J Anim Sci Technol 2023;65(5):1024-1039 https://doi.org/10.5187/jast.2023.e18

Check for updates

Received: Jan 17, 2023 Revised: Jan 31, 2023 Accepted: Feb 8, 2023

[#]These authors contributed equally to this work.

*Corresponding author

Soomin Lee Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea. Tel: +82-2-2077-7585 E-mail: slee0719@naver.com

Heeyoung Lee Food Standard Research Center, Korean Food Research Institute, Wanju 55365, Korea. Tel: +82-63-219-9333 E-mail: hylee06@kfri.re.kr

Copyright © 2023 Korean Society of Animal Sciences and Technology. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http:// creativecommons.org/licenses/bync/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Hyemin Oh https://orcid.org/0000-0002-8073-7242 Yohan Yoon https://orcid.org/0000-0002-4561-6218 Quantitative risk assessment of foodborne *Salmonella* illness by estimating cooking effect on eggs from retail markets

Hyemin Oh^{1,2#}, Yohan Yoon^{1,2#}, Jang Won Yoon³, Se-Wook Oh⁴, Soomin Lee^{2*} and Heeyoung Lee^{5*}

¹Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea ²Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea ³College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea ⁴Department of Food and Nutrition, Kookmin University, Seoul 02703, Korea

^⁴Department of Food and Nutrition, Kookmin University, Seoul 02703, Korea ^₅Food Standard Research Center, Korean Food Research Institute, Wanju 55365, Korea

Abstract

In this study, we performed a quantitative microbial risk assessment (QMRA) of Salmonella through intake of egg consumption after cooking (dry-heat, moist-heat, and raw consumption). Egg samples (n = 201) from retail markets were analyzed for the presence of Salmonella. In addition, temperature and time were investigated during egg transit, storage, and display. A predictive model was developed to characterize the kinetic behavior of Salmonella in eggs, and data on egg consumption and frequency were collected. Eventually, the data was simulated to estimate egg-related foodborne illnesses. Salmonella was not found in any of the 201 egg samples. Thus, the estimated initial contamination level was -4.0 Log CFU/ g. With R^2 values of 0.898 and 0.922, the constructed predictive models were adequate for describing the fate of Salmonella in eggs throughout distribution and storage. Eggs were consumed raw (1.5%, 39.2 g), dry-heated (57.5%, 43.0 g), and moist-heated (41%, 36.1 g). The probability of foodborne Salmonella illness from the consumption of cooked eggs was evaluated to be 6.8×10⁻¹⁰. Additionally, the probability of foodborne illness not applied cooking methods was 1.9×10⁻⁷, indicating that Salmonella can be reduced by cooking. Therefore, the risk of Salmonella infection through consumption of eggs after cooking might be low in S. Korea.

Keywords: Eggs, Salmonella, Quantitative microbial risk assessment (QMRA), Cooking method, Food safety

INTRODUCTION

Salmonella is harmful bacteria that causes foodborne illness in sensitive consumers like the elderly (> 65 years old), children (< 5 years old), pregnant women, and immune weakened people [1]. *Salmonella* infection can be transmitted by contaminated eggs or chicken meat, as well as transportation, cooking, and serving. After an incubation period of 6 to 48 h, symptoms such as vomiting, diarrhea, and

Jang Won Yoon https://orcid.org/0000-0002-6874-5290 Se-Wook Oh https://orcid.org/0000-0002-8580-6032 Soomin Lee https://orcid.org/0000-0003-1811-7365 Heeyoung Lee https://orcid.org/0000-0001-6115-9179

Competing interests

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

Funding sources

This research was supported by a grant (18162KFDA525) from the Korea Food and Drug Administration in 2020.

Acknowledgements Not applicable.

Availability of data and material

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

Authors' contributions

Conceptualization: Yoon Y, Lee S, Lee H. Data curation: Oh H, Oh SW, Lee S. Formal analysis: Oh H, Lee H. Methodology: Oh H, Yoon JW, Oh SW. Validation: Yoon Y, Lee H. Investigation: Yoon Y, Yoon JW. Writing - original draft: Oh H. Writing - review & editing: Oh H, Yoon Y, Yoon JW, Oh SW, Lee S, Lee H.

Ethics approval and consent to participate

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

fever occur when contaminated foods are ingested [2,3]. *Salmonella* causes roughly 1.35 million infections, 420 deaths, and 26,500 hospitalizations in the United States, according to the CDC [4]. In 2021, five European Union/European Economic Area (EU/EEA) nations and the United Kingdom (UK) reported 272 confirmed cases. There were two adult male deaths, and twenty-five individuals were hospitalized. Sixty of the interviewees specifically mentioned consuming eggs/ egg products [5]. In 2020, eggs and egg products are the most common foods linked to *Salmonella*, accounting for 5.3% of all the foodborne *Salmonella* outbreaks [6]. *Salmonella* can transmit an egg either from the inside of a chicken (vertical transmission of the pathogen) or from the outside (horizontal transmission from poultry feces) [7].

Although consumption of raw or incompletely cooked food is associated with the risk of salmonellosis, eggs are consumed either raw or cooked [8]. Salmonellosis is most commonly caused by consuming raw egg products such as sauces and spreads produced with raw eggs (e.g., whipped cream and egg butter), sweets created without an adequate cooking process (e.g., tiramisu and chocolate mousse), and drinks containing raw eggs (e.g., eggnog and raw egg high-protein smoothies) [9]. Avoiding undercooked or raw egg products reduces the risk of *Salmonella* illness [10,11]. As the last line of protection in the food system, consumers' cooking techniques reduce foodborne infections at home [12].

A quantitative microbial risk assessment (QMRA) can quantify risk levels and provide a basis for food safety. In addition, this assessment evaluates the risk probability of foodborne pathogens in food during the distribution from final products to consumption with cooking at home [13,14]. Changes in *Salmonella* cell counts by cooking can accurately estimate the *Salmonella* QMRA. In the present study, the reduction of load of *Salmonella* pathogens during cooking was examined, as well as the consumption frequency and patterns of egg-based-food in order to assess the risk of *Salmonella* illness due to the egg consumption. The purpose of this study was to assess the risk of foodborne *Salmonella* illness due to the consuming of raw and cooked egg samples obtained from the markets in S. Korea.

MATERIALS AND METHODS

Investigation of Salmonella prevalence in eggs and determination of initial contamination level

To monitor Salmonella in eggs throughout retail markets in S. Korea, 201 samples were collected and analyzed from two retail markets and thirteen traditional markets (four in the capital region, two in the Chungcheong region, three in the Gangwon region, three in the southwest region, and one in the southeast region). The isolation and identification method were used to detect Salmonella as described by 'Bacteriological test method for eggs' in the Food code [15]. All of the egg samples were taken aseptically and soaked for 10 s in a disinfectant solution containing 250 mL of Lugol's solution (an iodine/potassium iodide solution) and 750 mL of 70% alcohol. The purpose of disinfecting the eggshell is to kill microorganisms on the surface of the eggshell in order to check only the internal contamination of the egg samples [16]. Eggs were taken out to dry, and a piece of an egg was broken into 225 mL of buffered peptone water (BPW; Becton Dickinson and Company [BD], Franklin Lakes, NJ, USA) in a sterile filter bag (3M, St. Paul, MN, USA). The homogenates were then incubated at $36 \pm 1^{\circ}$ for 18–24 h after being mixed for 60 s using a BagMixer (Interscience, St. Nom, France). The 0.1-mL aliquot of the enriched suspension was placed into 10 mL of Rappaport-Vassiliadis medium (RV; BD) and incubated for 20-24 h at 42°C. One loop of the incubated RV culture was streaked onto Xylose Lysine Deoxycholate (XLD; BD) agar plates, which were then incubated at 37 °C for 24 h. 16s rRNA was analyzed identify typical *Salmonella* black colonies with clear membranes. *Salmonella* prevalence data (PR) from eggs were fitted to the beta distribution (α , β), where α is the number of positive samples plus one, and β is one plus the number of positive samples subtracted from the total samples [17]. The initial contamination level (CFU/mL) of *Salmonella* in egg samples was determined using the equation [-LN (1-PR) / mL], originally presented by Sanaa et al. [18].

Predictive model development

Salmonella inoculum preparation

Twelve poultry-isolated *Salmonella* strains (FKS001, FKS002, S2, S15, S22, S30, S39, S46, S50, S56, S66, and S72) and two reference strains (*S.* Typhimurium ATCC 70020 and *S.* Enteritidis ATCC 13076) were cultured at 37° C for 24 h in 10 mL of tryptic soy broth (TSB; BD). Following the inoculation, 1-mL aliquots of each culture were inoculated into 10 mL of TSB and incubated for 24 h at 37° C. After centrifugation at 1,912×g and 4°C for 15 min, the *Salmonella* bacteria were washed twice with phosphate buffered saline (PBS; 8.0 g NaCl, 1.5 g NaHPO₄, 0.2 g KH₂PO₄, and 0.2 g KCl in 1 L distilled water, pH 7.4). To obtain 6 Log CFU/mL of *Salmonella* inoculum, the optical density (OD) of the cell suspensions was adjusted to 2.0 at 600 nm. PBS was used to modify the cell concentrations so that the strains had similar cell counts. The suspensions were then mixed and used as the inoculum.

Determination of inoculation methods to develop the predictive model

Due to temperature differences between rinsing water and eggs, *Salmonella* can penetrate the shell and infect eggs [19,20]. To confirm the penetration of *Salmonella* into eggs due to the temperature difference, eggs at 42 °C, which is the body temperature of poultry, were immersed in 8–9 Log CFU/mL of *Salmonella* inoculum at 4°C for 1 min and then dried for 30 min. Additionally, 0.1 mL of the inoculum was added to the egg yolks and whites to confirm *Salmonella* growth. The samples were microbiologically examined after seven days at 15°C. Ten mililiters of 0.1% BPW were put into each infected egg yolk and egg white and pounded for 10 s with a pummeler (BagMixer®, Interscience, St. Nom, France). The homogenates were then serially diluted in 9 mL of 0.1% BPW, and 0.1-mL aliquots were spread-plated on XLD. The XLD plates were incubated at 37°C for 24 h under aerobic conditions.

Development of predictive models

To develop prediction models of *Salmonella* in eggs, each egg was directly injected into the egg yolk with 2–3 Log CFU/g of *Salmonella* inoculum, and the injection holes were sealed. Infected eggs were stored at 7, 15, 25, and 30 °C for 4–7 days. In this investigation, the average weight of the egg samples was 52.5 g. To enumerate the *Salmonella* cells, samples were placed in a sterile filter bag (3M) with 10 mL of 0.1% BPW then pummeled with a pummeler for 60 s. The aliquot (0.1 mL) of the diluted homogenates were spread-plated on XLD agar. The plates were incubated at 37 °C for 24 h. *Salmonella* cell counts were fitted to the Baranyi model [21] using DMfit (Institute of Food Research, Norwich, UK). The Baranyi model was as follows:

$$N_{t} = N_{0} + \mu_{\max} \times \ln\left[1 + \frac{\exp(\mu_{\max} \times A_{t}) - 1}{\exp(N_{\max} - N_{0})}\right]$$
$$A_{t} = t + \frac{1}{\mu_{\max}} \ln\left(\frac{\exp(-\mu_{\max}) + q_{0}}{1 + q_{0}}\right)$$

$$q_0 = \frac{1}{\exp(h_0) - 1}$$

The maximal specific growth rate (μ_{max} ; Log CFU/g/h) and lag-phase duration (*LPD*; h) were determined as kinetic parameters. Using the polynomial model, the *LPD* and $\sqrt{\mu_{max}}$ values were examined as a function of temperature to develop a secondary model:

$$LPD = a_0 + a_1T^1 + a_2T^2$$
 and $\sqrt{\mu_{\text{max}}} = a(T - T_{\text{min}})$

where a_i is the coefficient value, and *T* is storage temperature (°C). Additional experiments at 10°C and 20°C assessed the model's performance. For the observed values, *Salmonella* cells were counted during storage. The root mean square error (*RMSE*), bias factor (B_j), and accuracy factor (A_j) [22] were calculated to quantify the differences between the observed values and predicted data resulting from the constructed predictive models at 10°C and 20°C.

$$RMSE == \sqrt{\sum} (\text{ predicted value-observed value })^2 / n$$
$$B_f = 10^{(\sum \log(\text{ predicted / observed })/n)}$$
$$A_\ell = 10^{(\sum \log(\text{ predicted / observed })/n)}$$

where n is the total number of data points.

Evaluation of effect of cooking methods on reduction of Salmonella cell counts

Representative cooking methods for eggs have been investigated in previous studies [23,24]. The conditions of cooking time and temperature according to the cooking method (dry heat [fried], moist heat [boiled, steamed, and poached], and raw [whipping cream and butter cream]) were investigated, and the appropriate or inappropriate cooking times were applied. *Salmonella* inoculum was put into each egg at 3–4 Log CFU/g to investigate cooking methods' *Salmonella* reduction. The whipping cream and butter cream were prepared using raw eggs. Whipping cream is made by mixing egg yolk with milk, while butter cream is prepared by mixing egg white and butter. In this study, the whipping cream and butter cream inoculated with *Salmonella* were prepared and refrigerated for seven days. Appropriate cooking at dry and moist heat, which completely kills *Salmonella* inoculated into egg yolk, was performed for at least 1 min after reaching an internal temperature of 74°C [25]. When the internal temperature did not reach 74°C, the eggs were undercooked, and that duration was considered inappropriate cooking time. These effects on the reduction in *Salmonella* cell counts were included as input variables in the simulation model.

Investigation of egg storage conditions and consumption data

The temperature and time spent transporting, storing, and displaying of eggs in retail markets were obtained through communication with managers in retail markets and from previous studies [26,27]. The 24 h recall data from the 2016 Korea National Health and Nutrition Examination Survey (KNHNES) [28] were used to calculate the daily consumption amounts and ratios of eggs. Using SAS® (Version 9.3, SAS Institute, Cary, NC, USA), the raw data were analyzed. The egg consumption ratio was determined by dividing the total number of survey respondents (7,042 people) by the number of respondents who consumed eggs (4,230 people). @Risk (Palisade, Ithaca, NY, USA) was used to analyze the collected temperature, time, and consumption data to determine proper probabilistic distributions.

Model of dose-response and risk characterization

In previous data, we searched for a dose-response model to assess *Salmonella* exposure after consuming infected eggs. The MRA scenario was constructed according to Fig. 1. The initial *Salmonella* infection level in eggs, predictive models, probabilistic distributions for time and temperature from markets to homes, probabilistic distribution of consumption data, *Salmonella* reduction rate by cooking methods, and a dose-response model were used to create a simulation model in Excel[®] (Microsoft, Seattle, WA, USA). Monte Carlo simulation with @Risk was used to calculate egg-borne *Salmonella* risk.

RESULTS AND DISCUSSION

Salmonella prevalence and initial contamination level

Salmonella cell counts in all 201 egg samples were below the detection limit (0.1 Log CFU/g). Furthermore, Mahdavi et al. [29] found no Salmonella in 525 egg samples, and Safaei et al. [30] identified no Salmonella, Listeria monocytogenes, or Campylobacter jejuni contamination in 100 eggs. Other investigations found 0.1%–1.6% Salmonella infection in commercial eggs [31–33]. Since no Salmonella-positive samples were included in this study, the Beta distribution (RiskBeta [1, 202])



Fig. 1. Scheme of quantitative risk assessment for Salmonella in eggs.

was used to assess the prevalence of *Salmonella* in eggs. In addition, initial contamination level was determined to be -4.0 Log CFU/g (Fig. 2).

Predictive Salmonella kinetic model

Due to the temperature difference between rinsing water and eggs, Salmonella can enter the shell at rates of almost 2 Log CFU/g. However, the standard rinsing water temperature must be 5 °C higher than the egg temperature. If 150 ppm of sodium hypochlorite solution or a disinfectant with equivalent efficacy is used in rinsing water [15], no penetration of Salmonella through the egg shell is observed. When *Salmonella* cells were inoculated into egg yolk or egg white and stored at 15° C for 7 d, Salmonella cell counts in egg yolk samples increased, but egg whites did not show growth of Salmonella (data not shown). Therefore, egg yolk was selected for development of predictive models. Salmonella-infected eggs were used to develop these models, and they were stored at temperature of 7 °C, 15 °C, 25 °C, and 30 °C. The temperature for *Salmonella* growth is between 10 °C to 30 °C, however it can survive at 7°C. The primary models were used to obtain the kinetic parameters (LPD and $\sqrt{\mu_{max}}$), which are listed in Table 1. LPDs reduced (p < 0.05) from 22.2 to 2.1 h as the temperature increased (Table 1), demonstrating that Salmonella can grow quickly in eggs when the storage temperature increases. A polynomial model was used to assess the effect of temperature on LPD and $\sqrt{\mu_{\text{max}}}$ values. Fig. 3 illustrates the secondary models. Due to the relatively high R^2 values, the secondary models were appropriate for representing the relationship among temperature and LPD (R^2 =0.898) and $\sqrt{\mu_{max}}$ (R^2 = 0.922) values. In model performance validation, the RMSE values at 10 °C and 20°C were 0.176 and 0.294, respectively. B_f and A_f were respectively 0.97 and 1.07 at 10° C, and 0.98 and 1.06 at 20° C. These findings suggested that the developed models were suitable for predicting the number of Salmonella cells in eggs during storage.



Fig. 2. Probabilistic distributions for initial contamination levels of Salmonella in eggs.

Variable –				
variable –	7	15	25	30
Kinetic parameters				
μ_{max}	-0.01 ± 0.01	0.07 ± 0.02	0.20 ± 0.03	0.25 ± 0.10
LPD	22.2 ± 5.7	9.6 ± 5.0	1.9 ± 0.4	2.1 ± 2.2
No	2.1 ± 0.3	2.5 ± 0.4	2.1 ± 0.0	2.1 ± 0.0
N _{max}	1.6 ± 0.2	7.8 ± 0.4	8.1 ± 0.2	8.5 ± 0.3

Table 1. Kinetic parameters calculated by the Baranyi model for Salmonella in eggs during storage at 7, 15, 25, and 30° C

 μ_{max} maximum specific growth rate (Log CFU/g/h), indicating death and growth rates; LPD, lag phase duration (h), period of no cell count change in a growth/death curve; N_0 , initial bacterial cell counts (Log CFU/g).



Fig. 3. Secondary model for lag phase duration (A) and growth rate (B) of *Salmonella* in eggs as a function of storage temperature. Symbol, observed value; line, fitted line with the polynomial model. μmax, maximum specific growth rate; LPD, lag phase duration.

Effects of cooking methods on reducing Salmonella cell counts

Salmonella decreased by 2.1 ± 0.1 Log CFU/g in whipping cream and by 1.4 ± 0.0 Log CFU/g in butter cream after seven days (Figs. 4A and 4B). When the Salmonella-inoculated (3.8 ± 0.4 Log CFU/g) eggs were steamed, Salmonella was not detected for 1 min (Fig. 4C). When Salmonella-inoculated (3.8 ± 0.4 Log CFU/g) eggs were boiled, Salmonella was not detected after 6 min. When eggs were boiled for 4 min, only the surface of the egg yolk was cooked. Thus, Salmonella remained and was detected when the egg yolk was cooked for 4 min or less (Fig. 4D). Poached eggs are





eaten by pouring hot broth into the eggs and cooking them slightly. Although hot broth $(100^{\circ}C)$ was poured, 0.2 Log CFU/g of *Salmonella* was detected after 2 min in poached eggs that were not sufficiently cooked without additional cooking (Fig. 4E). When contaminated eggs (3.8 ± 0.4 Log CFU/g) were fried, *Salmonella* was decreased 99.5% and detected at 0.8 ± 0.1 Log CFU/g until 2 min, and *Salmonella* was completely dead after 4 min (complete cooking condition; Fig. 4F).

Time and temperature during distribution

Pert distribution (0.5, 4, and 9) was used to create a probabilistic distribution for egg transportation from manufacturing plants to the market, which was estimated to take 4 h with a minimum of 30 min and a maximum of 9 h. Park and Bahk [26] reported 2.12°C and 12.54°C minimum and maximum temperatures during transit to the market. Therefore, the transit temperature was fitted to the Uniform distribution (2.12, 12.54) to derive the probability distribution (Table 2). After being transported to the market, eggs were stored at 0° C-15 $^{\circ}$ C (often at 4 $^{\circ}$ C) for 0-24 h, using the Pert distribution (0, 4, 15) for storage temperature and the Uniform distribution (0, 24) for storage duration. The Uniform distribution was fitted using the parameters (0, 72) after the eggs were displayed at the market for 0–72 h. Eggs were refrigerated and stored at 0° C –15 $^{\circ}$ C in S. Korea [15]. To derive the probabilistic distribution for the market display, the Uniform distribution (0, 15) was used (Table 2). Jung [27] reported that the market-to-home commuting duration and temperature ranged from 10° to 25° and 0.325 to 1.643 h, respectively. The calculated average transport temperature was 18 °C. Thus, the Pert distribution (10, 18, 25) was used to model the transport temperature, while the Uniform distribution (0.325, 1.643) was used to model the transit duration (Table 2). Additionally, the data for at-home storage duration was fitted to the Uniform distribution (0, 540) because eggs were consumed within 540 h (about 3 weeks of shelf life). The temperature of eggs was calculated using the Loglogistic distribution (-29.283, 33.227, 26.666, RiskTruncate [-5, 10]) in relation to the temperature of household refrigerators, as described by Lee et al. [34] (Table 2).

Amount and ratio of egg consumption for consumers

The KNHNES [28] raw data on daily egg consumption levels were fitted to @Risk program. In S. Korea, the average daily consumption of raw eggs (consumed without additional cooking) was 39.2 g, with a consumption frequency of 1.5%. The Weibull distribution (RiskWeibull [1.2556, 41.992, RiskShift [0.067782]]) was found to be appropriate for the consumption of raw eggs. The average daily consumption of eggs by dry-heat cooking was 43.0 g by Exponential distribution (RiskExpon [42.896, RiskShift [0.065791]]) at 57.5% frequency. In addition, the average consumption of eggs by moist-heat cooking was 36.1 g by Exponential distribution (RiskExpon [36.061, RiskShift [-0.016726]]) at 41% frequency. This data indicates that the majority of S. Koreans consume eggs daily; nonetheless, the raw egg intake is very low. These results were used to calculate the final contamination level of *Salmonella* based on the ratio of intake patterns, according to the cooking method and the decreased amount of *Salmonella* after cooking (Table 2).

Dose-response model

The Beta Poisson model $[1 - (1 + D / \beta)^{-\alpha}]$ evaluated foodborne *Salmonella* illness after egg consumption by cooking method. Teunis et al. [35] created $\alpha = 0.89$ and $\beta = 4.4 \times 10^5$, where *D* is the number of viable *Salmonella* consumed and *D* (CFU) is determined as *Salmonella* cell count (CFU/g) × consumption amount (g).

Risk characterization

The simulation model was developed using the estimated Salmonella contamination level, predictive

Input model	Unit	Variable	Formula	Reference
Product				
Pathogens contamination level				
Salmonella prevalence		PR	= RiskBeta (1,202)	This research; [17]
Initial contamination level	CFU/g	С	= – LN (1 – PR) / 25g	[18]
	Log CFU/g	IC	= Log(C)	
Transportation				
Transportation				
Transportation time	h	Time _{trans}	= RiskPert (0.5,4,9)	Personal communica- tion ¹⁾ ; This research
Food temperature during transportation	C	Temp _{trans}	= RiskUniform (2.12,12.54)	[26]
Growth				
		h0	= Average (<i>LPD</i> × growth rate), Fixed 0.3198	This research; [21]
	Log CFU/g	Y0	= Average (Y_{0i}), Fixed 2.2	This research; [21]
	Log CFU/g	\mathbf{Y}_{end}	= Average (Y _{endi}), Fixed 6.5	This research; [21]
		ln(q)	= LN (1 / (EXP (h ₀) – 1))	This research; [21]
Growth rate	Log CFU/g/h	GR_{trans}	= IF (Temp _{trans} > 5.30841, (0.0214 × (Temp _{trans} – 5.30841)) ² , 0)	This research; [21]
Salmonella growth	Log CFU/g	C1	= IC + 1 / (1 + EXP ($-$ In(q))) × (1- (10- $^{ Y0-Yend }$ / LN(10))) × GR _{trans} × Time _{trans}	This research; [21]
Market				
Market storage				
Storage time	h	Time _{Mark-st}	= RiskUniform (0,24)	Personal communica- tion; This research
Food temperature during storage	C	Temp _{Mark-st}	= RiskPert (0,4,15)	Personal communica- tion; This research
Growth				
		h _o	= Average (<i>LPD</i> × growth rate), Fixed 0.3198	This research; [21]
	Log CFU/g	Y ₀	= Average (Y_{0i}), Fixed 2.2	This research; [21]
	Log CFU/g	Y_{end}	= Average (Y _{endi}), Fixed 6.5	This research; [21]
		ln(q)	= LN (1 / (EXP (h ₀) -1))	This research; [21]
Growth rate	Log CFU/g/h	$GR_{Mark-st}$	= IF (Temp _{Mark-st} > 5.30841, (0.0214 × (Temp _{Mark-st} - 5.30841)) ² , 0)	This research; [21]
Salmonella growth	Log CFU/g	C2	= C1 + 1 / (1 + EXP (-ln (q))) × (1 - (10 ^{-$$ Y0-Yend} / LN(10))) × GR _{Mark-st} × Time _{Mark-st}	This research; [21]
Market display				
Display time	h	Time _{Mark-dis}	= RiskUniform (0,72)	Personal communica- tion; This research
Food temperature during display	C	Temp _{Mark-dis}	= RiskUniform (0,15)	Personal communica- tion; This research
Growth				
		ho	= Average (LPD × growth rate), Fixed 0.3198	This research; [21]
	Log CFU/g	Y ₀	= Average (Y_{0i}) , Fixed 2.2	This research; [21]
	Log CFU/g	Y _{end}	= Average (Y _{endi}), Fixed 6.5	This research; [21]
	0 0	ln(q)	= LN (1 / (EXP (h0) - 1))	This research; [21]
Growth rate	Log CFU/g/h	GR _{Mark-dis}	= IF (Temp _{Mark-dis} > 5.30841, (0.0214 × (Temp _{Mark-dis} - 5.30841))2, 0)	This research; [21]
Salmonella growth	Log CFU/g	C3	= C2 + 1 / (1 + EXP (-ln (q))) × (1 - (10- ^{Y0-Yend} / LN (10))) × GR _{Mark-dis} × Time _{Mark-dis}	This research; [21]

Table 2. Simulation model and formulas for calculating the risk of Salmonella through egg intake prepared by different cooking methods with @Risk

Table 2. Continued

Input model	Unit	Variable	Formula	Reference
Transportation (vehicle)				
Transportation				
Transportation time	h	Time _{Veh}	= RiskUniform (0.325,1.643)	[27]
Food temperature during storage	°C	Temp _{Veh}	= RiskPer t(10,18,25)	[27]
Growth				
		h _o	= Average (<i>LPD</i> × growth rate), Fixed 0.3198	This research; [21]
	Log CFU/g	Y ₀	= Average (Y_{0i}) , Fixed 2.2	This research; [21]
	Log CFU/g	\mathbf{Y}_{end}	= Average (Y_{endi}), Fixed 6.5	This research; [21]
		ln(q)	= LN (1 / (EXP (h ₀) -1))	This research; [21]
Growth rate	Log CFU/g/h	GR_{Veh}	= IF(Temp _{Veh} > 5.30841, (0.0214 × (Temp _{Veh} -5.30841)) ² , 0)	This research; [21]
Salmonella growth	Log CFU/g	C4	= C3 + 1/(1 + EXP(-In(q))) × (1– (10– $ VO-Yend $ / LN(10))) × GR _{Veh} × Time _{Veh}	This research; [21]
Home				
Home storage				
Storage time	h	Time _{Home}	= RiskUniform (0,540)	Personal communica tion; This research
Food temperature during storage	C	Temp _{Home}	= RiskLogLogistic (-29.283,33.227,26.666,Risktruncate (-5,10))	[33]
Growth				
		h _o	= Average (<i>LPD</i> × growth rate), Fixed 0.3198	This research; [21]
	Log CFU/g	Y ₀	= Average (Y_{0i}) , Fixed 2.2	This research; [21]
	Log CFU/g	Y _{end}	= Average (Y _{endi}), Fixed 6.5	This research; [21]
		ln(q)	= LN (1 / (EXP (h ₀) -1))	This research; [21]
Growth rate	Log CFU/g/h	GR _{Home}	= IF(Temp _{Home} > 5.30841, (0.0214 × (Tem- p_{Home} -5.30841)) ² , 0)	This research; [21]
Salmonella growth	Log CFU/g	C5	= C4 + 1 / (1 + EXP (-ln (q))) × (1 - (10 - $ V0-Yend $ / LN (10))) × GR _{Home} × Time _{Home}	This research; [21]
	CFU/g	$C5_{CFU/g}$	= 10 ^{C5}	
Consumption				
Daily consumption frequency for	%	ConRatio	Fixed 60.1	[28]
eggs		CR(0)	= 1 - (60.1/100)	[28]
		CR(1)	= 60.1 / 100	[28]
		CR	= RiskDiscrete ({0,1},{CR(0),CR(1)})	[28]
Cooking method				
Dry heat cooking		Cook(dry)	= 57.5/100	[28]
Moist heat cooking		Cook(moist)	= 41/100	[28]
Raw (uncooked)		Cook(raw)	= 1.5/100	[28]
		Cook	= RiskDiscrete ({1,2,3}, {Cook (dry), Cook (moist), Cook (raw)})	
Consumption by dry heat cooking	g	Consump _{dry-cook}	= RiskExpon (42.896,RiskShift (0.065791), RiskTruncate (0.08,360))	This research; [28]
Consumption by moist heat cooking	g	Consump _{moist-cook}	= RiskExpon (36.061, RiskShift (−0.016726), RiskTruncate (0,340))	This research; [28]
Consumption by raw	g	Consum _{praw}	= RiskWeibull (1.2556,41.992, RiskShift (0.067782), RiskTruncate (0.32,153.9))	This research; [28]
	g	Consump	= IF (Cook = 1, Consump _{dry-cook} , IF (Cook = 2,Con- sump _{moist-cook} , IF (Cook = 3,Consump _{raw})))	
Total consumption	g	Amount	= IF (CR = 0,0,Consump)	

Table 2. Continued

Input model	Unit	Variable	Formula	Reference
Reduction				
Dry heat cooking		Reduce(_{dry})	= 57.5 / 100	[28]
Moist heat cooking		Reduce(moist)	= 41 / 100	[28]
Raw (uncooked)		Reduce(_{raw})	= 1.5 / 100	[28]
		Reduce	= RiskDiscrete ({1,2,3}, {Reduce (dry), Reduce (moist), Reduce (raw)})	
Reduce(dry) -dry heat cooking				
Cooking time	h	Time _{dry-cook}	= RiskPert (0.03,0.07,0.1)	This research
Food temperature during cooking	ື	Temp _{dry-cook}	= RiskPert (74 × 0.8,74,74 × 1.2)	This research; [14]
	CFU/g	Reduce _{dry-cook}	= IF (AND (Temp _{dry-cook} > 74,Time _{dry-cook} > 0.07), 0, $C5_{CFU/g} \times 0.01$)	
Reduce(moist) -moist heat cooking				
Cooking time	h	Time _{moist-cook}	= RiskPert (0.03,0.07,0.25)	This research
Food temperature during cooking	ິ	Temp _{moist-cook}	= RiskPert (74 × 0.8,74,74 × 1.2)	This research; [14]
	CFU/g	Reduce _{moist-cook}	= IF (AND (Temp _{moist-cook} > 74,Time _{moist-cook} > 0.07), 0, $C5_{CFU/g} \times 0.01$)	
Reduce(raw) –raw				
Cooking time	h	Time _{raw}	= RiskPert (0,0.02,0.03)	This research;
Food temperature during cooking	ິ	Temp _{raw}	= RiskUniform (0,60)	This research;
	CFU/g	Reduce _{raw}	= IF (AND (Temp _{raw} > 50, Time _{raw} > 0.02), 0, C5 _{CFU/g} \times 0.01)	
	CFU/g	Reduction	= IF (Reduce = 1, Reduce _{dny-cook} , IF (Reduce = 2, Reduce _{moist-cook} , IF (Reduce = 3, Reduce _{raw})))	
Final concentration	CFU/g	C6 (Cooked)	= IF (CR = 0,0,Reduction)	This research
Dose-Response				
Salmonella amount	CFU	D	= C6 × Amount	
Parameter of Beta Poisson		α	Fixed, 0.89	[35]
		β	Fixed, 4.4 × 10 ⁵	[35]
Risk				
Probability of illness/person/day		Risk	$= 1 - (1 + D / \beta) - \alpha$	[35]

¹⁾ Personal communication with manager in charge of products at retail store.

models simulating *Salmonella* cell counts with probabilistic distributions of temperature and time, probabilistic distributions of consumption amounts, consumption frequency, reduction by cooking, and a dose-response model, as shown in Table 2. *Salmonella* cell counts were predicted to have increased gradually from initial contamination (IC; -4.0 Log CFU/g) to home storage (C5; -3.6 Log CFU/g) using the cumulative density calculated by this simulation (Fig. 5). *Salmonella* cell counts increased significantly during market display (C3; -3.7 Log CFU/g) as a result of eggs being sold at 25 °C. The simulation showed that in S. Korea, the daily risk of *Salmonella* infection per person per day from consuming cooked eggs was estimated at 6.8×10^{-10} (Table 3). The simulation that did not include cooking procedures revealed that the risk of *Salmonella* infection from egg consumption in S. Korea was 1.9×10^{-7} (2.8×10^2 -fold increase) (Table 3). When fitted without cooking procedures, the risk of foodborne *Salmonella* disease is predicted to be higher. Most people in S. Korea consume eggs that have been cooked in the form of egg rolls, braised eggs, and egg drop soups. Thus, the scenario in which the cooking methods were used was determined to be more realistic and accurate when evaluating the risk of foodborne *Salmonella* disease from



Fig. 5. Changes in *Salmonella* contamination levels in eggs predicted by distributions during transportation, storage, and display in retail market.

Table 3. Probability of Salmonella foodborne illness per person per day with different scenarios in th	e
eggs cooking methods and ratios	

Scenario	Mean	Fold change
Baseline (applied cooking)	6.8 × 10 ⁻¹⁰	-
1. Not applied cooking	1.9 × 10 ⁻⁷	2.8×10 ² ↑
2. 33% of raw consumption	1.1 × 10 ⁻⁹	1.6 ↑
3. 50% of raw consumption with 50% dry-heat cooking	1.3 × 10 ⁻⁹	1.9 ↑
4. 50% of raw consumption with 50% moist-heat cooking	2.5 × 10 ⁻⁹	3.7 ↑



Fig. 6. Correlation coefficients for risk factors affecting the probability of *Salmonella* illness per person per day by eggs consumption.

egg consumption. Furthermore, raising the raw consumption ratio increased the probability of foodborne *Salmonella* disease compared to the baseline scenario (Table 3). When the ratio of raw egg intake was increased to 33%, the probability of foodborne *Salmonella* disease increased 1.6-fold over the baseline prediction (Table 3). When the raw egg consumption ratio was increased to 50%, the probability of foodborne *Salmonella* disease increased to 50%, the probability of foodborne *Salmonella* disease increased to 50%. The probability of foodborne *Salmonella* disease increased by 1.9- to 3.7-fold (Table 3). Consuming uncooked *Salmonella*-contaminated eggs increase the risk of foodborne *Salmonella* outbreaks. In addition, higher consumption frequency and prevalence increased the risk of foodborne *Salmonella* disease, while increased cooking time and temperature reduced the risk (Fig. 6).

CONCLUSION

In conclusion, it appears that the risk of foodborne *Salmonella* disease due to egg consumption in S. Korea is low. In the retail market, *Salmonella* prevalence in eggs is low, and disinfection procedures may reduce or eliminate the risks of contamination by *Salmonella* in the manufacturing step. However, the risk of foodborne *Salmonella* outbreaks increases, if eggs contaminated with *Salmonella* are not cooked. Consequently, consumption of raw eggs was the most influential input factor in risk estimations. Although this QMRA used insufficient data evaluated under certain assumptions, the risk of foodborne *Salmonella* illness can be re-estimated when additional data are collected.

REFERENCES

- CDC [Centers for Disease Control and Prevention]. People with a higher risk of food poisoning [Internet]. 2022 [cited 2022 Oct 10]. https://www.cdc.gov/foodsafety/people-atrisk-food-poisoning.html
- Kubo I, Kajiya M, Aramaki N, Furutani S. Detection of Salmonella enterica in egg yolk by PCR on a microfluidic disc device using immunomagnetic beads. Sensors. 2020;20:1060. https://doi.org/10.3390/s20041060
- Kogut MH, Arsenault RJ. Immunometabolic phenotype alterations associated with the induction of disease tolerance and persistent asymptomatic infection of Salmonella in the chicken intestine. Front Immunol. 2017;8:372. https://doi.org/10.3389/fimmu.2017.00372
- CDC [Centers for Disease Control and Prevention]. Salmonella [Internet]. 2022 [cited 2022 Oct 11]. https://www.cdc.gov/salmonella/index.html
- European Centre for Disease Prevention and Control, European Food Safety Authority. Multi-country outbreak of Salmonella Enteritidis sequence type (ST)11 infections linked to eggs and egg products – 8 February 2022. EFSA Support Publ. 2022;19:7180E. https://doi. org/10.2903/sp.efsa.2022.EN-7180
- European Food Safety Authority, European Centre for Disease Prevention and Control. The European Union One Health 2019 Zoonoses report. EFSA J. 2021;19:e06406. https://doi. org/10.2903/j.efsa.2021.6406
- CDC [Centers for Disease Control and Prevention]. Salmonella and eggs [Internet]. 2021 [cited 2022 Oct 10]. https://www.cdc.gov/foodsafety/communication/salmonella-and-eggs. html
- Mihalache OA, Teixeira P, Nicolau AI. Raw-egg based-foods consumption and food handling practices: a recipe for foodborne diseases among Romanian and Portuguese consumers. Food Control. 2022;139:109046. https://doi.org/10.1016/j.foodcont.2022.109046
- 9. NSW [New South Wales] Food Authority. Food safety guidelines for the preparation of raw

egg products [Internet]. 2016 [cited 2022 Oct 12]. https://www.foodauthority.nsw.gov.au/sites/ default/files/_Documents/retail/raw_egg_guidelines.pdf

- Cardoso MJ, Nicolau AI, Borda D, Nielsen L, Maia RL, Møretrø T, et al. Salmonella in eggs: from shopping to consumption—a review providing an evidence-based analysis of risk factors. Compr Rev Food Sci Food Saf. 2021;20:2716-41. https://doi.org/10.1111/1541-4337.12753
- Tomaszewska M, Trafialek J, Suebpongsang P, Kolanowski W. Food hygiene knowledge and practice of consumers in Poland and in Thailand - a survey. Food Control. 2018;85:76-84. https://doi.org/10.1016/j.foodcont.2017.09.022
- Redmond EC, Griffith CJ. Consumer food handling in the home: a review of food safety studies. J Food Prot. 2003;66:130-61. https://doi.org/10.4315/0362-028X-66.1.130
- MFDS [Ministry of Food and Drug Safety]. Risk assessment and reduction of Salmonella species in livestock product. Cheongju: MDFS; 2020. Report No. TRKO202000029897
- Jeong J, Chon JW, Kim H, Song KY, Seo KH. Risk assessment for salmonellosis in chicken in South Korea: the effect of Salmonella concentration in chicken at retail. Korean J Food Sci Anim Resour. 2018;38:1043-54. https://doi.org/10.5851/kosfa.2018.e37
- 15. MFDS [Ministry of Food and Drug Safety]. Food code. Cheongju: MFDS; 2022.
- Olsen R, Kudirkiene E, Thøfner I, Pors S, Karlskov-Mortensen P, Li L, et al. Impact of egg disinfection of hatching eggs on the eggshell microbiome and bacterial load. Poult Sci. 2017;96:3901-11. https://doi.org/10.3382/ps/pex182
- 17. Vose DJ. Risk analysis in relation to the importation and exportation of animal products. Rev Sci Tech. 1997;16:17-29. https://doi.org/10.20506/rst.16.1.997
- Sanaa M, Coroller L, Cerf O. Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Anal. 2004;24:389-99. https://doi.org/10.1111/j.0272-4332.2004.00440.x
- 19. Song KY, Lee SK, Seo KH. Producing safe egg. Safe Food. 2017;12:37-49.
- Messens W, Grijspeerdt K, Herman L. Eggshell penetration by Salmonella: a review. Worlds Poult Sci J. 2005;61:71-86. https://doi.org/10.1079/WPS200443
- Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23:277-94. https://doi.org/10.1016/0168-1605(94)90157-0
- Ross T. Indices for performance evaluation of predictive models in food microbiology. J Appl Bacteriol. 1996;81:501-8. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x
- 23. Yang JS, Oh BY. Characteristics of egg white. Food Sci Ind. 1999;32:42-55.
- Burtness CA, Brandt K, Driessen S. Handling eggs safely to prevent Salmonella [Internet].
 2021 [cited 2022 Oct 15]. https://extension.umn.edu/preserving-and-preparing/handlingeggs-prevent-salmonella#sources-2921810
- Health Canada. Safe internal cooking temperatures [Internet]. 2020 [cited 2022 Oct 15]. http://healthycanadians.gc.ca/eating-nutrition/safety-salubrite/cook-temperatures-cuissoneng.php
- Park MS, Bahk GJ. Current state for temperature management of cold and frozen food transportation vehicles in Jeonbuk province. J Food Hyg Saf. 2017;32:107-13. https://doi. org/10.13103/JFHS.2017.32.2.107
- 27. Jung H. Consumer survey and hazard analysis for the improvement of food hygiene and safety in purchase [Master's thesis]. Seoul: Korea University; 2011.
- KDCA [Korea Disease Control and Prevention Agency]. Korea health statistics 2016: Korea national health and nutrition examination survey (KNHANES). Cheongju: Korea Disease Control and Prevention Agengy; 2018.
- 29. Mahdavi M, Jalali M, Safaei HG, Shamloo E. Microbial quality and prevalence of Salmonella

and Listeria in eggs. Int J Env Health Eng. 2012;1:48. https://doi.org/10.4103/2277-9183.105347

- Safaei HG, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheimi E. The prevalence of bacterial contamination of table eggs from retails markets by Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. Jundishapur J Microbiol. 2011;4:249-53.
- 31. Jamshidi A, Kalidari GA, Hedayati M. Isolation and identification of Salmonella enteritidis and Salmonella typhimurium from the eggs of retail stores in Mashhad, Iran using conventional culture method and multiplex PCR assay. J Food Saf. 2010;30:558-68. https:// doi.org/10.1111/j.1745-4565.2010.00225.x
- Esaki H, Shimura K, Yamazaki Y, Eguchi M, Nakamura M. National surveillance of Salmonella Enteritidis in commercial eggs in Japan. Epidemiol Infect. 2013;141:941-3. https:// doi.org/10.1017/S0950268812001355
- Jones DR, Musgrove MT. Pathogen prevalence and microbial levels associated with restricted shell eggs. J Food Prot. 2007;70:2004-7. https://doi.org/10.4315/0362-028x-70.9.2004
- Lee H, Kim K, Choi KH, Yoon Y. Quantitative microbial risk assessment for Staphylococcus aureus in natural and processed cheese in Korea. J Dairy Sci. 2015;98:5931-45. https://doi. org/10.3168/jds.2015-9611
- Teunis PFM, Nagelkerke NJD, Haas CN. Dose response models for infectious gastroenteritis. Risk Anal. 1999;19:1251-60. https://doi.org/10.1111/j.1539-6924.1999.tb01143.x