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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