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Quantitative microbial risk assessment indicates very low risk for *Vibrio parahaemolyticus* foodborne illness from *Jeotgal* in South Korea

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Abstract

In this study, a microbial risk assessment was performed for the bacteria *Vibrio parahaemolyticus*, which causes a foodborne illness following the consumption of *Jeotgal*, a fermented seafood in South Korea. The assessment comprised of six stages: product, market, home, consumption, dose-response, and risk. The initial contamination level (IC) was calculated based on the prevalence of *V. parahaemolyticus* in 90 *Jeotgal* samples. The kinetic behavior of *V. parahaemolyticus* was described using predictive models. The data on transportation conditions from manufacturer to market and home were collected through personal communication and from previous studies. Data for the *Jeotgal* consumption status were obtained, and an appropriate probability distribution was established. The simulation models responding to the scenario were analyzed using the @RISK program. The IC of *V. parahaemolyticus* was estimated using beta distribution [Beta (1, 91)]. The cell counts during transportation were estimated using Weibull and polynomial models [$\delta = 1 / (0.0718 - 0.0097 \times T + 0.0005 \times T^2)$], while the probability distributions for time and temperature were estimated using Pert, Weibull, Uniform, and LogLogistic distributions. Daily average consumption amounts were assessed using the Pareto distribution [0.60284,1.32,Risk Truncate(0,155)]. The results indicated that the risk of *V. parahaemolyticus* infection through *Jeotgal* consumption is low in South Korea.

Keywords: Foodborne illness, *Jeotgal*, Risk assessment, Simulation, *Vibrio parahaemolyticus*

Introduction

Vibrio parahaemolyticus is a gram-negative, curved, rod-shaped, halophilic bacterium that can be found in marine, coastal, and estuarine environments (Broberg et al., 2011; Nelapati et al., 2012; Tran et al., 2013). It has two types of flagella that it uses

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to swim, swarm, and produce a capsule (Letchumanan et al., 2014; McCarter, 1999). The principal virulence factors of *V. parahaemolyticus* include adhesin, thermostable direct hemolysin (TDH; *tdh*), and TDH-related hemolysin (*trh*), which are involved in its pathogenesis (Letchumanan et al., 2014; Wang et al., 2015). *V. parahaemolyticus* foodborne illness is prevalent globally and evokes symptoms of gastroenteritis in humans (Broberg et al., 2011; Daniels et al., 2000).

V. parahaemolyticus-associated foodborne illnesses are mainly caused by the contamination of raw seafood. For example, sardines contaminated with this pathogen were first identified as the cause of seafood borne illnesses in Japan (Levin, 2006). According to the Korean official statistics by the Ministry of Food and Drug Safety (2020), 52 foodborne outbreaks in 873 patients reported between 2015 and 2019 in South Korea were caused by *V. parahaemolyticus*, followed by other pathogens such as *Escherichia coli* (221 outbreaks), *Salmonell*a (88 outbreaks), and *Campylobacter jejuni* (64 outbreaks). In South Korea, *V. parahaemolyticus* was detected in raw Korean oysters at retail outlets (Lee et al., 2008); Jun et al. (2012) isolated *V. parahaemolyticus* from corb shells, short neck clams, sea mussels, sorb shells, Pacific oysters, and charm abalone purchased in 2009 at fish markets in Seoul, South Korea. In addition, *V. parahaemolyticus*-associated foodborne illnesses caused by cross-contamination from squids were reported in 2018 in South Korea (Jung, 2018).

Jeotgal is a Korean, salt-fermented, seasoned seafood that is commonly consumed as a nutritious side dish in South Korea (Koo et al., 2016; Lee, 2013). *Jeotgal* is a raw seafood delicacy prepared using squid, oyster, clam, and octopus (Koo et al., 2016), and therefore, there is a potential food vector for *V. parahaemolyticus* transmission. Microbial quantitative risk assessment of food is a procedure commonly used for identifying health risks, establishing regulations, determining the research needed, and deciding whether the current standards are adequate; the ultimate goal of these assessments is to provide public health services through food safety management (Lammerding, 1997; USDA, FSIS & EPA, 2012). Studies on the risk assessment of *Jeotgal* related to ethyl carbamate (Lee, 2013) have been performed; however, risk assessment for *V. parahaemolyticus* contamination in *Jeotgal* has not been conducted yet. The purpose of this study was to analyze the risk of foodborne illnesses caused by the consumption of *Jeotgal* contaminated with *V. parahaemolyticus*.

Materials and Methods

Prevalence of *Vibrio parahaemolyticus* in *Jeotgal*

Among the various types, squid, clam, and oyster *Jeotgal* were the focus of this study. Ninety *Jeotgal* samples (30 squid , 30 clam, and 30 oyster) were purchased from markets in Seoul and Mokpo, as well as from online markets in South Korea. The *Jeotgal* samples (25 g) were placed in filter bags (3M, St. Paul, MN, USA) along with sterile 0.85% saline (225 mL) and homogenized at 230 rpm for 2 min using a stomacher (Seward, Worthing, UK). Aliquots of the homogenates (1 mL) and diluents (100 μL) were spread onto thiosulfate-citrate-bile salts-sucrose agar (TCBS; BD, Franklin Lakes, NJ, USA) and incubated at 35℃ for 24 h. Colonies on the plates that were predicted to be *V. parahaemolyticus* were analyzed using a compact VITEK2 GN card (BioMérieux, Craponne, France) and PCR targeting the thermolabile hemolysin (*tlh*) gene. The PCR conditions were as follows: initial denaturation at 94℃ for 3 min; 30 cycles of denaturation at 94℃ for 1 min, elongation at 58℃ for 1 min, extension at 72℃ for 1 min, and final extension at 72℃ for 5 min (Bej et al., 1999).

Based on the number of *V. parahaemolyticus*-positive *Jeotgal* samples, the prevalence was estimated using the beta distribution [RiskBeta (α: number of positive samples + 1, β: total number of samples – number of positive samples + 1)], and the initial contamination level (IC) was estimated as described by Vose (1997).

Selection of a model *Jeotgal*

Although there are several types of *Jeotgal*, only one can be used to establish a predictive model for microbial risk assessment. Thus, a predictive model should be developed using model food. The prediction from the model food should be higher than that from the other types of *Jeotgal*. Therefore, five types of *Jeotgal*, prepared using squid, octopus, oyster, pollack roe, and clam, were purchased from supermarkets in Seoul, South Korea. Colonies of *V. parahaemolyticus* ATCC 17802, ATCC 27519, ATCC 33844, and ATCC 43996 strains were inoculated in 10 mL of marine broth (BD) and incubated at 35℃ for 24 h. Aliquots (100 μL) were also inoculated in 10 mL of fresh marine broth and incubated at 35℃ for 24 h. The supernatant was removed following centrifugation at 1,912×g at 4℃ for 15 min using centrifuge (Combi R515; Hanil Science, Gimpo, Korea). The washed cell suspensions were then mixed and diluted with phosphate buffered saline (8.0 g of NaCl, 1.5 g of

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$Na_2HPO_4 \cdot 7H_2O$, 0.2 g of KH_2PO_4, and 0.2 g of KCl in 1 L of distilled water; pH 7.4) to obtain 6.0 Log CFU/mL. *Jeotgal* samples were inoculated with the prepared solution, to obtain a concentration of 4.0 Log CFU/g. Inoculated samples were stored at 20℃ for 96 h, during which alkaline peptone water (20 mL) (APW; BD) was placed in a conical tube (BD) containing 10 g of *Jeotgal*, that had been homogenized by vortexing for 1 min. The homogenates were decimally diluted in APW, followed by spread-plating on TCBS agar and incubation at 35℃ for 24 h. *V. parahaemolyticus* cell counts obtained in the five types of *Jeotgal* were compared. The type of *Jeotgal* that had the highest survival rate of *V. parahaemolyticus* was selected as the model food for developing predictive models.

Development of predictive models

Among the *Jeotgal*, squid *Jeotgal* retained the most live bacteria, and thus, squid *Jeotgal* was selected for predictive models. Ten-gram portions of squid *Jeotgal*, purchased from a supermarket in Seoul, South Korea, were inoculated with *V. parahaemolyticus* to obtain a 4.0 Log CFU/g concentration. The inoculated samples were stored at 7℃, 15℃, 25℃, and 35℃, considering the exposable temperature of *Jeotgal* during distribution and storage. During storage, 10 g of each *Jeotgal* sample was diluted with 20 mL of APW, and the samples were then homogenized by vortexing for 1 min. Homogenates (0.1 mL) diluted in APW were inoculated onto TCBS agar by spreading. The plates were incubated at 35℃ for 24 h, after which *V. parahaemolyticus* cell counts were confirmed. To describe the kinetic behavior of *V. parahaemolyticus* in *Jeotgal*, a primary model was developed by fitting the Weibull model [$\text{Log}(N) = \text{Log}(N_0) - (\text{time}/\delta)^{\rho}$; N, cell counts; N_0, initial cell counts; δ, treatment time for the first decimal reduction; ρ, curve shape parameter] (Mafart et al., 2002) to the *V. parahaemolyticus* cell counts. A secondary model was developed by fitting a polynomial model [$Y = 1 / (a + b \times T + c \times T^2)$; a, b, and c, constant; T, storage temperature] with δ values as a function of the storage temperature. This model was used to describe the effect of temperature on the kinetic parameters. To validate the model performance, squid *Jeotgal* was inoculated with *V. parahaemolyticus*, and the samples were exposed to the temperatures 10℃ and 23℃. *V. parahaemolyticus* cell counts (observed values) were compared to the values predicted by the developed models. The root-mean-square error (*RMSE*; Baranyi et al., 1996) was calculated as follows:

$$RMSE = \sqrt{\sum (observed\ value - predicted\ value)^2 / n}$$

Data collection of distribution condition

Distribution time and temperature data for the *Jeotgal*, including transportation, storage, and display at market, were collected via personal communication with an administrator who is in charge of *Jeotgal* sales at a major market in Korea. Storage time at home was collected via personal communication, and the distribution model for food temperature at home storage was constructed as described by Lee et al. (2015).

Data collection of consumption condition

Among the various types, squid *Jeotgal* is the most commonly consumed *Jeotgal* in South Korea; hence, the risk in the simulation may be overestimated if the data of consumption for all types of *Jeotgal* is applied in this scenario. Thus, only the consumption data for squid *Jeotgal* were collected and analyzed. Data on the daily consumption frequency and amount of squid *Jeotgal* in South Korea were collected from the 2016 Korea National Health and Nutrition Examination Survey (KNHNES; KCDC, 2018). The appropriate probability distribution from this data was analyzed using the @RISK program (version 7.6, Palisade, Ithaca, NY, USA).

Hazard characteristics and risk characterization

The probability of illness/person/day was calculated using a beta-Poisson dose-response model associated with *V. parahaemolyticus*, which was evaluated as a well-described model and generally used by FAO & WHO (2011) and Iwahori et al. (2010). The full simulation model included data on *V. parahaemolyticus* prevalence and initial concentration, the predictive models applied to distribution condition, consumption amount and frequency, and the dose-response model. The risk was estimated using a comprehensive simulation in the @RISK program.

Statistical analysis

Kinetic parameters (δ and ρ values) from the predictive models which were developed by three repetitions of experiments were established using the general linear model in SAS® (version 9.3, SAS Institute, Cary, NC, USA). The kinetic parameters at different storage temperatures were compared using pairwise *t*-test with least squared means at $\alpha = 0.05$.

Results and Discussion

Initial contamination levels of *Vibrio parahaemolyticus*

V. parahaemolyticus contamination was not detected in any of

the 90 *Jeotgal* samples; thus, the IC was estimated using a beta distribution [BetaRisk(1,91)] model. The average IC estimated was –3.6 Log CFU/g (Fig. 1).

Predictive model

In the preliminary experiments, *V. parahaemolyticus* in squid *Jeotgal* survived for the longest time compared to that in other *Jeotgal* (data not shown). The pH (6.0) of squid *Jeotgal* was relatively high compared to that of other types (octopus: 5.73, oyster: 5.45, pollock roe: 5.63, and clam: 5.61). The water activity (Aw) of squid *Jeotgal* was 0.980, which is similar to the values of other types of *Jeotgal* (octopus: 0.983, oyster: 0.989, pollock roe: 0.988, and clam: 0.981). The salinity of squid *Jeotgal* was 4.73%, which was different from those of the other types (octopus: 34.3%, oyster: 3.02%, pollock roe: 4.50%, clam: 7.80%). Considering the overall characteristics, including optimal NaCl concentration (2%–4%), pH (pH 7.6–8.6), and Aw (0.936–0.995), *V. parahaemolyticus* could survive the longest time in squid *Jeotgal* (Jay et al., 2005; Miles et al., 1997; Parveen et al., 2013).

Squid *Jeotgal* was ultimately selected to develop a predictive model (Table 1). *V. parahaemolyticus* δ values decreased with increasing temperature: 7℃ (δ = 56.77), 15℃ (δ = 25.11), 25℃ (δ = 7.23), and 35℃ (δ = 3.02) as the storage time progressed. The ρ values in the Weibull (primary) model were 0.72 (7℃), 0.56 (15℃), 0.53 (25℃), and 0.66 (35℃). The goodness of fit for the primary model was evaluated with an R^2 value of 0.899–0.936 (Table 1), which were close to the R^2 values calculated by Ha et al. (2019) that was the range of 0.869–0.967 in the Weibull models. Considering the correlation between the δ values and temperature, the polynomial (secondary) model for δ values was developed as $\delta = 1 / (0.0718 - 0.0097 \times T + 0.0005 \times T^2)$, resulting in an R^2 value of 0.860 (Fig. 2). The R^2 of the δ values derived from the polynomial (secondary) model developed by Lee et al. (2019) was 0.890, which is similar to the resulting values of this study; however, the ρ values showed no correlation with temperature, eliminating the need for a secondary model. The average ρ value (0.6158) from the primary model was used in the simulation. The predictive model was validated for performance at different temperatures (*RMSE*: 0.746 for 10℃ and 0.470 for 23℃) and in different types of *Jeotgal* (*RMSE*: 0.394). Ha et al. (2019) reported that an *RMSE* of 0.618 indicated appropriate model performance. The *RMSE* in this study indicates that the model appropriately describes the kinetic behavior of *V. parahaemolyticus* in *Jeotgal*.

Probability distributions for storage time and temperature

The *Jeotgal* products were transported from manufacturers to markets within 4, 5, or 7 h from manufacturing, which were

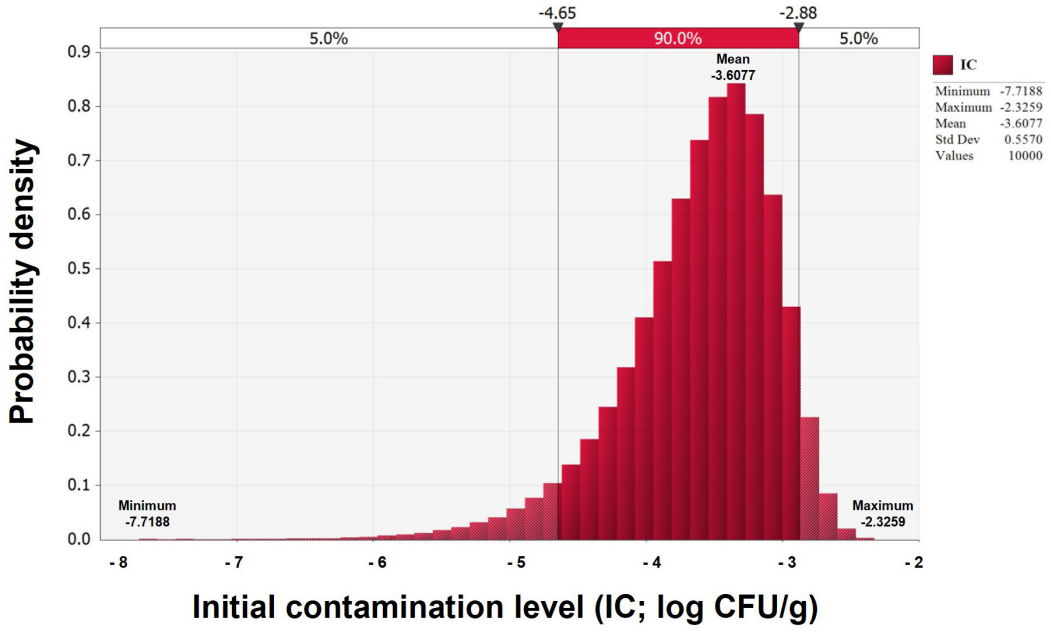

Fig. 1. Beta distribution of the initial contamination level (IC) of *Vibrio parahaemolyticus* in *Jeotgal*.

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Table 1. Kinetic parameters of *Vibrio parahaemolyticus* death in *Jeotgal* during storage at 7℃, 15℃, 25℃, and 35℃, calculated by the Weibull model (primary model)

Storage temperature (℃)	Kinetic parameters		R^2
	δ	ρ	
7	56.77 ± 34.58^{A}	0.72 ± 0.25^{A}	0.932 ± 0.027
15	25.11 ± 16.33^{AB}	0.56 ± 0.15^{A}	0.899 ± 0.033
25	7.23 ± 3.68^{B}	0.53 ± 0.03^{A}	0.936 ± 0.023
35	3.02 ± 0.74^{B}	0.66 ± 0.07^{A}	0.913 ± 0.054

Values are mean ± SD.
^{A,B} Values followed by different letters in a row are significantly different.
δ, treatment time for the first decimal reduction; ρ, curve shape parameter.

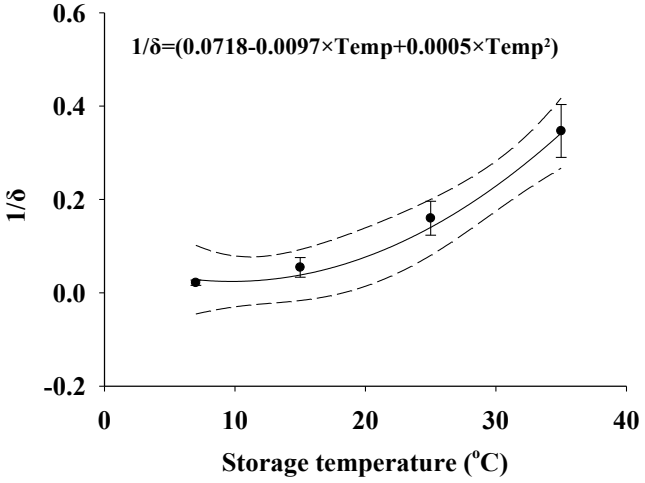

Fig. 2. δ values from the Weibull models (primary models) and fitted line developed by the polynomial model (secondary model) for *Vibrio parahaemolyticus* in *Jeotgal* made of squid.
•, observed value; line, fitted value from the polynomial model; ---, 95% confidence interval.

collected through personal communication with the person in charge of the products at the market, and thus facilitating the analysis of time using the Pert distribution [RiskPert(4,5,7)]. The data on food temperature during transport, which were collected through personal communication with the person in charge of the products at the market, were fitted with a Weibull distribution [RiskWeibull(1.3219,2.8404, RiskShift(3.1093), RiskTruncate(1,40))]. The *Jeotgal* products were stored at 0℃–10℃ for 0.17–0.5 h, and the uniform distribution was fitted using the parameters RiskUniform(0,10) and RiskUniform(0.17,0.5), respectively. At the markets, the *Jeotgal* products were displayed at 2.2281℃–20.272℃ for 0–4,320 h (mean: 720 h); thus, RiskUniform(2.2281,20.272) and RiskPert(0,720,4320) were applicable, respectively to the model. After purchase, the *Jeotgal* products were stored in refrigerators at home for 0–720 h (shelf life) until consumption. Thus, the data for the storage time at home were fitted to a uniform distribution with the parameter RiskUniform(0,720), and food temperature during home storage was described using the LogLogistic distribution [–29.283,33.227,26.666, RiskTruncate(–5,20)] (Lee et al., 2015; Table 2).

Jeotgal consumption in South Korea

According to the 2016 KNHNES (KCDC, 2018) dataset, the daily consumption frequency of squid *Jeotgal* was 80.0%, with an average mass consumption of 13.9 g in South Korea. For the consumption related data, the Pareto distribution [(0.60284,1.32, RiskTruncate(0,155)] was determined to be the optimal probabilistic distribution analyzed by the @RISK program (Table 2 and Fig. 3).

Vibrio parahaemolyticus dose-response model

The Beta-Poisson dose-response model [$1 - (1 + D / \beta)^{-\alpha}$, $\alpha = 0.17$, $\beta = 1.18 \times 10^5$] was selected for *V. parahaemolyticus* (FAO & WHO, 2011; Iwahori et al., 2010), where D is the viable *V. parahaemolyticus* cell count consumed and calculated from consumption amount (g) of *Jeotgal* × *V. parahaemolyticus* cell counts (CFU/g). Consumption amounts were calculated from the initial concentration to home storage derived from the predictive models, responding to the distribution and storage conditions (time and temperature) (Table 2).

Risk characterization

The simulation for *V. parahaemolyticus*-associated illness following the consumption of contaminated *Jeotgal* was performed for a scenario consisting of different stages categorized as product, market, home, consumption, dose-response, and risk (Table 2). Specifically, transportation, storage, and display were associated with the market stage, and daily consumption frequency and average daily consumption amount were associated with the consumption stage. The IC calculated from the product stage was applied to the predictive models at the market and home stages, as a response to the environment (time and temperature). In the consumption and dose-response stages, the final contamination level was applied. The probability of illness per person per day was calculated and applied to the scenario described above. The mean probability of illness per person per

Table 2. @RISK simulation model and scenario for calculation of the risk of *Vibrio parahaemolyticus* foodborne illness by consumption of *Jeotgal*

Input model	Variable	Formula	Reference
Product			
Pathogen contamination level			
V. parahaemolyticus prevalence	PR	= RiskBeta(1,91)	This study; Vose, 1997
Concentration (CFU/g)	C	= –LN (1 – PR) / 25 g	Sanaa et al., 2004
Initial contamination level (Log CFU/g)	IC	= Log(C)	
Market			
Market transportation			
Transportation time (h)	$Mark\text{-}time_{trans}$	= RiskPert(4,5,7)	Personal communication[1)]
Food temperature during transportation (℃)	$Mark\text{-}Temp_{trans}$	= RiskWeibull(1.3219,2.8404,RiskShift(3.1093),RiskTruncate(1,40))	Personal communication
Death			
Treatment time for the first decimal reduction	δ	$= 1 / (0.0718 - 0.0097 \times Mark\text{-}Temp_{trans} + 0.0005 \times Mark\text{-}Temp_{trans}^2)$	This study
Curve shape parameter	ρ	Fixed 0.6158	This study
V. parahaemolyticus survival model	C1	$= IC - (Mark\text{-}time_{trans} / \delta)^{\rho}$	Mafart et al., 2002
Market storage			
Storage time (h)	$Mark\text{-}time_{st}$	= RiskUniform(0.17,0.5)	Personal communication
Food temperature during storage (℃)	$Mark\text{-}Temp_{st}$	= RiskUniform(0,10)	Personal communication
Death			
Treatment time for the first decimal reduction	δ	$= 1 / (0.0718 - 0.0097 \times Mark\text{-}Temp_{st} + 0.0005 \times Mark\text{-}Temp_{st}^2)$	This study
Curve shape parameter	ρ	Fixed 0.6158	This study
V. parahaemolyticus survival model	C2	$= C1 - (Mark\text{-}time_{st} / \delta)^{\rho}$	Mafart et al., 2002
Market display			
Display time (h)	$Mark\text{-}time_{dis}$	= RiskPert(0,720,4320)	Personal communication
Display temperature in market (℃)	$Mark\text{-}Temp_{dis}$	= RiskUniform(2.2281,20.272)	Personal communication
Death			
Treatment time for the first decimal reduction	δ	$= 1 / (0.0718 - 0.0097 \times Mark\text{-}Temp_{dis} + 0.0005 \times Mark\text{-}Temp_{dis}^2)$	This study
Curve shape parameter	ρ	Fixed 0.6158	This study
V. parahaemolyticus survival model	C3	$= C2 - (Mark\text{-}time_{dis} / \delta)^{\rho}$	Mafart et al., 2002
Home			
Home storage			
Storage time (h)	$Home\text{-}time_{st}$	= RiskUniform(0,720)	Personal communication
Food temperature during storage (℃)	$Home\text{-}Temp_{st}$	= RiskLogLogistic(–29.283,33.227,26.666,RiskTruncate(–5,20))	Lee et al., 2015
Death			
Treatment time for the first decimal reduction	δ	$= 1 / (0.0718 - 0.0097 \times Home\text{-}Temp_{st} + 0.0005 \times Home\text{-}Temp_{st}^2)$	This study
Curve shape parameter	ρ	Fixed 0.6158	This study
V. parahaemolyticus survival model	C4	$= C3 - (Home\text{-}time_{st} / \delta)^{\rho}$	Mafart et al., 2002
Consumption			
Daily consumption average amount (g)	Consump	= RiskPareto(0.60284,1.32,RiskTruncate(0,155))	KCDC, 2018
Daily consumption frequency (%)	ConFre	Fixed 0.8	KCDC, 2018
Daily non-consumption frequency (rate)	CF(0)	= 1 – 0.8 / 100	KCDC, 2018
Daily consumption frequency (rate)	CF(1)	= 0.8 / 100	KCDC, 2018

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Table 2. Continued

Input model	Variable	Formula	Reference
Distribution for consumption frequency	CF	= RiskDiscrete ({0,1},{CF(0),CF(1)})	KCDC, 2018
Daily consumption average amount considered frequency	Amount	= IF(CF = 0,0,Consump)	KCDC, 2018
Dose-response			
V. parahaemolyticus amount (CFU)	*D*	= 10^{C4} × Amount	
Parameter	α	Fixed 0.17	FAO & WHO, 2011; Iwahori et al., 2010
Parameter	β	1.18×10^{5}	FAO & WHO, 2011; Iwahori et al., 2010
Risk			
Probability of illness/person/day	Risk	$=1-(1+D/\beta)^{-\alpha}$	FAO & WHO, 2011; Iwahori et al., 2010

[1] Personal communication with a person in charge of products at the market.
IC, initial contamination level; C1, market transportation; C2, market storage; C3, market display; C4, home storage.

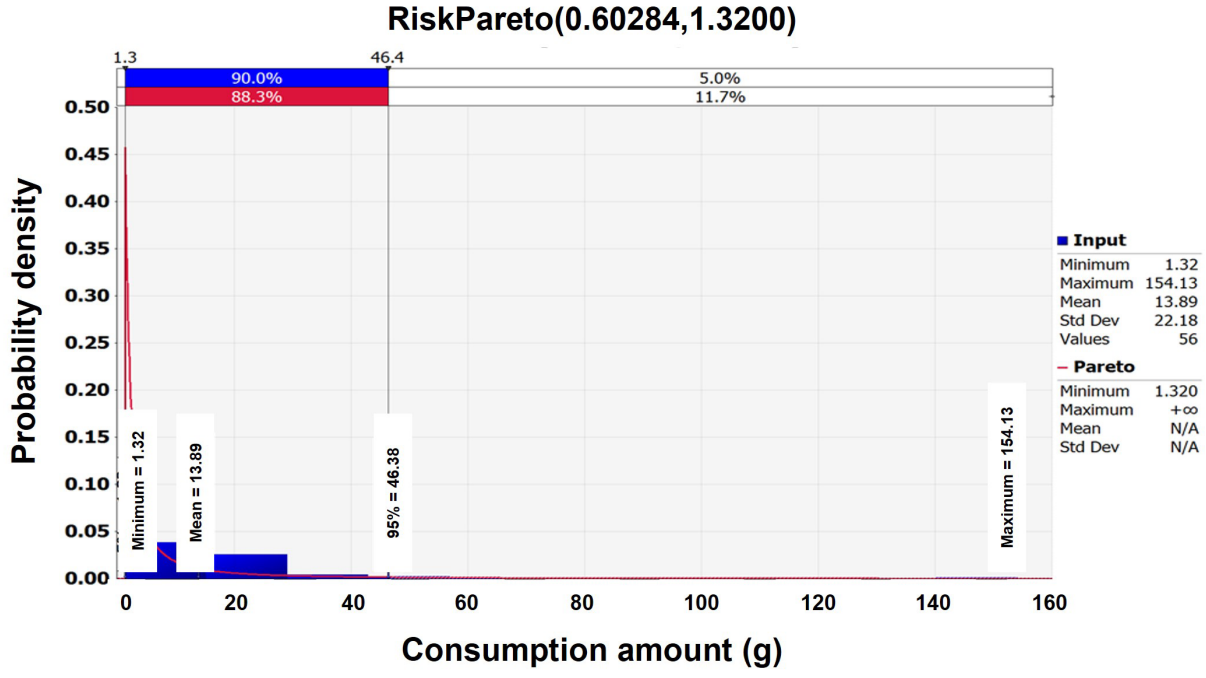

Fig. 3. Pareto distribution of daily consumption of squid *Jeotgal* without additional heating.

day was zero. This low risk was derived from the continuously decreasing cumulative contamination levels of *V. parahaemolyticus*: –3.61 (IC), –3.94 (market transportation), –4.01 (market storage), –14.11 (market display), and –23.37 Log CFU/g (home storage) (Fig. 4).

Conclusion

Jeotgal containing raw seafoods could be a vector of *V. parahaemolyticus*-associated foodborne illness. In this study, *V. parahaemolyticus* was not detected in all *Jeotgal* and the contamination level was decreased as the distribution carried on in the

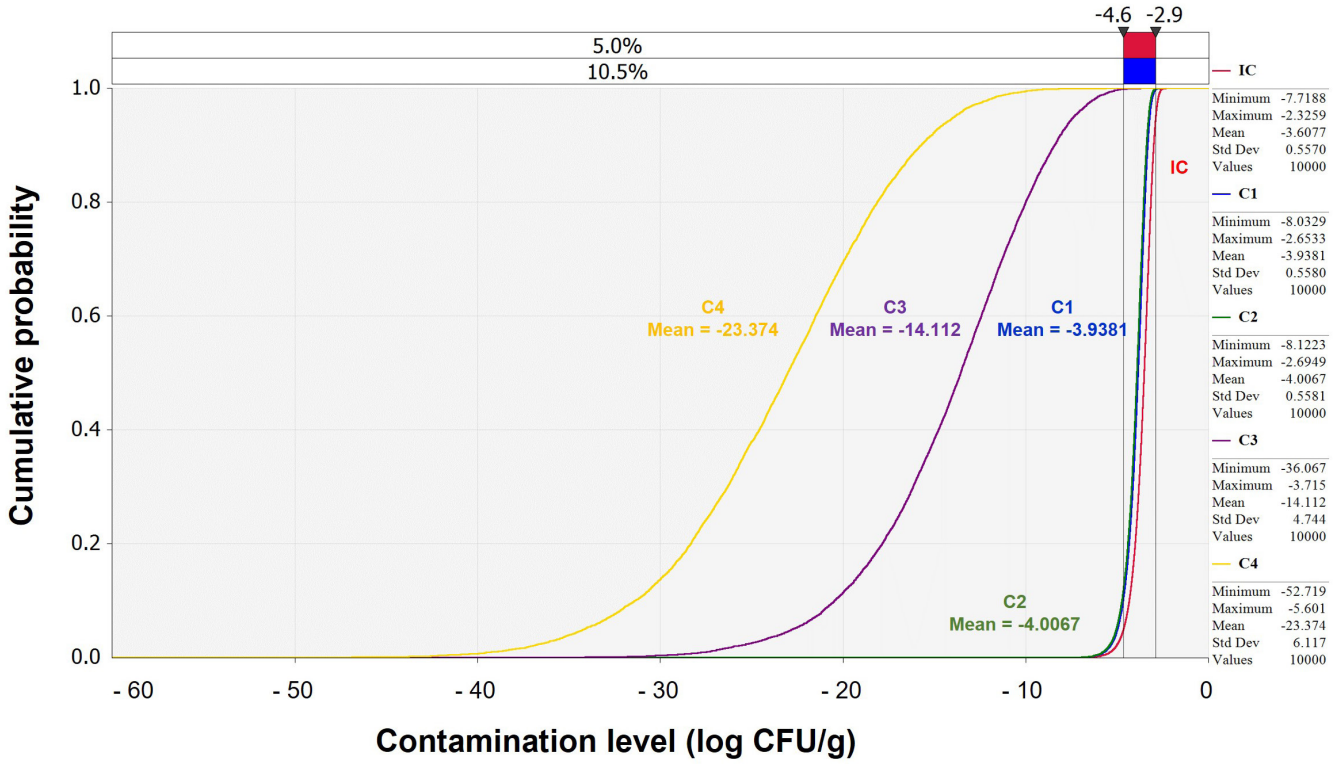

Fig. 4. Changes in *Vibrio parahaemolyticus* contamination level of *Jeotgal* during C1, C2, C3, and C4, predicted by distribution models. IC, initial contamination level; C1, market transportation; C2, market storage; C3, market display; C4, home storage.

simulation. The risk of *V. parahaemolyticus* infection in *Jeotgal* was very low, as analyzed by quantitative risk assessment, with respect to the distribution in South Korea. The result suggested that the low contamination level and distribution environment could be important factors to decrease the probability of *V. parahaemolyticus*-associated foodborne illnesses.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

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