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Author	Hyemin Oh ^{1,2†} , Yohan Yoon ^{1,2†} , Jang Won Yoon ³ , Se-Wook Oh ⁴ , Soomin Lee ^{2*} and Heeyoung Lee ^{5*} †These authors contributed equally to this work.
Affiliation	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, Gangwon, 24341, Korea 4Department of Food and Nutrition, Kookmin University, Seoul 02703, Korea 5Food Standard Research Center, Korean Food Research Institute, Wanju 55365, Korea
ORCID (for more information, please visit https://orcid.org)	Oh Hyemin (https://orcid.org/0000-0002-8073-7242) Yoon Yohan (https://orcid.org/0000-0002-4561-6218) Jang Won Yoon (https://orcid.org/0000-0002-6874-5290) Oh Se-Wook (https://orcid.org/0000-0002-8580-6032) Lee Soomin (https://orcid.org/0000-0003-1811-7365) Lee Heeyoung (https://orcid.org/0000-0001-6115-9179)
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4 CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Soomin Lee / Heeyoung Lee
Email address – this is where your proofs will be sent	slee0719@naver.com / hylee06@kfri.re.kr
Secondary Email address	
Address	Soomin Lee : Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea Heeyoung Lee : Food Standard Research Center, Korean Food Research Institute, Wanju 55365, Korea
Cell phone number	
Office phone number	(82)2-2077-7585 / (82)63-219-9333
Fax number	(82)2-710-9479 / (82)63-219-9333

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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* spp. In addition, temperature and time were investigated during egg transit, storage, and display. The development of predictive models to characterize the kinetic behavior of *Salmonella* in eggs and the collection of data on the amount and frequency of egg consumption. The data was simulated to estimate egg-related foodborne illnesses. *Salmonella* was not found in any of the 201 egg samples that were tested for it. Thus, the estimated initial contamination level was -4.0 Log CFU/g. With R^2 values of 0.898 and 0.922, respectively, the constructed prediction models were adequate for explaining the fate of *Salmonella* spp. 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 is low in S. Korea.

Key words: Eggs, *Salmonella*, 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 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. 60 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, chocolate mousse), and drinks containing raw eggs (e.g., eggnog, 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 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 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 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 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 and southwest regions, 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 in a sterile way 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 $36\pm1^{\circ}\text{C}$ 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 used to isolate and identify black *Salmonella*-like 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 $[-\text{LN}(1-\text{PR}) / \text{mL}]$, originally presented by Sanaa et al. (2004) [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 incubation, 1-mL aliquots of each culture were inoculated into 10 mL of TSB and incubated for 24 h at 37°C. After centrifugation at $1,912 \times g$ 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 densities 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. 10 mL of 0.1% BPW was put into each infected egg yolk and egg white and pounded for 10 s with a BagMixer. The homogenates were then serially diluted in 9 mL of 0.1% BPW, and 0.1-mL aliquots were spread-plated on XLD. 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, USA) with 10 mL of 0.1% BPW then pummeled with a BagMixer for 60 s. 0.1-mL aliquots of the diluted homogenates were spread-plated on XLD agar. The plates were inoculated at 37°C for 24 h. *Salmonella* cell counts were fitted to the Baranyi model [21] using DMfit (Institute of Food Research, Norwich, UK) on typical black colonies with clear membranes. Equation:

$$N_t = N_0 + \mu_{max} \times \ln \left[1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} - N_0)} \right]$$

$$A_t = t + \frac{1}{\mu_{max}} \ln \left(\frac{\exp(-\mu_{max}) + q_0}{1 + q_0} \right)$$

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

The polynomial model was fitted to $\sqrt{\mu_{max}}$ and *LPD* values to determine how storage temperature affected the kinetic parameters.

$$LPD = a_0 + a_1 T^1 + a_2 T^2 \quad \text{and} \quad \sqrt{\mu_{max}} = a (T - T_{min})$$

where a_i is the coefficient value, and T is storage temperature (°C). Additional experiments at 10 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_f), and accuracy factor (A_f) [22] were calculated to quantify the differences between the observed values and predicted data resulting from the constructed predictive models at 10 and 20°C:

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

$$B_f = 10^{\sum \text{Log}(\text{predicted}/\text{observed})/n}$$

$$A_f = 10^{\sum |\text{Log}(\text{predicted}/\text{observed})|/n}$$

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 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) was used to calculate the daily consumption amounts and ratios of eggs. Using SAS® (Version 9.3, SAS Institute Inc., Cary, NC, USA), we analyzed the raw data. 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 Corp, 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 Figure 1. The initial *Salmonella* infection level in eggs, prediction models, probabilistic distributions for time and temperature from markets to homes, probabilistic distribution of consumption data, reduction rate by cooking methods, and a dose-response model were used to create a simulation model in Excel® (Microsoft Corp, 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. (2012) [28] found no *Salmonella* in 525 egg samples, and Safaei et al. (2011) [29] identified no *Salmonella*, *Listeria monocytogenes*, or *Campylobacter jejuni* contamination in 100 eggs. Other investigations found 0.1–1.6% *Salmonella* infection in commercial eggs [30–32]. Since no *Salmonella*-positive samples were included in this study, the Beta distribution (RiskBeta (1, 202)) was used to assess the prevalence of *Salmonella* in eggs. In addition, initial contamination levels were determined to be –4.0 Log CFU/g (Figure 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. *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, 15, 25, and 30°C during storage. The optimal temperature for *Salmonella* growth is between 10 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 1.4 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 how temperature affects *LPD* and $\sqrt{\mu_{max}}$ values. Figure 3 illustrates the secondary models. Due to the relatively high R^2 values, the secondary models were appropriate for characterizing the association between 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 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 finding suggested that the developed models are suitable for predicting the number of *Salmonella* cells in eggs during storage.

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 (Figure 4A and 4B). When the *Salmonella*-inoculated (3.8 ± 0.4 Log CFU/g) eggs were steamed, *Salmonella* was not detected in a microwave for 1 min (Figure 4C). When *Salmonella*-contaminated (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 (Figure 4D). Poached eggs are eaten by pouring hot broth into the eggs and cooking slightly. Although hot broth (100°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 (Figure 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; Figure 4F).

Time and temperature during distribution

Pert distribution (0.5, 4, 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 et al. (2017) [26] reported 2.12 and 12.54°C minimum and maximum temperatures during transit to 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 0–15°C (often at 4°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 0–15°C in Korea [15]. To derive the probabilistic distribution for the market display, the Uniform distribution (0, 15) was used (Table 2). Jung (2011) [27] reported that the market-to-home commuting duration and temperature ranged from 10 to 25°C 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. (2015) [33] (Table 2).

Amount and ratio of egg consumption for consumers

The KHNHES [34] raw data on daily egg consumption levels were fitted to @Risk. 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% of 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% of frequency. This data indicates that 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. (1999) [35] created $\alpha=0.89$ and $\beta=4.4\times10^5$, where D is the number of viable *Salmonella* consumed and D (CFU) is determined as *Salmonella* cell count (CFU/g) \times consumption amount (g).

Risk characterization

The simulation model was developed using the estimated *Salmonella* contamination level, predictive models simulating *Salmonella* cell counts with probabilistic distributions of temperature and time, probabilistic distributions of consumption amounts, consumption frequency, reduction by cooking, and 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 (Figure 5). *Salmonella* cell counts increased significantly in the 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 6.8×10^{-10} (Table 3). The simulation that did not include cooking procedures revealed that the risk of *Salmonella* infection from egg consuming in S. Korea was 1.9×10^{-7} (2.8 $\times10^2$ -fold) (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 which the cooking methods were used was determined to be more realistic and accurate when evaluating the risk of foodborne *Salmonella* disease from egg consumption. Furthermore, raising the raw consumption ratio increased the probability of foodborne *Salmonella* disease compared to the baseline scenario (Table 3). When the raw egg intake ratio 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 by 1.9- to 3.7-fold (Table 3). Uncooked *Salmonella*-contaminated eggs increase the risk of foodborne *Salmonella* outbreaks. In addition, consumption frequency and prevalence increased the risk of foodborne *Salmonella* disease, while raising the cooking time and temperature decreased it (Figure 6).

Conclusion

In conclusion, it appears that the risk of foodborne *Salmonella* disease due to egg consumption in S. Korea was 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 on 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.

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References

- 279 1. Centers for disease control and prevention (CDC), People with a higher risk of food poisoning.
280 2022a [cited 2022 Oct 10]. <https://www.cdc.gov/foodsafety/people-at-risk-food-poisoning.html>
- 281 2. Kubo I, Kajiya M, Aramaki N, Furutani S. Detection of Salmonella Enterica in Egg Yolk by PCR
282 on a microfluidic disc device using immunomagnetic beads. *Sensors*. 2020;20:1060.
283 <https://doi.org/10.3390/s20041060>
- 284 3. Kogut MH, Arsenault RJ. Immunometabolic phenotype alterations associated with the induction of
285 disease tolerance and persistent asymptomatic infection of Salmonella in the chicken intestine.
286 *Front. Immunol*. 2017;8:1–7. <https://doi.org/10.3389/fimmu.2017.00372>
- 287 4. Centers for disease control and prevention (CDC), Salmonella. 2022b [cited 2022 Oct 11].
288 <https://www.cdc.gov/salmonella/index.html>
- 289 5. EFSA, Multi-country outbreak of Salmonella Enteritidis sequence type (ST)11 infections linked to
290 eggs and egg products – 8 February 2022. 19(2), EN-7180. [cited 2022 Oct 11]
291 <https://doi.org/10.2903/sp.efsa.2022.EN-7180>
- 292 6. European food safety authority and European centre for disease prevention and control (EFSA &
293 ECDC), The European union one health 2019 zoonoses report. 2021 [cited 2022 Oct 11]. *EFSA*
294 *Journal*. 19:6406. 10.2903/j.efsa.2021.6406
- 295 7. Centers for disease control and prevention (CDC), Salmonella and eggs. 2021 [cited 2022 Oct 10].
296 <https://www.cdc.gov/foodsafety/communication/salmonella-and-eggs.html>
- 297 8. Mihalache OA, Teixeira P, Nicolau AI. Raw-egg based-foods consumption and food handling
298 practices: A recipe for foodborne diseases among Romanian and Portuguese consumers. *Food*
299 *Control*. 2022;139:109046. <https://doi.org/10.1016/j.foodcont.2022.109046>
- 300 9. New South Wales (NSW) Food Authority. Food safety guidelines for the preparation of raw egg
301 products. 2016 [cited Oct 12].
302 [https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/retail/raw_egg_guidelines.p](https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/retail/raw_egg_guidelines.pdf)
303 [df](https://www.foodauthority.nsw.gov.au/sites/default/files/_Documents/retail/raw_egg_guidelines.pdf)
- 304 10. Cardoso MJ, Nicolau AI, Borda D, Nielsen L, Maia RL, Moretro T, Ferreira V, Knochel S, Langsrud
305 S, Teixeira P. Salmonella in eggs: From shopping to consumption—A review providing an
306 evidence-based analysis of risk factors. *Compr. Rev. Food Sci. Food Saf*. 2021;20:2716-2741.
307 <https://doi.org/10.1111/1541-4337.12753>
- 308 11. Tomaszewska M, Trafialek J, Suebpongsang P, Kolanowski W. Food hygiene knowledge and
309 practice of consumers in Poland and in Thailand - a survey. *Food Control*, 2018;85:76-84.
310 <https://doi.org/10.1016/j.foodcont.2017.09.022>

- 311 12. Redmond EC, Griffith CJ. Consumer food handling in the home: a review of food safety studies. *J.*
312 *Food Prot.* 2003;66:130-161. <https://doi.org/10.4315/0362-028X-66.1.130>
- 313 13. Ministry of food and drug safety (MFDS). Risk assessment and reduction of Salmonella species in
314 livestock product. 2020.
- 315 14. Jeong J, Chon JW, Kim H, Song KY, Seo KH. Risk assessment for salmonellosis in chicken in
316 South Korea: The effect of Salmonella concentration in chicken at retail. *Korean J Food Sci Anim*
317 *Resour.* 2018;38:1043-1054. <https://doi.org/10.5851/kosfa.2018.e37>
- 318 15. Ministry of food and drug safety (MFDS). Food Code. 2022.
- 319 16. Olsen R, Kudirkiene E, Thofner I, Pors S, Karlskov-Mortensen P, Li L, Papasolomontos S,
320 Angastiniotou C, Christensen J. Impact of egg disinfection of hatching eggs on the eggshell
321 microbiome and bacterial load. *Poultry Sci.* 2017;98:3901-3911. <https://doi.org/10.3382/ps/pex182>
- 322 17. Vose DJ. Risk analysis in relation to the importation and exportation of animal products. *Rev. Sci.*
323 *Tech.* 1997;16:17-29. <http://dx.doi.org/10.20506/rst.16.1.997>
- 324 18. Sanaa M, Coroller L, Cerf O. Risk assessment of listeriosis linked to the consumption of two soft
325 cheeses made from raw milk: Camembert of Normandy and Brie of Meaux, *Risk Anal.*
326 2004;24:389-399. <https://doi.org/10.1111/j.0272-4332.2004.00440.x>
- 327 19. Song KY, Lee SK, Seo KH. Producing safe egg. *Safe Food.* 2017;12:37-49.
- 328 20. Messens W, Grijspeerdt K, Herman L. Eggshell penetration by Salmonella: a review. *Worlds Poult.*
329 *Sci. J.* 2005;61:71-86. <https://doi.org/10.1079/WPS200443>
- 330 21. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. *Int. J. Food*
331 *Microbiol.* 1994;23:277-294. [https://doi.org/10.1016/0168-1605\(94\)90157-0](https://doi.org/10.1016/0168-1605(94)90157-0)
- 332 22. Ross T. Indices for performance evaluation of predictive models in food microbiology. *J. Appl.*
333 *Microbiol.* 1996;81:501-508. <https://doi.org/10.1111/j.1365-2672.1996.tb03539.x>
- 334 23. Yang JS, Oh BY. Characteristics of egg white. *Food Sci. Ind.* 1999;32:42-55.
- 335 24. Burtness CA, Brandt K, Driessen S. Handling eggs to prevent Salmonella. 2021 [cited Oct 15].
336 [https://extension.umn.edu/preserving-and-preparing/handling-eggs-prevent-salmonella#sources-](https://extension.umn.edu/preserving-and-preparing/handling-eggs-prevent-salmonella#sources-2921810)
337 [2921810.](https://extension.umn.edu/preserving-and-preparing/handling-eggs-prevent-salmonella#sources-2921810)
- 338 25. Health Canada. Healthy Canadians: Safe internal cooking temperatures. 2020 [cited Oct 15].
339 <http://healthycanadians.gc.ca/eating-nutrition/safety-salubrite/cook-temperatures-cuisson-eng.php>

26. Park MS, Bahk GJ. Current state for temperature management of cold and frozen food transportation vehicles in Jeonbuk province. *J. Food Hyg. Saf.* 2017;32:107-113. <https://doi.org/10.13103/JFHS.2017.32.2.107>
27. Jung HK. Consumer survey and hazard analysis for the improvement of food hygiene and safety in purchase. Master's thesis. Korea University, Seoul, Korea. 2011 [cited Oct 15].
28. 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>
29. Safaei HG, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheimi E. The prevalence of bacterial contamination of table eggs from retail markets by *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. *Jundishapur J. Microbiol.* 2011;4:249-253.
30. 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 Safety*, 2010;30:558–568. <https://doi.org/10.1111/j.1745-4565.2010.00225.x>
31. Esaki H, Shimura K, Yamazaki Y, Eguchi M, Nakamura M. National surveillance of *Salmonella* Enteritidis in commercial eggs in Japan. *Epidermiol. Infect.* 2013;141:941-943. <https://doi.org/10.1017/S0950268812001355>
32. Jones DR, Musgrove MT. Pathogen prevalence and microbial levels associated with restricted shell eggs. *J. Food Prot.* 2007;70:2004–2007. <https://doi.org/10.4315/0362-028x-70.9.2004>
33. 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;98:5931-5945. <https://doi.org/10.3168/jds.2015-9611>
34. Korea disease control and prevention agency (KDCA). 2016 Korea national health and nutrition examination survey (KNHNES). 2018.
35. Teunis PF, Nagelkerke NJ, Haas CN. Dose response models for infectious gastroenteritis, *Risk anal.* 1999;19:1251-1260. <https://doi.org/10.1023/a:1007055316559>
36. 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. An.* 2018;38:1043-1054. <https://doi.org/10.5851/kosfa.2018.e37>

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

		Temperature (°C)			
		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	22.18±5.67	9.55±4.96	1.90±0.40	2.10±2.18
N_0		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

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)

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

Input model	Unit	Variable	Formula	Reference
PRODUCT				
Pathogens contamination level				
Salmonella prevalence		PR	=RiskBeta(1,202)	This research; [17]
Initial contamination level	CFU/g	C	=LN(1-PR)/25g	[18]
	Log CFU/g	IC	=Log(C)	
TRANSPORTATION				
Transportation				
Transportation time	h	Time _{trans}	=RiskPert(0.5,4,9)	Personal communication ^a ; This research
Food temperature during transportation	°C	Temp _{trans}	=RiskUniform(2.12,12.54)	[26]
Growth				
		h ₀	=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]
		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(-ln(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 communication; This research

Food temperature during storage	°C	Temp _{Mark-st}	=RiskPert(0,4,15)	Personal communication; This research
Growth				
		h ₀	=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]
		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]
<i>Salmonella</i> growth	Log CFU/g	C2	=C1+1/(1+EXP(-ln(q)))*(1-(10 ^{- Y₀-Y_{end}} /LN(10)))*GR _{Mark-st} *Time _{Mark-st}	This research; [21]
Market display				
Display time	h	Time _{Mark-dis}	=RiskUniform(0,72)	Personal communication; This research
Food temperature during display	°C	Temp _{Mark-dis}	=RiskUniform(0,15)	Personal communication; This research
Growth				
		h ₀	=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]
		ln(q)	=LN(1/(EXP(h ₀)-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]
<i>Salmonella</i> growth	Log CFU/g	C3	=C2+1/(1+EXP(-ln(q)))*(1-(10 ^{- Y₀-Y_{end}} /LN(10)))*GR _{Mark-dis} *Time _{Mark-dis}	This research; [21]
TRANSPORTATION (vehicle)				
Transportation				
Transportation time	h	Time _{Veh}	=RiskUniform(0.325,1.643)	[27]

Food temperature during storage	°C	Temp _{Veh}	=RiskPert(10,18,25)	[27]
Growth				
		h ₀	=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]
		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]
<i>Salmonella</i> growth	Log CFU/g	C4	=C3+1/(1+EXP(-ln(q)))*(1-(10 ^{- Y0-Yend} /LN(10)))*GR _{Veh} *Time _{Veh}	This research; [21]
HOME				
Home storage				
Storage time	h	Time _{Home}	=RiskUniform(0,540)	Personal communication; This research
Food temperature during storage	°C	Temp _{Home}	=RiskLogLogistic(-29.283,33.227,26.666,Risktruncate(-5,10))	[33]
Growth				
		h ₀	=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]
		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*(Temp _{Home} -5.30841)) ² , 0)	This research; [21]
<i>Salmonella</i> growth	Log CFU/g	C5	=C4+1/(1+EXP(-ln(q)))*(1-(10 ^{- Y0-Yend} /LN(10)))*GR _{Home} *Time _{Home}	This research; [21]
	CFU/g	C5 _{CFU/g}	=10 ^{C5}	
CONSUMPTION				
Daily consumption frequency for eggs	%	ConRatio	Fixed 60.1	[34] ^b
		CR(0)	=1-(60.1/100)	[34]
		CR(1)	=60.1/100	[34]

		CR	=RiskDiscrete({0,1},{CR(0),CR(1)})	[34]
COOKING METHOD				
Dry heat cooking		Cook(dry)	=57.5/100	[34]
Moist heat cooking		Cook(moist)	=41/100	[34]
Raw (uncooked)		Cook(raw)	=1.5/100	[34]
		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; [34]
Consumption by moist heat cooking	g	Consump _{moist-cook}	=RiskExpon(36.061,RiskShift(-0.016726),RiskTruncate(0,340))	This research; [34]
Consumption by raw	g	Consump _{raw}	=RiskWeibull(1.2556,41.992,RiskShift(0.067782),RiskTruncate(0.32,153.9))	This research; [34]
	g	Consump	=IF(Cook=1,Consump _{dry-cook} , IF(Cook=2,Consump _{moist-cook} , IF(Cook=3,Consump _{raw})))	
Total consumption	g	Amount	=IF(CR=0,0,Consump)	
REDUCTION				
Dry heat cooking		Reduce _(dry)	=57.5/100	[34]
Moist heat cooking		Reduce _(moist)	=41/100	[34]
Raw (uncooked)		Reduce _(raw)	=1.5/100	[34]
		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	°C	Temp _{dry-cook}	=RiskPert(74*0.8,74,74*1.2)	This research; [36]
	CFU/g	Reduce _{dry-cook}	=IF(AND(Temp _{dry-cook} >74,Time _{dry-cook} >0.07),0,C5 _{CFU/g} *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	°C	Temp _{moist-cook}	=RiskPert(74*0.8,74,74*1.2)	This research; [36]

	CFU/g	Reduce _{moist-cook}	=IF(AND(Temp _{moist-cook} >74,Time _{moist-cook} >0.07),0,C5 _{CFU/g} *0.01)	
Reduce(raw) -raw				
Cooking time	h	Time _{raw}	=RiskPert(0,0.02,0.03)	This research;
Food temperature during cooking	°C	Temp _{raw}	=RiskUniform(0,60)	This research;
	CFU/g	Reduce _{raw}	=IF(AND(Temp _{raw} >50,Time _{raw} >0.02),0,C5 _{CFU/g} *0.01)	
	CFU/g	Reduction	=IF(Reduce=1,Reduce _{dry-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				
<i>Salmonella</i> 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/ β)- α	[35]

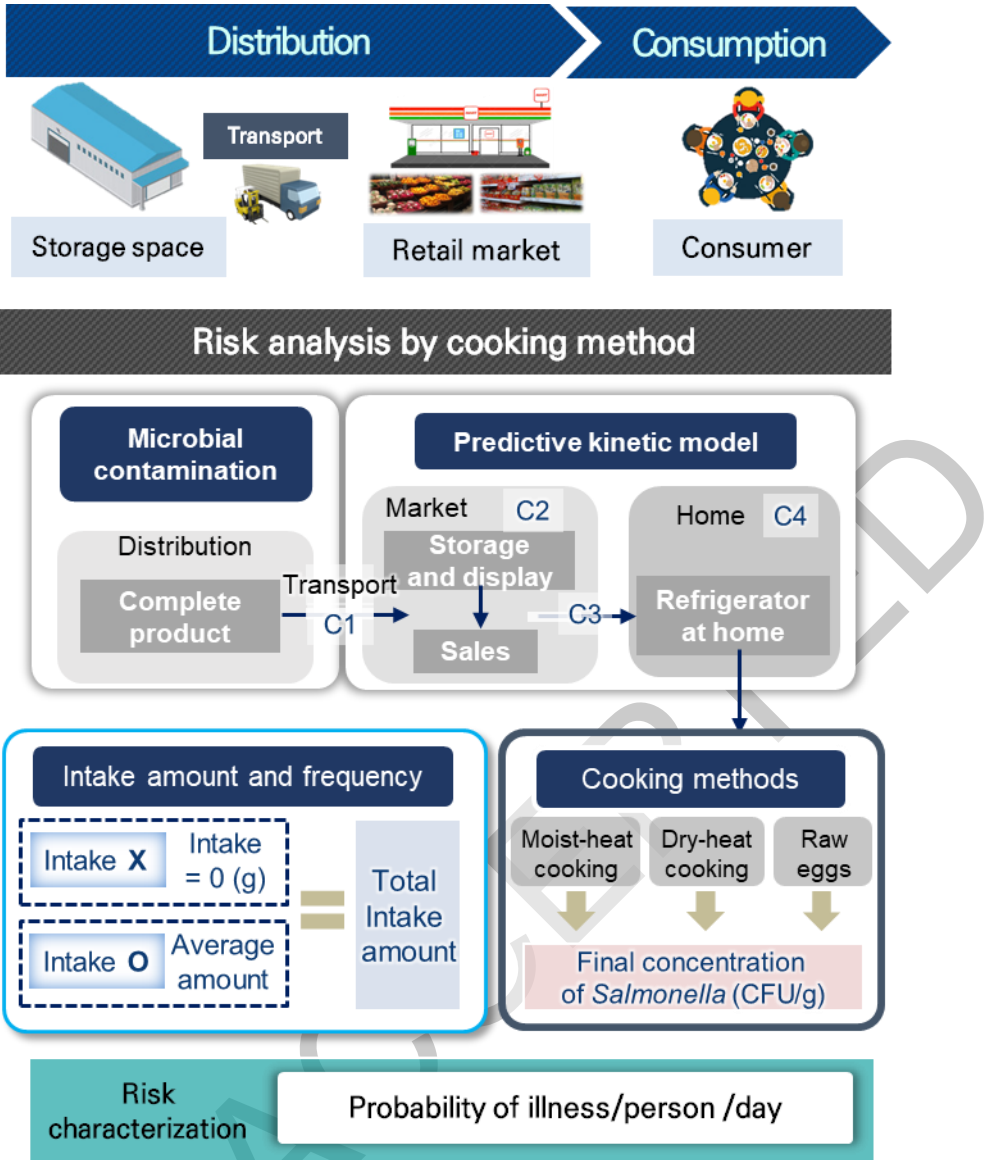
^a Personal communication with manager in charge of products at retail store

^b Korea Disease Control and Prevention Agency

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

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

377



379 **Figure 1.** Scheme of *Salmonella* risk assessment in eggs.

Figure 2.

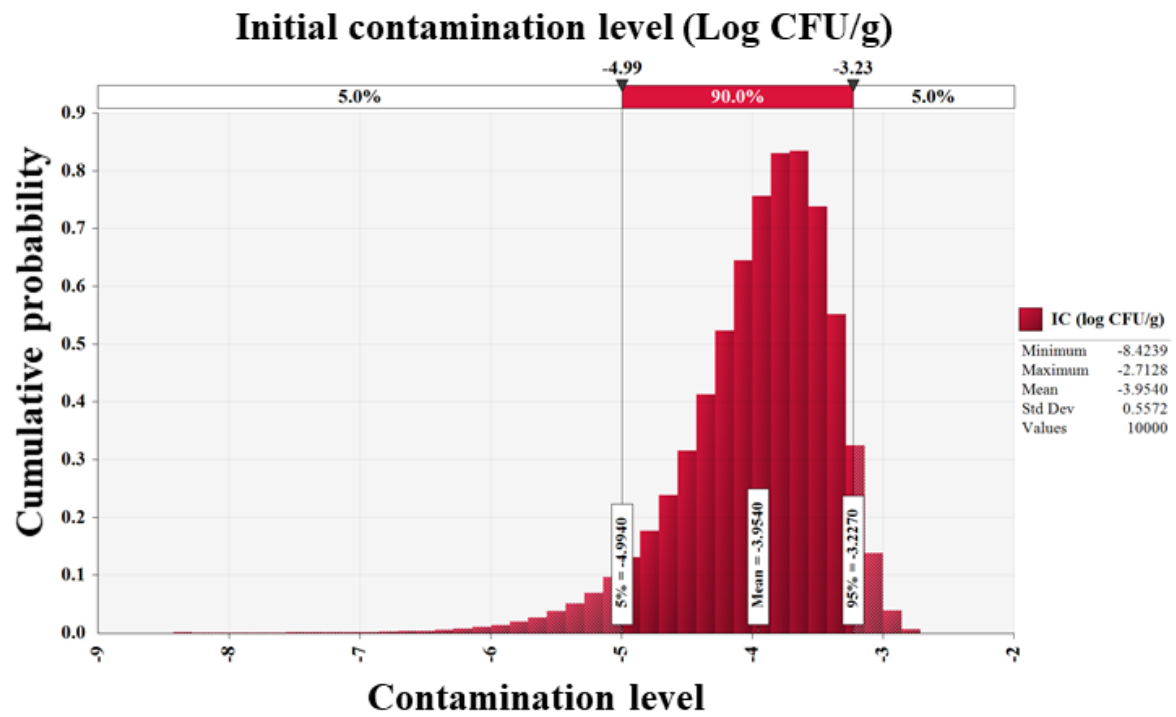
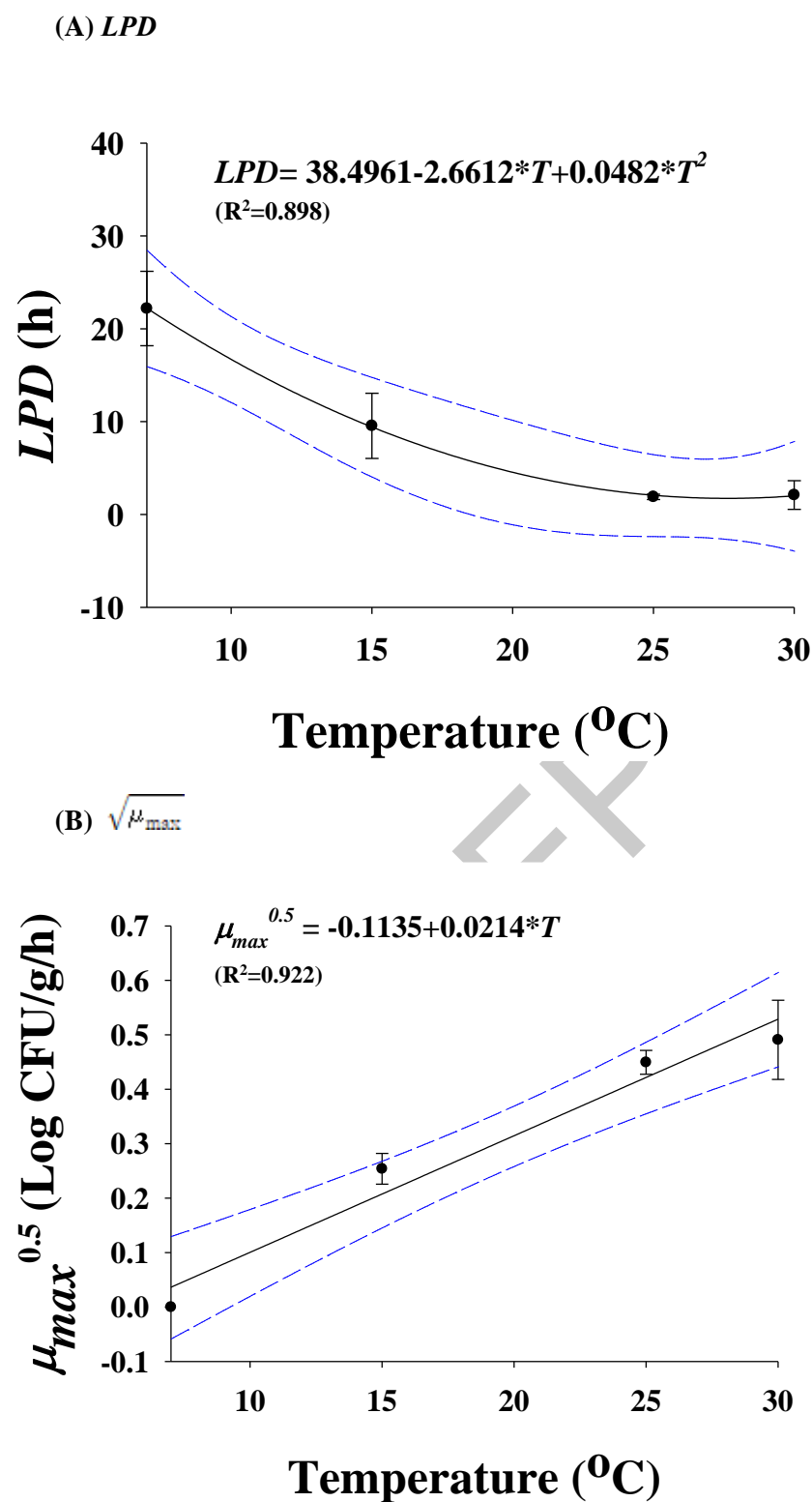


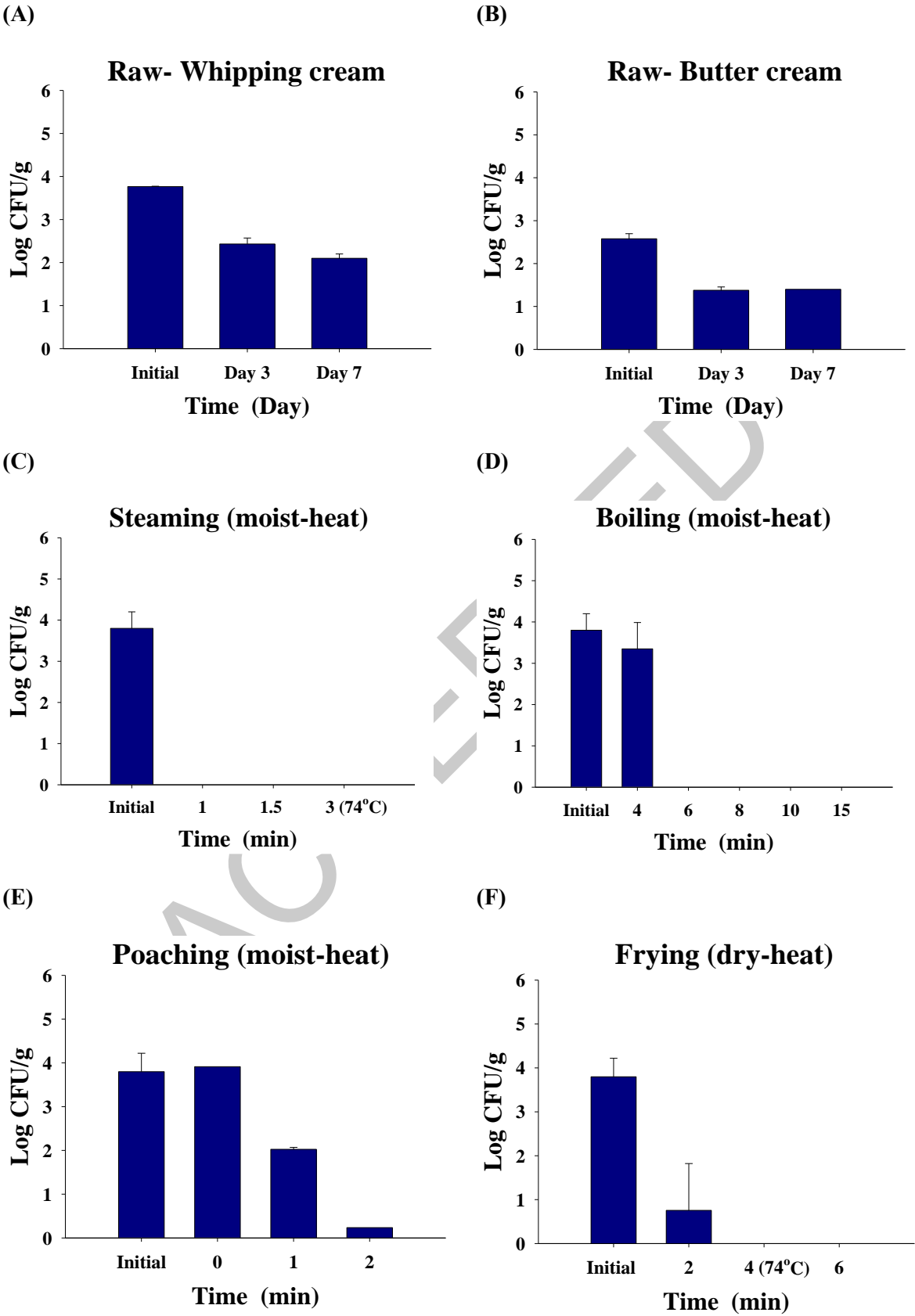
Figure 2. Probability density for initial contamination level of *Salmonella* in eggs.

385 **Figure 3.**



386 **Figure 3.** Secondary model for lag phase (A) and growth rate (B) of *Salmonella* in eggs as a function
387 of storage temperature. Symbol, observed value; line, fitted line with the polynomial model.

388



390 **Figure 4.** Reduction of *Salmonella* cell counts by cooking methods (raw, moist-heating, and dry-heating)

Figure 5.

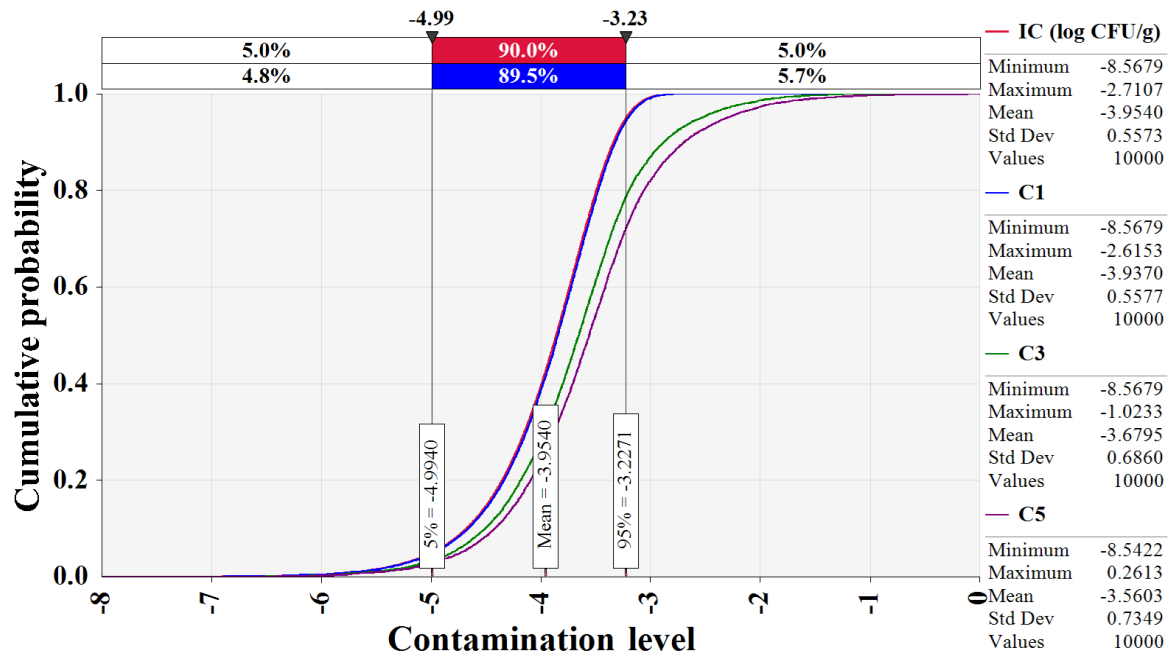


Figure 5. Changes in *Salmonella* contamination levels in eggs predicted by distributions during transportation, storage, and display in retail market

Figure 6.

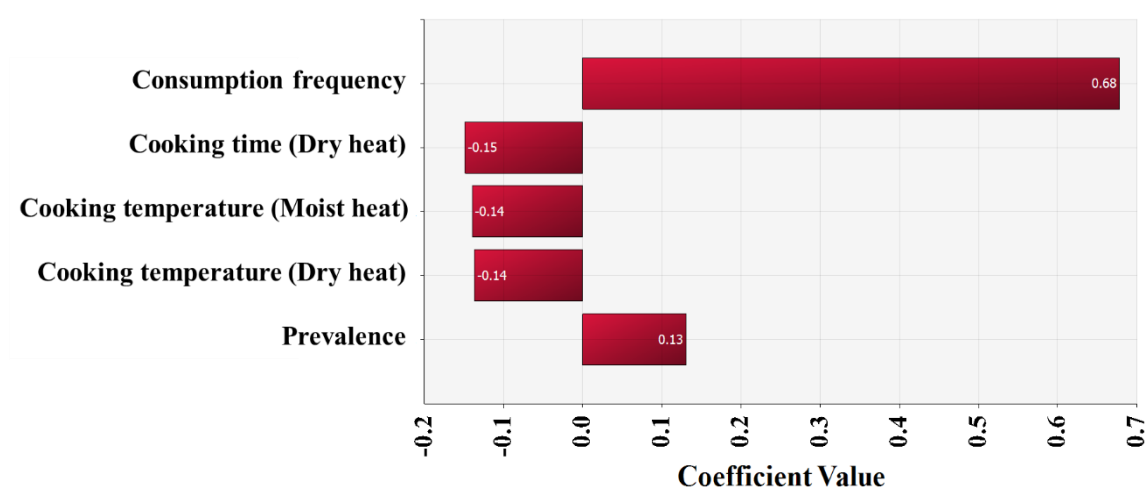


Figure 6. Correlation coefficients for risk factors affecting the probability of *Salmonella* illness per person per day by eggs consumption