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Quantitative assessment of the impact of cross-contamination during the washing step of ready-to-eat leafy greens on the risk of illness caused by *Salmonella*

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ABSTRACT

The aim of this study was to develop a quantitative microbial risk assessment (QMRA) model to estimate the risk of illness caused by *Salmonella* in ready-to-eat (RTE) leafy greens, based on common practices in Brazilian processing plants. The risk assessment model considered five modules: in field, washing step, retail storage, home storage and dose-response. Fifty thousand iterations of a @Risk model built in Excel were run for each of sixty scenarios. These scenarios considered different initial pathogen concentrations, fractions of contaminated produce and chlorine concentrations. For chlorine, seven pre-set concentrations (0, 5, 10, 25, 50, 150 and 250 mg/L) and three triangular distributions were considered [RiskTriang(0,5,10 mg/L), RiskTriang(0,80,250 mg/L) and RiskTriang(10,120,250 mg/L)]. The outputs were risk of infection, estimated number of illnesses and estimated percent of illnesses arising from cross-contamination. The QMRA model indicated quantitatively that higher chlorine concentrations resulted in lower risk of illness. When simulation was done with <5 mg/L of chlorine, most (>96%) of the illnesses arose from cross-contamination, but when a triangular distribution with 10, 120 and 250 mg/L of chlorine was simulated, no illnesses arising from cross-contamination were predicted. Proper control of the sanitizer in the washing step is essential to reduce initial contamination and avoid cross-contamination.

1. Introduction

An increased number of foodborne disease outbreaks have been associated with fresh and fresh-cut produce during the past decade concomitant with an increased consumption of these products (Doyle & Erickson, 2008; Jung, Jang, & Matthews, 2014; Lynch, Tauxe, & Hedberg, 2009). Ready-to-eat (RTE) fresh-cut produce is often consumed raw and typically requires no further preparation before consumption, increasing risk of infection if pathogens are present (Berger et al., 2010).

Considering all steps in the RTE fresh cut vegetables production chain, washing at processing is the primary step for removal of dirt and debris and reduction of microbial populations in the incoming vegetables. However, pathogens, such as *Escherichia coli* O157:H7, *Salmonella* and noroviruses can be transferred from contaminated to non-contaminated vegetables in this step, evidencing that wash water can be a source of cross-contamination if not properly sanitized (Allende, Selma, Lopez-Galvez, Villaescura, & Gil, 2008; Holvoet et al., 2014; Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2015; López-Gálvez, Allende, Selma, & Gil, 2009; Luo et al., 2011; Perez-Rodriguez et al., 2014; Tomás-Callejas et al., 2012, Zhang, Ma, Phelan, & Doyle, 2009).

Danyluk and Schaffner (2011) developed a quantitative assessment of the microbial risk of leafy greens, showing that occurrence of cross-contamination in the washing step could explain 95% to 100% of the cases caused by *E. coli* 0157:H7 in the spinach outbreak occurred in the USA in 2006. Chardon, Swart, Evers, and Franz (2016) constructed a mathematical model simulating the dispersion of contamination with *E. coli* O157 and *Salmonella* from a load of leafy greens during industrial washing, and compared the contribution of the contamination caused by direct transfer of the pathogens from contaminated to non-contaminated products to that caused by cross-contamination in the washing water. The authors observed that when the level of contamination was up to 10^6 CFU per batch, the direct route was more important that cross contamination in terms of number of illnesses. The two studies differed in some aspects, as the Chardon et al. (2016) model did not consider storage time, and was deterministic and did not consider variability in transfer coefficients.

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Despite the lack of information associating outbreaks to consumption of RTE vegetables in Brazil, *Salmonella* accounted for ~38% of foodborne outbreaks reported between 2000 and 2014 (Anonymous, 2014). Ceuppens et al. (2014) performed a microbiological quality of lettuce during primary production in Brazil and found the presence of enteric pathogens *Salmonella* (5) and *E. coli* O157:H7 (2) from 260 samples, of which only one was lettuce and the others were manure, soil and water. The prevalence of *Salmonella* was 5.6% in manure, 2.6% in soil, 1.9% in water and 1.3% in lettuce. *E. coli* O157:H7 was only isolated from water samples (3.8%).

Some other studies reported the occurrence of *Salmonella* in RTE minimally processed vegetables marketed in the country (Froder et

Table 1
Overview of simulation variables and parameters.

Cell	Variable	Value	Unit	Reference
B2	In field			
B3	Initial contamination level	_	Log CFU/g	Authors input
B4	Days in the field after contamination	=RiskUniform(1,60)	Days	Authors input
B5	Log reduction in field	=RiskNormal(−0.0175,0.00862)	Log CFU/g/day	Islam et al. (2004)
B6	Level at harvest	=B3–(B4*B5)	Log CFU/g	Calculated
B7	Fraction contaminated on incoming leafy greens	_	Percent	Authors input
B8	Fraction non-contaminated	=1–B7	Percent	Calculated
B9	Washing step			
B10	Chlorine concentration	=RiskTriang(0,80,250)	mg/L	Maffei, Alvarenga et al. (2016)
B11	Log reduction on contaminated portions after washing <10 mg/L	=B10*0.04 + 0.3	Log CFU/g	Maffei, Sant'Ana et al. (2016)
B12	Log reduction on contaminated portions after washing ≥10 mg/L	=B10*0.0056 + 0.5952	Log CFU/g	Maffei, Sant'Ana et al. (2016)
B13	Log reduction on contaminated portions after washing, chosen	=IF(B10 < 10,B11,B12)	Log CFU/g	Maffei, Sant'Ana et al. (2016)
B14	Log reduction SD	0.175	Log CFU/g	Maffei, Sant'Ana et al. (2016)
B15	Log reduction on contaminated portions after washing, with sd	=RiskNormal(B13,B14)	Log CFU/g	Maffei, Sant'Ana et al. (2016)
B16	Log % Transfer to non-contaminated portions (cross-contamination), upper	=−0.6798*B10–0.6	Log percent	Maffei, Sant'Ana et al. (2016)
B17	Log % Transfer to non-contaminated portions (cross-contamination), lower	=−1.3596*B10–0.6	Log percent	Maffei, Sant'Ana et al. (2016)
B18	Log % Transfer to non-contaminated portions (cross-contamination), actual	=RiskUniform(B17,B16)	Log percent	Maffei, Sant'Ana et al. (2016)
B19	Level on contaminated portions after washing	=B6–B13	Log CFU/g	Calculated
B20	Level on cross-contaminated portions after washing	=B6 + B18	Log CFU/g	Calculated
B21	Choose contaminated or non-contaminated	=RiskBinomial(1,B7)	Log CFU/g	Calculated
B22	Chosen level	=IF(B21 = 0,B20,B19)	Log CFU/g	Calculated
B23	Retail storage			
B24	Min retail temperature	8.1	°C	Maistro et al. (2012)
B25	Max retail temperature	11.3	°C	Maistro et al. (2012)
B26	Mean retail temperature	=RiskUniform(B24,B25)	°C	Calculated
B27	sd min retail temperature	1	°C	Maistro et al. (2012)
B28	sd most likely retail temperature	1	°C	Maistro et al. (2012)
B29	sd max retail temperature	2.7	°C	Maistro et al. (2012)
B30	sd retail temperature	=RiskTriang(B27,B28,B29)	°C	Calculated
B31	Retail temperature act	=RiskNormal(B26,B30)	°C	Calculated
B32	Time	=RiskTriang(3.5,7.7)	Days	Maffei, Alvarenga et al. (2016)
B33	Growth model *b* parameter	0.0243	Log CFU/h/°C	ComBase Predictor
B34	Growth model T_0 parameter	2.66	°C	ComBase Predictor
B35	Square root growth rate	=B33*(B31–B34)	sq rt. (log CFU/h)	Calculated
B36	Growth rate	=B35*B35	Log CFU/h	Calculated
B37	Below min temp corrected growth rate	=IF(B35 > 0;B35*B35;0)	Log CFU/h	Calculated
B38	Hours to days corrected growth rate	=B37*24	Log CFU/day	Calculated
B39	Change during retail storage	=B38*B32	Log CFU/g	Calculated
B40	Level after retail storage	=B22 + B39	Log CFU/g	Calculated
B41	Home storage			
B42	Temperature	=RiskGamma(7.15,1.03)	°C	Marklinder et al. (2004)
B43	Time	=RiskTriang(0,1,4)	Days	Marklinder et al. (2004)
B44	Growth model *b* parameter	0.0243	Log CFU/h/°C	ComBase Predictor
B45	Growth model T_0 parameter	2.66	°C	ComBase Predictor
B46	Square root growth rate	=B44*(B42–B45)	sq rt.(log CFU/h)	Calculated
B47	Growth rate	=B46*B46	Log CFU/h	Calculated
B48	Below min temp corrected growth rate	=IF(B46 > 0;B46*B46;0)	Log CFU/h	Calculated
B49	Hours to days corrected growth rate	=B48*24	Log CFU/day	Calculated
B50	Change during home storage	=B49*B43	Log CFU/g	Calculated
B51	Level after home storage	=B40 + B50	Log CFU/g	Calculated
B52	Consumption, dose-response and risk of infection			
B53	Serving size	=RiskNormalAlt(20%,45,80%,90)	g	Agudo (2004)
B54	Level of pathogen (non-log)	=10^B51	CFU/g	Calculated
B55	Level per serving, uncorrected	=B54*B53	CFU	Calculated
B56	Level per serving, with zeros	=IF(B55 < 1.0,TRUNC(B55))	CFU	Calculated
B57	Dose-response alpha	0.1324	No unit	WHO/FAO (2002)
B58	Dose-response beta	51.45	No unit	WHO/FAO (2002)
B59	Probability of infection single dose	=1-(1 + B56/B58)^-B57	Percent	Calculated
B60	Exposure (number of servings per iteration)	1	Serving	Authors input
B61	Risk of infection per number of servings per iteration (illness)	=RiskBinomial(B60,B59)	Illness	Calculated
B62	Occurrence of illness	=IF(B61 > 0.1,0)	No unit	Calculated
B63	Occurrence of cross-contamination	=IF(B21 = 0.1,0)	No unit	Calculated
B64	Number of illness due to cross-contamination	=IF(B63 + B62 = 2,B61,0)	Illness	Calculated
B65	Population of Sao Paulo city	11,896,893	Inhabitants	IBGE (2014)
B66	% of population consuming RTE leafy greens	64.3	%	Sato et al. (2007)
B67	Population of Sao Paulo consuming RTE leafy greens	=B65*B66	Inhabitants	Calculated
B68	Number of cases in population exposed	=B61*B67	Cases	Calculated

al., 2007; Maistro, Miya, Sant'Ana, & Pereira, 2012; Oliveira, Souza, Bergamini, & Martinis, 2011; Sant'Ana, Landgraf, Destro, & Franco, 2011). As industrial practices for production of RTE vegetables may vary from country to country, such as prohibition of use of chlorine and other antimicrobial agents in certain European countries (Chardon et al., 2016), risk estimates in different countries may differ. The purpose of this study was to develop a quantitative microbial risk assessment model to estimate the impact of cross-contamination during washing of RTE leafy greens on the risk of illness by *Salmonella*, based on the most common practices in Brazilian RTE vegetables processing plants.

2. Materials and methods

The risk assessment model considered five modules: (i) in field, (ii) washing step, (iii) retail storage, (iv) home storage and (v) consumption, dose-response and risk of infection. Table 1 provides an overview of the simulation variables and distributions used in the model, discussed in detail in the Results and Discussion Section. The first column in the spreadsheet indicates the cell reference of the variable or module on the corresponding line of the table. The second column describes the variable or module describing each line of the risk assessment. The third column indicates the value of the variable (a number, a formula or a @Risk function). The fourth column shows the unit of the variable, and the last column indicates the source of the information used to establish the variable (authors input, literature citation or calculated from other cells in the spreadsheet).

The risk assessment model was built in an Excel spreadsheet (Microsoft, Redmond, WA) and simulated using @Risk software version 6 (Palisade Corporation, Ithaca, NY). Fifty thousand iterations were run for each scenario. Latin Hypercube sampling was used and random number seed fixed at 1 to ensure that results could be directly compared. Initial pathogen concentration in the produce (0 and 1 log CFU/g), fraction of contaminated produce (0.01, 0.1 and 1.0%) and concentration of chlorine in the washing water were chosen to simulate sixty unique scenarios. Chlorine concentrations were defined using seven pre-set concentrations (0, 5, 10, 25, 50, 150 and 250 mg/L) and three selected triangular distributions [RiskTriang(0,5,10 mg/L), RiskTriang(0,80,250 mg/L) and RiskTriang(10,120,250 mg/L)].

3. Results and discussion

The first module of Table 1 describes the model for *Salmonella* in leafy greens in the field. Variables were derived from authors input (initial contamination level, days in the field after contamination and fraction of contaminated produce) or determined by calculation (contamination level at harvest and fraction of non-contaminated produce). Starting prevalence and concentration of *Salmonella* were assumed to be similar to those reported by Danyluk and Schaffner (2011) for *E. coli* O157:H7 and represent worst-case assumptions (e.g. an overt contamination event in the field). Data from Islam et al. (2004) regarding the persistence of *Salmonella* on lettuce grown in fields contaminated by poultry manure, two types of dairy manure or irrigation water were used to create a normal distribution to estimate the log reduction per day for *Salmonella* in the field.

The second module in Table 1 (washing step) is based on data from Maffei, Alvarenga, Sant'Ana, and Franco (2016) on the quality of RTE vegetables in Brazilian processing plants. A triangular distribution was created, considering the minimum, most likely and maximum concentration of chlorine used in the washing step (Maffei, Alvarenga et al., 2016). Data from another study of Maffei, Sant'Ana, Monteiro, Schaffner, and Franco (2016) were used to assess the relationship between chlorine concentration and log reduction of *Salmonella* in the washing step and to evaluate the transfer rate of the pathogen from contaminated to non-contaminated leaves washed in the same water.

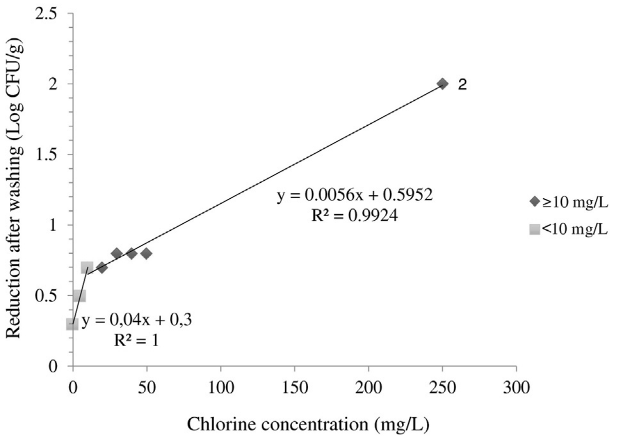

Fig. 1. Reduction of *Salmonella* in RTE leafy greens after washing contaminated portions with water containing <10 mg/L and ≥10 mg/L of chlorine.

To evaluate the relationship between chlorine concentration in the washing water and log reduction of *Salmonella*, data from Maffei, Sant'Ana et al. (2016) were divided into two regions: <10 and ≥10 mg/L of chlorine (Fig. 1). The average log reduction values were predicted by linear regression as a function of chlorine concentration. The standard deviations from each log reduction from Maffei, Sant'Ana et al. (2016) were combined to create a standard deviation for all chlorine concentrations. The means obtained from the linear regression and the overall standard deviation were used to create a normal distribution of log reduction as a function of chlorine concentration (Table 1, washing step).

Linear regression was also used to predict upper and lower limits for *Salmonella* transfer between contaminated and non-contaminated leaves (cross-contamination), based on data by Maffei, Sant'Ana et al. (2016). Cross-contamination at 0 mg/L of chlorine corresponded to the counts of *Salmonella* in the leaves. Upper and lower limits for cross-contamination at 5 mg/L of chlorine corresponded to the detection limit of the counting method (upper limit) and the detection limit of the enrichment method (lower limit). Cross-contamination at 10 mg/L of chlorine corresponded to the limit of the enrichment method. A uniform distribution was used to estimate the cross contamination, where the upper and lower limits were based on the corresponding regression equations (Fig. 2).

The third module in Table 1 (retail storage) represents the expected change in *Salmonella* level according to storage temperature and time at retail. Maistro et al. (2012) observed that the storage temperature of minimally processed vegetables sold in Brazilian supermarkets ranged from 8.1 to 11.3 °C, with standard deviations ranging from 1 to 2.7 °C.

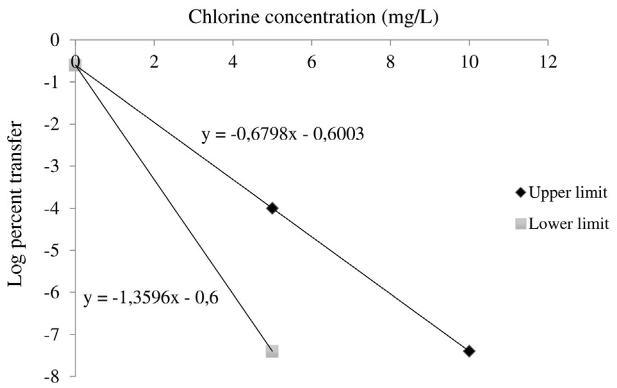

Fig. 2. Log percent transfer of *Salmonella* in RTE leafy greens via cross-contamination during washing.

Table 2
Number of servings needed to cause 1 illness.

Assumed prevalence of *Salmonella*	1.0%		0.1%		0.01%	
Initial contamination level (log CFU/g)	0	1	0	1	0	1
Chlorine concentration (mg/L)						
0	1.4	1.2	1.4	1.2	1.4	1.2
5	4.7	3.3	4.8	3.4	4.8	3.4
10	31.4	20.5	39.2	23.8	40	24.2
25	140.0	125.3	1666	1351	8333	8333
50	143.6	127.5	1785	1428	12,500	12,500
150	151.9	135.8	1851	1562	12,500	12,500
250	165.0	144.5	1923	1785	16,666	12,500

Table 4
Number of servings needed to cause 1 illness (using RiskTriang for chlorine concentration).

Assumed prevalence of *Salmonella*	1.0%		0.1%		0.01%	
Initial contamination level (log CFU/g)	0	1	0	1	0	1
Chlorine concentration (mg/L)						
RiskTriang(0,5,10)	4	3	4	3	4	3
RiskTriang(0,80,250)	133	118	684	537	1162	819
RiskTriang(10,120,250)	152	137	1562	1428	16,666	16,666

These data were used to calculate the mean of a normal distribution for retail temperature and standard deviation (Table 1, B24–31).

Data from Maffei, Alvarenga et al. (2016) for the labeled shelf life of leafy greens produced by Brazilian processing plants were used to represent retail storage time, via a triangular distribution (Table 1, B32). The growth rate of *Salmonella* as a function of temperature (Table 1, B33–34) was determined using data from ComBase Predictor [http://modelling.combase.cc/] with the following assumptions: pH 6.8, a_w 0.995, CO_2 15% and temperature 7, 10, 13 and 16 °C. The pH, a_w and CO_2 parameters were chosen considering data published by Sant'Ana, Franco, and Schaffner (2012). The level of *Salmonella* at the end of storage time and the changes in the level of contamination during storage were determined by calculation (Table 1, B35–40).

The fourth module in Table 1 (home storage, B41–51) represents the expected change in *Salmonella* level according to storage temperature and time at the consumer's home, based on data from Marklinder, Lindblad, Eriksson, Finnson, and Lindqvist (2004). These authors measured the temperature at which RTE salads were stored in Swedish households and modeled those data using a Gamma distribution (7.15,1.03). In Brazil, Silva, Celidonio, and Oliveira (2008) collected data on the temperature of domestic refrigerators in Brazilian households but reported minimum and maximum temperatures exclusively (3.0 and 10.8 °C, respectively). These data were not enough for the model, and only data from Marklinder et al. (2004) were considered. The home storage time (days) was expressed as a triangular distribution, where “0” means that the vegetables are consumed immediately after purchase. The growth of *Salmonella* during home storage was obtained using the same growth model used for retail storage.

The fifth module in Table 1 (B52–68) refers to consumption, dose-response and risk of infection. As the serving size of RTE leafy greens in Brazil is unknown, the data for “mixed salad” of Agudo (2004) were used, and modeled using a RiskNormalAlt distribution, considering two percentiles (20% at 45 g and 80% at 90 g) to represent the serving size.

The level of *Salmonella* per serving was calculated multiplying the amount consumed by the concentration of the pathogen in the vegetable. The dose-response was estimated using the Beta-Poisson model (Table 1, B57–58) proposed by the World Health Organization/Food and Agriculture Organization of the United Nations WHO/FAO (World Health Organization/Food and Agriculture Organization of the United Nations (2002). The exposure (number of servings per iteration) was chosen to be 1. The probability of illness by a single dose was calculated using a binomial distribution where probability of illness is from the dose response function and the number of serving was set to 1 (Table 1, B61).

The main outputs of the QMRA model were risk of infection, estimated number of illnesses and estimated percent of illnesses arising from cross-contamination. The results showed that the higher the chlorine concentration, the lower the risk of illness, regardless the initial contamination level of *Salmonella* in the leafy greens. In scenarios where chlorine concentrations at 0 and 5 mg/L were simulated (Tables 2 and 3), less than five servings were needed to cause 1 illness and most (96.5% to 99.9%) of the illnesses would arise from cross-contamination. When washing with 10 mg/L of chlorine was simulated, from 20 to 40 servings were needed to cause 1 illness and from 77.6% to 99.8% of the illnesses were predicted to arise from cross-contamination. On the other hand, when washing with ≥25 mg/L of chlorine was simulated, the number of servings needed to cause 1 illness was much higher (Table 2).

As antimicrobial activity of chlorine in the washing water is influenced by many factors, such as batch size and organic load, three additional scenarios for chlorine concentration were simulated, using a triangular distribution with minimum, most likely and maximum chlorine concentrations [RiskTriang(0,5,10), RiskTriang(0,80,250) and RiskTriang(10,120,250)]. Results shown in Table 5 indicate that RiskTriang(0,5,10) predicted that 97.1% to 99.9% of the cases of salmonellosis would arise from cross-contamination. When concentration was raised to RiskTriang(0,80,250), the predicted number of serving needed to cause 1 illness increased substantially and no illnesses arising from cross-contamination were observed when RiskTriang(10,120,250) was simulated (Tables 4 and 5).

The findings in Tables 3 and 5, showing that lower prevalence of *Salmonella* in the incoming servings results in higher percentage of illness arising from cross-contamination, can be understood by realizing that a higher concentration of pathogens in a single dose may be more likely to cause an individual illness, but if those cells are distributed over multiple servings, even if the probability of illness per serving is lower, the total number of potential illnesses will be higher.

Similar results were obtained by Danyluk and Schaffner (2011) for *E. coli* 0157:H7. Based on data published by Zhang et al. (2009), Danyluk and Schaffner (2011) developed a QMRA for leafy greens and concluded that 95% to 100% of the cases caused by *E. coli* 0157:H7 in the spinach

Table 3
Number of illness per 50,000 servings, where each iteration is a serving. Percent of illnesses arising from cross-contamination is shown in parenthesis.

Assumed prevalence of *Salmonella*	1.0%		0.1%		0.01%	
Initial contamination level (log CFU/g)	0	1	0	1	0	1
Chlorine concentration (mg/L)						
0	36,286 (98.9)	39,815 (98.9)	36,277 (99.9)	39,810 (99.9)	36,272 (99.9)	39,807 (99.9)
5	10,610 (96.5)	15,118 (97.3)	10,367 (99.6)	14,879 (99.7)	10,349 (99.9)	14,859 (99.9)
10	1589 (77.6)	2440 (83.6)	1276 (98.5)	2095 (98.3)	1252 (99.7)	2064 (99.8)
25	357 (0.6)	399 (0.5)	30 (6.6)	37 (5.4)	6 (33.3)	6 (33.3)
50	348 (0)	392 (0)	28 (0)	35 (0)	4 (0)	4 (0)
150	329 (0)	368 (0)	27 (0)	32 (0)	4 (0)	4 (0)
250	303 (0)	346 (0)	26 (0)	28 (0)	3 (0)	4 (0)

Table 5
Number of illness per 50,000 servings, where each iteration is a serving, using RiskTriang for chlorine concentration. Percent of illness arising from cross-contamination shown in parenthesis.

Assumed prevalence of *Salmonella*	1.0%		0.1%		0.01%	
Initial contamination level (log CFU/g)	0	1	0	1	0	1
Chlorine concentration (mg/L)						
RiskTriang(0,5,10)	12,524 (97.1)	16,538 (97.6)	12,302 (99.7)	16,320 (99.7)	12,281 (99.9)	16,298 (99.9)
RiskTriang(0,80,250)	374 (10.4)	423 (13.4)	73 (54.7)	93 (62.3)	43 (93)	61 (95)
RiskTriang(10,120,250)	328 (0)	364 (0)	32 (0)	35 (0)	3 (0)	3 (0)

outbreak in the USA in 2006 could have been attributed to cross-contaminated pieces. It was predicted that the lower the prevalence of *E. coli* 0157:H7 in the incoming vegetables, the higher the percentage of cross contaminated pieces associated with predicted cases of infection caused by this bacterium. The QMRA developed by Danyluk and Schaffner (2011) was based on unchlorinated water, while the present model considered a range of chlorine levels, which is of critical importance in determining appropriate sanitizer levels, and the consequences of low levels on occurrence of illness.

Outbreaks involving *Salmonella* and fresh produce have been reported worldwide (Berger et al., 2010; Callejón et al., 2015; Kozak, MacDonald, Landry, & Farber, 2013), becoming a major concern for consumers, governments and the food industry. Despite avoided in some countries, especially member states of the European Union, the use of sanitizers during washing of vegetables is an important tool for reducing microbial contamination and avoiding cross-contamination between clean and contaminated products (Gil, Selma, López-Gálvez, & Allende, 2009; Prado-Silva, Cadavez, Gonzales-Barron, Rezende, & Sant'Ana, 2015).

The risk of *Salmonella* in leafy greens is reduced if disinfectants are properly used within the washing tank. According to the European Food Safety Authority (EFSA, 2014), disinfectants and their concentrations as well as the mode of washing vary depending on the processor. Application of free chlorine at 50–200 mg/L with a variable contact time from 1 to 10 min is the most widely used technique for disinfection of fresh produce, but this procedure has limitations, such as inactivation of active agents by high level of organic load and dependence on neutral pH for optimal activity (FAO/WHO, 2008; Goodburn & Wallace, 2013; Tirpanalan, Zunabovic, Domig, & Kneifel, 2011).

Some other studies have recommended low levels of free chlorine (Gómez-López, Lannoo, Gil, & Allende, 2014; Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). A study conducted by Luo et al. (2011) found that the presence of 1 mg/L of free chlorine in the wash solution was insufficient to prevent *E. coli* O157:H7 survival and transfer during washing, because the introduction of cut lettuce to the wash system quickly depleted the free chlorine. These findings suggest that a continuous control of disinfectants and water quality in fresh-cut processing plants is very important to ensure the safety of these products.

Information regarding consumption of RTE vegetables by the Brazilian population was found in only two studies. Perez et al. (2008) reported that 23% of the interviewed individuals in Belo Horizonte, MG, consumed this type of vegetables, but a much higher consumption (64.3%) was reported in Sao Paulo, SP (Sato, Martins, & Bueno, 2007).

The number of cases of infection by the consumption of RTE leafy greens contaminated with *Salmonella* was estimated using the most recent data on the population of Sao Paulo city Brazil (IBGE, 2014). The simulated number of cases of infection per month, assuming that 64.3% of the population eats these products at least once per month (Sato et al., 2007), is shown in Table 6. Considering a scenario simulating the triangular distribution with 10, 120 and 250 mg/L of chlorine, which corresponds to the most representative for Brazilian processing plants, (Maffei, Alvarenga et al., 2016), and the initial prevalence and concentration of *Salmonella* in leafy greens, the population of Sao Paulo city would experience from 459 to 55,837 illnesses per month. These predictions seem quite high, but it should be noted that the current QMRA considered worst-case assumptions for pathogen prevalence and concentration.

Validation of these findings using Brazilian public health data is not possible as reports regarding foodborne diseases in Brazil are scarce and inconsistent. Only a few Brazilian Federative States have a structured foodborne diseases surveillance system and regular reports to the health authorities (Gomes, Franco, & Martinis, 2013). Consequently, it is not possible to determine the degree to which the obtained results overestimate or underestimate the risk.

Although validation with Brazilian public health data is not feasible at this moment, some comparisons with similar risk assessments are possible. Sant'Ana, Franco, and Schaffner (2014) developed a QMRA model to estimate the risks of infection due to consumption of RTE leafy vegetables contaminated with *Salmonella* and *Listeria monocytogenes* in Sao Paulo, Brazil, focusing on the retail and consumption steps. Their model did not include in field and processing steps and prevalence and concentration levels of pathogens were based on published data. Sant'Ana et al. (2014) predicted that the mean risk of *Salmonella* infection per month in the exposed population is 5.7E-03 (0.0057) per serving, with 14,958 cases of infection per month. It should be highlighted that Sant'Ana et al. (2014) did not focus the entire RTE

Table 6
Estimated number of cases of infection by the consumption of RTE leafy-greens contaminated with *Salmonella* in the population of Sao Paulo city per month.

Assumed prevalence of *Salmonella*	1.0%		0.1%		0.01%	
Initial contamination level (log CFU/g)	0	1	0	1	0	1
Pre-set chlorine concentration (mg/L)						
0	5,464,073	6,374,752	5,464,073	6,374,752	5,464,073	6,374,752
5	1,627,596	2,318,092	1,593,688	2,249,912	1,593,688	2,249,912
10	243,621	373,156	195,145	321,416	191,416	316,103
25	54,641	61,051	4592	5662	918	918
50	53,271	59,998	4286	5357	612	612
150	50,360	56,331	4133	4897	612	612
250	46,362	52,939	3978	4286	459	612
Triangular distribution for chlorine concentration (mg/L)						
0,5,10	1,961,462	2,549,901	1,912,426	2,549,901	1,865,781	2,549,901
0,80,250	57,517	64,828	11,184	14,245	6583	9340
10,120,250	50,327	55,837	4897	5357	459	459

leafy vegetables chain and calculated the probability of infection, not the probability of illness. It is well known that not all cases of infection result in disease. The predictions of illness in the present study as a function of chlorine concentrations, considering RiskTriang(0,80,250) and RiskTriang(10,120,250), are consistent with the predicted number of illness by *Salmonella* in the exposed population obtained by Sant'Ana et al. (2014).

Epidemiological data have indicated that salmonellosis is one of the most common types of food poisoning worldwide and historically outbreaks were associated with foods of animal origin. However, a recent trend on the occurrence of outbreaks linked to consumption of fresh produce has been observed (CDC, 2016; Lynch et al., 2009). According to the Centers for Disease Control and Prevention, *Salmonella* is estimated to cause one million foodborne illnesses in the United States, with 19,000 hospitalizations and 380 deaths. Among these, a significant number of illnesses have been linked to the consumption of fresh produce (CDC, 2016). In the European Union (EU), over 100,000 human cases of salmonellosis are reported each year (EFSA, 2016).

Despite limited available public health data in Brazil related to foodborne diseases associated to RTE leafy greens, our model contributes to risk assessment. The results are useful for food processing plants, as our results indicate that proper control of the sanitizer in the washing step is essential to reduce initial contamination and avoid cross-contamination. Our results also show that concentration of chlorine should be kept above 10 mg/L in order to minimize the risk of illness due to consumption of these products.

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