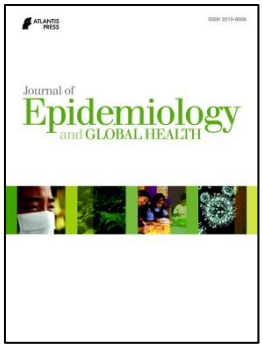

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REVIEW ARTICLES

Growth and inactivation of *Salmonella* at low refrigerated storage temperatures and thermal inactivation on raw chicken meat and laboratory media: Mixed effect meta-analysis

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KEYWORDS
Refrigeration; Thermal inactivation; *Salmonella*; Broiler chicken; Mixed effect; Meta-analysis

Abstract Growth and inactivation regression equations were developed to describe the effects of temperature on *Salmonella* concentration on chicken meat for refrigerated temperatures (≤10 °C) and for thermal treatment temperatures (55—70 °C). The main objectives were: (i) to compare *Salmonella* growth/inactivation in chicken meat versus laboratory media; (ii) to create regression equations to estimate *Salmonella* growth in chicken meat that can be used in quantitative risk assessment (QRA) modeling; and (iii) to create regression equations to estimate *D*-values needed to inactivate *Salmonella* in chicken meat. A systematic approach was used to identify the articles, critically appraise them, and pool outcomes across studies. Growth represented in density (Log_{10} CFU/g) and *D*-values (min) as a function of temperature were modeled using hierarchical mixed effects regression models. The current meta-analysis analysis found a significant difference ($P \leqslant 0.05$) between the two matrices — chicken meat and laboratory media — for both growth at refrigerated temperatures and inactivation by thermal treatment. Growth and inactivation were significantly influenced by temperature after controlling for other variables; however, no consistent pattern in growth was found. Validation of growth and inactivation equations against data not used in their development is needed.

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Contents

1. Introduction

Salmonellosis is one of the main bacterial food-borne illnesses in Canada and worldwide [1]. In humans, salmonellosis is primarily a disease confined to the gastrointestinal tract, but may cause serious extra-intestinal tract disease, especially in the very young, the aged and those that are immunologically compromised [2]. The symptoms of salmonellosis include nausea, vomiting, abdominal cramps, fever and headaches with a duration that ranges from days to weeks [3]. It is primarily transmitted from infected carrier animals to humans through contaminated food [4]. Meat in general and poultry in particular are the most common sources of food-borne illness by *Salmonella* [5]. Contamination of poultry can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling/preparation [6].

Microbial quantitative risk assessment (QRA) has been incorporated in the decision-making process of the Codex Alimentarius Commission (CAC) to manage public health risks associated with microbial hazards [7]. Mathematical models for use in QRA to predict *Salmonella* growth as a function of temperature, pH and water activity are available in the literature [8—10]. However, most predictive models designed for *Salmonella* growth have used laboratory media, such as brain/heart infusion broth and not actual chicken to develop prediction models for *Salmonella* growth [11]. This might overestimate growth owing to the absence of competitive micro flora usually available in raw chicken meat [12].

Effects of environmental conditions such as temperature and its impact on growth kinetics of *Salmonella* in real food products have been studied less extensively. To address this gap, Oscar studied the impact of temperature on *Salmonella* growth on cooked chicken breast [13—16], and raw chicken [12,17]. For raw chicken, the studied temperature range was 10—40 °C [12,17]. Considering the broad range of temperature where *Salmonella* can grow (5—47 °C) [18], growth at temperatures $\leqslant$10 °C in raw chicken still needs to be investigated. As far as this research is concerned, meta-analysis to pool the available data on the effect of temperatures $\leqslant$10 °C has not been conducted using data based on real chicken products or laboratory media.

Furthermore, an important contributing factor that leads to salmonellosis is inadequate temperature/time exposure during the cooking process to kill the pathogenic bacteria [19]. Insufficient cooking has been identified as one of the most important factors contributing to food-borne disease in Canada [20]. As a result, cooking is considered to be a primary means of eliminating pathogens from contaminated meat products and hence serves as a protective method for preventing food-borne illnesses [21]. Previous researchers have conducted thermal inactivation studies of *Salmonella* spp. in aqueous media and foods [18]. However, few

researchers have addressed the question of whether *Salmonella* inactivation in food products such as beef, pork, turkey, or chicken is the same as inactivation in laboratory media. The common approach is to use the estimated *D*-values, which is the time required at a given temperature to reduce the number of pathogenic bacteria by 90% [22] during heat treatment, in laboratory media and apply these values to food products. Such estimation might underestimate risk as the bacteria attached to meat tissues may be more heat resistant than bacteria suspended in a liquid medium [23]. Therefore, it is important to evaluate the thermal inactivation of individual pathogens in food products.

The objectives of this paper were: (i) to compare *Salmonella* density (Log_{10} colony forming units [CFU]/g) and inactivation (*D*-values) from primary studies available to investigate *Salmonella*

Table 1 Search terms used to identify potentially relevant literature to address quantitative effect of refrigerated storage and thermal inactivation temperatures on the enumeration of *Salmonella* on raw chicken meat and laboratory media.[a]

Population	
Chicken meat	*Laboratory media*
(poultry or chicken)	(laboratory)
(broiler or meat)	(laboratory media or matrix)
(raw chicken)	(nutrient agar)
(chicken or broiler or flocks)	(BHI or brain—heart infusion)
(chicken production or processing)	(agar)
(chicks or chicken or parts or flocks)	(agar medium)
(chicken legs or wings or breasts or thighs or liver)	(TSA or TSB)
(minced or ground meat)	(nutrient or laboratory)
(skin-on or skin-off or chicken)	
Refrigerated storage	
(refrigeration)	
(refrigeration time or temperature)	
(chilling or chilled storage)	
(refrigerated poultry or foods)	
(refrigerated chicken or poultry)	
(growth temperature)	
(chilled foods)	
(refrigeration or cooling)	
(storage time or temperature)	
(refrigerated storage)	
(growth or survival)	
Thermal inactivation	
(cooking time or temperature)	
(roasting or heating)	
(*D*-values or *Z*-values)	
(thermal treatment)	
(thermal lethality)	
(thermal or cooking inactivation)	
(thermal inactivation or thermal resistance)	
(cook or cooking)	
Outcome	
(enteric illness)	
(foodborne disease or poisoning)	
(disease or illness or risk)	
(bacterial or pathogenic count or enumeration)	
(infection or illness)	
(*Salmonella* or concentration)	
(bacteria or *Salmonella* or zoonoses)	
(bacterial or bacteria load or level or counts)	
(mesophilic counts or cfu)	

[a] Search terms were combined within each category using ''OR'' and between different categories using ''AND''.

growth/inactivation in chicken meat with that in laboratory media using a meta-analysis approach, and if there is a significant difference between the two matrices; then (ii) to conduct meta-analysis of chicken data to estimate *Salmonella* growth at refrigerated storage conditions below 10 °C and to create a mathematical equation that could be used in QRA modeling to estimate *Salmonella* growth at this temperature range; and (iii) to conduct meta-analysis to create a mathematical model for *Salmonella* inactivation using *D*-values in chicken products. The meta-analysis approach combines data from a number of individual studies to produce a more precise estimate of the summary outcome [24].

2. Materials and methods

The review carried out the following steps: a comprehensive literature search to identify all potentially relevant research, relevance screening of abstracts identified by the search, full text screening and quality assessment of relevant abstracts, and data extraction. Mixed-effect regression models using SAS PROC MIXED (version 9.1.3 SAS) were conducted on extracted data to predict growth (Log_{10} CFU) and Log_{10} *D*-values as a function of the characteristics of individual studies.

2.1. Literature search

The identification of potentially relevant research began by compiling a comprehensive list of search terms (Table 1). Experimental designs and observational studies were eligible for inclusion to allow for the investigation of different study designs, i.e., methodological heterogeneity. The search terms were entered into six electronic databases to identify abstracts published between January 1960 and 2008: MEDLINE, PubMed, EMBASE, The AGRICultural OnLine Access (AGRICOLA), INGENTA, and ISI Web of Knowledge. The search was limited to words in the title or abstracts and an English language limit was imposed. In addition, hand searching was completed using the search terms presented in Table 1 for the references listed in the reference section of all relevant and review articles identified by the electronic database search. To identify ongoing research, a search was completed for the inventory of Canadian Agri-Food Research [25] and TEKTRAN, which is a database that contains recent articles of published or soon-to-be published research results of the Agricultural Research Service [26] — the U.S. Department of Agriculture's chief scientific research agency — but no related papers were found.

2.2. Title and abstract screening

Abstracts were screened by one reviewer (H. Smadi) for relevance to the study objectives. An abstract was considered relevant if it described primary (original) research and evaluated *Salmonella* in fresh chicken meat or laboratory media, and *Salmonella* concentration during refrigerated storage or at different thermal inactivation temperatures (CFU/g or *D*-values) rather than prevalence was the focus of the research.

2.3. Full text screening

Full articles were obtained for all relevant abstracts and underwent full text screening by one reviewer (H. Smadi). Inclusion criteria for *Salmonella* growth studies were: (i) the study included one or more trials evaluating growth at temperatures $\leqslant$10 °C; (ii) the study was conducted under normal storage conditions (e.g., air); and (iii) the study provided growth curves or data to calculate a growth curve (e.g., initial inoculation level, time or testing intervals and their corresponding log CFU/g) and storage temperatures were provided to model lag, exponential and stationary phases of the growth curves. The whole data set was used to model *Salmonella* density (Log_{10} CFU/g) at the studied temperatures. If only generation time (GT) or growth rate (GR) were given to represent the exponential part of the growth curve, then the article was excluded. For inactivation studies, the inclusion criteria were: (i) initial inoculation level; (ii) thermal inactivation temperatures; and (iii) their corresponding *D*-values.

2.4. Quality assessment

Full papers were obtained for all relevant articles and underwent quality assessment. Standardized quality assessment forms were created for experimental studies. Only challenge trials, where *Salmonella* was inoculated directly onto the chicken meat and experiments took place in a controlled laboratory setting, and field trials with a natural exposure to *Salmonella* were found. No observational studies were identified. The following criteria were used to assess the study quality: description of randomization, blinding of the outcome assessor, numbers lost to follow-up, and measures of outcome variability or sufficient data to calculate one (standard deviation, standard er-

ror, confidence interval or *P*-value for post hoc calculation).

2.5. Data extraction

For all studies that passed the relevant screening stage, data extraction was conducted. When multiple trials were included within the same publication, data were extracted separately for each trial. After reviewing the articles, it was found that the longest testing intervals for chicken were 15 days, whereas it was 50 days for laboratory media. Therefore, in this analysis, only data up to 15 days were included for laboratory media to allow comparisons to chicken data. Repeated measures designs were used for all laboratory media studies, whereas none of the chicken data studies used repeated measures, i.e., different chicken pieces were used to enumerate *Salmonella* levels at each of the time periods evaluated.

For studies evaluating thermal inactivation of *Salmonella*, data on whether or not *Salmonella* Senftenberg was part of the inoculum were included because this serotype is more heat resistant than other *Salmonella* serotypes commonly used in thermal tests [27,28]. Two studies [27,28] reported the inoculation level as a range of Log_{10} 7—8 and one study [29] reported it as a range of Log_{10} 7—7.5. As a conservative assumption, in the statistical analysis, it was assumed that the inoculation level for these studies was Log_{10} 7 CFU/g as typically this is the spoilage level for bacterial pathogens in raw poultry [30]. True replicates were used at each of the testing temperatures for both chicken and laboratory data.

2.6. Statistical methods

Weighted least squares regression analyses were performed independently for Log_{10} *Salmonella* CFU and Log_{10} *D*-values as the dependent variables for growth and inactivation, respectively. For growth, the outcome modeled was the change in *Salmonella* density (Log_{10} CFU/g). Refrigerated storage temperatures (in Celsius), time (in days), and media type (chicken versus laboratory media) were modeled as the independent variables (fixed effects). Inoculation level was analyzed as part of the lag phase at time zero. Studies, trials within studies, and repeated measurements at a given temperature within an experiment were modeled as random effects. The covariance structure type was variance component. For repeated measures, different covariance structures were tried; however, the regression models did not converge. As a result, repeated measures were treated as a random effect to account partially for the correlation between measurements over time. For inactivation, Log_{10} *D*-values were the dependent variable of interest. Temperature (in Celsius), media type (chicken versus laboratory media), inoculation levels (Log_{10} CFU/g), and use of *Salmonella* Senftenberg (yes or no) were modeled as fixed effects and the study was included as a random effect.

For growth and inactivation, the assumption of heterogeneity between studies (or homogeneity within the same study) implied by the use of random effect is plausible due to different laboratory settings among different studies. To check the degree of similarity of growth of *Salmonella* between studies, the intra-class correlation coefficient ρ was computed, which estimates the proportion of the total variance that is due to the heterogeneity among studies [31].

Initially, all second and third order interaction terms and quadratic polynomial functions were evaluated for significance. The significance level used was 0.05. If the coefficient of a variable was not significant ($P > 0.05$), it was removed from the model using a backward regression. For the computation of the pooled effect estimates, each primary study was given a weight equal to the reciprocal of its sample size (SS) since the variance or data for post hoc calculation of the variance (standard error or standard deviation) were not provided in most studies. A normal distribution assumption was considered for the log of measured bacterial counts (and Log_{10} *D*-values) for all treatment groups in all studies included in the meta-analysis. Normality tests of residuals, such as Shapiro—Wilk (W), Kolmogorove—Smirnov (D), Cramer—von Mises (W-Sq) and Anderson—Darling (A-Sq), and diagnostic plots of the residuals were used to test this assumption along with tests for skewness and kurtosis of the residuals.

3. Results

3.1. Identification and assessment of relevant literature

The search located 449, 395, 207, and 225 records for *Salmonella* growth in chicken, growth in laboratory media, thermal inactivation in chicken, and thermal inactivation in laboratory media, respectively. After relevance screening of titles and abstracts and removal of duplicated records, there were 18, 16, 6, and 4 records for each of these topic areas, respectively. Additional articles were excluded upon full text screening as follows. For growth in chicken, 10 articles were excluded

Table 2 Summary of primary studies evaluating *Salmonella* growth at refrigerated storage temperatures (chicken meat).

Author	Study type (country)	Food type	*Salmonella* spp.	Enumeration method	Sample size/# time points tested	Temperature (inoculation level Log_{10} CFU/g)
Pintar et al. [46]	Challenge (Canada)	Retail raw chicken meat (skinless, boneless chicken breast)	Typhimurium	USDA/FSIS MPN[a] described in the Microbiology Laboratory Guidebook (sensitive to 0.3 MPN/g of sample)	14 (3)	4 °C (3.29)
CCFRA [41]	Challenge (U.K.)	Fresh boneless chicken thighs	Typhimurium, and Enteritidis	Selective media: XLD and MLCB	5 (6—9)	0, 4, 6, 8, 10 °C (3.79—4.54)
Baker et al. [40]	Challenge (U.S.) Field trial	Minced chicken meat Chicken parts (chicken breast muscle, and leg quarters with skin)	Typhimurium Enteritidis	Pour plate method Plate count agar (DIFCO)	2 (6) 2 (6)	2, 7 °C (4) 2, 7 °C (N/A)
Gray et al. [45]	Challenge (U.S.)	Fresh chicken thighs	Enteritidis	Brilliant green agar (DIFCO)	2 (4)	10 °C (3.3)
Cunningham [44]	Challenge (U.S.)	Fresh broiler drumsticks	Typhimurium NRRL B-411	*Salmonella*-Shigella agar (SS agar)	10 (4)	10 °C (4.3)
To and Robach [43]	Challenge (U.S.)	Fresh whole broilers	Enteritidis ATCC 13076, Heidelberg ATCC 8326, Infantis 2-13, and Typhimurium ATCC 13311	Bismuth Sulfite Agar (DIFCO)	3 (5—6)	3 °C (1.9—2)
Robach and Ivey [42]	Challenge (U.S.)	Chicken breasts	Typhimurium 13311, Heidelberg 8326, and Montevideo 8387	Trypticase soy broth (DIFCO)	6 (3—4)	10 °C (1.5—3.6)

[a] Most probable number (microbiological testing method).

Table 3 Summary of primary studies evaluating *Salmonella* growth at refrigerated storage temperatures (laboratory media).

Author	Study type (country)	Media type	*Salmonella* spp.	Enumeration method	Sample size/# time points tested	Temperature (inoculation level Log_{10} CFU/g)	pH/NaCl
Alcock [48]	Challenge (U.K.)	Broth medium	Anatum, Montevideo, Napoli, Panama, Saint-paul, Stanley, Agona, Bredeney, Enteritidis, Hadar, Infantis, Senftenberg, Typhimurium, and Virchow	Nutrient agar plate	2 (9)	8 °C (4.5)	6.4/NR[a]
Baker et al. [40]	Challenge (U.S.)	Trypticase soy broth	Typhimurium	Nutrient broth (DIFCO)	2 (6)	2, 7 °C (4.2)	NR/NR
Alcock [63]	Challenge (U.K.)	Broth medium	Agona, Bredeney, Enteritidis, Hadar, Infantis, Senftenberg, Typhimurium, and Virchow	Nutrient agar plate	2 (5—7)	6.2, 6.7, 7.6, 8.6 °C (4)	NR/NR
Elliott and Gray [49]	Challenge (Norway)	Trypticase soy agar	Enteritidis	TSA plates	2 (6)	10 °C (7)	6/NR
Matches and Liston [64]	Challenge (U.S.)	Nutrient broth	Heidelberg, Typhimurium, and Derby	Samples at 8 °C by TSA plates. At 12, 22, 37, and 41 °C by Bausch and Lomb Spectronic 20 spectrophotometer	2 (6)	8 °C (5.5)	NR/NR
Matches and Liston [65]	Challenge (U.S.)	Trypticase soy broth and agar	Derby, Heidelberg, Typhimurium, Aertrycke, Montevideo, Newport, and Thompson	TSA plates	2 (4)	5.1, 5.9, 6.7, 7.5, 8.3 °C (6.7)	NR/NR

[a] NR: not reported.

Table 4 Summary of primary studies evaluating *Salmonella* inactivation at thermal treatment temperatures (chicken meat).

Author	Study type (country)	Food type	*Salmonella* spp.	Enumeration method	Sample size per testing temperature	Temperature (inoculation level Log_{10} CFU/g)
Murphy et al. [28]	Challenge (U.S.)	Chicken patties, and chicken tenders	Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo and California	Food and Drug Administration procedures	3	55, 57.5, 60, 62.5, 65, 67.5, 70 °C (7–8)
Juneja et al. [50]	Challenge (U.S.)	Ground chicken.	Kentucky, Heidelberg, Hadar and Thompson	TSA surface plating	2	58, 60, 62.5, 65 °C (8)
Juneja et al. [52]	Challenge (U.S.)	Ground chicken	Thompson, Enteritidis, Typhimurium, Hadar, Copenhagen, Montevideo and Heidelberg	TSA surface plating	2	58, 60, 62.5, 65 °C (8)
Mazzotta [51]	Challenge (U.S.)	Ground chicken breast meat	Typhimurium, Enteritidis, Montevideo, Mbandaka, Heidelberg and Thompson	TSA surface plating	3	56, 60, 62, 63 °C (7)
Murphy et al. [29]	Challenge (U.S.)	Ground chicken breast meat	Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo and California	Food and Drug Administration procedures	3	55, 57.5, 60, 62.5, 65, 67.5, 70 °C (7–7.5)
Murphy et al. [27]	Challenge (U.S.)	Ground chicken breast meat	Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo and California	Food and Drug Administration procedures	3	67.5, 70 °C (7–8)

Table 5 Summary of primary studies evaluating *Salmonella* inactivation at thermal treatment temperatures (laboratory media).

Author	Study type (country)	Media type	*Salmonella* spp.	Enumeration method	Sample size per testing temperature	Temperature (inoculation level Log_{10} CFU/g)
Juneja et al. [50]	Challenge (U.S.)	Broth medium	Thompson, Enteritidis, Typhimurium, Hadar, Copenhagen, Montevideo and Heidelberg	TSA surface plating	2	55, 58, 60, 62 °C (8)
Murphy et al. [29]	Challenge (U.S.)	Liquid medium (0.1% peptone-agar solution)	Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo and California	Food and Drug Administration procedures	3	55, 57.5, 60, 62.5, 65, 67.5, 70 °C (7–7.5)
Murphy et al. [27]	Challenge (U.S.)	Liquid medium (0.1% peptone-agar solution)	Senftenberg, Typhimurium, Heidelberg, Mission, Montevideo and California	Food and Drug Administration procedures	3	67.5, 70 °C (7–8)
Xavier and Ingham [66]	Challenge (Canada)	Casein soymeal peptone-yeast extract broth medium	Enteritidis (ATCC4931)	Nutrient agar (BDH)	2	52, 54, 56, 58 °C (7)

because they reported prevalence only rather than concentration data and one [32] was excluded because it investigated growth in chicken à la king rather than raw chicken. For growth in laboratory media, four studies were excluded [33—36] because they reported prevalence rather than concentration data, and an additional four studies were excluded [10,22,37—38] because the temperatures evaluated were higher than the range of temperatures of interest in this review. Two additional studies were excluded: one study [39] gave only the generation time (GT) and the other [9] gave only the growth rate (GR) instead of full details of the growth curve. Therefore, there were 7, 6, 6, and 4 records for each of growth in chicken, growth in laboratory media, inactivation in chicken, and inactivation in laboratory media, respectively, included in the analysis (Tables 2—5).

3.1.1. Refrigerated storage

Tables 2 and 3 summarize characteristics of the studies that met the inclusion criteria for *Salmonella* growth on chicken and laboratory media at refrigerated storage temperatures, respectively. For chicken, all articles were challenge trials, except one [40], which had both a challenge trial and a field trial within the same publication. Combining results from different study designs was not performed, as an invalid effect estimate may arise due to the methodological heterogeneity of different designs [24]. As a result, the field trial was excluded from the meta-analysis. Among the seven studies included, three trials had more than one challenge experiment within the same publication [41—43]. CCFRA [41] tested *Salmonella* growth in chicken using two different enumeration media and had two sets of five replicates for each medium. Results from all replicates in different media were included separately in the analysis. To and Robach [43] tested *Salmonella* growth at two poultry processing plants. Results from both plants were included separately in the analysis. Robach and Ivey [42] reported two trials with two different levels of *Salmonella* inoculation. Both trials were included in our analysis. For studies investigating the effect of different interventions, such as carbon dioxide and potassium sorbate on *Salmonella* growth [40,42—45], only control growth curves (e.g., air) were included in this analysis. All studies reported the outcome as a continuous outcome, e.g., at each storage temperature; testing intervals and their corresponding Log_{10} CFU/g were reported. Only one study reported growth of *Salmonella* as most probable number (MPN)/g [46] which was converted into CFU/g for analysis [47].

Use of randomization was explicitly stated in only one trial [46]. Blinding of the person assessing the outcome and loss to follow-up were not reported in any trial. Standard deviation or variability measures for post hoc calculation were reported only in one study [41]. Therefore, evaluating the impact of these factors was not possible. For laboratory media, only a few studies controlled for the potential confounding effect of pH value [48,49], and none of the studies reported NaCl level, which is a potentially confounding variable, when examining the relationship between temperature and growth of *Salmonella*. Enumeration methods used to count *Salmonella* were all standard cultural methods.

3.1.2. Thermal inactivation

Tables 4 and 5 summarize characteristics of the studies that met the inclusion criteria for thermal inactivation studies in chicken and laboratory media, respectively. All studies reported the initial inoculation level, testing temperatures and their corresponding *D*-values. All were challenge trials and estimated *D*-values over a range of temperatures.

One study explicitly stated random allocation to treatment [50]. Blinding of the person assessing the outcome and loss to follow-up were not reported in any of the studies. Standard deviation or variability measures for post hoc calculation were provided in three of six trials [27,50—51]. Three studies [27,50—51] reported standard deviation within each treatment group and three studies [28—29,52] did not report the SD or data needed for post hoc calculation. One study that did report the SD used *S*. Senftenberg [27]; the other two studies that reported the SD did not use *S*. Senftenberg [50,51]. Among the studies that did not report the SD [28—29,52], only one did not use *S*. Senftenberg [52].

3.2. Meta-analysis equations

3.2.1. Refrigerated storage

Significant predictors of *Salmonella* growth (Log_{10} CFU) in chicken meat versus laboratory media are shown in Table 6. The intra-class correlation coefficient, ρ, calculated as the ratio of the estimate of the study random effect divided by the sum of the estimates of all variance components was 0.51. This indicated that there was a similarity in growth of *Salmonella* within the same study and therefore the study should be included as a random effect. The residual in this table was significant (<0.001), meaning that growth varied significantly within studies and trials even after controlling for the other effects in the model.

Table 6 Results of meta-analysis equation for growth of *Salmonella* in chicken meat and laboratory media over 15 days at temperatures ⩽10 °C.

Cov parameter	Estimate	Standard error	*Z* value	Pr > *Z*	Alpha	Lower CI[a]	Upper CI
Estimates of covariance parameters							
Study(Media)	1.03	0.92	1.13	0.13	0.05	0.31	20.67
Trial(Study × Media)	0.11	0.095	1.13	0.13	0.05	0.032	2.18
Repeated(Study × Media × Temperature × Trial)	0.66	0.11	6.19	<0.0001	0.05	0.49	0.93
Residual	0.23	0.045	5.05	<0.0001	0.05	0.16	0.35

Effect	Num DF	Den DF	*F* value	Pr > *F*
Estimates of fixed effects				
Temperature	12	87	0.89	0.56
Time	1	50	65.02	<0.0001
Media	1	4	0.12	0.74
Time × Temperature	12	50	25.67	<0.0001
Media × Temperature	2	87	1.27	0.29
Time × Media	1	50	0.48	0.49
Time × Media × Temperature	2	50	6.39	0.0034
Time × Time	1	50	8.74	0.0047

[a] CI: confidence interval.

The significant three-way interaction term Time × Media × Temperature indicated that there was a significant difference in the growth of *Salmonella* on chicken versus that on laboratory media under the same storage time and temperature. The ratio of the variance estimate between the two media (chicken divided by laboratory) was 6.79 (data not shown). This means that *Salmonella* growth on chicken varied 6.79 times more than in laboratory media. Thus *Salmonella* growth in the two media differed in the average value, and there was more variation on the pattern of growth across different testing intervals between the two media types.

Therefore, chicken data were analyzed alone to estimate the growth equations at different temperatures. Table 7 shows the parameter estimates for the random effect covariance, fixed effects, and solutions for fixed effects for chicken data. There was a lack of a consistent pattern in growth with the increase in temperature. Growth at temperatures 4 °C and 7 °C was significantly different than growth at other temperatures ($P = 0.005$), otherwise there were no differences among the remaining temperatures. Therefore, it is more appropriate to model temperatures separately rather than combining them in a single estimate, and the results are presented as such.

Statistical normality tests were all <0.05 indicating that the residual data were not normally distributed (e.g., *P*-values for W, D, W-Sq, and A-Sq were <0.0001, <0.01, <0.005, and <0.005, respectively). Skewness and Kurtosis were −1.51 and 5.70, respectively, indicating that the residuals were skewed to the left with a peaked curve. To adjust for this non-normality in the residuals distribution log−log CFU/g and square root of CFU/g transformations were evaluated. However, none of these transformations resulted in normally distributed residuals and were therefore not used in the final model.

3.2.2. Thermal inactivation

A comparison of the parameter estimates for temperature at different inoculation levels (7 or 8), media type (Chicken = C, Laboratory = L), whether *Salmonella* Senftenberg was part of the serotypes mix (Yes = Y, or No = N), and significance of second and third interaction terms and their effect on Log_{10} *D*-values during heat treatment is summarized in Table 8.

For thermal inactivation equations, at different combinations, the intercepts and slopes, respectively were estimated to be: (7, C, Y) 6.016 and −0.043 (7, C, N), −27.84 and 1.12 (7, L, Y). 6.65 and −0.056 (7, L, N), −25.27 and 1.066 (8, C, Y), 3.78 and 0.0057 (8, C, N), −30.074 and 1.168 (8, L, Y), 4.418 and −0.0077 (8, L, N), −27.51 and 1.115. From this data, *D*-values were consistently higher in chicken meat than in laboratory media, when *S.* Senftenberg was part of the inoculation and when the inoculation level was 8 in comparison to 7. Also, as the temperature increased, *D*-values decreased. Normality tests were all >0.05, meaning that the residuals from the inactivation model did not show any significant normality (e.g., *P*-values for W, D,

Table 7 Results of meta-analysis equation for growth of *Salmonella* in chicken meat over 15 days at temperatures ⩽10 °C.

Cov parameter	Estimate	Standard error	*Z* value	Pr > *Z*	Alpha	Lower CI	Upper CI
Estimates of covariance parameters							
Study	0.87	0.801	1.09	0.14	0.05	0.25	21.23
Trial (Study)	0.096	0.094	1.02	0.15	0.05	0.026	3.44
Residual	3.45	0.53	6.54	<0.0001	0.05	2.61	4.77

Effect	Num DF	Den DF	*F* value	Pr > *F*
Estimates of fixed effects				
Temperature	5	84	1.65	0.15
Time	1	84	29.2	<0.0001
Time × Temperature	5	84	2.17	0.065
Time × Time	1	84	15.82	0.0001

Effect	Temperature	Estimate	Standard error	DF	*t* value	Pr > l *t* l
Solution for fixed effects						
Temperature	2	4.02	1.37	84	2.94	0.004
Temperature	3	1.43	1.11	84	1.28	0.203
Temperature	4	3.21	1.09	84	2.94	0.004
Temperature	7	4.27	1.37	84	3.12	0.003
Temperature	8	4.91	0.62	84	7.93	<0.0001
Temperature	10	4.63	0.55	84	8.46	<0.0001
Time × Temperature	2	0.45	0.32	84	1.43	0.16
Time × Temperature	3	0.53	0.12	84	4.6	<0.0001
Time × Temperature	4	0.22	0.10	84	2.18	0.03
Time × Temperature	7	1.005	0.32	84	3.18	0.002
Time × Temperature	8	0.47	0.099	84	4.72	<0.0001
Time × Temperature	10	0.45	0.071	84	6.36	<0.0001
Time × Time		−0.027	0.007	84	−3.98	0.0001

W-Sq, and A-Sq were 0.28, >0.15, >0.25, and >0.25, respectively). Skewness and Kurtosis were −0.27 and −0.57 indicating that the data were a bit skewed to the left with small flatness in the curve.

4. Discussion

This review found a significant difference between growth/inactivation in chicken meat versus that in laboratory media. As a result, the use of laboratory media as an alternative to chicken meat in QRA modeling may not be appropriate. Meta-analysis equations for *Salmonella* growth and inactivation in chicken meat were developed in this study and could be used to support future risk assessment modeling to estimate growth and inactivation of *Salmonella* at different temperatures. For chicken, there was no consistent pattern in growth of *Salmonella* as a function of temperature. No specific explanation could be found considering that all the studied temperatures were at the lower scale of *Salmonella* growth temperatures (e.g., ⩽10 °C). However, there was evidence of growth of *Salmonella* at temperatures of less than 10 °C. Ignoring growth in this temperature range may therefore underestimate the total number of *Salmonella* predicted in chicken that can cause the illness.

In this review, growth data (starting from zero days until the maximum of 15 days) were modeled rather than growth rate, which is commonly used to model bacterial growth [53]. This can have several advantages: (i) this approach avoids the use of subjective measures to decide on cut-points between the end and start of different growth phases, especially when growth does not follow the traditional sigmoidal shape and (ii) it includes all data points available to model different growth phases (including the lag phase), which makes biological sense as bacteria require time to adapt when moved from one environment to another, while using growth rate will overestimate bacterial numbers predicted in the lag phase.

As the temperature increased, *D*-values for *Salmonella* inactivation decreased. For thermal inactivation modeling in chicken meat, it is recommended to use the thermal inactivation equations presented with *Salmonella* Senftenberg included in the inoculum, as the high thermal resistance of this serotype will provide a conservative assumption of killing other serotypes contaminating the chicken meat.

Table 8 Results of meta-analysis equation for thermal inactivation of *Salmonella* in chicken meat and laboratory media at temperatures ranging from 55 to 70 °C.

Cov parameter	Estimate	Standard error	*Z* value	Pr > *Z*	Alpha	Lower CI[a]	Upper CI
Estimates of covariance parameters							
Study(Media × Senftenberg)	0.008	0.007	1.18	0.12	0.05	0.003	0.13
Residuals	0.019	0.003	6.26	<0.0001	0.05	0.014	0.03

Effect	Num DF	Den DF	*F* value	Pr > *F*
Estimates of fixed effects				
Media	1	5	8.69	0.03
Senftenberg	1	5	18.37	0.008
Inoculation	1	79	5.38	0.02
Temperature	1	79	15.97	0.0001
Media × Senftenberg	1	5	3.15	0.14
Temperature × Media	1	79	13.44	0.0004
Temperature × Senftenberg	1	79	18.77	<0.0001
Temperature × Media × Senftenberg	1	79	4.76	0.03
Temperature × Temperature	1	79	26.55	<0.0001
Temperature × Inoculation	1	79	8.81	0.004
Temperature × Temperature × Senftenberg	1	79	20.16	<0.0001

Effect	Media/ Senftenberg[b]	Inoculation	Estimate	Standard error	DF	*t* value	Pr > l *t* l
Solution for fixed effects							
Inoculation		7	6.65	2.65	79	2.51	0.01
Inoculation		8	4.42	2.82	79	1.57	0.12
Media × Senftenberg	C/N		−34.49	8.06	5	−4.28	0.008
Media × Senftenberg	C/Y		−0.64	0.45	5	−1.42	0.21
Media × Senftenberg	L/N		−31.92	7.31	5	−4.37	0.007
Media × Senftenberg	L/Y		0				
Temperature × Media × Senftenberg	C/N		1.17	0.27	79	4.38	<0.0001
Temperature × Media × Senftenberg	C/Y		0.006	0.09	79	0.07	0.95
Temperature × Media × Senftenberg	L/N		1.11	0.25	79	4.41	<0.0001
Temperature × Media × Senftenberg	L/Y		−0.008	0.086	79	−0.09	0.93
Temperature × Inoculation		7	−0.05	0.016	79	−2.97	0.004
Temperature × Inoculation		8	0				
Temperature × Temperature × Senftenberg	N		−0.01	0.002	79	−5.05	<0.0001
Temperature × Temperature × Senftenberg	Y		−0.00075	0.0007	79	−1.12	0.27

[a] CI: confidence interval.
[b] C: Chicken media, L: Laboratory media, Y: (Yes) multiple *Salmonella* serotypes including *Salmonella* Senftenberg, N: (No) multiple *Salmonella* serotypes without *Salmonella* Senftenberg.

However, the three studies pooled for *Salmonella* Senftenberg were from the same author. This might enhance consistency of the testing environment, such as the laboratory setting, source of chicken pieces tested, reliability of tools used to measure the outcome, and source of bacteria (age of culture) used to contaminate the chicken.

4.1. Methodological issues/sources of heterogeneity

The current review may be subject to selection bias as the focus was only on English language articles [54]. However, an attempt was made to reduce selection bias in the identification of primary research studies by: (i) searching six databases that are among the most commonly used in the food safety area; (ii) expanding the publication period of the included trials; and (iii) contacting experts in the field to identify unpublished work. Furthermore, most of the studies found were challenge studies in which case it would not be expected to find different results in studies from different geographic areas. However, challenge trials do not provide evidence of a high quality for real world application as do natural disease outcomes [55],

and hence having more studies with natural exposure to *Salmonella* to address these types of questions is an area to consider when designing future studies.

Methodological concerns were identified for several studies in the quality assessment stage. Randomization, blinding and loss-to-follow-up were generally not reported, raising the possibility of selection bias (at the chicken parts level). It is possible that random allocation was performed, but not explicitly reported. Blinding may not have been used because objective laboratory techniques were used to determine the outcome, so the laboratory technicians' knowledge of the treatment temperatures would not likely affect the measured outcome, and loss-to-follow-up (due, for example, to mishandling practices or spoilage) may not have been reported since this type of research lasts from a few days to a few weeks at most due to the short shelf life of the tested products. Nonetheless, the use of guidelines in food safety research, such as the CONSORT and REFLECT statements, may assist in ensuring complete reporting of essential design features for RCTs [56—59] and similarly for challenge trials.

Clinical heterogeneity might exist in the combined studies due to variability in the chicken characteristics. For example, in refrigerated storage studies, different types of chicken meat were investigated, such as skinless, boneless chicken breast, chicken thighs, and chicken muscles. Similarly, the thermal inactivation studies included chicken patties, chicken tenders, ground chicken, and ground chicken breast. Different chicken parts may vary in their pH values [41]. Thigh chicken meat, for example, has a higher pH value (6.4—6.7) than breast chicken meat (5.8) [41] which makes the former closer to the optimum pH required for *Salmonella* to grow which lies between 6.5 and 7.5 [18]. Subgroup analysis to compare growth/inactivation in different chicken types was not performed due to the limited number of studies.

Sub-group analysis to investigate the impact of potential confounders and effect modifiers was not performed as the number of trials available was insufficient. Potential confounding variables include pH and water activity level, and an example of an effect modifier is the percentage of fat level in the tested product. The higher the percentage fat, the higher the time (*D*-value) needed to heat the product to a certain temperature [60,61]; and the higher percentage fat, the higher the protection for bacterial cells against heat [62]. Other examples might be the history and age of the *Salmonella* mixture inoculated and level of nutrients available for *Salmonella* to grow. Control for such factors, by measuring the composition of the tested products and deriving separate equations to apply to different levels of the effect variables, minimizes the possibility of invalid effect estimates.

5. Conclusion

The current meta-analysis approach provided a structured method for finding and pooling data to increase precision of estimates for *Salmonella* growth and inactivation at different temperatures. A significant difference was found between *Salmonella* growth/inactivation on chicken meat versus laboratory media. The growth and inactivation meta-analysis equations detailed in this review should be used in QRA to model growth and inactivation of *Salmonella* in chicken meat. Parameter estimates for growth of *Salmonella* in chicken meat at temperatures $\leqslant$10 °C and inactivation at temperatures between 55 °C and 70 °C were provided and should be used when modeling *Salmonella* growth and inactivation in chicken meat. Validation of growth/inactivation equations created in this review against independent data is an area to be considered for future research, keeping in mind the methodological recommendations made in this paper to enhance the quality of reported data in the food safety area.

Conflict of interest

There are no competing interests.

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