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Risk of infection with *Salmonella* and *Listeria monocytogenes* due to consumption of ready-to-eat leafy vegetables in Brazil

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ABSTRACT

The current study was carried out to estimate the risks of infection due to consumption of RTE vegetables contaminated with *Salmonella* and *Listeria monocytogenes* in Brazil. The risk assessment model was composed of five different modules comprising the retail-consumption steps. Scenarios were simulated using prevalence and concentration levels reported in RTE vegetables in Brazil as well as considering values 10 times lower. In addition, scenarios in which temperature during transportation and storage are maintained below 5 °C were also evaluated. Models built in Excel spreadsheets were run (100,000 iterations) using @Risk software. The two outputs were risk of infection per month (probability of infection per month due to consumption of RTE vegetables) and number of infections per month (number of people that consumed RTE vegetables and get infected per month). The QMRA models predicted that the mean risk of *Salmonella* infection per month is 5.7E-03, while the mean risk of infection for *L. monocytogenes* was 8.1E-06 per month. The reduction of prevalence of *Salmonella* from 1.7% to 0.17% resulted in a decrease of risk of infection per month by about 6 times. In the case of *L. monocytogenes*, the reduction of prevalence from 2.2% to 0.22% resulted in decrease of risk of infection from 8.1E-06 to 1.0E-06. The risks and number of cases predicted in scenarios in which temperature was kept below 5 °C were reduced for both pathogens studied when compared to scenarios where this was not the case. The scenario where prevalence and concentration of pathogens was reduced and where temperature was <5 °C led to the lowest number of infections due by *Salmonella* and *L. monocytogenes* (187 and 3.3E-05 cases, respectively). The results suggest that effective mitigation strategies need to be adopted. The strict control of temperature during transportation, storage and consumption was more effective to reduce risk and number of cases due to *L. monocytogenes* than to *Salmonella*. More data is needed to improve the accuracy of risk assessment models developed.

1. Introduction

The increased association of fresh produce with foodborne disease outbreaks in the last 15 years concerns consumers, governments and the food industry worldwide. Although this increase may also be due to improvements in microbiological methods and surveillance programs (Harris et al., 2003), it highlights the need for efforts to increase the microbiological safety of fresh produce. Among all the types of fresh produce currently available, ready-to-eat (RTE) vegetables play a central role as several outbreaks have been linked to these products (Harris et al., 2003; Little & Gillespie, 2008; Lynch, Tauxe, & Hedberg, 2009; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004).

Epidemiological investigations have shown that *Salmonella* and pathogenic *Escherichia coli* are the predominant foodborne bacterial pathogens involved in produce outbreaks (Friesema et al., 2007; Little & Gillespie, 2008; Lynch et al., 2009; Sivapalasingam et al., 2004; Takkinen et al., 2005). *Salmonella* is the most important foodborne pathogen in Brazil, accounting for 47% of foodborne disease outbreaks notified, while pathogenic *E. coli* (enteropathogenic and enterotoxigenic) is responsible for less than 0.1% of outbreaks (Anonymous, 2010). *Listeria monocytogenes* has not been directly associated with foodborne illness in Brazil to date (Martins et al., 2010) although it represents an important challenge for the safety of RTE foods which should not be overlooked.

The prevalence of *Salmonella* in RTE vegetables marketed in Brazil has been reported in the literature (Fröder et al., 2007; Oliveira, Souza, Bergamini, & Martinis, 2011; Sant'Ana, Landgraf,

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Destro, & Franco, 2011). Although the occurrence of Shiga toxin-producing *E. coli* has been reported in Brazilian food-producing animals (Oliveira et al., 2008), the prevalence of this microorganism in Brazilian vegetables (Silva, Silveira, Yokoya, & Okazaki, 2003) and in meat products in Brazil is very low (Bergamini, Simões, Irino, Amaral, & Guth, 2007; Silva et al., 2001). Shiga toxin-producing *E. coli* has occasionally been isolated from food commodities and ill persons in the country (Barancelli et al., 2011; Lemes-Marques, Cruz, & Destro, 2007; Miyasaki et al., 2009; Oliveira, Abeid Ribeiro, Morato Bergamini, & Martinis, 2010;).

A total of 6062 foodborne disease outbreaks were reported in Brazil between 1999 and 2008, and 144 of those outbreaks were linked to consumption of vegetables (Anonymous, 2010). Although the epidemiological association of foodborne diseases and consumption of RTE vegetables in Brazil is not clear, this relationship has been reported in other countries (Friesema et al., 2007; Little & Gillespie, 2008; Lynch et al., 2009; Takkinen et al., 2005). This lack of association in Brazil may be due to the inherent complexities for the attribution of disease outbreaks, even under the best of circumstances (Greig & Ravel, 2009; Pires et al., 2009).

Quantitative microbial risk assessment (QMRA) allows the quantitative estimation of the risks posed to public health by a food–pathogen combination (Oscar, 2011). The outputs of QMRA can be used in the development of scientific-based strategies to manage risks and safeguard public health. Quantitative microbial risk assessment (QMRA) consists of four steps: 1) hazard identification; 2) exposure assessment; 3) hazard characterization and 4) risk characterization (Codex, 1999 , pp. 1–6). The development of QMRA models has increased in the last 15-years, however, few models on produce–pathogens combinations are available (Danyluk & Schaffner, 2011; Franz, Tromp, Rijgersberg, & Van Der Fels-Klerx, 2010; Tromp, Rijgersberg, & Franz, 2010). The development of QMRA models focusing on fresh produce is of foremost importance because RTE vegetables are mostly eaten raw, without a definitive cooking step before consumption. Given the above and considering the increasing consumption of RTE vegetables in Brazil (Sato, Martins, & Bueno, 2007), the current study was carried out to estimate the risks of infection due to consumption of RTE vegetables contaminated with *Salmonella* and *L. monocytogenes*.

2. Models development

The risk assessment model comprised five different modules from finished product leaving the produce processing facility through consumption (Table 1).

2.1. Transportation from produce processing facilities to retail

Data on prevalence of *Salmonella* and *L. monocytogenes* on RTE vegetables were gathered from published literature. Only information on leafy green vegetables was considered (Table 2), and the prevalence of both pathogens was represented in the model by Beta distribution (Table 1). Few data on populations of both pathogens in RTE vegetables in Brazil were available in the literature. Only one study enumerated *Salmonella* in RTE leafy vegetables (Sant'Ana et al., 2011). In that study, *Salmonella* was recovered from two out of 477 packages of RTE leafy vegetables, with populations of 8.8×10^2 and 2.4×10^2 CFU/g, respectively. Populations of 1.0×10^1 and 1.6×10^1 CFU/g of *L. monocytogenes* were reported in two samples from a total of 477 packages of RTE vegetables analyzed (Sant'Ana, Igarashi, Landgraf, Destro, & Franco, 2012). Few other studies presented enumeration data of *L. monocytogenes* in RTE vegetables in Brazil (Oliveira et al. 2010; Porto & Eiroa, 2001), however, the populations found (0.4, 1.2 and 3.2 MPN/g) were lower than the levels reported by Sant'Ana, Igarashi, et al. (2012). Given the above, the concentration of both pathogens was modeled using Pert distribution with the average counts found by Sant'Ana et al. (2011, 2012) inserted as maximum values. The minimal value (−3 log CFU/g) was an user input, while the most likely value (−1.4 CFU/g) was estimated considering that 1 CFU was present in 25 g of positive samples according to the prevalence study (Table 2).

Temperature and time during transportation of RTE vegetables from industry to retail were modeled based on data obtained from Pereira (2008) , 173 p. and Pereira, Doria, Carvalho, Neves Filho, and Silveira (2010). These authors recorded temperature of chilled and frozen food products during deliveries for 9 different retail shops over a 9 h time period (Pereira, 2008 , 173 p.; Pereira et al., 2010). Considering that RTE vegetables that are consumed in the large cities of Brazil originate from production sites located in areas that surround the metropolitan regions (<100 Km), maximum transportation time was assumed to be 9 h. The minimum time to delivery was 2 h and corresponded to average time for the first delivery in a working day. The most likely value (5 h) was the average value for the remaining (seven) deliveries done after the first and last deliveries, respectively (Pereira, 2008 , 173 p.). A pert distribution was used to model transportation time from processing facilities to retail (Table 1). Temperature was based on the data collected with thermocouples attached outside the primary package of foods used as models (Pereira, 2008 , 173 p.). Maximum (10.3 °C), minimum (3 °C) and mean (most probable) (7.6 °C) values reported by this author were used to describe transportation temperature from industry to retail using a pert distribution (Table 1).

The growth of *Salmonella* and *L. monocytogenes* was described by the relationship between growth rate and temperature represented by the linear regression model proposed by Ratkowsky, Olley, McMeekin, and Ball (1982):

$$\sqrt{r} = b(T - T_0) \quad (1)$$

Where: $\sqrt{r}$ is the square root of maximum growth rate (μ), b is the slope of the regression line, T is temperature and T_0 is a conceptual minimum temperature for microbial growth, where T is given in °C. The growth of *Salmonella* (Equation (2)) and *L. monocytogenes* (Equation (3)) during transportation was modeled using previous published data (Sant'Ana, Franco, & Schaffner, 2012) (Table 1):

$$\sqrt{\mu} = 0.0178(T - 4.6) \quad (2)$$

$$\sqrt{\mu} = 0.0144(T - 1.6) \quad (3)$$

The growth of both pathogens during transportation was calculated by multiplying predicted growth by time of transportation at a given temperature. The concentration after transportation was the sum of initial concentration of each pathogen and subsequent growth during this step.

2.2. Arrival and storage at retail

In a survey of microbiological quality of RTE vegetables carried out by Maistro, Miya, Sant'Ana, and Pereira (2012) temperature of displays in the supermarkets were recorded. Although the integrated data ditting tool of @Risk version 5.7.0 (Palisade Corporation, Ithaca, NY) was used to fit statistical distributions to observed temperature, a suitable distribution to fit these data was not obtained (data not shown). Instead, frequencies of temperature recorded at retail level were calculated and a discrete distribution was included in the risk assessment model to represent this event (Fig. 1, Table 1). A maximum shelf life of RTE vegetables of 8 days was assumed, based on the labeled shelf life of these products. The

Table 1
The risk assessment models of infection by *Salmonella* and *L. monocytogenes* due to consumption of RTE vegetables in Brazil.

Notation	Event	Values	Unities	Source
1 − Transportation from produce processing facility to retail module				
P_i	Prevalence	*Salmonella* = RiskBeta(20,1098) *L. monocytogenes* = RiskBeta(27,1181)	%	see Table 2
C_i	Concentration	*Salmonella* = RiskPert(−3,−1.4,2.74) *L. monocytogenes* = RiskPert(−3,−1.4,1.1)	log CFU/g	Sant'Ana et al. (2011, Sant'Ana, Igarashi, et al., 2012)
T_1	Temperature during transportation	=RiskPert(3,7.6,10.3)	°C	Pereira et al. 2010, Pereira, 2008 , 173 p.
t_1	Time of transportation	=RiskPert(2,5,9)	h	Pereira et al. 2010, Pereira, 2008 , 173 p.
b	Parameter b growth model	*Salmonella*: 0.0178/*L. monocytogenes*: 0.0144	√ Log CFU/day/°C	Sant'Ana, Franco, et al. (2012)
T_0	Parameter T_0 growth model	*Salmonella*: 4.6/*L. monocytogenes*: 1.6	°C	Sant'Ana, Franco, et al. (2012)
Lg_1	Logarithmic growth	*Salmonella* = $(0.0178 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$ *L. monocytogenes* = $(0.0144 \times (\text{If}(T_1 - T_0) < 0{,}0, 9T_1 - T_0)))^2$	log CFU/g/h	Sant'Ana, Franco, et al. (2012)
G_{tra1}	Growth during transportation 1	$=t_1 \times Lg_1$	log CFU/g	Calculated
L_{at1}	Level after transportation 1	$=C_i + G_{tra}$	log CFU/g	Calculated
2 − Retail storage module				
T_2	Storage temperature	=RiskDiscrete({5\7\8\9\10\11\12\13\15}, {0.017\0.051\0.33\0.10\0.17\0.12\0.16\0.017\0.017})	°C	Maistro (2006)
$t_2$70	Storage time at retail I − 70%	=RiskUniform(0,120)	h	Assumption
$t_2$30	Storage time at retail II − 30%	=RiskUniform(0,72) + 120	h	Assumption
b	Parameter b growth model	*Salmonella*: 0.0178/*L. monocytogenes*: 0.0144	√ Log CFU/day/°C	Sant'Ana, Franco, et al. (2012)
T_0	Parameter T_0 growth model	*Salmonella*: 4.6/*L. monocytogenes*: 1.6	°C	Sant'Ana, Franco, et al. (2012)
Lg_2	Logarithmic growth	*Salmonella* = $(0.0178 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$ *L. monocytogenes* = $(0.0144 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$	log CFU/g/h	Sant'Ana, Franco, et al. (2012)
G_{St}70	Growth during retail storage − 70%	$=t_2$70 × Lg_2	log CFU/g	Calculated
G_{St}30	Growth during retail storage − 30%	$=t_2$30 × Lg_2	log CFU/g	Calculated
L_{ar}	Level after retail storage	=RiskDiscrete(G_{St}70:G_{St}30,{0.7\0.3})	log CFU/g	Calculated
3 − *Transportation from retail to home module*				
T_3	Temperature	=RiskPert(7,12,20)	°C	Assumption
t_3	Time	=RiskGamma(5.24,8.17)/60	h	Nauta et al. (2003)
b	Parameter b growth model	*Salmonella*: 0.0178/*L. monocytogenes*: 0.0144	√ Log CFU/day/°C	Sant'Ana, Franco, et al. (2012)
T_0	Parameter T_0 growth model	*Salmonella*: 4.6/*L. monocytogenes*: 1.6	°C	Sant'Ana, Franco, et al. (2012)
Lg_3	Logarithmic growth	*Salmonella* = $(0.0178 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$ *L. monocytogenes* = $(0.0144 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$	log CFU/g/h	Sant'Ana, Franco, et al. (2012)
G_{tra2}	Growth during transportation	$=t_3 \times Lg_3$	log CFU/g	Calculated
L_{at2}	Level after transportation 2	$=G_{tra2}$	log CFU/g	Calculated
4 − Home storage module				
T_4	Storage temperature	=RiskPert(3.04,6,10.8)	°C	Silva et al., 2008
t_4	Storage time	=RiskUniform(0,192)	h	Assumption
t_{p1}	Time of purchase I	$=t_1 + t_2$70 + t_3	h	Calculated
t_{p2}	Time of purchase II	$=t_1 + t_2$30 + t_3	h	Calculated
$\%^{pur70}$	% of packages purchased < 5 days	=0.7	%	Assumption
$\%^{pur30}$	% of packages purchased > 5 days	=0.3	%	Assumption
PD^{usd}	Time of purchase used	=RiskDiscrete(t_{p1}: t_{p2}, $\%^{pur70}$: $\%^{pur30}$)	h	Calculated
PSL	Product shelf-life	192	h	Assumption
b	Parameter b growth model	*Salmonella*: 0.0178/*L. monocytogenes*: 0.0144	√ Log CFU/day/°C	Sant'Ana, Franco, et al. (2012)
T_0	Parameter T_0 growth model	*Salmonella*: 4.6/*L. monocytogenes*: 1.6	°C	Sant'Ana, Franco, et al. (2012)
Lg_4	Logarithmic growth	*Salmonella* = $(0.0178 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$ *L. monocytogenes* = $(0.0144 \times (\text{If}(T_1 - T_0) < 0{,}0, (T_1 - T_0)))^2$	log CFU/g/h	Sant'Ana, Franco, et al. (2012)
G_{hs}	Growth during home storage	$=\text{If}(t_4 + PD^{usd} > \text{PSL}, 0, Lg_4)$	log CFU/g	Calculated
L_{ahSt}	Level after home storage	$=G_{hs}$	log CFU/g	Calculated
T_{lcons}	Total level before consumption	$=L_{at1} + L_{ar} + L_{at2} + L_{ahSt}$	log CFU/g	Calculated
5 − Consumption, dose−response and risk of infection module − *Salmonella*				
S	Serving size	=RiskPert(25,50,75)	g	Assumption
CFU	Level of pathogen (non-log)	$=10^{T}_{\text{lcons}}$	CFU/g	Calculated
D	Dose per serving	$=S \times$ CFU	CFU	Calculated
α	Parameter alpha	=RiskPert(0.0763,0.1324,0.2274)	−	WHO/FAO (2002) , 329 p.
β	Parameter beta	=RiskPert(38.4,51.4,57.9)	−	WHO/FAO (2002) , 329 p.
P_{id}	Probability of infection single dose	$=1 - (1 + D/\alpha)^{-\beta}$	−	Calculated
E	Exposure (number of servings/month)	=RiskDiscrete({1\2\12\30},{0.43\0.23\0.27\0.07})	Servings	Perez et al. (2008)
R_m	Risk of infection per month	$=\text{RiskOutput}() + 1 - (1 - P_i \times P_{id})^E$	−	Calculated
P_{sp}	Population Sao Paulo city	=1.13E+07	Inhabitants	IBGE (2011)
$\%_{eat}$	% of population eating RTE vegetables	=23	%	Perez et al., 2008
P_{eat}	Population of Sao Paulo eating RTE vegetables	=2.60E + 06	Inhabitants	Calculated
N_c	Number of cases in population exposed	$=R_m \times P_{eat}$	Cases	Calculated

(*continued on next page*)

Table 1 (*continued*)

6 – Consumption, dose–response and risk of infection module – *L. monocytogenes*				
S	Serving size	=RiskPert(25,50,75)	g	Assumption
CFU	Level of pathogen (non-log)	$=10^{T_{lcons}}$	CFU/g	Calculated
D	Dose per serving	$=S \times CFU$	CFU	Calculated
r	Parameter r	=RiskPert(1.11×10^{-15}, 4.47×10^{-11},1.36×10^{-9})	–	Mataragas et al. (2010)
P_{id}	Probability of infection single dose	$=1-e^{(-r \times D)}$	–	Calculated
E	Exposure (number of servings/month)	=RiskDiscrete({1\2\12\30},{0.43\0.23\0.27\0.07})	Servings	Perez et al. (2008)
R_m	Risk of infection per month	$=1-(1-P_i \times P_{id})^E$	–	Calculated
P_{sp}	Population Sao Paulo city	=1.13E + 07	Inhabitants	IBGE (2011)
$\%_{eat}$	% of population eating RTE vegetables	=23	%	Perez et al., 2008
P_{eat}	Population of Sao Paulo eating RTE vegetables	=2.60E + 06	Inhabitants	Calculated
N_c	Number of cases in population exposed	$=R_m \times P_{eat}$	–	Calculated

storage time was modeled using uniform distributions, where it was assumed that 70% of RTE vegetables are purchased within 5 days and the remaining packages (30%) are purchased in the last 3 days of shelf-life. This approach was based on the study of Nauta, Litman, Barker, and Carlin (2003) for *Bacillus cereus* and cooked chilled vegetables. The growth of *Salmonella* and *L. monocytogenes* and their respective levels after retail storage were calculated as described in Section 2.1.

2.3. Transportation from retail to home

No consumer time and temperature transportation data in Brazil is currently available. A pert distribution was used in this module and the values were based on assumptions by the authors (Table 1). Minimum, most likely and maximum temperatures in this module were assumed to be 7 °C, 12 °C and 20 °C, respectively. Time of transportation was modeled as described by Nauta et al. (2003) (mean: 42.8 min; standard deviation: 18.7 min), using a Gamma distribution with 5.24 and 8.17 as parameters (Table 1). The growth during transportation from retail to home was calculated as described in Section 2.1.

2.4. Home storage module

Temperature during storage in home refrigerators was modeled using a pert distribution with minimum, most likely and maximum values of 3.1 °C, 6 °C and 10.8 °C, respectively, as extracted from Silva, Celidonio, and Oliveira (2008). Storage time was modeled by assuming that consumer behavior on storage of foods in the home refrigerator is influenced by shelf-life shown in the label in the moment of purchase as proposed by Nauta et al. (2003). A uniform distribution with 0 and 192 h as minimum and maximum values was used to model storage time at home. As the time of purchase and maximum shelf life are considered in the calculations proposed by Nauta et al. (2003), times of purchase (I and II) was calculated using time of transportation, storage time at retail and time of transportation from retail to home. Time of purchase used in the calculations of the model was determined using a discrete distribution. The logarithmic growth and level after home storage were calculated as described in Section 2.1.

Table 2
Data published in literature and selected for this study on the prevalence of *Salmonella* and *L. monocytogenes* in RTE leafy vegetables in Brazil.

Microorganisms	Samples		Reference
	Total	Positive	
Salmonella	166	4	Simões et al. (2001)
	172	1	Maistro (2006)
	111	4	Fröder et al. (2007)
	56	6	Tresseler et al. (2009)
	134	2	Oliveira et al., (2011)
	477	2	Sant'Ana et al. (2011)
Total	1116	19	–
L. monocytogenes	150	8	Porto and Eiroa (2001)
	172	2	Maistro (2006)
	111	1	Fröder et al. (2007)
	162	2	Oliveira et al. (2010)
	134	2	Oliveira et al. (2011)
	477	11	Sant'Ana et al. (2011)
Total	1206	26	–

2.5. Consumption of RTE vegetables node, determination of dose–response relationship, probability of illness and number of cases

The typical serving size of RTE vegetables as consumed by the Brazilian population is unknown. We assumed 25 g, 50 g and 75 g, as minimum, most likely and maximum serving sizes, respectively. The level of pathogens was calculated by summing their levels at the end of each module of the QMRA model (Table 1). The dose of pathogens per serving was calculated by multiplying amounts of vegetables consumed and the level of pathogen (Table 1). The exposure (number of servings of RTE vegetables intake per month) was obtained from Perez et al. (2008). The dose–response

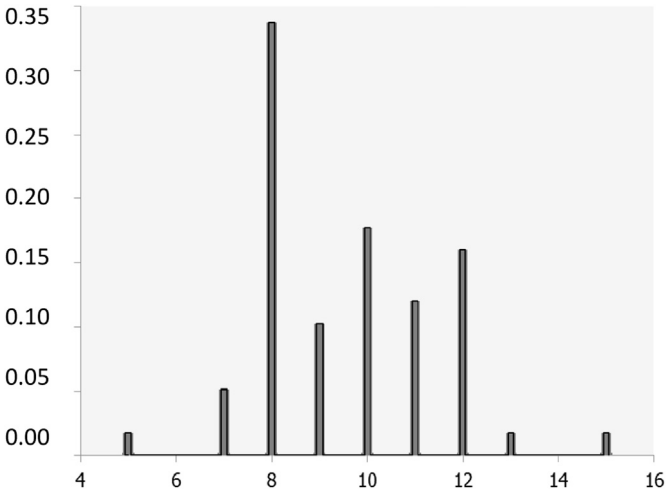

Fig. 1. Discrete distribution representing the temperature of retail storage of RTE vegetables as recorded by Maistro (2006).

relationship for infection by *Salmonella* was estimated using a beta-Poisson model as proposed by (WHO/FAO, 2002 , 329 p.). The parameters α and β were modeled using pert distribution. Minimum, most likely and maximum values were obtained from WHO/FAO (2002) , 329 p.. The dose–response relationship for infection by *L. monocytogenes* was determined using an exponential model (Buchanan, Damert, Whiting, & Van Schothorst, 1997). The r parameter was represented in the model by a pert distribution with values described by Mataragas, Zwietering, Skandamis, and Drosinos (2010). The outputs of the QMRA model were the risk of infection per month (probability of infection per month due to consumption of RTE vegetables) and number of cases (number of people that consumed RTE vegetables and get infected per month) in the exposed population (Table 1). The determination of number of cases of infection due to *Salmonella* and *L. monocytogenes* was calculated considering the population of Sao Paulo city, Brazil (IBGE, 2011) and assumption that approximately 23% of population eats RTE vegetables (Perez et al., 2008).

2.6. Evaluation of different scenarios

The QMRA model was used to simulate risk of infection and number of cases due to consumption of RTE vegetables contaminated with *Salmonella* and *L. monocytogenes* starting with lower prevalence of these pathogens (0.17% and 0.22%, respectively) and/or lower initial populations (−1.04 and −0.39 log CFU/g, respectively) (Table 3). In order to model the concentration of these pathogens, the values described above were used to replace the maximum value of a Pert distribution. The minimum and most likely values remained the same as in the real world scenario (scenario #1), *i.e.*, −3 log CFU/g (user input) and −1.4 CFU/g (estimated considering that 1 CFU was present in 25 g of positive samples according to prevalence study). Scenarios evaluating the impact of stricter control of temperature (maximum temperature always below 5 °C) from transportation to retail until home storage on risks and number of cases of infection were also studied (Table 3).

2.7. Simulation settings and analysis of models outputs

The QMRA model was built in an Excel spreadsheet (Microsoft, Redmond, WA) and simulated using @Risk software version 5.7.0 (Palisade Corporation). A total of 100,000 iterations for each scenarios created were run using Monte Carlo sampling and with the random generator seed fixed at 1 to ensure that results could be repeated, allowing comparisons of different scenarios.

Table 3
Outputs of the QMRA model depicting the risk of infection per month per serving and number of cases of infection per month in the population exposed due to consumption of RTE vegetables contaminated with *Salmonella* in Sao Paulo, Brazil[a].

Scenarios[b]	Prevalence (%)	Maximum population (log CFU/g)	Risk of infection per month per serving		Number of cases of infection per month in the population exposed	
			Mean	Upper 95%	Mean	Upper 95%
1	1.7	2.74	5.7E-03	3.1E-02	14,958	80,952
2	0.17	2.74	8.8E-04	4.3E-03	2294	11,226
3	1.7	−1.04	5.0E-04	2.2E-03	1313	5809
4	0.17	−1.04	7.6E-05	3.4E-04	197	903
5	1.7	2.74	5.5E-03	3.0E-02	14,556	78,253
6	0.17	2.74	8.6E-04	4.1E-03	2232	10,832
7	1.7	−1.04	4.7E-04	2.1E-03	1244	5495
8	0.17	−1.04	7.2E-05	3.3E-04	187	857

[a] Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.
[b] Scenario 1 was run with data representing the real world, while scenarios 2–8 represent changes in prevalence and concentration of pathogen. Scenarios 5–8 represent strict temperature conditions during steps of processing and storage of RTE vegetables studied. Stricter temperature control was modeled using a Pert distribution with 1 °C, 3 °C and 5 °C as minimum, most likely and maximum values.

3. Results and discussion

The current study was carried out to estimate the risks of infection by *Salmonella* and *L. monocytogenes* due to contamination of RTE vegetables consumed in Brazil. The five modules composing the QMRA model are shown in Table 1. Few studies were found reporting the prevalence and levels of foodborne pathogens in the field, and none in Brazil (Johnston et al., 2005; Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004; Schwaiger, Helmke, Hölzel, & Bauer, 2011). Moreover, information on the behavior of foodborne pathogens in leafy vegetables during field operations is scarce (Fonseca, Fallon, Sanchez, & Nolte, 2011; Islam, Doyle, Phatak, Millner, & Jiang, 2004, Islam, Morgan, et al., 2004; Tomás-Callejas et al., 2011). Thus, the fate of *Salmonella* and *L. monocytogenes* in the field and processing operations was not assessed in the current model. As noted recently by Danyluk and Schaffner (2011) the lack of data in these phases of the operation highlights an important data need. Because of the lack of data, it was assumed that RTE leafy vegetables leaving the processing facilities were contaminated with *Salmonella* and *L. monocytogenes* either in the field or during processing operations. Although the current model does not include these operations in the calculations, research should be done to generate these data because it is known that field and processing contamination seem to play an important role in the occurrence of foodborne disease outbreaks (Tauxe, 1997). These data would be very useful for improving accuracy of the QMRA models developed in this study as well as an aid in the development of risk management strategies.

As can be seen in Table 1, in the first module of QMRA model (transportation from produce facility to retail) data on prevalence and concentration of both pathogens were gathered from surveys carried out in Brazil (Fröder et al., 2007; Maistro et al., 2012; Oliveira et al., 2010, 2011; Porto & Eiroa, 2001; Sant'Ana, Igarashi, et al., 2012; Simões et al., 2001; Tresseler et al., 2009). Although these samples were collected in retail shops, in the QMRA model assumes these data represent prevalence and levels of pathogens (Table 2) as found in RTE vegetables just before transportation from processing facilities to retail (Table 1). In the current study, prevalence data were represented by beta distribution with α ($s + 1$; where s represents the number of positive samples) and β ($n - s + 1$; where n represents the number of samples analyzed) parameters being 20 and 1098 for *Salmonella*, and 27 and 1181 for *L. monocytogenes*, respectively (Table 1). Surveys carried out in Brazil have reported that the prevalence of *Salmonella* in RTE vegetables is well below 3% (Table 2). The prevalence of *L. monocytogenes* has been reported in levels of up to 5%, with most surveys showing prevalence of about 2% (Table 2). In addition, studies reporting levels of *Salmonella* and *L. monocytogenes* in RTE vegetables were sought. Data on concentration of *Salmonella* in RTE vegetables was found in only one study in Brazil (Sant'Ana et al., 2011), whereas *L. monocytogenes* have been reported in countable levels more frequently (Oliveira et al., 2010; Porto & Eiroa, 2001; Sant'Ana, Igarashi, et al., 2012). The maximum levels of *Salmonella* (2.7 log CFU/g) and *L. monocytogenes* (1.1 log CFU/g) found in these studies were used in the QMRA model to represent the possibility that few packages might harbor high levels of these microorganisms (Table 1). It is known that few packages presenting high levels of pathogens (at the extremes of statistical distributions) might be responsible for greatest portion of risks (Miller, Whiting, & Smith, 1997). Although the QMRA models were created with prevalence and concentration data available currently

(Table 2), it should be highlighted that more surveys to determine *Salmonella* and *L. monocytogenes* concentrations in fresh produce should be done using MPN or PCR-based techniques (Oliveira et al., 2010). These data would be very useful to improve QMRA models and may reduce uncertainty involved in their predictions (Lammerding, Fazil, & Paoli, 2001).

The only Brazilian study in which temperature was fully recorded during food transportation and delivery are those from Pereira (2008) , 173 p., and Pereira et al. (2010), who collected data on cooked ham. The temperature recorded at surface of primary packages were selected and used in the models because it was observed that temperature of products did not change in the same magnitude of the containers, although large variations occurred between deliveries, mostly when the doors of the truck were opened to unload the products (Pereira, 2008 , 173 p.; Pereira et al., 2010).

The increase in pathogen concentration in the modules of RTE vegetables commercialization and consumption chain were modeled using the predictive models generated in experiments to consider the variability in growth rate of three different strains of *Salmonella* and *L. monocytogenes* isolated from RTE vegetables Equations (2) and (3) (Sant'Ana, Igarashi, et al., 2012). Using this approach, no growth of *Salmonella* and *L. monocytogenes* in RTE vegetables is assumed if product temperature is below 4.6 °C or 1.6 °C, respectively. Thus, everywhere in the QMRA model when a temperature below T_0 was selected during iterations, zero growth was assigned and no increase in the initial concentration (module 1) was assumed (Table 1).

The temperature in retail storage module was represented by data recorded by Maistro et al. (2012). As can be seen in Fig. 1, a great percentage of data is above 7 °C, which indicates the storage of Brazilian RTE vegetables in retail stores is seldom what experts would recommend. Although these data are limited in scope, they do represent the single best effort to date to provide data on temperature of displays of RTE vegetables in Brazilian retail shops. The events involving purchase and consumption of RTE vegetables were based on the approach developed by Nauta et al. (2003). In our study, the relation between purchase and consumption of RTE vegetables was assessed considering that 70% of vegetables are consumed in less than 5 days, while the 30% remaining are consumed the last 3 days of shelf-life (total shelf-life of 8 days).

Table 4
Outputs of the QMRA model depicting the risk of infection per month per serving and number of cases of infection per month in the population exposed due to consumption of RTE vegetables contaminated with *L. monocytogenes* in Sao Paulo, Brazil[a].

Scenarios[b]	Prevalence (%)	Maximum population (log CFU/g)	Risk of infection per month per serving		Number of cases of infection per month in the population exposed	
			Mean	Upper 95%	Mean	Upper 95%
1	2.2	1.11	8.1E-06	2.1E-07	21	5.4E-01
2	0.22	1.11	1.0E-06	2.1E-08	2.7	5.5E-02
3	2.2	−0.39	1.6E-06	7.8E-08	4.1	2.0E-01
4	0.22	−0.39	1.9E-07	7.9E-09	4.9E-01	2.0E-02
5	2.2	1.11	5.2E-10	1.9E-09	1.3E-03	5.0E-03
6	0.22	1.11	5.6E-11	2.1E-10	1.5E-04	5.4E-04
7	2.2	−0.39	1.1E-10	5.3E-10	2.9E-04	1.4E-03
8	0.22	−0.39	1.2E-11	5.7E-11	3.3E-05	1.5E-04

[a] Each scenario was run in @Risk using 100,000 iterations with generator seed fixed at 1.

[b] Scenario 1 was run with data representing the real world, while scenarios 2–8 represent changes in prevalence and concentration of pathogen. Scenarios 5–8 represent strict temperature conditions during steps of processing and storage of RTE vegetables studied. Stricter temperature control was modeled using a Pert distribution with 1 °C, 3 °C and 5 °C as minimum, most likely and maximum values.

The main outputs of the QMRA models (risks of infection per month per serving and numbers of cases of infection in the population exposed) developed are shown in Tables 3 and 4. The first scenario (#1) represents the current knowledge regarding RTE vegetable and the pathogens studied, while scenarios #2–4 consider a reduction of prevalence and concentrations of each pathogen (Table 3). In the case of pathogen concentration, the reduction was only applied on maximum values. In actual practice, the intervention scenarios (#2–4) would represent the application of intervention measures either in the field or during processing to reduce the prevalence and populations of pathogens from those reported in Table 2. The lower prevalence and concentrations of pathogens tested in these intervention scenarios (#2–4) consider a reduction of in these parameters by 10 fold, e.g. a reduction of prevalence of *Salmonella* from 1.7% to 0.17%, or for *L. monocytogenes* prevalence from 2.2% to 0.22% (Table 3). It was assumed that measures to reach these targets need to be applied either in the field or processing. Despite this, it should be clear that the impact of field operations were not included in this model.

The QMRA simulations show that overall risks of foodborne disease due to consumption of RTE vegetables are higher for *Salmonella* than for *L. monocytogenes*. For example, in scenario #1 the mean risk of infection per serving per month for *Salmonella* is 5.7E-03, while for *L. monocytogenes* the risk is ~1000 lower or 8.1E-06 was predicted (Table 3). According to the models developed, scenario #1 would result in the highest number of cases of infection (14,958 and 21, for *Salmonella* and *L. monocytogenes*, respectively). Although these numbers can be considered high, it should be highlighted that the current QMRA model did not evaluate the probability of illness (*i.e.*, any derangement in the whole body function or any of its parts), but the probability of infection (*i.e.*, the invasion of pathogens in the body of hosts), which involves a series of complex events dependent upon interaction of infective agent, host and food determinants, among others (Putt, Shaw, Woods, Tyler, & James, 1988). Therefore, the results should be evaluated carefully as not all cases of infection predicted might result in disease.

Outbreaks involving *Salmonella* and fresh produce have been reported world-wide (Friesema et al., 2007; Little & Gillespie, 2008; Lynch et al., 2009; Sivapalasingam et al., 2004; Takkinen et al., 2005). Although the results of the simulations indicated that the risk of infection caused by *L. monocytogenes* is low (scenario #1: 8.1E-06), the potential of this food–pathogen combination should not be underestimated because *L. monocytogenes* is widely spread in the environment (Gandhi & Chikindas, 2007) and can be resistant to chlorine, a common sanitizer used in produce washing (Aarnisalo, Lundén, Korkeala, & Wirtanen, 2007). *L. monocytogenes* is also able to form biofilms which may allow its persistence in food processing environments (Harvey, Keenan, & Gilmour, 2007) and it also grows under chilled temperatures (Gandhi & Chikindas, 2007). RTE vegetables have been considered a category of low risk for listeriosis (Warriner & Namvar, 2009), however, the recent outbreak involving *L. monocytogenes* and cantaloupe in the USA might change this evaluation (CDC, 2011).

The analysis of scenarios #2–4 shows that the reduction of prevalence of *Salmonella* from 1.7% to 0.17% would lead to reduction in risk of infection per month of up to 6 times (Table 3). In the case of *L. monocytogenes*, the reduction of prevalence from 2.2% to 0.22% resulted in decrease of risk of infection by this microorganism from 8.1E-06 to 1.0E-06. The reduction of population of *L. monocytogenes* from 1.11 log CFU/g to −0.39 log CFU/g with a prevalence of 1.7% would mean a decrease of risk of infection per serving per month from 8.1E-06 to 1.6E-06 (Table 4).

Among the first set of scenarios studied (#1–4), the lowest number of cases of infection were predicted in scenario #4 (Tables 3

and 4). This indicates that interventions to reduce both prevalence and populations of pathogens would appear to be effective to reduce potential threats to food safety due to consumption of RTE vegetables. Danyluk and Schaffner (2011) reported that according to predictions of their risk assessment model for leafy greens and *E. coli* 0157:H7 up to 95.4% of cases found could be due to cross-contamination during washing step. Thus, cross-contamination during washing seems to play a critical role in RTE vegetable safety because depending on operating conditions, this step might serve as a point of contamination rather than an inactivation step (Gil, Selma, López-Gálvez, & Allende, 2009).

A further action to reduce the risk of infection by *Salmonella* and *L. monocytogenes*, would be to ensure that temperature is kept <5 °C during transportation and storage, and this was evaluated in scenarios #5–8. These scenarios were evaluated because it is known that maintenance of temperature during commercialization and transportation of food products is important, although food handlers and consumers may not be aware on how slight changes in temperature impact microbial growth and food safety. The risks and number of cases predicted by these temperature control scenarios (#5–8) were less for both pathogens studied when compared to scenarios #1–4 (Tables 3and 4). The reduction in risk and number of cases of infection was less pronounced for *Salmonella* than for *L. monocytogenes.* As *L. monocytogenes* is a microorganism showing markedly psychrotrophic behavior, the cold chain seems to have a greater contribution to reduce the risks associated with this pathogen and RTE vegetables (Table 4). Strategies aiming to reduce *Salmonella* prevalence and concentration in the raw vegetables in the field or during processing (washing) are expected to have more impact in risk mitigation for this pathogen (Table 3). Thus, depending on the food–pathogen combination, measures chosen will be more effective in reducing the risks of infection and predicted number of cases.

Epidemiological data have indicated that most outbreaks of salmonellosis and listeriosis are associated with foods of animal origin (Greig & Ravel, 2009). Despite this a recent trend on the occurrence of outbreaks linked to consumption of fresh produce has been observed (Lynch et al., 2009). Currently, few risk assessment models focusing on pathogens and fresh produce have been developed (Danyluk & Schaffner, 2011; Franz et al., 2010; Tromp et al., 2010). However, as these products normally have close contact with soil during field operations (Harris et al., 2003), and knowing that their microbiological safety relies mainly on washing (actually the only step able to reduce pathogen concentration) (Gil et al., 2009), the association of these products with foodborne disease outbreaks should not be discounted. More studies that take into account the diverse variables and factors affecting the fate of pathogens in produce should be developed. This need is confirmed by the recent large and severe outbreak involving *L. monocytogenes* and consumption of cantaloupes in the USA (CDC, 2011).

More data are always needed to improve the accuracy of risk assessment models, including those developed here. Despite this need, the results obtained here, even with the limited data available show that *Salmonella* (the most common foodborne pathogen linked to foodborne disease outbreaks in Brazil) does pose a measurable risk in Brazilian produce. Our results suggest that not only are more data needed, but that *Salmonella* mitigation strategies in fresh Brazilian produce need to be developed.

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