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Risk of infection with *Salmonella* and *Escherichia coli* O157: H7 due to consumption of lettuce in southern Brazil

Abstract

This study was carried out to estimate the risks of infection due to consumption of lettuce contaminated with these pathogens in Southern Brazil. The quantitative microbial risk assessment (QMRA) model comprised nine modules from storage of lettuce in producer farms until consumption. Scenarios were simulated using prevalence, concentration, and exposure levels lower than found in Brazil. Different procedures of washing and disinfection as well as cold chain (≤5ºC) in all distribution steps were also tested. Models built in Excel spreadsheet were simulated using @Risk® software. The QMRA simulations show that overall risks of foodborne disease due to consumption of lettuce are higher for *Salmonella* than for *E. coli* O157:H7. All alternative scenarios to clean lettuce, increase the risk (the best procedure was washing leaves with potable water followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 15 minutes and rinsing with potable water). The major risk reduction was due to cold chain scenario. Sensitivity analyses indicated that in addition to the maintenance of the cold chain and the washing and disinfection procedures, it is important to reduce the prevalence and concentration of pathogens on lettuce in fields, in order to decrease the risk of infection by these bacteria.

Keywords: QMRA, foodborne pathogen, leafy green, bacteria, vegetable, *Lactuca sativa*

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Introduction

Lettuce is the main produced and consumed leafy vegetable crop in Brazil and the Grand Rapids (crispa) type represents the main varietal segment growing in this country. This Brazilian preference for the crispa type is a unique event in world market of lettuce.[1] Although healthy benefits are attributed to the consumption of lettuce, foodborne diseases can happen if this vegetable is contaminated by pathogens, and this fact has been highlighted by the increased number of foodborne outbreaks linked with the consumption of this fresh produce worldwide.[2,3]

Salmonella spp. and *Escherichia coli* O157:H7 are the most common pathogenic bacteria that contaminate lettuce, causing many outbreaks in the world.[4] In Brazil, a total of 7170 foodborne disease outbreaks were reported between 2007 and 2017, and *E. coli* was the most identified foodborne pathogen, accounting for 525 of foodborne disease outbreaks, while *Salmonella*, which occupied the second place, was responsible for 515 outbreaks.[5] Rio Grande do Sul (RS), the southernmost State of Brazil, is one of the Brazilian states that most investigates and reports foodborne diseases outbreaks. Shigatoxin-producing *E. coli* (*E. coli* O157:H7) and *Salmonella* has been isolated from lettuce cultivated in this state, and from water used to irrigate this lettuce, indicating risk of illnesses due to consumption of this vegetable.[6-9]

The contamination of lettuce can occur at any point, from the farm to plate. As fresh produce is normally consumed raw or with minimum processing, it is important to keep the microbial load of fresh produce as low as possible to prevent foodborne illnesses.[10] Then, quantitative microbial risk assessment (QMRA) can be a very useful tool to help in this prevention. QMRA allows the quantitative estimation of the risks posed to public health by a food-pathogen combination.[11] The outputs of QMRA can be used in the development of scientific-based strategies in order to manage risks and safeguard public health.[12] Given the above and considering the increasing consumption of these vegetable in Brazil,[1] this study was carried out to estimate the risks of infection due to consumption of lettuce contaminated with *Salmonella* and *E. coli* O157:H7 in Southern Brazil.

Materials and methods

Models development

The risk assessment model comprised nine different modules from storage of lettuce in the producer farms until consumption. Table 1 summarizes the cells on Excel spreadsheet used for subsequent risk calculations. The first column (symbol) represents the spreadsheet cell designation of the variable on that line of the table. This label is needed to understand how the variable links to the other variables in the risk assessment. The next column (event) is a text description of the variable. The third column (values) is either a number, a simple formula, or an @RISK formula representing the value of the cells. The fourth column (unities) represents the units of the variables. The last column (source) represents the source of the information used to determine the value of the variables. The source can be an assumption or a literature citation or was calculated from other cells in the spreadsheet.

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Table 1 The risk assessment models of infection by Salmonella and *E. coli* O157:H7 due to consumption of lettuce in Southern Brazil

Symbol	Event	Values	Unities	Source
1- Producer storage				
Pi	Prevalence	Salmonella RiskBeta (127, 1589)	%	Elias et al.[13]
		E. coli O157:H7 RiskBeta (4, 102)		
Ci	Concentration	Salmonella RiskPert (0, 0.66, 2.34)	Log MPN/g	Elias et al.[13]
		E. coli O157:H7 RiskPert (0, 0.47, 3.04)		
T1	Temperature during storage1	RiskPert (3.55,18.12, 33.56)	°C	INMET[15]
t1	Time of storage1	RiskPert (1, 10, 12)	h	Ascal[14]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg1	Logarithmic growth	Salmonella (0.0339x(If(T1 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T1 - T0) < 0,0, (T1 - T0)))2		
G1	Growth during storage1	t1xLg1	log cfu/g	Calculated
L1	Level after storage1	Ci+G1	log cfu/g	Calculated
2- Transportation from producer to distribution center				
T2	Temperature during transportation1	RiskPert (3.55,18.12, 33.56)	°C	INMET[15]
t2	Time of transportation1	RiskPert(0.5, 1.8, 4)	h	Ascal[14]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg2	Logarithmic growth	Salmonella (0.0339x(If(T2 - T0) < 0,0, (T2 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T2 - T0) < 0,0, (T2 - T0)))2		
G2	Growth during transportation1	t2xLg2	log cfu/g	Calculated
L2	Level after transportation1	L1+G2	log cfu/g	Calculated
3- Arrival and storage at distribution center				
T3	Temperature during dc storage	RiskPert (10, 15, 20)	°C	Ascal[14]
t3	Time of dc storage	RiskPert (1, 10, 12)	h	Ascal[14]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg3	Logarithmic growth	Salmonella (0.0339x(If(T3 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T3 - T0) < 0,0, (T1 - T0)))2		
G3	Growth during dc storage	t3xLg3	log cfu/g	Calculated
L3	Level after dc storage	L2+G3	log cfu/g	Calculated

Table Continued...

Symbol	Event	Values	Unities	Source
4- Transportation from distribution center to market				
T4	Temperature during transportation2	RiskPert (3.55,18.12, 33.56)	°C	INMET[15]
t4	Time of transportation2	RiskPert(0.05, 0.65, 4)	h	Ascal[14]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg4	Logarithmic growth	Salmonella (0.0339x(If(T4 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T4 - T0) < 0,0, (T1 - T0)))2		
G4	Growth during transportation2	t4xLg4	log cfu/g	Calculated
L4	Level after transportation2	L3+G4	log cfu/g	Calculated
5- Market storage				
T5	Temperature during market storage	RiskPert(20, 22.5 , 25)	°C	Ascal[14]; Missiae[n]8
t5	Time of market storage	RiskUniform(0,17)	h	Ascal[14]; Missiaen[8]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg5	Logarithmic growth	Salmonella (0.0339x(If(T5 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T5 - T0) < 0,0, (T1 - T0)))2		
G5	Growth during market storage	t5xLg5	log cfu/g	Calculated
L5	Level after market storage	L4+G5	log cfu/g	Calculated
6- Transportation from retail to home				
T6	Temperature during transportation3	RiskPert(10,20,30)	°C	Assumption
t6	Time of transportation3	RiskGamma(5.24,8.17)/60	h	Nauta et al.[16]
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg6	Logarithmic growth	Salmonella (0.0339x(If(T6 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T6 - T0) < 0,0, (T1 - T0)))2		
G6	Growth during transportation3	t6xLg6	log cfu/g	Calculated
L6	Level after transportation3	L5+G6	log cfu/g	Calculated
7- Home storage				
T7	Temperature during home storage	RiskPert(3.04,6,10.8)	°C	Silva et al.[17]
t7	Time of home storage	RiskUniform(0,120)	h	Borghi et al.[18]

Table Continued...

Symbol	Event	Values	Unities	Source
b	Parameter b growth model	Salmonella 0.0339	$\sqrt{\text{Log CFU/day/°C}}$	Elias et al.[37]
		E. coli O157:H7 0.025		
T0	Parameter T0 growth model	Salmonella 1.92	°C	Elias et al.[37]
		E. coli O157:H7 0.408		
Lg7	Logarithmic growth	Salmonella (0.0339x(If(T7 - T0) < 0,0, (T1 - T0)))2	log CFU/g/h	Elias et al.[37]
		E. coli O157:H7 (0.025x(If(T7 - T0) < 0,0, (T1 - T0)))2		
G7	Growth during home storage	t7xLg7	log cfu/g	Calculated
L7	Level after home storage	L6+G7	log cfu/g	Calculated
8- Washing and disinfection				
Rw	Log reduction by washing and disinfection	Salmonella RiskNormal(5.83,0.82)	log cfu/g	Silveira et al.[20]; de Paula[21]
		E. coli O157:H7 RiskNormal(6.27,0.50)		
L8	Log concentration after washing	L7-Rw	log cfu/g	Calculated
9- Consumption of lettuce, determination of dose-response relationship, probability of illness and number of cases				
S	Serving size	RiskTriangle (20; 30; 50)	g	Carlos et al.[22]
CFU	Level of pathogen (non-log)	10^L8	CFU/g	Calculated
D	Dose per serving	SxCFU	CFU	Calculated
α	Parameter alpha	Salmonella 0.1324	No units	WHO/FAO[26], Cassin et al.[27]
		E. coli O157:H7 0.267		
β	Parameter beta	Salmonella 51.45	No units	WHO/FAO[26], Cassin et al.[27]
		E. coli O157:H7 229.2928		
Pisd	Probability of infection single dose	1-(1+D/β)ˆ-α	%	Calculated
E	Exposure (number of servings/month)	RiskDiscrete({28\30\31},{0.083\0.417\0.5}	Servings	Mattos et al.[24]; Krzyzanowski et al.[23]; Souza et al.[25]
Rim	Risk of infection per month	RiskOutput() + 1 - (1 - Pi x Pisd)^E		Calculated
	Population RS	11322895	Inhabitants	IBGE[29]
%eat	% of population eating lettuce	16	%	Souza et al.[25]
Peat	Population RS eating lettuce	1811663	Inhabitants	Calculated
Nc	Number of cases in population exposed	RimxPeat		Calculated

Producer storage: Data on prevalence and levels of *Salmonella* and *E. coli* O157:H7 on lettuce were gathered from a systematic review metanalysis.[13] Few data on prevalence and populations of both pathogens on lettuce in Brazil were available in the literature. Because of this, the values chosen to be used represent the developing countries in the systematic review metanalysis. The prevalence of both pathogens was represented in the model by Beta distribution, while the concentration was modeled using Pert distribution (Table 1). Temperature and time during producer storage of lettuce were modeled based on data obtained from Ascal.[14] This author studied the distribution chain of lettuce from farms until retail in an important supermarket network in Southern Brazil. The data from time storage depends on when the lettuce was harvested, being that the most common process is doing the harvest at evenings and transporting it in the next morning. Then, generally, the lettuce remains at environment temperature for approximately 10h (most probable), waiting to be transported. Sometimes lettuce is harvested and transported in the same day, then the minimum transport period was considered 1h, while the maximum period was assumed 12h. These values were reported by Ascal[14] and were used to describe storage time in the farms using a Pert distribution (Table 1). The temperatures were based on the data collected by INMET (National Institute of Meteorology)[15], and represent the annual averages of temperature in RS that were: 33.56ºC (maximum), 3.55ºC (minimum) and 18.12ºC (most probable). These values were used to describe storage temperature in the farms using a Pert distribution (Table 1). The growth of *Salmonella* and *E. coli* O157:H7 was described by the relationship between growth rate and temperature represented by the models in the study of Elias et al. (2018). The growth of both pathogens during storage was calculated by multiplying predicted growth by time of storage at a given temperature. The concentration after transportation was the sum of initial concentration of each pathogen and subsequent growth during this step.

Transportation from producer to distribution center: Time and temperature during transportation of lettuce from producers to distribution center were modeled based on data obtained from Ascal.[14] Considering different producers in different cities that produce lettuce to the referred supermarket network the mean distance from farm to the main distribution center was 133.11 km, while the maximum route was 289 km, and the minimum was 16 km. Then, the transportation time was considered 1.8h (most probable), 0.5h (minimum), and 4h (maximum). A Pert distribution was used to model transportation time from farms to distribution center (Table 1). The temperature during the transportation was based on the data collected by INMET, already described in Section 2.1.1, because some vehicles do not have refrigeration. The growth of *Salmonella* and *E. coli* O157:H7 and their respective levels after transportation were calculated as described in Section 2.1.1

Arrival and storage at distribution center

Time and temperature during storage of lettuce at distribution center were modeled based on data obtained from Ascal.[14] The storage time reported in this study was 10h (most probable), ranging from 1h (minimum) to 12h (maximum). A Pert distribution was used to model storage time at distribution center (Table 1). The temperature during the storage was 15ºC (most probable temperature inside cold chambers), 10ºC (minimum), and 20ºC (maximum). A Pert distribution was used to model storage temperature at distribution center (Table 1). The growth of *Salmonella* and *E. coli* O157:H7 and their respective levels after storage were calculated as described in Section 2.

Transportation from distribution center to markets

Time and temperature during transportation of lettuce from distribution center to markets were modeled based on data obtained from Ascal.[14] Considering all markets of this network in the RS the mean distance from distribution center to them was 39 km, while the maximum route was 291 km, and the minimum was 1.6 km. Then, the most probable transportation time was considered 0.65h, 0.05h (minimum), and 4h (maximum). A Pert distribution was used to model transportation time from distribution center to markets (Table 1). The temperature during the transportation was based on the data collected by INMET, the same as cited in Section 2.1.1, because the vehicles do not have refrigeration. The growth of *Salmonella* and *E. coli* O157:H7 and their respective levels after transportation were calculated as described in Section 2.1.1

Market storage: Time and temperature during storage of lettuce at market were modeled based on data obtained from Ascal[14] and Missiaen.[8] The latter studied lettuces marketed in Southern Brazil. The storage time at markets reported in these studies were one day, all markets are supplied of lettuces every day. A uniform distribution was used to model storage time at market (Table 1). The most probable temperature during the storage was considered 22.5ºC, while the minimum was 20ºC, and the maximum 25ºC. A Pert distribution was used to model storage temperature at markets (Table 1). The growth of *Salmonella* and *E. coli* O157:H7 and their respective levels after storage were calculated as described in Section 2.1.1.

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 10ºC, 20ºC and 30ºC, respectively. Time of transportation was modeled as described by Nauta et al.[16] (mean: 42.8 min; standard deviation: 18.7 min), using a Gamma distribution with 5.24 and 8.17 as parameters (Table 1). The bacterial growth during transportation from retail to home was calculated as described in Section 2.1.1.

Home storage: Temperature during storage in home refrigerators was modeled using a Pert distribution with minimum, most likely and maximum values of 3.1ºC, 6ºC and 10.8ºC, respectively, as extracted from Silva et al.[17] Storage time was modeled by assuming that consumers behavior on storage of foods in their home refrigerator is influenced by the organoleptic properties. Borghi et al.[18] studied the storage of lettuce *in natura*, and found that the maximum shelf-life considering organoleptic properties of lettuce in these cited conditions was approximately 120h. A uniform distribution with 0 and 120h as minimum and maximum values, respectively, was used to model storage time at home. The logarithmic growth and level after home storage were calculated as described in Section 2.1.1.

Washing and disinfection: It was assumed that lettuce is washed and disinfected before the consumption at home, using the same procedures preconized to the Brazilian food services described by the RS regulation Portaria 78/2009.[19] The process considered the washing of lettuce using potable water followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 15 minutes and rinsing with potable water. Silveira et al.[20] studied the *Salmonella* reduction, using this procedure, reported levels of 5.83±0.82 log CFU/g, while De Paula[21] demonstrated reductions levels of 6.27±0.50 log CFU/g for *E. coli* O157:H7, using the same process; in both studies bacteria were artificially inoculated on lettuce. These levels of reductions were used in the present study. A normal distribution was used to model the washing and disinfection of lettuce. The concentration after sanitation was calculated by subtracting the concentration after home storage of each pathogen and subsequent reduction after washing and disinfection steps.

Consumption of lettuce, determination of dose-response relationship, probability of illness and number of cases: The typical serving size of lettuce as consumed by the Brazilian population was studied by Carlos et al.[22]; the values were 20g, 30g and 50g, as minimum, most likely and maximum serving sizes, respectively. The distribution used was triangular.[23] The level of pathogens was calculated by summing or subtracting 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 lettuce intake per month) was obtained from Mattos et al.[24], Krzyzanowski et al.[23], and Souza et al.[25], who reported that the consumption of lettuce in Brazil occurs every day. The dose-response relationship for infection by *Salmonella* was estimated using a beta-Poisson model as proposed by WHO/FAO.[26] Also, the values of parameters α and β were obtained from WHO/FAO.[26] The dose-response model for *E. coli* O157:H7 was based on that developed by Cassin et al.[27], using *E. coli* O157:H7 in ground beef. Cassin et al.[27] proposed a beta-binomial model that predicts the probability of illness from a particular dose. We simplified the Cassin et al.[27] beta-binomial model, converting it back into a simple beta-Poisson model[26] that specifies a mean population risk.[28] The outputs of the QMRA model were the risk of infection per month (probability of infection per month due to consumption of lettuce) and number of cases (number of people that consumed lettuce and get infected per month) in the exposed population (Table 1). The determination of number of cases of infection due to *Salmonella* and *E. coli* O157:H7 was calculated considering the population of RS, Brazil[29] and assuming that approximately 16% of population eats lettuce.[25]

Evaluation of different scenarios: The QMRA model was used to simulate risk of infection and number of cases due to consumption of lettuce contaminated with *Salmonella* and *E. coli* O157:H7 in several scenarios showed in Tables 2 & 3, respectively. The scenario 1 was composed by all inputs of Table 1 and represent the real-world conditions. The scenarios 2-4 represented the decrease in prevalence and/or levels of *Salmonella* and *E. coli* O157:H7; scenario 2 used half of the prevalence of these pathogens (3,2% and 1,2%, respectively). Scenario 3 used half of the concentrations values for *Salmonella* was used: RiskPert (0, 0.33, 1.17), while for *E. coli* O157:H7 was used: RiskPert (0, 0.235, 1.52). Scenario 4 combined the reductions of both scenarios 2 and 3. Also, scenario 5 considered less consumption frequency of lettuce by population. The exposure adopted by scenario 5 was half of the exposure used in the real-world scenario (15 days per month).

Table 2 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 lettuce contaminated with *Salmonella* in Southern Brazil[a]

Scenarios		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	Real	0.017183	0.023348	31129.81	42298.71
2	-50% prevalence	0.007467	0.009571	13527.69	17339.43
3	-50% concentration	0.016629	0.023228	30126.15	42081.31
4	-50% prevalence and concentration	0.007233	0.009527	13103.76	17259.72
5	-50% exposure	0.008493	0.011531	15386.46	20890.29
6	Only washing	0.02105	0.024645	38135.51	44648.44
7	1 min disinfection	0.018075	0.023609	32745.81	42771.56
8	5 min disinfection	0.018881	0.023844	34206.01	43197.3
9	Cold chain	3.16E-07	1.03E-06	0.572486	1.866013
10	Cold chain and only washing	0.00185	0.004781	3351.577	8661.562

[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. Scenario 2 represents change in prevalence of pathogen; scenario 3 represents change in concentration of pathogen; scenario 4 represents change in prevalence and concentration of pathogen. Scenario 5 represents change in exposure of population. Scenario 6 represents only lettuce washing; scenarios 7-8 represent change in time of disinfection. Scenario 9 represent strict temperature conditions during all steps of lettuce chain studied. Scenario 10 represent strict temperature conditions and the only lettuce washing

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 lettuce contaminated with *E. coli* O157:H7 in Southern, Brazil

Scenarios		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	Real	0.006093	0.016284	11038.46	29501.12
2	-50% prevalence	0.003218	0.003713	5829.932	6726.705
3	-50% concentration	0.005446	0.015584	9866.318	28232.96
4	-50% prevalence and concentration	0.003162	0.003713	5728.479	6726.705
5	-50% exposure	0.002988	0.007985	5413.25	14466.13
6	Only washing	0.010835	0.020848	19629.37	37769.55
7	1 min disinfection	0.009852	0.019787	17848.51	35847.38
8	5 min disinfection	0.010228	0.020158	18529.69	36519.51
9	Cold chain	2.27E-08	7.81E-08	0.041125	0.141491
10	Cold chain and washing	0.000461	0.001892	835.1767	3427.667

[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. Scenario 2 represents change in prevalence of pathogen; scenario 3 represents change in concentration of pathogen; scenario 4 represents change in prevalence and concentration of pathogen. Scenario 5 represents change in exposure of population. Scenario 6 represents only lettuce washing; scenarios 7-8 represent change in time of disinfection. Scenario 9 represent strict temperature conditions during all steps of lettuce chain studied. Scenario 10 represent strict temperature conditions and the only lettuce washing

Furthermore, different washing and disinfection procedures were tested: scenario 6 considered washing of lettuce using only potable water [reducing 0.97±0.18 for *Salmonella*, and 1,49±0.18 for *E. coli* O157:H7 according to Silveira et al.,[20] and De Paula et al.[21])]; scenario 7 considered washing with potable water followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 1 minute and rinsing with potable water [reducing 5.11±0.82 for *Salmonella*, and 3.27±0.27 for *E. coli* O157:H7 according to Silveira et al.,[20] and De Paula et al.[21]]; and scenario 8 considered washing of lettuce with potable water followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 5 minutes and rinsing with potable water [reducing 4.41±0.48 for *Salmonella*, and 2.76±0.26 for *E. coli* O157:H7 according to Silveira et al.,[20] and De Paula et al.[21]]. The scenario 9 evaluated the impact of control of temperature (maximum temperature 5ºC) from storage at farms until home storage on risks and on number of cases of infection, it was modeled using a Pert distribution with 1ºC, 3ºC and 5ºC as minimum, most likely and maximum values. The last scenario (10) considered lettuces submitted to entire cold chain control with the "only washing" procedure.

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 7.5 (Palisade Corporation). A total of 100,000 iterations for each scenario created was 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. Spearman's correlation coefficients were used for sensitivity analysis of the real-world model and the scenario 9 (cold chain) to determine the effect of input variables on the probability of illness per serving and on the number of illness cases in RS per month.

Results and discussion

The present study was carried out to estimate the risks of infection by *Salmonella* and *E. coli* O157:H7 due to contamination of lettuce consumed in Southern Brazil, based on the distribution chain of lettuces of RS. The nine modules composing the QMRA model are shown in Table 1. Although there are some studies that identified these pathogens in the field in RS, no of them reported the behavior of these bacteria in leafy vegetables during field operations.[6,7,9] Thus, the fate of *Salmonella* and *E. coli* O157:H7 in the field operations was not assessed in the current model. The risk factors influencing the pathogens occurrence and growth in primary production of leafy greens are: temperature, precipitation, flooding, the presence domestic and wild animals, irrigation water soucers, topography, fertilizers, and hygiene practices of farmers.[30-32] However, the scarcity of data about this issue in Brazil do not permit its consideration in the present model. These data would be useful for improving the accuracy of QMRA models developed in this study, as well as could help in the development of risk management strategies. Moreover, to the best of our knowledge, this is the first study examining microbial growth and reductions during distribution chain of lettuce in Brazil.

In the first module of QMRA model (producer storage) we presented the prevalence and concentration of pathogens. These data were extracted from the Elias et al.,[13] that reported the prevalence and levels of pathogens on lettuce. Few studies were found describing this data in the fields in Brazil, because of this it was considered all developing countries data in the present QMRA. Besides this, some samples of Elias et al.,[13] study were collected in retail shops, but the current QMRA model assumes that these data represent prevalence and levels of pathogens as found on lettuce just after harvest. In relation to temperatures, the only Brazilian study in which temperature was measured on the lettuce head was Missiaen.[8] In the other cases it was used environmental or equipment (cold chamber, refrigerators) temperatures. Even with these data limitations, the present study is important, since few QMRA have been performed in developing countries.[33,34] Furthermore, risk assessment is a valuable alternative when surveillance data are nonexistent or sparse, and the development of a QMRA offers a scientific basis approach for risk management, providing ranks of the most effective risk management options.[35,36]

The increase in pathogen concentration in the modules of current study were modeled using the predictive models generated in experiments in order to consider the variability of growth rates of six strains of *Salmonella* and four strains of *E. coli* O157:H7 isolated from food in Brazil.[37] Using this approach, no growth of *Salmonella* and *E. coli* O157:H7 on lettuce was assumed if product temperature was below 1.92ºC or 0.408ºC, respectively. Thus, in the QMRA model, when a temperature below T0 was selected during iterations, zero growth was assigned and no increase in the initial concentration (module 1) was assumed (Table 1). 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 2 (*Salmonella* results) and 3 (*E. coli* O157:H7 results). In general, the QMRA simulations show that overall risks of foodborne disease due to consumption of lettuce are higher for *Salmonella* than for *E. coli* O157:H7. This can be explained, because the prevalence of *Salmonella* on lettuce is higher than *E. coli* O157:H7. Also, the growth rates of *Salmonella* (in most tested temperatures) are slightly higher than *E. coli* O157:H7 ones. The first scenario (real-world) represents the current knowledge regarding lettuce and the pathogens studied, while scenarios 2-4 consider a reduction of prevalence and/or concentrations of each pathogen.

These scenarios (2-4) would represent the application of intervention measures in the field before harvest to reduce the prevalence and levels of pathogens. Table 2 shows in scenario 2 the reduction of prevalence of *Salmonella* from 6.4% to 3.2%, representing 56.5% of reduction in the risk of infection per month per serving in relation to scenario 1. A similar reduction was seen (58%) when the prevalence and the concentration were reduced by half (scenario 4). However, less than 5% of reduction was observed when only the concentration was reduced by half (scenario 3). In Table 3 is possible to observe that reductions in the scenarios 2-4 were similar. In the scenario 2, *E. coli* O157:H7 prevalence was reduced from 2.4% to 1.2% and the number of cases of infection per month in the population exposed reduced 47,2%. When prevalence and concentration were reduced (scenario 4) by half, the number of cases reduced 48,1%, and in scenario 3 the reduction was nearly 10%. This indicates that interventions to reduce prevalence of pathogens would be more effective than measures to reduce the concentration of pathogens on lettuces, regarding the risk of illnesses caused by both microorganisms.

Scenario 5 simulated a reduction of approximately 50 % in the population exposure, reducing lettuce consumption to 3-4 times per week instead of eating lettuce every day. A decrease of 50.6% in the risk of infection by *Salmonella* per month per serving was observed in relation to scenario 1 (Table 2). Similarly, the number of cases of infection by *E. coli* O157:H7 per month in the population exposed was reduced 51% when the consumption was reduced by half (Table 3). It means that people who consume lettuce fewer times a week have a lower risk of contamination by these pathogens. It is important to highlight that the number of infection cases do not represent the number of illnesses, but the number of people contaminated by the microorganisms. The development of disease will depend on the immunity of the person contaminated with the pathogen, the infections dose and the severity of the strain.[38]

The scenarios 6-8 showed different procedures to clean lettuce before eating. The scenario 1 represents the procedure recommended to food services described by the regulation of RS (washing leaves with potable water followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 15 minutes and rinsing with potable water). The scenario 6 considered only the procedure of washing the lettuce using potable water; while the scenario 7 comprehend the washing followed by immersion in 200 ppm of sodium hypochlorite sanitizer for only 1 minute and rinsing with potable water; and scenario 8 considered washing followed by immersion in 200 ppm of sodium hypochlorite sanitizer for 5 minutes and rinsing. In Tables 2 & 3, is possible to observe the scenarios 6-8 increased the risk of infection by *Salmonella* and *E. coli* O157:H7 per month per serving in relation to scenario 1. Thus, it is strongly recommended to adopt the procedure recommended by Portaria 78/2009,[19] for being the most effective method to reduce counts of both pathogens.

The scenario 9 considered that lettuce was kept from 1ºC to 5ºC during all steps of the QMRA model, simulating a well-controlled cold chain. In Tables 2 & 3, it is possible to observe that this condition was very effective to decrease the risk of infection, since the number of cases of infection by *Salmonella* and *E. coli* O157:H7 per month in the population exposed was reduced to less than 1 case per month. In other words, the number of *Salmonella* cases would be approximately 7 per year, while for *E. coli* O157:H7 would be nearly 1 case every 2 years. In majority of cities of Brazil, lettuce is not kept refrigerated, from the harvest to the final consumer, which makes this vegetable distribution one of the major bottlenecks to the expansion of this sector.[1] Even considering that ready-to-eat (RTE) vegetables that must be kept under cold chain in Brazil, it is noticed that a great percentage of temperature of displays in the supermarkets is above 7ºC, which indicates the storage of Brazilian RTE vegetables in retail stores is seldom what experts would recommend.[12] Then, it is strongly recommended to maintain the lettuce until 5ºC to kept it safe to consume, also increasing the quality and shelf-life of this vegetable. Besides this, the adoption of cold chain could contribute to the increase of acceptance and sales of lettuce.

The last scenario (10) joined the cold chain with the "only washing" procedure to clean lettuce representing the safer and the riskier procedures, respectively. In Tables 2 & 3 it is possible to observe that this scenario showed the lowest risk of infection by *Salmonella* and *E. coli* O157:H7 per month per serving when compared to the others excepted scenario 9 (cold chain). Based on our results, the best manner to reduce the risk of infection by *Salmonella* and *E. coli* O157:H7 is to keep lettuce refrigerated ≤5ºC and carry out washing and disinfection procedure stablished by Portaria 78/2009 (Rio Grande do Sul, 2009). The sensitivity of the baseline model (scenario 1) outcomes to input values and model parameters, determined by Spearman's rank order correlation, revealed that the mean number of illness cases per year was most sensitive to time of market storage (t5), for both pathogens (Figures 1A & 2A). In second place was temperature during producer storage (T1), followed by the *Salmonella* prevalence on lettuce (Figure 1A), while to *E. coli* O157:H7 occurred the opposite, prevalence in second place, followed by temperature during producer storage (T1) (Figure 2A). The same sensitivity analysis was applied to scenario 9 (cold chain), and revealed that the mean number of illness cases per year was most sensitive to reduction by washing and disinfection (Rw) and concentration of pathogens on lettuce for both bacteria (Figures 1B & 2B). Then, in addition to the maintenance of the cold chain and the washing and disinfection procedures, it is important to reduce the prevalence and concentration of pathogens on lettuce in fields, in order to decrease the risk of infection by these bacteria.

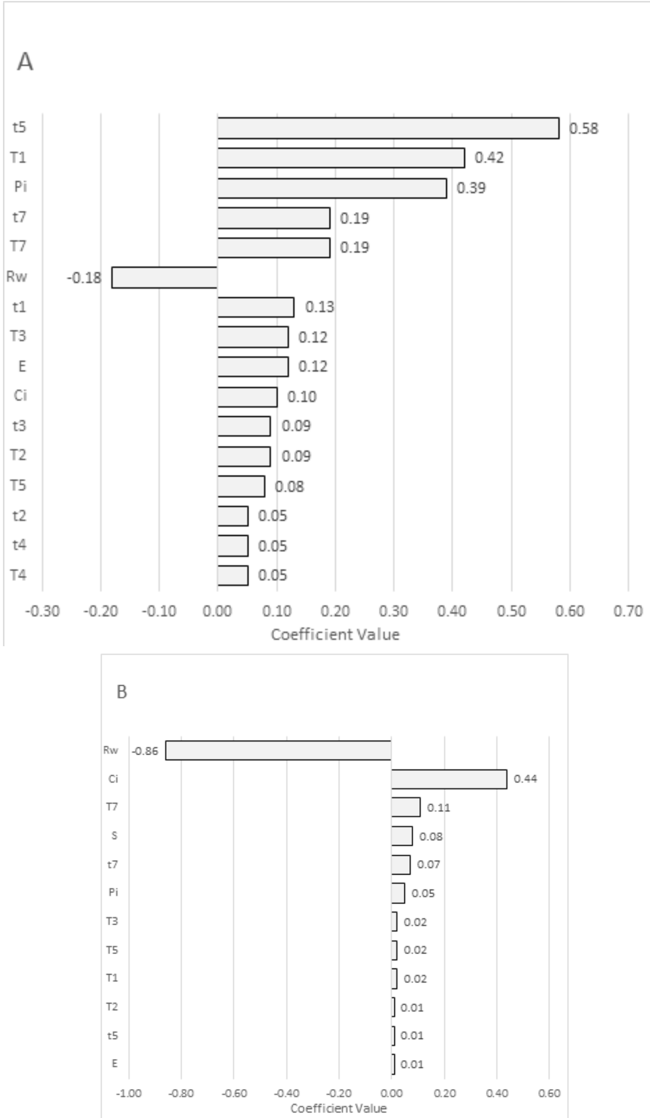
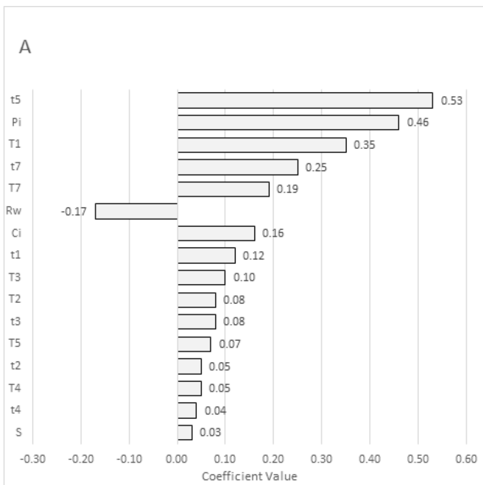


Figure 1 Tornado graph showing the most important parameters and variables (Table 1 symbols) affecting the estimated number of illness cases per month due to consumption of *Salmonella*–contaminated lettuce. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar.
A: real-world scenario; B: cold-chain scenario.



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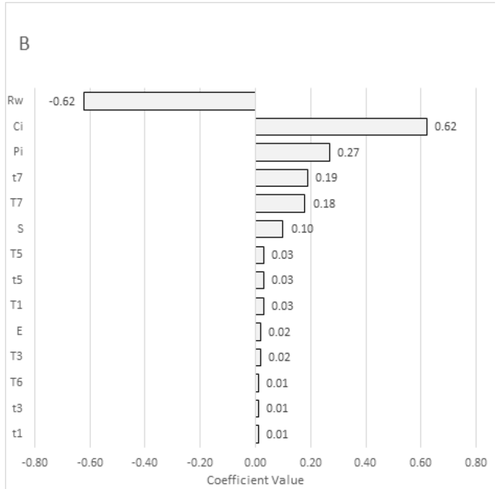

Figure 2 Tornado graph showing the most important parameters and variables (Table 1 symbols) affecting the estimated number of illness cases per month due to consumption of *E. coli* O157:H7–contaminated lettuce. Spearman's correlation coefficients were obtained from @Risk sensitivity analyses and were shown next to each bar.

A: real-world scenario; B: cold-chain scenario.

Conclusion

The developed QMRA model estimated the risk associated with consumption of *Salmonella* and *E. coli* O157:H7 contaminated lettuce. However, more data are always needed to improve the accuracy of risk assessment models,[12] including these developed here. Despite this need, the results obtained by our study demonstrated that *Salmonella* and *E. coli* O157:H7 represent measurable risks in lettuce of Southern Brazil, guiding intervention strategies to mitigate the risk. Our results suggest that *Salmonella* and *E. coli* O157:H7 risk of infection by lettuces can be best mitigated adopting cold chain to all steps of lettuce distribution and by performing correct washing and disinfection procedure before eating.

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Conflicts of interest

None.

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