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ARTICLE

Microbial Risk Assessment of Non-Enterohemorrhagic *Escherichia coli* in Natural and Processed Cheeses in Korea

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Abstract

This study assessed the quantitative microbial risk of non-enterohemorrhagic Escherichia coli (EHEC). For hazard identification, hazards of non-EHEC E. coli in natural and processed cheeses were identified by research papers. Regarding exposure assessment, non-EHEC E. coli cell counts in cheese were enumerated, and the developed predictive models were used to describe the fates of non-EHEC E. coli strains in cheese during distribution and storage. In addition, data on the amounts and frequency of cheese consumption were collected from the research report of the Ministry of Food and Drug Safety. For hazard characterization, a doseresponse model for non-EHEC E. coli was used. Using the collected data, simulation models were constructed, using software @RISK to calculate the risk of illness per person per day. Non-EHEC E. coli cells in natural- (n=90) and processed-cheese samples (n=308) from factories and markets were not detected. Thus, we estimated the initial levels of contamination by Uniform distribution \times Beta distribution, and the levels were -2.35 and -2.73 Log CFU/g for natural and processed cheese, respectively. The proposed predictive models described properly the fates of non-EHEC E. coli during distribution and storage of cheese. For hazard characterization, we used the Beta-Poisson model (α =2.21×10⁻¹, N₅₀=6.85×10⁷). The results of risk characterization for non-EHEC E. coli in natural and processed cheese were 1.36×10-7 and 2.12×10⁻¹⁰ (the mean probability of illness per person per day), respectively. These results indicate that the risk of non-EHEC E. coli foodborne illness can be considered low in present conditions.

Keywords microbial risk assessment, Escherichia coli, cheese, exposure assessment

Introduction

Cheese consumption has been increasing gradually in Korea since the 1990s (KDC, 2016), but the cases of contamination with *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* have been reported (Jo *et al.*, 2007; Tekinsen and Özdemir, 2006; Thayer *et al.*, 1998). Especially, *E. coli* has been isolated from various cheeses in many countries (Haran *et al.*, 2011; Zinke *et al.*, 2012).

E. coli, a facultative anaerobic Gram-negative bacillus, is commonly found in the intestinal flora of humans and animals, and certain strains are pathogenic (MFDS, 2010; Olsvik *et al.*, 1991). According to infection symptoms and pathogenesis, pathogenic *E. coli* strains are classified e.g., enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC) (Nataro and

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Yohan Yoon Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea Tel: +82-2-2077-7585 Fax: +82-2-710-9479 E-mail: yyoon@sookmyung.ac.kr Kaper, 1998; Yoon, 2009). Among the pathogenic *E. coli* strains, *E. coli* O157:H7 is one of the major concerns in the dairy industry, and the survival of the pathogens in various cheeses has been well documented (Griffin and Tauxe, 1991; Reitsma and Henning, 1996). Thus, several countries (EU, USA, and Canada) have a quantitative standard or "zero tolerance" policy for control of the pathogens in cheese (EC, 2005; FDA, 2009; Health Canada, 2008); several microbiological risk assessments for *E. coli* O157:H7 in cheese have also been conducted (FSANZ, 2009; Perrin *et al.*, 2015). However, microbial risk assessment for non-EHEC *E. coli* in cheese has not been conducted. Hence, there is a lack of scientific evidence to determine microbial risk of non-EHEC *E. coli*.

EPA (2012) recommends microbiological risk assessment to evaluate the risk posed by bacteria, to prevent foodborne illnesses, and to identify environmental factors influencing microbial growth. The microbiological risk assessment should include hazard identification, exposure assessment, hazard characterization, and risk characterization (Codex, 1999).

The objective of this study was to conduct microbial risk assessment for non-EHEC *E. coli* in natural cheese which is manufactured from milk fermentation by adding start culture enzyme, and salt and processed cheeses which are manufactured from natural cheese using emulsifiers in Korea.

Materials and Methods

Hazard identification

To identify the hazards of *E. coli*, the general characteristics and foodborne-illness outbreaks linked to *E. coli* in cheese were collected from other studies.

Exposure assessment

Prevalence of E. coli

To evaluate non-EHEC *E. coli* prevalence and the contamination level, natural- (n=90) and processed-cheese samples (n=308) were collected from various cheese factories and markets. At two factories, samples were collected throughout the manufacturing process from raw milk to packaged cheese. Natural-cheese samples were collected from raw milk, pasteurized milk, cheese before ripening, cheese after packaging, cheese before shipping, and markets. Processed-cheese samples were also collected after packaging, before shipping, and in markets. In addition, distributed cheeses were collected from local markets in five cities in Korea. Cheese samples were evaluated in both summer and winter to reduce the effect of external environmental factors such as temperature, humidity and contamination levels of the pathogen. The collected samples were placed in an ice cooler and were transported to a laboratory. One-milliliter samples of raw milk and pasteurized milk were serially diluted with 0.1% buffered peptone water (BPW; Becton, Dickinson, and Company, USA). The diluents were then surface-plated on tryptic soy agar (TSA; Becto, Dickinson, and Company) and *E. coli* /Coliform Count petrifilm (3M[™], USA) to quantify total bacteria, and non-EHEC E. coli and coliform counts, respectively. In addition, 25 g or 1 slice of cheese was aseptically transferred into a sample bag (3M[™]), and 25 mL of BPW was added, and the mixture was homogenized for 120 s with a pummeler (Bag-Mixer[®], Interscience, France). One milliliter of the homogenate was serially diluted with BPW, and 0.1-mL diluents for TSA and 1-mL diluents for non-EHEC E. coli / Coliform Count petrifilm $(3M^{TM})$ were then surface-plated, respectively. The plates and petrifilms were incubated at 35°C for 24 h, and then the colonies were manually counted.

Initial level of contamination with non-EHEC E. coli

Beta distribution is a continuous probability distribution parametrized by two shape parameters (α_1 and α_2), and the interval of the distribution is zero to one (Johnson et al., 1995). When the number of positive samples is low, beta distribution can be used to estimate bacterial prevalence. The data on non-EHEC E. coli prevalence in cheese were fitted to a Beta distribution ($\alpha_1 \alpha_2$), where α_1 is the number of positive samples + 1, and α_2 is the number of all tested samples - positive samples + 1 (Vose, 1998). Uniform distribution is also a continuous probability distribution defined by the two parameters (a and b), and the distribution indicates equal probability in the range of two parameters. Because non-EHEC E. coli were detected under detection limit, initial concentration was assumed in the range of zero to detection limit. Thus, the data on the non-EHEC E. coli contamination level in cheese from cheese factory storage were fitted to a Uniform distribution (a, b), where a is the minimal contamination level, and b is the maximal contamination level. Finally, the initial contamination level (Log CFU/g) was calculated by prevalence \times contamination level using the @RISK software, version 5.7 (Palisade Corp., USA).

Non-EHEC *E. coli* growth during distribution and storage

To calculate non-EHEC *E. coli* growth during distribution and storage, predictive models for natural and processed cheeses from a study by MFDS (2013) were used as follows.

<Natural cheese>

$$\mu_{\max} = \left[0.00268 \times (T - 5.4235)\right]^2 \tag{1}$$

$$LPD = \left[\frac{1}{(-0.0522 + 0.0142 \times T)}\right]^2$$
(2)

<Processed cheese>

$$\mu_{\rm max} = 0.0036 - 0.0030 \times T + 0.0004 \times T^2 \tag{3}$$

$$LPD = \left[\frac{1}{-0.0826 + 0.0275 \times T}\right]^2 \tag{4}$$

The μ_{max} (Log CFU/g) is the maximum specific growth rate, *LPD* (h) is lag phase duration, and *T* (°C) is temperature. In addition, to simulate non-EHEC *E. coli* growth under changing temperature and time, probabilistic distributions for temperature and time from a study by Lee *et al.* (2015) were used.

Cheese consumption

Data on cheese consumption and intake frequency of cheese were taken from the study of Lee et al. (2015) to calculate the non-EHEC E. coli risk as a result of cheese consumption in Korea. According to a study by Lee et al. (2015), the mean consumption amounts of natural cheese and processed cheese are 12.40±19.43 g/d (95% confidence interval: 0.915-34.90 g/d) and 19.46±14.39 g/d (95% confidence interval: 2.6-40.0 g/d), respectively, and the consumption frequencies of cheese are 0.0389 and 0.0232 for natural and processed cheese, respectively. The ratios were fitted to the Discrete distribution $\{(0,1), [1, 1)\}$ - (daily frequency of consumption), daily frequency of consumption]} (Lee et al., 2015). Finally, ingested E. coli cell counts were calculated as a result of consuming natural or processed cheese from the final concentration at the time of consumption taking into account the consumption amount and frequency.

The dose-response model

Twenty-eight dose-response models for *E. coli* infection were surveyed from other studies. Because about 90% of *E. coli* foodborne illness in Korea occurred by EPEC (Hong *et al.*, 2005), the following dose-response model developed by Powell *et al.* (2000) for EPEC was used in this study.

$$P = 1 - \left\{ 1 + \left(\frac{D \times [(2^{1/\alpha} - 1)]}{N_{50}} \right) \right\}^{-\alpha}$$
(5)

Where *P* is the probability of illness, *D* is the ingested *E*. *coli* cell number (CFU/serving), N_{50} is the dose infecting 50% of the population with *E*. *coli*, and α is a coefficient.

Risk characterization

The results of the exposure assessment, dose-response model, and cheese consumption amount and frequency were used to estimate the risk of non-EHEC *E. coli* in cheese by means of a simulation in software @RISK according to the scheme of the simulation model in Fig. 1. In the simulation for risk characterization, the sampling type was Median Latin Hypercube, and the generator seed was random with settings for 10,000 iterations. Tables 1 and 2 show simulation models and formulas for calculating the risk of non-EHEC *E. coli* in natural and processed cheeses by means of @RISK. Sensitivity analysis to determine factors influencing the risk was also conducted in @RISK.

Results and Discussion

Hazard identification of E. coli in cheese

Pathogenic E. coli causes diarrhea in infants or acute enteritis in adults (MFDS, 2010). Although ground beef and fresh vegetables are considered major vectors for pathogenic E. coli (MFDS, 2010), there are several reports about E. coli isolated from various cheeses in many countries. The most frequently isolated E. coli serotype in cheese is E. coli O157:H7 in many countries (BCCDC, 2013; CDC, 2010; Honish et al., 2005), but other pathotypes such as EPEC, ETEC, and EAEC were also isolated from various cheeses (Baranceli et al., 2014; Bonyadian et al., 2014; Najand and Ghanbarpour, 2006). In addition, the most frequently isolated pathotype in Korea in various foods is EPEC (Hong et al., 2015). Thus, after non-EHEC E. coli was identified as a hazard in cheese, subsequent quantitative microbial risk assessment for natural and processed cheeses was conducted.

Initial level of non-EHEC E. coli

Non-EHEC *E. coli* cell counts were found to be below the detection limit (natural cheese: 2 CFU/g; processed



Fig. 1. Fitted Beta distribution (A) and probability density (B) of the simulated initial level of contamination with *Escherichia* coli in natural cheese.

Table 1. The simulation model and formulas in a Microsoft Excel [®] s	spreadsheet used to calculate the risk of illness of Esche-
richia coli in natural cheese by means of the @RISK software	

Input Model	Unit	Code	Formula	References				
PRODUCT								
Product								
Pathogen Contamination								
level								
Non-EHEC <i>E. coli</i> prevalence		PR	=RiskBeta(1,91)	Vose (1998)				
Concentration	CFU/g	С	=RiskUniform(0,2)	Vose (1998)				
Initial contamination level	CFU/g	IC	=PR×C	Vose (1998)				
	Log CFU/g	log(IC)	$=\log(PR \times C)$					
			TRANSPORTATION					
Transportation time	h	time _{trans}	=RiskPert(1,3,6)	Personal communication ^a				
Food temperature during transportation	°C	Temp _{trans}	=RiskPert(0,4,10)	Personal communication ^a				

Input Model	Unit	Code	Formula	References
Growth				
\mathbf{h}_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
Y _{end}	Log CFU/g	\mathbf{Y}_{end}	=average($Y_{end}i$), Fixed 9.04	MFDS (2013), Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h_0)-1]}	MFDS (2013), Baranyi and Roberts (1994)
Lag time	h	Trans_{Lt}	=IF {Temp _{trans} >4, [1/(-0.0522+0.0142×Temp _{trans})] ² , 1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Trans _{Gr}	=IF {Temp _{trans} >5.4235, $[0.0268 \times (Temptrans-5.4235)]^2, 0$ }	MFDS (2013), Ratkowsky <i>et al.</i> (1982)
Non-EHEC <i>E. coli</i> growth	Log CFU/g	C1	=IC+1/{1+EXP[-ln(q)]}× [1-10 ^{- Y0-Yend} /LN(10)]×Trans _{Gr} ×time _{trans}	MFDS (2013), Baranyi and Roberts (1994)
			MARKET	
Market storage				
Storage time	h	$Mark-time_{st}$	=RiskPert(0,2,48)	Personal communication ^b
during storage Growth	°C	Mark-Temp _{st}	=RiskUniform(2,4)	Personal communication ^b
\mathbf{h}_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
\mathbf{Y}_{end}	Log CFU/g	\mathbf{Y}_{end}	=average($Y_{end}i$), Fixed 9.04	MFDS (2013), Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h_0)-1]}	MFDS (2013), Baranyi and Roberts (1994)
Lag time	h	$Mark_{st}$ -Time _{Lt}	=IF {Mark-Temp _{st} >4, [1/(-0.0522+0.0142×Mark-Temp _{st})] ² ,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{st}$ - R_{Gr}	=IF {Mark-Temp _{st} > 5.4235 , [0.0268×(Mark-Temp _{st} - 5.4235)] ² ,0}	MFDS (2013), Ratkowsky <i>et al.</i> (1982)
Non-EHEC <i>E. coli</i> growth	Log CFU/g	C2-1	=C1+1/{1+EXP[-ln(q)]}×[1-10 ^{- Y0-end /} LN(10)]×Mark _{st} -R _{Gr} ×Mark-time _{st}	MFDS (2013), Baranyi and Roberts (1994)
Storage time	h	Mark-time _{dis}	=RiskPert(0,48,168)	Personal communication ^b
Food temperature during storage Growth	°C	Mark-Temp _{dis}	=RiskTriang(0.60703,4.1000,15.18)	Lee <i>et al</i> . (2015)
h_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 2.26	MFDS (2013), Baranyi and Roberts (1994)
\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.36	MFDS (2013), Baranyi and Roberts (1994)
\mathbf{Y}_{end}	Log CFU/g	\mathbf{Y}_{end}	=average($Y_{end}i$), Fixed 9.04	MFDS (2013), Baranyi and Roberts (1994)
ln(q)		ln(q)	=LN{1/[EXP(h_0)-1]}	MFDS (2013), Baranyi and Roberts (1994)
Lag time	h	$Mark_{dis}$ -Time _{Lt}	=IF {Mark-Temp _{dis} >4, [1/(-0.0522+0.0142×Mark-Temp _{dis})] ² ,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	$Mark_{dis}$ -R _{Gr}	=IF {Mark-Temp _{dis} >5.4235, $[0.0268 \times (Mark-Temp_{dis}-5.4235)]^2, 0$ }	MFDS (2013), Ratkowsky <i>et al.</i> (1982)
Non-EHEC <i>E. coli</i> growth	Log CFU/g	C2	=(C2-1)+1/{1+EXP[-ln(q)]}×[1-10 ^{- Y0-end} / LN(10)]×Mark _{dis} - R_{Gr} ×Mark-time _{dis}	MFDS (2013), Baranyi and Roberts (1994)

Table 1. The simulation model and formulas in a Microsoft Excel[®] spreadsheet used to calculate the risk of illness of *Escherichia coli* in natural cheese by means of the @RISK software (Continued I)

Input Model	Unit	Code	Formula	References
		TRA	NSPORTATION (CAR)	
Transportation				
(CAR) storage				
Transportation time	h	time _{car}	=RiskPert(0.325,0.984,1.643)	Jung (2011)
Food temperature	°C	Temp	=RickPert(10, 18, 25)	Jung(2011)
during transportation	C	remp _{car}	-KISKI EI(10,18,23)	Julig (2011)
Growth				
h	Lag CEU/a	h	-avanage (growth notavlag time) Fined 2.26	MFDS (2013),
Π_0	Log CrU/g	п ₀	-average(growth rate×lag time), rixed 2.20	Baranyi and Roberts (1994)
X /	I CEU/	V	(V_{1}) Γ'_{1} 12.26	MFDS (2013),
Υ ₀	Log CFU/g	Υ ₀	=average($Y_0 l$), Fixed 3.36	Baranyi and Roberts (1994)
				MFDS (2013),
Y _{end}	Log CFU/g	Y _{end}	=average($Y_{end}i$), Fixed 9.04	Baranyi and Roberts (1994)
				MFDS (2013).
ln(q)		ln(q)	$=LN\{1/[EXP(h_0)-1]\}$	Baranyi and Roberts (1994)
			=IF{Temn >4	Durung Fund Roberts (1991)
Lag time	h	Car-Time _{Lt}	$[1/(-0.0522+0.0142\times\text{Temp})]^2$ 1320)	MFDS (2013)
			$=IF{Temn} > 5.4735$	MEDS (2013)
Growth rate	Log CFU/g/h	Car-R _{Gr}	$[0.0268\times(Temp - 5.4235)]^2$ (1)	Patkowsky at al. (1082)
Non-FHFC			$=C2+1/(1+EXP[-ln(a)]) \times [1-10^{-[Y0-Yend]})$	MFDS (2013)
E_{i} and E_{i} growth	Log CFU/g	C3	$\frac{1}{10} \frac{1}{10} \frac$	Paranyi and Paharta (1004)
E. con glowin			$Lin(10)$]×Cai $-n_{Gr}$ ×time _{car}	Barallyl allu Roberts (1994)
Home storage			HOME	
Home storage			=RiskNormal[250 1742 176 0175	
Storage time	h	Home-time _{st}	-RiskNormal[250.1742, 170.0175, BigleTrupoete(0.4220)]	Lee <i>et al.</i> (2015)
Food temperature			$= \operatorname{RiskI}_{\operatorname{ord}} \operatorname{oristic}[-29,283,33,227,26,666]$	
during storage	°C	Home-Temp _{st}	-RiskLogLogistic[-29.265, 55.227, 20.000,	Lee <i>et al.</i> (2015)
Crosseth			Kisk Truncale(-5,20)]	
Glowin				MEDS (2012)
\mathbf{h}_0	Log CFU/g	h_0	=average(growth rate×lag time), Fixed 2.26	Paranyi and Paharta (1004)
				MEDS (2012)
\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average($Y_0 i$), Fixed 3.36	Denenvi and Dabarta (1004)
				MEDS (2013)
\mathbf{Y}_{end}	Log CFU/g	Y _{end}	$=$ average($Y_{end}i$), Fixed 9.04	Denervi and Deberts (1004)
				MEDS (2012)
ln(q)		ln(q)	$=LN\{1/[EXP(h_0)-1]\}$	MFDS(2013),
				Baranyi and Roberts (1994)
Lag time	h	Home-T ₁	$=1F\{Home-Iemp_{st}>4,$	MFDS (2013)
C			$[1/(-0.0522+0.0142\times\text{Home-lemp}_{st})]^2, 1320\}$	
Growth rate	Log CFU/g/h	Home-R _C	=IF {Home-lemp _{st} > 5.4235 ,	MFDS (2013),
	8 8	0i	$[0.0268 \times (\text{Home-Temp}_{st}-5.4235)]^2,0\}$	Ratkowsky <i>et al.</i> (1982)
Non-EHEC	Log CFU/g	C4	$=C3+1/{1+EXP[-ln(q)]}\times[1-10^{110} mat/$	MFDS (2013),
E. coli growth	8 8		LN(10)]×Home–R _{Gr} ×Home–time _{st}	Baranyi and Roberts (1994)
			CONSUMPTION	
Daily consumption	g	Consump	$= R_{15k} Pearson 5[2.6488, 25.81,]$	MFDS (2013)
average amount	8	T	RiskTruncate(0,100),RiskShift(-3.2572)]	
Daily consumption	%	ConFre	Fixed 3.894	MFDS (2013)
frequency		CE(0)	-1.2.904/100	MEDS (2012)
		CF(0)	-1-5.894/100 -2.804/100	MEDS (2013)
		CF(1)	-3.694/100 -DisleDiscrete[(0,1),(CE(0),CE(1))]	MFDS(2013) $MFDS(2013)$
		ConFre	$-\text{KISKDISCICIC}[\{0,1\},\{Cr(0),Cr(1)\}]$ $-\text{IE}(CE=0,0,Consump)$	MFDS (2013) $MFDS (2013)$
		Comite	DOSE-RESPONSE	MI DS (2013)
Non-EHEC E coli amount		D	$=10^{C4} \times ConFre$	
Parameter of a		a	=Fixed 2.21×10^{-1}	Powell (2000)
Parameter of N.		Ň	=Fixed 6.85×10^7	Powell (2000)
RISK		* '50		2000
Probability of				
illness/person/dav		Rısk	=1-(1+{D×[(2 ^{1/α})-1]/N ₅₀ }) ^{-α}	Powell (2000)
·····				

Table 1. The simulation model and formulas in a Microsoft Excel[®] spreadsheet used to calculate the risk of illness of *Escherichia coli* in natural cheese by means of the @RISK software (Continued II)

^aWith a supervisor of a cheese manufacturing plant

^bWith a manager in charge of cheese products at markets

PRODUCTPRODUCTProductParbogic ContaminationNon-EHEC E. collPRRiskBeta(1,309)Vose (1998)ConcentrationCFU/gICPRCVose (1998)Initial contamination levelCFU/gCCRiskPert(1,3,6)Personal communication*Transportation timehmersonal communication*Food temperatureMEDS (2013), Baranyi and Roberts (1994)MEDS (2013), MEDS (2013), ME	Input Model	Unit	Code	Formula	References
ProductPathogen Contamination levelPR=RiskBeta(1,309)Vose (1998)Non-EHEC C E coli prevalenceCFU/gC=RiskUniform(0,2,8)Vose (1998)ConcentrationCFU/gLC=PR×CVose (1998)Itidal contamination levelCFU/gLC=PR×CVose (1998)Transportation timehtime, taskBeta(1,3,6)Personal communication*Food temperature during transportation Growth"CTemp, average(growth ratextag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994)NgLog CFU/gNu=average(growth ratextag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994)NgLog CFU/gYu=average(Yu,d), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/gYu=average(Yu,d), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/g/hTransy, (10(0) acd254-0.0278-tempcar)]; 1320)MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/g/hTransy, (10(0) acd254-0.0278-tempcar)]; 1320)MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/g/hTransy, (11(0.00824-0.0278-tempcar)]; 1320)MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/gNu=average(growth ratextag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/g/hTransy, (11(0.00824-0.0278-tempcar)], 1200MFDS (2013), Baranyi and Roberts (1994)MarboxLog CFU/gMark-time, average(growth ra				PRODUCT	
Pathogen Contamination levelNon-EHEC E. coliPR-RiskBeta(1,309)Vose (1998)ConcentrationCFU/gIC-PR-CVose (1998)Transportation timehTRANSPORTATIONTransportation timehmetrosynamication*Transportation timehmetrosynamication*Transportation timehmetrosynamication*Transportation timehmetrosynamication*Transportation timehmetrosynamication*Transportation timehmetrosynamication*food temperature during transportationTRANSPORTATIONTransportation timehmetrosynamication*AUC CFU/gTransportation timehMEPS (2013)Baranyi and Roberts (1994)MEPS (2013)MEPS (2013)Baranyi and Roberts (1994)MEPS (2013)MEPS (2013)MEPS (2013)MEPS (2013)MEPS (2013)MEPS (2013)Colspan="2">MEPS (2013)MEPS (2013)MEPS (2013)<	Product				
$\begin{array}{c c c c } \mbox{left} C E. coli \\ \mbox{prevalence} CFU/g CFU/g (C = RiskBeta(1,309) Vose (1998) \\ \mbox{log} CFU/g log (CFU/g (C = RiskDinform(0,2,8) Vose (1998) \\ \mbox{log} CFU/g log (CFU/g (C = RiskDinform(0,2,8) Vose (1998) \\ \mbox{log} CFU/g log (CFU/g (C = RiskDinform(0,2,8) Vose (1998) \\ \mbox{log} CFU/g log (CFU/g (C = RiskDinform(0,2,8) Vose (1998) \\ \mbox{log} CFU/g log (CFU/g (C = RiskDinform(0,2,8) Vose (1998) \\ \mbox{log} CFU/g (C = RiskDinform(0,2,8) Vose (1993) \\ \mbox{log} CFU/g (C = RiskDinform(0,2,8) Vose (1994) \\ \mbox{log} CFU/g (C = RiskDinform(2,2,4) Vose (1994) \\ \mbox{log} CFU/g (C = RiskDinform(2,4) Vose (1994) \\ \mbox{log} CFU/g (C = RiskDinform(2,4) Vose (1994) \\ \mbox{log} CFU/g (C = RiskDinform(2,4) Vose (1994) \\ \mbox{log} CFU/g (RiskDinform(2,4) Vose (1994) \\ \mbox{log} CFU/g (RiskDinform(2,4) Vose (1994) \\ \mbox{log} CFU/g (RiskDinform(2,4) Vose (1,1) \\ \mbox{log} CFU/g (RiskDi$	Pathogen Contamination				
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	level				
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	non-Effec <i>E. con</i>		PR	=RiskBeta(1,309)	Vose (1998)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Concentration	CFU/g	С	=RiskUniform(0.2.8)	Vose (1998)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Initial contamination level	CFU/g	IC	=PR×C	Vose (1998)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Log CFU/g	log(IC)	=log(PR×C)	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $				FRANSPORTATION	
$ \begin{array}{c c c c c c } \hline Food temperature during transportation for the second communication for the second$	Transportation time	h	time _{trans}	=RiskPert(1,3,6)	Personal communication ^a
	Food temperature	°C	Temp	=RiskPert(0.4.10)	Personal communication ^a
GrowthMFDS (2013), Baranyi and Roberts (1994) h_0 Log CFU/g V_0 =average(growth ratexlag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) V_{oud} Log CFU/g V_{uod} =average(Y_{uod}), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) $h(q)$ $ln(q)$ =LN {1/[EXP(h_0)-1]}Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Lag timehTransc.=IF {Temp_{uots}>4, [1/4-0.08264-0.0275×Tempcar)]^2,1320}MFDS (2013), Baranyi and Roberts (1994)Growth rateLog CFU/gC1 $rarsc.=IF {Temp_{uots}>4,[1/4-0.08264-0.0275×Tempcar)]^2,1320}MFDS (2013),Baranyi and Roberts (1994)Market storageStorage timehMark-timeacMarket storage=RiskPert(0,2,48)Personal communicationbMarket storageGrowthhLog CFU/gh_p=average(growth ratexlag time), Fixed 0.65MFDS (2013),Baranyi and Roberts (1994)V_{ad}Log CFU/gh_p=average(growth ratexlag time), Fixed 0.65MFDS (2013),Baranyi and Roberts (1994)Y_{ad}Log CFU/gY_{out}=average(growth ratexlag time), Fixed 0.65MFDS (2013),Baranyi and Roberts (1994)Y_{ad}Log CFU/gY_{out}=average(growth ratexlag time), Fixed 0.65MFDS (2013),Baranyi and Roberts (1994)Y_{ad}Log CFU/gY_{out}=average(Y_{out}), Fixed 3.11MFDS (2013),Baranyi and Roberts (1994)Y_{ad}Log CFU/g$	during transportation		r trans		
	Growth				MEDC (2012)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	\mathbf{h}_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2015), Baranyi and Roberts (1994)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $					MFDS (2013).
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.11	Baranyi and Roberts (1994)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	V		V	-average(V i) Fixed 7.22	MFDS (2013),
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	1 end	Log CrU/g	I end	$-average(Y_{end}l), Fixed 7.52$	Baranyi and Roberts (1994)
Lag timehTrans Trans GrIF {Temp (1/-0.0826+0.0275×Tempcar)]^2,1320} 	$\ln(q)$		ln(a)	$=LN\{1/[EXP(h_0)-1]\}$	MFDS (2013),
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	(-1)		(-)		Baranyi and Roberts (1994)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Lag time	h	Trans_{Lt}	=IF{ $\text{Iemp}_{\text{trans}} > 4$, [1/(-0.0826+0.0275×Tempcar)] ² ,1320}	MFDS (2013)
Chown rateEng Cr O'g'nHans (1) $0.0004 \times Temp_{mas}^2, 0)$ Ratkowsky et al. (1982) MFDS (2013), Baranyi and Roberts (1994)Non-EHEC 	Growth rate	Log CEU/g/b	Trans	=IF(Temp _{trans} >8.6,0.0036-0.0030×Temp _{trans} +	MFDS (2013),
Non-EHEC E. coli growthLog CFU/gC1 $=IC+1/{\{1+EXP[-In(q)]\}\times[1-10]^{+1.0+10+10+10+10+10+10+10+10+10+10+10+10+10$	Growth rate	Log CI O/g/II	11ans _{Gr}	$0.0004 \times \text{Temp}_{\text{trans}}^2, 0)$	Ratkowsky et al. (1982)
L coti growthCLN(10) × Irans_G×time transBaranyi and Roberts (1994)Market storage Storage timehMark-time st=RiskPert(0,2,48)Personal communication ^b Food temperature during storage Growth°CMark-Temp st=RiskUniform(2,4)Personal communication ^b h_0Log CFU/gh_0=average(growth rate×lag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994)Y_0Log CFU/gY_0=average(Y_0i), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994)Y_endLog CFU/gY_end=average(Y_end), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994)In(q)In(q)=LN{1/[EXP(h_0)-1]}Baranyi and Roberts (1994)Lag timehMark_ar-Time Lt=IF{Mark-Temp<×4, [1/(-0.0826+0.0275×Mark-TempMFDS (2013), Baranyi and Roberts (1994)Growth rateLog CFU/ghMark Mark arR Gr=IF{Mark-Temp<×6,0.0030× Mark-TempMFDS (2013), Baranyi and Roberts (1994)Non-EHEC E. coli growth Market displayLog CFU/gC2-1=C1+1/{1+EXP[-In(q)]}×[1-10]^{10/10-rend}/ LN(10]×Mark-R GrMFDS (2013), Baranyi and Roberts (1994)Market displayhMark-time dis=RiskPert(0,48,168)Personal communication ^b	Non-EHEC	Log CFU/g	C1	$=IC+1/{1+EXP[-ln(q)]} \times [1-10^{- Y 0-Y end }/$	MFDS (2013),
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<i>E. coli</i> growth	0 0		LN(10) × I rans _{Gr} ×time _{trans}	Baranyi and Roberts (1994)
Number of storage Storage timehMark-time st e=RiskPert(0,2,48)Personal communication ^b Food temperature during storage Growth°CMark-Temp st=RiskUniform(2,4)Personal communication ^b h0Log CFU/gh0=average(growth rate×lag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Y0Log CFU/gY0=average(Y0,i), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)YendLog CFU/gYend=average(Yendi), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)In(q)In(q)=LN {1/[EXP(h0)-1]}MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Lag timehMarkst-Timet (1/(-0.08264+0.0275×Mark-Tempst)) ² ,1320} [F(Mark-Tempst=0.0045×Mark-Tempst) ² ,0)MFDS (2013), MFDS (2013), Baranyi and Roberts (1994)Non-EHEC E. coli growth Market displayLog CFU/gC2-1=IF {Mark-Tempst=0.0045×Mark-Tempst2,0) (LN(10)]×Mark-R _{Gr} ×Mark-timestMFDS (2013), MFDS (2013), Ratkowsky et al. (1982) MFDS (2013), Baranyi and Roberts (1994)Market display Storage timehMark-time dis=RiskPert(0,48,168)Personal communication ^b	Market storage			MARKET	
Food temperature during storage Growth°CMark-Temp st=RiskUniform(2,4)Personal communication h_0 Log CFU/g h_0 =average(growth rate×lag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) Y_0 Log CFU/g Y_0 =average(Y_0i), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) Y_{end} Log CFU/g Y_{end} =average($Y_{end}i$), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) $ln(q)$ $ln(q)$ =LN {1/[EXP(h_0)-1]}Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Lag timehMark str Time Lt=IF {Mark-Temp_s>4, [1/(-0.0826+0.0275×Mark-Temp_s])^2,1320} =IF(Mark-Temp_s+0.0004×Mark-Temp_si)^2,0)MFDS (2013), MFDS (2013), Ratkowsky <i>et al.</i> (1982) MFDS (2013), Baranyi and Roberts (1994)Non-EHEC <i>E. coli</i> growth Market display Storage timehMark-time dis=RiskPert(0,48,168)Personal communication ^b	Storage time	h	Mark-time.	=RiskPert(0.2.48)	Personal communication ^b
during storage GrowthCMark-Temp st=RiskUniform(2,4)Personal communication h_0 Log CFU/g h_0 =average(growth rate×lag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994) Y_0 Log CFU/g Y_0 =average(Y_0i), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994) Y_{end} Log CFU/g Y_{end} =average($Y_{end}i$), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994) $h(q)$ $h(q)$ =LN {1/[EXP(h_0)-1]}MFDS (2013), Baranyi and Roberts (1994)Lag time h Mark_st-TimeLt=IF {Mark-Temp_s>4, [1/(-0.0826+0.0275×Mark-Temp_s])^2,1320} =IF(Mark-Temp_s+0.0004×Mark-Temp_s1^2,0)MFDS (2013), MFDS (2013), Baranyi and Roberts (1994)Non-EHEC E. coli growth Market displayLog CFU/gC2-1 $C2-1$ $C2-1$ MFDS (2013), Baranyi and Roberts (1994)Market display Storage timehMark-time dis=RiskPert(0,48,168)Personal communication ^b	Food temperature	80	MIT		
GrowthMethodLog CFU/g h_0 =average(growth rate×lag time), Fixed 0.65MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Y_0Log CFU/gY_0=average(Y_0i), Fixed 3.11MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Y_{end}Log CFU/gY_{end}=average(Y_{end}i), Fixed 7.32MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)In(q)In(q)=LN {1/[EXP(h_0)-1]}MFDS (2013), Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Lag timehMark_{st}-Time_L $IF {Mark-Temp_{st}>4, [1/(-0.0826+0.0275 \times Mark-Temp_{st})^2, 1320)}IF[(Mark-Temp_{st}>4, (1/0.004 \times Mark-Temp_{st}^2, 0))MFDS (2013), Baranyi and Roberts (1994)MFDS (2013), Baranyi and Roberts (1994)Non-EHECLog CFU/g/hMark_{st}-R_{Gr}IF[(Mark-Temp_{st}>4, (1/0.004 \times Mark-Temp_{st}^2, 0))Un(10] \times Mark-Temp_{st}^2, 0)MFDS (2013), Baranyi and Roberts (1994)MFDS (2013), Baranyi and Roberts (1994)MFDS (2013)Mark_st-R_{Gr}IF[(Mark-Temp_{st}>4, (1/0.004 \times Mark-Temp_{st}^2, 0))Un(10] \times Mark-R_{Gr} \times Mark-time_{st}MFDS (2013), Baranyi and Roberts (1994)MFDS (2013), Baranyi and Roberts (1994)MArbert displayhMark-time_{dis}=RiskPert(0,48,168)Personal communicationb$	during storage	Ċ	Mark-Temp _{st}	= RiskUniform(2,4)	Personal communication
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Growth				
H_0 $Log CFU/g$ Y_0 =average(Y_0i), Fixed 3.11Baranyi and Roberts (1994) Y_0 $Log CFU/g$ Y_0 =average(Y_0i), Fixed 3.11MFDS (2013), Y_{end} $Log CFU/g$ Y_{end} =average($Y_{end}i$), Fixed 7.32Baranyi and Roberts (1994) $In(q)$ $In(q)$ $In(q)$ =LN {1/[EXP(h_0)-1]}Baranyi and Roberts (1994) $Lag time$ h $Mark_{st}$ -Time_Lt=IF {Mark-Temp_st>4, [1/(-0.0826+0.0275 \times Mark-Temp_{st})]^2, 1320}Baranyi and Roberts (1994) $Growth rate$ $Log CFU/g/h$ $Mark_{st}$ -R _{Gr} =IF {Mark-Temp_st>4, [1/(-0.0826+0.0275 \times Mark-Temp_{st})]^2, 1320}MFDS (2013), $Branyi and Roberts (1994)$ $MFDS (2013)$ Baranyi and Roberts (1994) $MFDS (2013)$ =IF {Mark-Temp_{st}>8.6,0.0036-0.0030 \times MFDS (2013),MFDS (2013), $MFDS (2013)$ =IF (Mark-Temp_{st}) + 0.0004 \times Mark-Temp_{st}^2, 0)MFDS (2013), $Non-EHEC$ $Log CFU/g$ $C2-1$ =C1+1/{1+EXP[-In(q)]} \times [1-10^{- Y0-Yend /} LN(10)] \times Mark-R_{Gr} \times Mark-time_{st}Baranyi and Roberts (1994) $MFDS (2013),$ $MFDS (2013),$ Baranyi and Roberts (1994) $Market display$ h $Mark-time_{dis}$ =RiskPert(0,48,168)Personal communication ^b	h	Log CFU/g	h _o	=average(growth rate×lag time), Fixed 0.65	MFDS (2013),
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0		0		Baranyi and Roberts (1994)
Y_{end} $Log CFU/g$ Y_{end} =average($Y_{end}i$), Fixed 7.32Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994) $ln(q)$ $ln(q)$ = $LN\{1/[EXP(h_0)-1]\}$ Baranyi and Roberts (1994) MFDS (2013), Baranyi and Roberts (1994)Lag timehMark_st-Time_Lt= $IF\{Mark-Temp_{st}>4,$ [1/(-0.0826+0.0275×Mark-Temp_{st})]^2,1320\}MFDS (2013) MFDS (2013)Growth rateLog CFU/g/hMark_st-R_Gr= $IF\{Mark-Temp_{st}>8.6,0.0036-0.0030\times$ Mark-Temp_st+0.0004×Mark-Temp_st^2,0) Mark-Temp_st+0.0004×Mark-Temp_st^2,0)MFDS (2013), MFDS (2013), Ratkowsky <i>et al.</i> (1982) MFDS (2013), Baranyi and Roberts (1994)Non-EHEC <i>E. coli</i> growth Market displayLog CFU/gC2-1= $C1+1/\{1+EXP[-ln(q)]\}\times[1-10^{ Y0-Yend /}LN(10)]\timesMark-R_{Gr}\timesMark-time_{st}$ Baranyi and Roberts (1994)MFDS (2013), Baranyi and Roberts (1994)= $RiskPert(0,48,168)$ Personal communication ^b	\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.11	MFDS (2013), Demonsi and Deborts (1004)
Y_{end} Log CFU/g Y_{end} =average(Y_{end}), Fixed 7.32IMI DS (2013), Baranyi and Roberts (1994) $h(q)$ $h(q)$ =LN{1/[EXP(h_0)-1]}Baranyi and Roberts (1994)Lag timehMark_{st}-Time_{Lt}=IF{Mark-Temp_{st}>4, [1/(-0.0826+0.0275×Mark-Temp_{st})]^2,1320}MFDS (2013), Baranyi and Roberts (1994)Growth rateLog CFU/g/hMark_{st}-R_{Gr}=IF{Mark-Temp_{st}>4, 					MEDS (2013)
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$\mathrm{Y}_{\mathrm{end}}$	Log CFU/g	\mathbf{Y}_{end}	$=$ average($Y_{end}i$), Fixed 7.32	Baranvi and Roberts (1994)
In(q)In(q)=LN {I/[EXP(n_0)-1]}Baranyi and Roberts (1994)Lag timehMark_st-Time_Lt=IF {Mark-Temp_st>4, [1/(-0.0826+0.0275×Mark-Temp_st)]^2,1320}MFDS (2013)Growth rateLog CFU/g/hMark_st-R_Gr=IF (Mark-Temp_st>8.6,0.0036-0.0030× Mark-Temp_st>8.6,0.0036-0.0030×MFDS (2013), Ratkowsky <i>et al.</i> (1982)Non-EHECLog CFU/gC2-1=C1+1/{1+EXP[-In(q)]}×[1-10 ^{-1Y0-Yend]/} LN(10)]×Mark-R_Gr×Mark-time_{st}MFDS (2013), Baranyi and Roberts (1994)Market displayhMark-time_dis=RiskPert(0,48,168)Personal communication ^b	1 ()		1 ()	IN(1/(EVD(1)) 1)	MFDS (2013),
Lag timehMark_{st}-Time_Lt=IF {Mark-Temp_{st}>4, [1/(-0.0826+0.0275 \times Mark-Temp_{st})]^2, 1320} [1/(-0.0826+0.0275 \times Mark-Temp_{st})]^2, 1320} =IF (Mark-Temp_{st}>8.6, 0.0036-0.0030 \times MFDS (2013), MrDS (2013), Mark-Temp_{st}+0.0004 \times Mark-Temp_{st}^2, 0) Mark-Temp_{st}+0.0004 \times Mark-Temp_{st}^2, 0)MFDS (2013), Ratkowsky et al. (1982) MFDS (2013), Ratkowsky et al. (1982) MFDS (2013), Baranyi and Roberts (1994)Non-EHEC E. coli growth Market displayLog CFU/gC2-1 $C2-1$ C	ln(q)		ln(q)	$= LN\{I/[EXP(n_0)-I]\}$	Baranyi and Roberts (1994)
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Lagtime	h	Mark -Time	=IF {Mark-Temp _{st} >4,	MEDS (2013)
Growth rateLog CFU/g/hMark_st-R_Gr $=IF(Mark-Temp_{st}>8.6,0.0036-0.0030\times)$ MFDS (2013),Non-EHECLog CFU/gC2-1 $=IF(Mark-Temp_{st}>8.6,0.0036-0.0030\times)$ MFDS (2013),Non-EHECLog CFU/gC2-1 $=C1+1/\{1+EXP[-ln(q)]\}\times[1-10^{-[Y0-Yend]/})$ MFDS (2013),Market displayMark-time _{dis} $=RiskPert(0,48,168)$ Baranyi and Roberts (1994)	Eug time	11	What K _{st} Time _{Lt}	$[1/(-0.0826+0.0275 \times Mark-Temp_{st})]^2, 1320\}$	MI D5 (2015)
Non-EHEC E. coli growth Market display Storage timeLog CFU/g $C2-1$ Mark-Temp _{st} +0.0004×Mark-Temp _{st} -0) = $C1+1/{1+EXP[-ln(q)]}×[1-10-[Y0-Yend]/LN(10)]×Mark-RGr×Mark-timestRatkowsky et al. (1982)MFDS (2013),Baranyi and Roberts (1994)Storage timehMark-timedis=RiskPert(0,48,168)Personal communicationb$	Growth rate	Log CFU/g/h	Mark _{st} -R _{Gr}	=IF(Mark-Temp _{st} >8.6,0.0036-0.0030×	MFDS (2013),
Non-ErricLog CFU/gC2-1 $-C1+1/{1+EAF}[-III(q)] \times (1-10^{-1/4})^{-1/4}$ IMPDS (2013),E. coli growthMarket displayLN(10)]×Mark-R _{Gr} ×Mark-time _{st} Baranyi and Roberts (1994)Market displayhMark-time _{dis} =RiskPert(0,48,168)Personal communication ^b	Non EHEC			$\operatorname{Mark-lemp}_{st} + 0.0004 \times \operatorname{Mark-lemp}_{st}, 0)$	Ratkowsky <i>et al.</i> (1982)
DeterminingDeterminingDeterminingDeterminingMarket display Storage timehMark-time dis=RiskPert(0,48,168)Personal communication	E coli growth	Log CFU/g	C2-1	LN(10) Mark-R _o × Mark-time	Baranyi and Roberts (1994)
Storage time h Mark-time _{dis} =RiskPert(0,48,168) Personal communication ^b	Market display				
	Storage time	h	Mark-time _{dis}	=RiskPert(0,48,168)	Personal communication ^b
Food temperature $^{\circ}$ C Mark-Temp = Risk Triang(0.60703.4.1000.15.18) Leg at al. (2015)	Food temperature	°C	Mark-Temp	=RiskTriang(0 60703 4 1000 15 18)	Lee $pt al (2015)$
during storage	during storage	C	totar k- tomp _{dis}	Kisk mang(0.00703,7.1000,13.10)	Lee ei ul. (2015)
Growth	Growth				MEDG (2012)
h_0 Log CFU/g h_0 =average(growth rate×lag time), Fixed 0.65 MFDS (2013), Baranyi and Roberts (1994)	\mathbf{h}_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 0.65	NITDS (2013), Baranyi and Roberts (1994)

Table 2. The simulation model and formulas in a Microsoft Excel[®] spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software

Input Model	Unit	Code	Formula	References
Y	Log CFU/g	Y	=average($Y_0 i$), Fixed 3.11	MFDS (2013),
U	0 0	Ū		Baranyi and Roberts (1994)
\mathbf{Y}_{end}	Log CFU/g	Y_{end}	=average($Y_{end}i$), Fixed 7.32	MFDS (2013), Paranyi and Paharta (1004)
				MEDS (2013)
ln(q)		ln(q)	$=LN\{1/[EXP(h_0)-1]\}$	Baranyi and Roberts (1994)
Lag time	h	Mark _{dis} -Time _{Lt}	=IF {Mark-Temp _{dis} >4, $[1/(-0.0826+0.0275 \times Mark-Temp_{10})]^2$,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Mark _{dis} -R _{Gr}	$= IF(Mark-Temp_{dis}>8.6,0.0036-0.0030\times Mark-Temp_{dis}+0.0004\times Mark-Temp_{dis}^{2}.0)$	MFDS (2013), Ratkowsky <i>et al.</i> (1982)
Non-EHEC		C	= $(C2-1)+1/{1+EXP[-ln(q)]}\times[1-10^{- Y0-Yend }/$	MFDS (2013),
E. coli growth	Log CFU/g	C2	LN(10)]×Mark _{dis} -R _{Gr} ×Mark-time _{dis}	Baranyi and Roberts (1994)
		TRA	ANSPORTATION (CAR)	
Transportation				
(CAR) storage				
Transportation time	h	time _{car}	=RiskPert(0.325,0.984,1.643)	Jung (2011)
Food temperature	°C	Temp _{car}	=RiskPert(10,18,25)	Jung (2011)
during transportation		2 Cur		
Growin				MEDS (2012)
\mathbf{h}_0	Log CFU/g	\mathbf{h}_0	=average(growth rate×lag time), Fixed 0.65	MFDS (2013), Baranyi and Roberts (1994)
				MFDS (2013)
\mathbf{Y}_{0}	Log CFU/g	\mathbf{Y}_{0}	=average(Y_0i), Fixed 3.11	Baranyi and Roberts (1994)
				MFDS (2013),
Y _{end}	Log CFU/g	Y _{end}	=average($Y_{end}i$), Fixed 7.32	Baranyi and Roberts (1994)
1(-)		1 ()	$-\mathbf{I} \mathbf{N} (1/\mathbf{F} \mathbf{Y} \mathbf{D} (\mathbf{L}) 1)$	MFDS (2013),
m(q)		m(q)	$-LN\{I/[EAP(II_0)-I]\}$	Baranyi and Roberts (1994)
Lag time	h	$Car-Time_{Lt}$	=IF {Temp _{car} >4, [1/(-0.0826+0.0275×Temp _{car})] ² ,1320}	MFDS (2013)
Growth rate	Log CFU/g/h	Car-R _{Gr}	=IF(Temp _{car} >8.6, 0.0036-0.0030×Temp _{car} +0.0004×Temp _{car} ² ,0)	MFDS (2013), Ratkowsky <i>et al.</i> (1982)
Non-EHEC	Log CEU/g	C3	$=C2+1/{1+EXP[-ln(q)]}\times [1-10^{- Y0-Yend }/$	MFDS (2013),
E. coli growth	Log of ofg	05	$LN(10)$]×Car– R_{Gr} ×time _{car}	Baranyi and Roberts (1994)
			HOME	
Home storage				
Storage time	h	Home-time _{st}	=RiskNormal[250.1742, 176.0175, RiskTruncate(0.4320)]	Lee et al. (2015)
Food temperature			=RiskLogLogistic[-29.283, 33.227, 26.666.	
during storage	°С	Home-Temp _{st}	RiskTruncate(-5,20)]	Lee <i>et al.</i> (2015)
Growth				
h	Lag CEU/a	h	-average (growth rate) (log time) Fixed 0.65	MFDS (2013),
n ₀	Log Cr U/g	Π_0	-average(growin rate×iag time), Fixed 0.05	Baranyi and Roberts (1994)
Y	Log CFU/g	Y	=average(Y_{i}). Fixed 3.11	MFDS (2013),
- 0	209 01 0,9	- 0		Baranyi and Roberts (1994)
Yand	Log CFU/g	Yand	=average($Y_{and}i$), Fixed 7.32	MFDS (2013),
ena	- 6 6	end		Baranyi and Roberts (1994)
ln(q)		ln(q)	$=LN\{1/[EXP(h_0)-1]\}$	MFDS (2013), Baranyi and Roberts (1994)
			=IF{Home_Temp >4	Daranyi anu Kuucits (1794)
Lag time	h	Home-T _{Lt}	$[1/(-0.0826+0.0275 \times \text{Home}-\text{Temp})]^2.1320\}$	MFDS (2013)
	t (7777/ "		$=IF(Home-Temp_{st}>8.6.0.0036-0.0030\times$	MFDS (2013),
Growth rate	Log CFU/g/h	Home-R _{Gr}	Home-Temp _{st} + $0.0004 \times$ Home-Temp _{st} ² ,0)	Ratkowsky et al. (1982)
Non-EHEC	Log CEU/a	C^{A}	$=C3+1/{1+EXP[-ln(q)]}\times [1-10^{- Y0-Yend })$	MFDS (2013),
<i>E. coli</i> growth		$LN(10)$]×Home- R_{Gr} ×Home-time _{st}	Baranyi and Roberts (1994)	

Table 2. The simulation model and formulas in a Microsoft Excel[®] spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software (Continued I)

Table 2. The simulation model and formulas in a Microsoft Excel[®] spreadsheet used to calculate the risk of *Escherichia coli* in processed cheese by means of the @RISK software (Continued II)

Input Model	Unit	Code	Formula	References
			CONSUMPTION	
Daily consumption	a	Consump	=RiskWeibull[1.3482, 20.932,	MEDS (2013)
average amount	g	Consump	RiskShift(0.26384),RiskTruncate(0,100)]	WIPDS (2013)
Daily consumption	0/	ConFro	Fixed 2 222	MEDS (2012)
frequency	/0	Comite	11xcu 2.323	WIPDS (2013)
		CF(0)	=1-2.323/100	MFDS (2013)
		CF(1)	=2.323/100	MFDS (2013)
		CF	=RiskDiscrete{[0,1],[CF(0),CF(1)]}	MFDS (2013)
		ConFre	=IF(CF=0,0,Consump)	MFDS (2013)
			DOSE-RESPONSE	
Non-EHEC E. coli amount		D	$=10^{C4}$ ×ConFre	
Parameter of α		α	=Fixed 2.21×10^{-1}	Powell (2000)
Parameter of N ₅₀		N ₅₀	=Fixed 6.85×10^7	Powell (2000)
RISK				
Probability of		Diale	$-1 (1 + (D \times I(2^{1/\alpha}) + 1) N =))^{-\alpha}$	Douvel1 (2000)
illness/person/day		KISK	$-1-(1+\{D\times[(2)-1]/(N_{50})\})$	rowen (2000)

^aWith a supervisor of a cheese manufacturing plant

^bWith a manager in charge of cheese products at markets



Fig. 2. Fitted Beta distribution (A) and probability density (B) of the simulated initial level of contamination with *Escherichia* coli in processed cheese.

cheese: 2.8 CFU/g) in all samples. Thus, it was assumed that non-EHEC *E. coli* cell counts in cheese to be above 0 CFU/g, but below the detection limit (2 CFU/g), and then we described contamination levels of the pathogen with Uniform distribution (0,2) and Uniform distribution (0,2.8) for natural and processed cheese, respectively (Figs. 2 and 3). Therefore, using the @RISK software, the initial contamination level of non-EHEC *E. coli* were calculated by Beta distribution(1,91) × Uniform distribution(0, 2), and Beta distribution(1,309) × Uniform distribution(0, 2.8) for natural and processed cheese, respectively. As a result of the simulation, the initial level of contamination with non-EHEC *E. coli* in cheese was 2.35 and -2.73 Log CFU/g for natural and processed cheese, respectively (Figs. 2 and 3).

Non-EHEC *E. coli* growth and cheese consumption

The cumulative distributions of non-EHEC *E. coli* growth during distribution and storage (initial concentration, concentration after transportation, concentration after storage in a market, concentration at the time of purchase, concentration when at home, and concentration at the time of consumption) were analyzed. As a result of the simulation, in natural cheese, the initial concentration was -2.35 Log CFU/g, and concentration at the final stage (at the time of consumption) was -2.31 Log CFU/g (data not shown). This result indicates that non-EHEC *E. coli* in natural cheese may not grow during distribution and storage under the conditions in Korea. In addition, non-EHEC *E. coli* growth probability in processed cheese was simi-



Fig. 3. The scatter plots of the initial concentration level versus the home consumption level in terms of *Escherichia coli* in natural (A) and processed cheese (B).

lar to that in natural cheese (data not shown). Moreover, the results of comparison of the initial concentration with final concentration indicate that none of the 10,000 iterations could yield more than 0 Log CFU/g at the point of final concentration (Fig. 4).

The dose-response model and risk characterization

After cheese consumption, to estimate the probability of non-EHEC *E. coli* foodborne illness, the Beta-Poisson model ($\alpha = 2.21 \times 10^{-1}$, N₅₀ = 6.85×10⁷) was used (Powell *et al.*, 2000). Subsequently, the simulation model was prepared with the values of input variables such as non-EHEC *E. coli* prevalence, temperature, and time for distribution and display in markets, and home storage, the amount of cheese consumption, and intake frequency as presented Tables 1 and 2. The simulations were conducted by random sampling from the distribution described above for 10,000 iterations, and the mean probabilities of a non-EHEC *E. coli* outbreak as a result of cheese consumption per person per day in Korea were 1.36×10^{-7} and 2.12×10^{-10} for natural and processed cheese, respectively (Table 3), which are higher than the risk (7.84×10^{10}) of *S. aureus* foodborne illness per person per day as a result of natural cheese consumption and the risk (3.64×10^{-9} to 1.30×10^{-7}) of listeriosis per person per day as a result of eating lettuce at a restaurant in Korea (Ding *et al.*, 2013; Lee *et al.*, 2015). These results indicate that natural cheese poses a high risk of a non-EHEC *E. coli* outbreak as compared to processed-cheese-related and *S. aureus*-related



Fig. 4. The regression coefficient (A) and the correlation coefficient (B) values for the sensitivity risk factor affecting the probability of illness per person per day as a result of consumption of natural cheese.

Probability of illness/ (person · d)	5%	25%	50%	95%	99%	Maximum	Mean
Natural cheese	0	0	0	0	3.34×10 ⁻⁶	2.26×10 ⁻⁴	1.36×10 ⁻⁷
Processed cheese	0	0	0	0	4.59×10 ⁻⁹	1.20×10 ⁻⁷	2.12×10 ⁻¹⁰

Table 3. Probability of foodborne illness caused by *Escherichia coli* per person per day as a result of consumption of natural and processed cheeses

foodborne illnesses as a result of natural cheese consumption and listeriosis as a result of lettuce consumption in Korea. In addition, sensitivity analysis revealed that intake frequency was the most influential factor for this risk, whereas the other factors such as storage temperature and time were not obviously related (Fig. 5).

Thus, our results indicate that non-EHEC *E. coli* cannot grow in natural and processed cheeses under the present

distribution and storage conditions, and that a different factor is more important for the risk of illness. Consumption frequency of processed cheese is lower than that of natural cheese, if we assume that the consumption amount of natural and processed cheese is similar. Accordingly, processed cheese poses a lower risk than natural cheese for non-EHEC *E. coli*.



Fig. 5. The regression coefficient (A) and the correlation coefficient (B) values for the sensitivity risk factor affecting the probability of illness per person per day as a result of consumption of processed cheese.

Conclusion

The risk of a non-EHEC *E. coli* outbreak via cheese consumption seems to be low for natural and processed cheese in Korea, and the intake frequency of cheese is the most influential factor for this risk. In addition, the microbial risk assessment model that we developed in this study can be useful for quantitative risk assessment.

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