

Quantitative Microbial Risk Assessment for *Campylobacter* spp. on Ham in Korea

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Abstract

The objective of this study was to evaluate the risk of illness from *Campylobacter* spp. on ham. To identify the hazards of *Campylobacter* spp. on ham, the general characteristics and microbial criteria for *Campylobacter* spp., and campylobacteriosis outbreaks were investigated. In the exposure assessment, the prevalence of *Campylobacter* spp. on ham was evaluated, and the probabilistic distributions for the temperature of ham surfaces in retail markets and home refrigerators were prepared. In addition, the raw data from the Korea National Health and Nutrition Examination Survey (KNHNES) 2012 were used to estimate the consumption amount and frequency of ham. In the hazard characterization, the Beta-Poisson model for *Campylobacter* spp. infection was used. For risk characterization, a simulation model was developed using the collected data, and the risk of *Campylobacter* spp. on ham was estimated with @RISK. The *Campylobacter* spp. cell counts on ham samples were below the detection limit (<0.70 Log CFU/g). The daily consumption of ham was 23.93 g per person, and the consumption frequency was 11.57%. The simulated mean value of the initial contamination level of *Campylobacter* spp. on ham was -3.95 Log CFU/g, and the mean value of ham for probable risk per person per day was 2.20×10^{-12} . It is considered that the risk of foodborne illness for *Campylobacter* spp. was low. Furthermore, these results indicate that the microbial risk assessment of *Campylobacter* spp. in this study should be useful in providing scientific evidence to set up the criteria of *Campylobacter* spp..

Keywords: *Campylobacter* spp., ham, quantitative microbial risk assessment

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Introduction

Foodborne illness caused by meat and processed meat products occurs steadily worldwide (Pépin *et al.*, 1997). In particular, the consumption of meat is increasing due to the fact that the grain-oriented diet has been converted to a meat-oriented diet in Korea (KREI, 2012). According to the Korea National Health and Nutrition Examination Survey 2012, the intake of meat has increased from 67.8 g in 1997 to 113.8 g in 2012 (MHW, 2012). In addition, the consumption of fermented-cured ham, which is consumed without further processing such as cooking or heating, has been increased. The increased intake of meat and processed meat products may cause increased foodborne outbreaks related to animal products (MFDS, 2013a).

The most common pathogenic microorganisms in meat

and processed meat products are *Campylobacter* spp., *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* (Borch and Arinder, 2002). Among these, *Campylobacter* spp. has been recognized as a foodborne illness bacterium related to livestock (Doyle and Erickson, 2006). *Campylobacter* spp. can survive well in fresh poultry, pork, beef, other processed meat products, and dairy products (Jacobs-Reitsma, 2000; Sammarco *et al.*, 2010; Taremi *et al.*, 2006; Wong *et al.*, 2007).

Campylobacter jejuni and *Campylobacter coli* are the major species of *Campylobacter* spp. (Ge *et al.*, 2013), and they cause sporadic gastrointestinal infection (Blaser, 1997; Gormley *et al.*, 2008). In particular, *C. jejuni* became the cause of approximately 90% of acute gastroenteritis cases (Park, 2001). In the United States, *C. jejuni* is the main pathogenic bacterium responsible for diarrhea, and foodborne illness caused by *Campylobacter* spp. is more frequent than foodborne illness caused by *Salmonella* spp. and *E. coli* O157:H7 (USDA, 2010). Also, human campylobacteriosis in Japan has been reported more than 2,000 cases per year since 2001 (Haruna *et al.*, 2012).

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Kubota *et al.* (2008) showed that estimated patients for campylobacteriosis in Japan might be 241,925 persons in 2005. In Korea, foodborne outbreaks related to *C. jejuni* were 13 cases (329 hospitalizations) in 2011 (MFDS, 2014).

Microbial risk assessment (MRA) is one of the scientific methods to evaluate the risk levels of microbial risk factors. MRA is composed of four stages such as hazard identification, exposure assessment, hazard characterization and risk characterization (CAC, 1999). In hazard identification, the level of potential hazards for microorganism is defined (Notermans and Teunis, 1996). For exposure assessment, the possible populations of microorganism are estimated at the point of consuming certain foods (Lammerding and Fazil, 2000; Notermans and Teunis, 1996). The populations of contamination, growth or death of microorganisms, consumed pattern of foods and cross-contamination are examined through the food distribution process. Dose response models are used in hazard characterization, and the risk of microorganism is calculated by the data from exposure assessment and hazard characterization for risk characterization (Notermans and Teunis, 1996). Then, microbiological criteria may be established by the result of MRA.

Even though foodborne illness of *Campylobacter* spp. by food consumption has been increasing in Korea, microbiological criteria for the pathogens are not well-established for each food. Therefore, the objective of this study was to evaluate the microbial risk of *Campylobacter* spp. on ham in Korea and present a scientific basis for the criteria.

Materials and Methods

Hazard identification

To identify the hazards of *Campylobacter* spp., the general characteristics, microbial criteria for *Campylobacter* spp., and outbreaks of foodborne illness for consumption of *Campylobacter* spp. were investigated by literatures.

Exposure assessment

Prevalence

To evaluate the prevalence and contamination levels of *Campylobacter* spp., pressed hams with antimicrobials (ham A, N=80), pressed hams without antimicrobials (ham B, N=80), and fermented-cured hams (ham C, N=40) were purchased from local grocery stores in Seoul and from a manufacturer in Hongseong, S. Korea. Twenty five-gram

portions of the samples were aseptically transferred to a sterilized bag. A hundred milliliter Bolton broth (Oxoid Ltd., UK), formulated with laked-horse blood, was added to the bag, and homogenized in a pummeler (BagMixer[®], Interscience, St. Nom) for 90 s.

The homogenates were incubated for 5 h at 37°C for the first enrichment, followed by incubation at 42°C for 44 h for the second enrichment in a microaerobic environment. The cultures were then streaked onto *Campylobacter* blood-free selective agar plates (modified CCDA-Preston, mCCDA; Oxoid Ltd.) and incubated microaerobically at 42°C for 48 h. Following incubation, one presumptive *Campylobacter* spp. colony was streaked onto two Columbia agar plates (bioMérieux, Marcy-l'Étoile) and incubated at 42°C for 48 h in the aerobic and microaerobic conditions, respectively. When colonies appeared only in the microaerobic environment, these were further analyzed to identify *Campylobacter* spp., using the PCR method described by Yamazaki-Matsune *et al.* (2007). One-milliliter portions of the homogenates were also surface-plated onto mCCDA (Oxoid Ltd.) to enumerate *Campylobacter* spp. cell counts, and the plates were incubated at 42°C for 48 h under microaerobic conditions. Following incubation, all presumptive *Campylobacter* spp. colonies were counted. Five randomly selected colonies of presumptive *Campylobacter* spp. were analyzed to identify *Campylobacter* spp., using the method described above. To obtain *Campylobacter* spp. cell counts, the ratio of identified *Campylobacter* spp. was multiplied by the number of presumptive *Campylobacter* spp. colonies.

Initial contamination level and *Campylobacter* spp. fate

The prevalence data (PR) of *Campylobacter* spp. was fitted to a beta distribution (α_1 , the number of positive samples+1; α_2 , tested total samples-positive samples+1), and the contamination level (CFU/g) was calculated using the equation: $[-\text{LN}(1-\text{PR})/\text{weight}]$ (Sanaa *et al.*, 2004; Vose, 1998) to estimate the initial contamination level. During the distribution and storage, the growth or death of *Campylobacter* spp. cells were estimated by a predictive model developed by González *et al.* (2009) (Table 1).

Temperature for distribution and storage

To simulate the storage environment, the temperatures were measured with a non contact infrared thermometer (HS-33CT, Hansung, Seoul) at eight retail markets, and results from the MFDS (2013b) were used for home refrigerator temperature profiles.

Table 1. Predictive model for *Campylobacter* spp. developed by González *et al.* (2009) to estimate the δ and p values

$\delta(T) = 10^{AT^3+BT^2+CT+D}$				
A	B	C	D	R ²
-6×10^{-5}	-7×10^{-4}	2.97×10^{-2}	1.07	0.963
$p(T) = 10^{ET^3+FT^2+GT+H}$				
E	F	G	H	R ²
2×10^{-5}	-5×10^{-4}	-1.1×10^{-3}	-0.15	0.880

Consumption of ham in Korea

To obtain the amounts of ham consumption in Korea, 24-h recall data from the Korea National Health and Nutrition Examination Survey (KNHNES) 2012 were analyzed by the SAS[®] program version 9.3 (SAS Institute

Inc., USA). Each data was then fitted to the @RISK program version 6 (Palisade Corp., Ithaca) to obtain appropriate data distributions. To estimate the consumption frequencies for ham, the number of people who consumed ham was divided into the total respondents.

Table 2. Simulation model and formulas in Excel[®] spreadsheet used to calculate the risk of *Campylobacter* spp. on ham with @RISK

Input Model	Unit	Variable	Formula	References
PRODUCT				
Pathogens contamination level				
<i>Campylobacter</i> prevalence		PR	=RiskBeta(1, 201)	Vose (1998)
Initial contamination level	CFU/g	C _i	=-LN(1-PR)/25g	Sanaa <i>et al.</i> (2004)
	Log CFU/g	IC	=Log(C _i)	
MARKET				
Market storage				
Storage time	h	Mark-time _{st}	=RiskPert(0, 1.5, 3)	Personal communication
Food temperature during storage	°C	Mark-Temp _{st}	=RiskUniform(0, 10)	Personal communication
Growth				
delta		delta	$=10^{\{(-6 \times 10^{-5} \times \text{Mark} - \text{Temp}_{st}^3 \} + \{ -7 \times 10^{-4} \times \text{Mark} - \text{Temp}_{st}^2 \} + \{ 2.97 \times 10^{-2} \times \text{Mark} - \text{Temp}_{st} \} + 1.07 \}}$	González <i>et al.</i> (2009)
p		p	$=10^{\{(2 \times 10^{-5} \times \text{Mark} - \text{Temp}_{st}^3 \} + \{ -5 \times 10^{-4} \times \text{Mark} - \text{Temp}_{st}^2 \} + \{ -1.1 \times 10^{-3} \times \text{Mark} - \text{Temp}_{st} \} - 0.15 \}}$	González <i>et al.</i> (2009)
<i>Campylobacter</i> growth model	Log CFU/g	C1	=IC-(Mark-time _{st} /delta) ^p	Mafart <i>et al.</i> (2002)
Market display				
Storage time	h	Mark-time _{dis}	=RiskPert(0, 72, 120)	Personal communication
Food temperature during storage	°C	Mark-Temp _{dis}	=RiskBetaGeneral(2.1385, 2.4086, 3.2875, 12.9912)	This research
Growth				
delta		delta	$=10^{\{(-6 \times 10^{-5} \times \text{Mark} - \text{Temp}_{dis}^3 \} + \{ -7 \times 10^{-4} \times \text{Mark} - \text{Temp}_{dis}^2 \} + \{ 2.97 \times 10^{-2} \times \text{Mark} - \text{Temp}_{dis} \} + 1.07 \}}$	González <i>et al.</i> (2009)
p		p	$=10^{\{(2 \times 10^{-5} \times \text{Mark} - \text{Temp}_{dis}^3 \} + \{ -5 \times 10^{-4} \times \text{Mark} - \text{Temp}_{dis}^2 \} + \{ -1.1 \times 10^{-3} \times \text{Mark} - \text{Temp}_{dis} \} - 0.15 \}}$	González <i>et al.</i> (2009)
<i>Campylobacter</i> growth model	Log CFU/g	C2	=C1-(Mark-time _{dis} /delta) ^p	Mafart <i>et al.</i> (2002)

Table 2. Simulation model and formulas in Excel® spreadsheet used to calculate the risk of *Campylobacter* spp. on ham with @RISK (Continued)

Input Model	Unit	Variable	Formula	References
TRANSPORTATION (CAR)				
Transportation (car) storage				
Transportation time	h	Trans-time _{car}	=RiskPert(0.325, 0.984, 1.643)	Jung (2011)
Food temperature during transportation	°C	Trans-Temp _{car}	=RiskPert(10, 18, 25)	Jung (2011)
Growth				
delta		delta	$\frac{\{(-6 \times 10^{-5} \times \text{Trans-Temp}_{\text{car}}^3) + (-7 \times 10^{-4} \times \text{Trans-Temp}_{\text{car}}^2) + (2.97 \times 10^{-2} \times \text{Trans-Temp}_{\text{car}}) + 1.07\}}{10}$	González <i>et al.</i> (2009)
p		p	$\frac{\{(2 \times 10^{-5} \times \text{Trans-Temp}_{\text{car}}^3) + (-5 \times 10^{-4} \times \text{Trans-Temp}_{\text{car}}^2) + (-1.1 \times 10^{-3} \times \text{Trans-Temp}_{\text{car}}) - 0.15\}}{10}$	González <i>et al.</i> (2009)
<i>Campylobacter</i> growth model	Log CFU/g	C3	=C2-(Trans-time _{car} /delta) ^p	Mafart <i>et al.</i> (2002)
HOME				
Home storage				
Storage time	h	Home-time _{st}	=RiskPert(0, 168, 504)	Personal communication
Food temperature during storage	°C	Home-Temp _{st}	=RiskLogLogistic(-29.283, 33.227, 26.666)	MFDS (2013b)
Growth				
delta		delta	$\frac{\{(-6 \times 10^{-5} \times \text{Home-Temp}_{\text{st}}^3) + (-7 \times 10^{-4} \times \text{Home-Temp}_{\text{st}}^2) + (2.97 \times 10^{-2} \times \text{Home-Temp}_{\text{st}}) + 1.07\}}{10}$	González <i>et al.</i> (2009)
p		p	$\frac{\{(2 \times 10^{-5} \times \text{Home-Temp}_{\text{st}}^3) + (-5 \times 10^{-4} \times \text{Home-Temp}_{\text{st}}^2) + (-1.1 \times 10^{-3} \times \text{Home-Temp}_{\text{st}}) - 0.15\}}{10}$	González <i>et al.</i> (2009)
<i>Campylobacter</i> growth model	Log CFU/g	C4	=C3-(Home-time _{st} /delta) ^p	Mafart <i>et al.</i> (2002)
CONSUMPTION				
Daily consumption average amount	g	Consump	=RiskLogLogistic(0.032518, 11.282, 1.4216, RiskTruncate(0, 1000))	MHW (2012)
Daily consumption frequency	%	ConFre	Fixed 11.57	MHW (2012)
		CF(0)	=1-11.57/100	MHW (2012)
		CF(1)	=11.57/100	MHW (2012)
		CF	=RiskDiscrete({0,1}, {CF(0), CF(1)})	MHW (2012)
		Amount	=IF(CF=0,0,Consump)	MHW (2012)
DOSE-RESPONSE				
<i>Campylobacter</i> amount		n	=10 ^{C4} ×Amount	
Parameter		α	Fixed 0.145	Teunis and Havelaar (2000)
		β	Fixed 7.59	Teunis and Havelaar (2000)
		p ₁	=RiskBeta(α, β)	Teunis and Havelaar (2000)
RISK				
Probability of infection		P _{inf} (n)	=1 - (1 - p ₁) ⁿ	Nauta <i>et al.</i> (2007)
Probability of illness given infection		P _{ill\ inf}	Fixed 0.33	Nauta <i>et al.</i> (2007)
Probability of illness/person/day		Risk	=P _{inf} (n)×P _{ill\ inf}	Nauta <i>et al.</i> (2007)

Hazard characterization

Dose-response model

To estimate the probability of illness per person per day from the consumption of *Campylobacter* cells, the Beta-Poisson model for the dose-response of *Campylobacter* developed by Teunis and Havelaar (2000) was used. The probability of infection by consumed *Campylobacter* cells, p_1 , is the value described by the beta distribution [$p_1 \sim \text{beta}(\alpha, \beta)$]. Subsequently, the probability of infection (P_{inf}) due to the consumption of *Campylobacter* cells (n) is described as $P_{inf}(n) = 1 - (1 - p_1)^n$. The probability of illness given infection ($P_{ill|inf}$) was assumed by another study (Nauta *et al.*, 2007). Eventually, the probability of illness per person per day for *Campylobacter* spp. ($P_{ill}(n)$) can be calculated by multiplying $P_{inf}(n)$ and $P_{ill|inf}$.

Risk characterization

To estimate the probability of illness per person per day due to the consumption of ham, a simulation model was developed in an Excel® (MicroSoft Excel 2007, MicroSoft Corp.) spreadsheet (Table 2) based on data for prevalence, contamination level, storage temperature distribution, consumption amounts and frequencies of ham, the dose-response model and the opinion of employees, and analyzed using the @RISK program with settings for 10,000 iterations.

Results and Discussion

Hazard identification of *Campylobacter* spp.

Campylobacter spp. are zoonotic bacteria for humans

and animals. This bacterium is hard to detect and control because of its VBNC (viable but non-culturable) characteristics (Blackburn and McClure, 2009). The clinical symptoms by *Campylobacter* spp. are mainly fever, diarrhea, vomiting, Reiter syndrome, rarely Guillain-Bare syndrome, and severely death (Fugimoto *et al.*, 1992). The minimum infectious doses are 400-500 CFU, and general infectious doses are 10,000 CFU (Heyndrickx *et al.*, 2001; Luning *et al.*, 2006; MFDS, 2012). *Campylobacter* spp. are usually isolated from fresh meat, especially poultry, and they can be cross-contaminated during food preparation, resulting in *Campylobacter* spp. foodborne illness (Nauta *et al.*, 2007; Nauta *et al.*, 2009). Cytolethal distending toxin (CDT) is the main toxin produced by *Campylobacter* spp., and the toxin prevents cell division and induces cell death (Yamasaki *et al.*, 2006).

In Korea, the foodborne illness related to *Campylobacter* spp. resulted in 68 outbreaks and 2,858 cases in the last 10 years (MFDS, 2014). Although foodborne illness caused by *Campylobacter* spp. has been increased in Korea, a risk assessment for *Campylobacter* spp. has not been conducted. Thus, a microbial risk assessment for *Campylobacter* spp. is necessary.

Initial contamination level of *Campylobacter* spp.

To collect the prevalence data for *Campylobacter* spp., three types of ham were analyzed at the retail markets. For all samples ($n=200$), *Campylobacter* spp. cells were below the detection limit (0.70 Log CFU/g). Hence, the initial contamination level of *Campylobacter* spp. was predicted by beta-distribution Vose (1998) and the equation developed by Sanaa *et al.* (2004). As a result, the mean

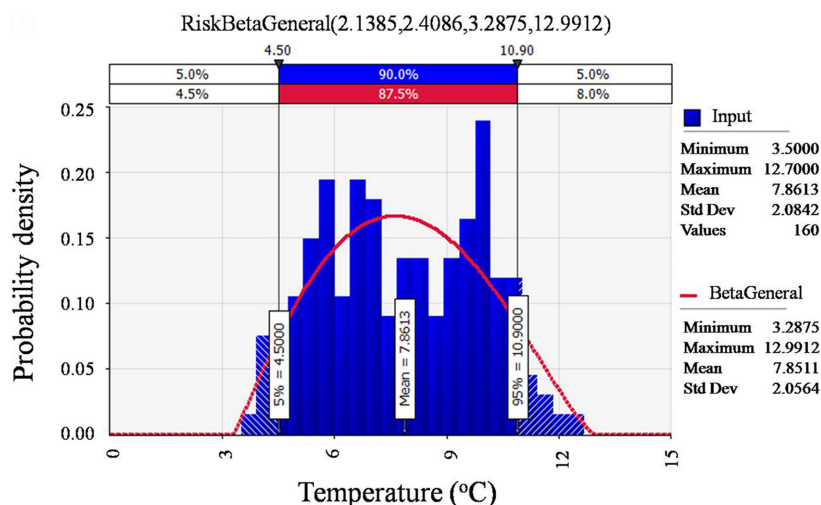


Fig. 1. Probabilistic distributions for temperature at retail markets with @RISK.

value of initial contamination level of *Campylobacter* spp. on ham was 3.95 ± 0.56 Log CFU/g, calculated from the prevalence and contamination level of *Campylobacter* spp.. It was indicated that *Campylobacter* spp. were present at an average of 0.11 CFU per 1 kg of ham. Therefore, the initial contamination level of *Campylobacter* spp. was very low.

Campylobacter spp. usually was found in poultry and other raw meats due to the fact that meats, especially poultry, are the main reservoirs of contamination of this bacterium (Nielsen *et al.*, 2006). *Campylobacter* spp. were present in 94 out of 289 poultry samples (32.5%) in Belgium in 2002 (Uyttendaele *et al.*, 2006), and 16% (almost *C. jejuni*) of 636 carcasses in Sweden (Lindblad *et al.*, 2006). Also, 259 samples of total 1,011 raw meats (chicken, pork, unweaned veal, lamb, mutton, and beef samples) were contaminated with *Campylobacter* spp. (Wong *et al.*, 2007). Compared to these results, it is considered that prevalence of *Campylobacter* spp. on ham was very low.

***Campylobacter* spp. fate during display, storage and consumption of ham**

To evaluate the fate of *Campylobacter* spp. during the display, storage and consumption of ham, in the exposure assessment, a predictive model for *Campylobacter* spp. was referred to in a study by González *et al.* (2009). The results show that *Campylobacter* spp. cell counts decreased during the distribution process of ham such as display, grocery store storage and home storage. In addition, temperatures in surface of ham at display and storage

were measured approximately 20 times, each for 30 min, at eight retail markets. The mean of the temperature profile at retail was $7.39 \pm 1.96^\circ\text{C}$, and the minimum and maximum temperatures were 3.5 and 12.7°C , respectively. Collected temperature data from eight retail markets were fitted by @RISK, and its appropriate probabilistic distribution was 'BetaGeneral distribution (2.1385, 2.4086, 3.2875, 12.9912)'. The mean, minimum and maximum values of the temperature were 7.85, 3.29 and 12.99°C , respectively (Fig. 1). In home refrigerators, the mean temperature profile was $4.06 \pm 2.28^\circ\text{C}$, and the minimum and maximum temperatures were 3.50 and 10.8°C , respectively (MFDS, 2013a). The appropriate probabilistic distribution for home refrigerators was 'LogLogistic distribution (-29.283, 33.227, 26.666)', and the mean value was $4.02 \pm 2.27^\circ\text{C}$.

Ham consumption pattern in Korea

The daily consumption amount of ham was extracted from the KNHNES 2012 using the SAS[®] version 9.3 and fitted by @RISK. The 'LogLogistic distribution (0.032518, 11.282, 1.4216)' was appropriate to explain the consumption amount of ham, and the mean value of the daily consumption of ham was 31.10 g per day (Fig. 2). The ratio of total respondents (7,208 people) and the respondents who consumed ham (834 people) was calculated to estimate the frequency of consumption, which was found to be 11.57%. The Discrete distribution was used to estimate the consumption level of *Campylobacter* spp. per person per day.

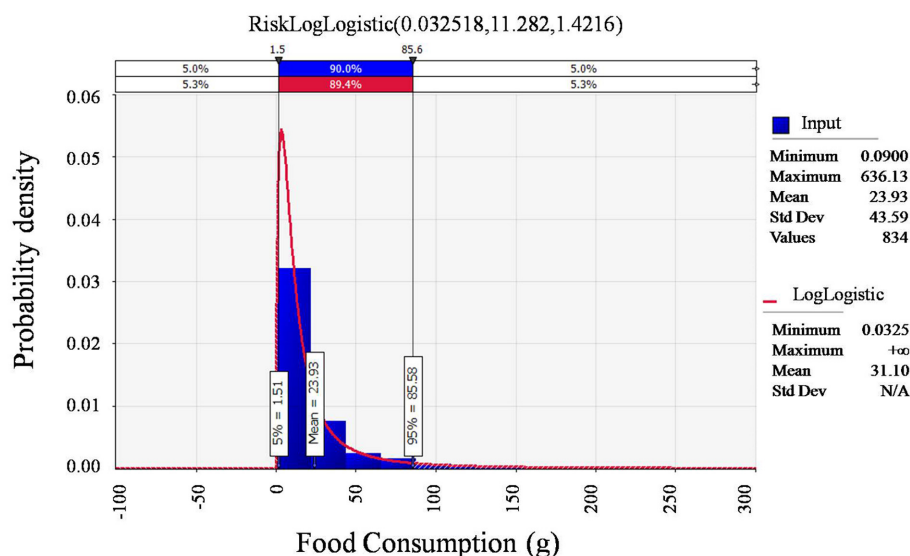


Fig. 2. Probabilistic distribution for ham intake in the Korea National Health and Nutrition Examination Survey (KNHNES) 2012 with @RISK.

Table 3. Probability of foodborne illness by *Campylobacter* spp. per person per day with consumption of ham

Ham	5%	50%	95%	99%	Maximum	Mean
Probability of illness/person/day	0	0	6.23×10^{-16}	1.68×10^{-12}	7.96×10^{-9}	2.20×10^{-12}

Dose-response

The values of α and β in *Campylobacter* were 0.145 and 7.59, respectively, then p_f was 0.019, as the Beta distribution [$p_f \sim \text{Beta}(\alpha, \beta)$]. According to other research (Nauta *et al.*, 2007), $P_{ill_{inf}}$ was 0.33 because 29 of 89 individuals who were infected developed the illness.

Risk characterization

To estimate the probability of illness per person per day caused by ham consumption, a simulation model was developed with the results listed above. The simulation model showed that *Campylobacter* spp. cell counts decreased during distribution and storage. The ingested dose of *Campylobacter* spp. by consumption of ham was estimated by final contamination level, consumption amount per day and frequency of consumption. The mean and maximum value of the probability of a *Campylobacter* spp. foodborne illness for ham per person per day were 2.20×10^{-12} and 7.96×10^{-9} , respectively (Table 3). From these results it can be assumed that the mean and maximum values for the probability of *Campylobacter* spp. foodborne illness were 8.03×10^{-10} and 2.91×10^{-6} per person per year for ham. In other literature, the mean probabilities of campylobacteriosis ingested from a chicken meal were 3.1×10^{-2} for children, and 1.5×10^{-2} for adults in Dakar, Senegal (Pouillot *et al.*, 2012). In addition, the mean probability of illness caused by *E. coli* O157:H7 was 5.1×10^{-5} for adults and 3.7×10^{-5} for children from the consumption of hamburgers (Cassin *et al.*, 1998). Ross *et al.* (2009) showed that the predicted mean values for risk of listeriosis per serving for processed meats, pâtés, and cooked sausages were 1.00×10^{-8} , 2.28×10^{-9} , and 7.06×10^{-9} , respectively. Compared with these studies, it is considered that the risk of *Campylobacter* spp. on ham is very low.

In the sensitivity analysis, the consumption amount of ham, dose-response model, prevalence of *Campylobacter* spp., and storage temperature at home were positive factors for the probable level of risk, while time for market display and transportation in a car were negative factors. According to these results, the temperature conditions were more important factor than time, to reduce the risk of foodborne illness caused by *Campylobacter* spp..

In conclusion, the risk of *Campylobacter* spp. through ham intake is very low in Korea. However, the control of foodborne illness caused by *Campylobacter* spp. on ham

is necessary due to the fact that the consumption amount of ham has increased. In addition, the results indicate that the microbial risk assessment of *Campylobacter* spp. in this study should be useful in providing scientific evidence to set up the criteria of *Campylobacter* spp. on ham.

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