H	Change in *E. coli* O157:H7 level	$IF(Q_H = 1, \mu_H \times t_H, -k \times t_H)$	Log CFU/g	Calculated
C_H	Concentration of *E. coli* O157:H7 at home	$C_R + \Delta C_H$	Log CFU/g	

Table 4. Overview of inputs and distributions for baseline model for *Escherichia coli* O157:H7 levels on Australian lettuce consumption behavior.

Symbol	Description	Distribution or Value	Unit	Reference
		Consumption		
M_{body}	Body mass	=RiskLogNorm(61.429, 13.362)	kg	Hamilton et al. (2006)
Mi	Per capita consumption of lettuce	=RiskTriang(0.123404,0.123404,0.170155)	$g(kg.day)^{-1}$	Hamilton et al. (2006)
D	Dose per day	$= C_H * M_{body} * Mi$	Log CFU/day	Calculated
		Dose response parameters		
α	Dose response parameter	0.267	-	Cassin et al. (1998)
β	Dose response parameter	229.2928	-	Cassin et al. (1998)
$P_I(\lambda)$	Probability of illness per day	$= 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \times \text{Prev}_{AP}$		Calculated
$P_I(A)(\lambda)$	Annual probability of illness	$= 1 - [1 - P_I(\lambda)]^{n=365}$		Haas (1983)
		Risk characterization		
N_{Aus}	Population in Australia	25469889		ABS (2020)

N_{cases}	Number of cases per year	$= N_{Aus} \times P_I(A)(\lambda)$	Calculated

Table 5. Overview of the changes made in the baseline scenario for the different scenarios.

Symbol	Description	Distribution or Value	Unit	Reference
		Autumn		
$D_{Growing}$	Growing time	=Round(RiskPert(60,65,70),0)	Day	Local data
D_{Rain}	Rainy days	= Round (RiskPert(5,10,17),0)	Day	Local data
h_{Sun}	Sun hours per day	=RiskPert(6,8,12)	h	Calculated
k_{Autumn}	Solar decay rate	0.47	Log CFU/g/day	Calculated
$Prev_{PP}$	Prevalence when entering processing plant	0.28	%	Local data
		Winter		
$D_{Growing}$	Growing time	= Round (RiskPert(70,75,80),0)	Day	Local data
D_{Rain}	Rainy days	=Round(RiskPert(3,5,8),0)	Day	Local data
h_{Sun}	Sun hours per day	=RiskPert(5,7,10)	h	Calculated
k_{Winter}	Solar decay rate	0.45	Log CFU/g/day	Calculated
$Prev_{PP}$	Prevalence when entering processing plant	0.23	%	Local data
		Spring		
$D_{Growing}$	Growing time	= Round (RiskPert(55,60,65),0)	Day	Local data
D_{Rain}	Rainy days	= Round(RiskPert(4,8,12),0)	Day	Local data
h_{Sun}	Sun hours per day	=RiskPert(7,9,13)	h	Calculated
k_{Spring}	Solar decay rate	0.61	Log CFU/g/day	Calculated
$Prev_{PP}$	Prevalence when entering processing plant	0.21	%	Local data
		Irrigation water sources		
$C_{IW\text{-}Tap\ water}$	IW quality	0	CFU/ml water	Local data
$Prev_{IW\text{-}Tap\ water}$	Prevalence in IW	0	-	Local data
$C_{IW\text{-}Surface\ water}$	IW quality	=RiskPert(7,96,390)	CFU/ml water	Local data
$Prev_{IW\text{-}Surface\ water}$	Prevalence in IW	0.25	-	Local data
$C_{IW\text{-}Reclaimed\ water}$	IW quality	=RiskPert(260,860,10300)	CFU/ml water	Local data
$Prev_{IW\text{-}\ Reclaimed\ water}$	Prevalence in IW	0.6	-	Local data
		Irrigation types		

Tr_{FI}	Amount of IW transferred to produce during irrigation via furrow irrigation	=RiskUniform(0.00007,0.00011)	ml/g produce	Stine et al. (2005)
Tr_{DI}	Amount of IW transferred to produce during irrigation via drip irrigation	=RiskUniform(0.0000006,0.00000088)	ml/g produce	Stine et al. (2005)
		Washing		
$W_{Chlorine}$	Chlorine	=RiskNormal(0.87,0.32,RiskTruncate(0.36,1.38))	Log CFU/g	This study
W_{PAA}	Peroxyacetic acid	=RiskPert(0.46,1.12,1.34)	Log CFU/g	This study

Table 6. Overview of preharvest conditions influencing *E. coli* O157:H7 distribution on lettuce at harvest (Log CFU/g) in different simulated scenarios.

Scenario	Mean	Std	P50	P75	P90	P95	P99	P99.9	P100	%Log-fold change
Baseline (Summer)	-1.1	2.4	-1.0	0.6	1.9	2.6	3.8	4.8	6.6	1.0
Autumn	0.3	2.3	0.4	1.9	3.2	3.9	5.0	6.0	7.6	-0.2
Winter	-0.6	2.3	-0.6	1.0	2.3	3.0	4.1	5.0	6.4	0.6
Spring	-3.4	2.5	-3.3	-1.6	-0.2	0.5	1.7	2.8	4.5	3.1
No solar	5.8	1.9	5.9	7.3	8.3	8.8	9.6	10.2	11.1	-5.3
No rain	-4.7	2.0	-4.7	-3.3	-2.2	-1.6	-0.7	0.1	1.2	4.3
No manure	-7.1	2.3	-7.0	-5.5	-4.2	-3.5	-2.4	-1.4	0.2	6.5
Tap water	-3.9	2.1	-3.7	-2.4	-1.3	-0.7	0.2	1.1	2.8	3.5
Surface water	0.8	2.1	-0.7	0.7	1.8	2.3	3.3	4.3	5.9	-0.7
Reclaimed water	0.4	2.1	0.6	1.9	3.0	3.6	4.6	5.5	7.2	-0.4
Drip irrigation	-8.2	2.3	-8.1	-6.5	-5.2	-4.5	-3.4	-2.4	-0.7	7.5
Furrow irrigation	-6.1	2.3	-6.0	-4.5	-3.2	-2.4	-1.3	-0.3	1.3	5.6

Table 7. Comparison of number of illness cases per year and probability of illness per serving due to consumption of *E. coli* O157:H7 contaminated lettuce in Australia predicted by the baseline model and other intervention scenarios.

Scenario	Number of illnesses per year				Probability of illness per year				
	Mean	**P90**	**P95**	**P99**	**Mean**	**P90**	**P95**	**P99**	**%Log fold change**
Water washing (Baseline)	13	43	99	179	4.9×10^{-7}	1.7×10^{-6}	3.9×10^{-6}	7.0×10^{-6}	-
Chlorine	9	20	73	162	3.5×10^{-7}	7.8×10^{-7}	2.9×10^{-6}	6.4×10^{-6}	1.4
Peracetic acid	8	13	59	152	3.0×10^{-7}	4.9×10^{-7}	2.3×10^{-6}	6.0×10^{-6}	1.6
Water washing + Chlorine	6	4	38	137	2.3×10^{-7}	1.7×10^{-7}	1.5×10^{-6}	5.4×10^{-6}	2.2
Water washing + Peracetic acid	5	2	26	123	1.8×10^{-7}	7.7×10^{-8}	1.0×10^{-6}	4.8×10^{-6}	2.7

Highlights:

- Development of a QMRA model for *E. coli* O157:H7 on cos lettuce based on Australian practices.
- The usage of manure and quality of irrigation water were found to be very relevant on pathogen level.
- *E. coli* O157:H7 levels over seasons influenced primarily by solar radiation and rainfall.
- The implementation of GAP is an important mitigation strategy within growers' control.
- Postharvest washing, and temperature control were identified as important risk control steps.
- Interpretation of food safety guidelines for lettuce production varies between countries.
- Risk management strategies should be based on local data for reliable risk characterization.

Conflict of Interest and Authorship Conformation Form

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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