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#### Original article

6456

# Systematic review and meta-analysis of the impact of decontamination interventions on the prevalence and concentration of *Salmonella* in broiler chickens during primary processing

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#### Summary

Systematic review and meta-analysis aggregate quantitative data from different studies into unified effect size estimates with better statistical power in risk assessment model parameterisation. This study uses systematic review and meta-analysis to estimate Salmonella decontamination during broiler slaughter from scalding to post-chilling, with meta-regression applied to explore modifier variables. Data from 161 studies published between 1998 and 2022 was extracted from thirty-five articles identified in the systematic review process with meta-analysis and meta-regression performed using the metafor package (version 2.0-0) in R statistical environment (version 3.6.0). The analysis revealed carcass wash (1.31 log<sub>10</sub> CFU/carcass reduction in odds; P < 0.01) and chilling (121.50% reduction in relative risk; P < 0.01) had significant reduction on Salmonella concentration and prevalence, respectively. Chemical additives reduced the concentration  $(0.98 \log_{10} \text{CFU/carcass}; P < 0.01)$  and prevalence (64.74% relative risk; P < 0.01) but the efficacy of physical methods was not conclusive. Application of decontaminants through immersion was superior (0.90 log<sub>10</sub> CFU/carcass; P < 0.01) to spraying (0.72  $\log_{10}$  CFU/carcass; P < 0.01). Adjusting the pH sequentially of electrolysed water, acetic acid and trisodium phosphate reduced the odds of Salmonella concentration by more than 2 log cycles and the relative risk by more than 100%. The results provide trends in the concentration and prevalence of Salmonella during the broilers slaughter process with application of decontamination interventions and provide a basis for control decision-making and quantitative microbial risk assessment.

#### Keywords

Abattoir, Gallus gullus, meta-regression, microbial decontamination.

#### Introduction

Recent global trends in salmonellosis indicate a considerable increase in incidence rates over the past three decades. Estimates show an annual incidence of 80.3 million foodborne illnesses and 155 000 deaths with higher disease burden in low-and middle-income settings (Ao et al., 2015; Als et al., 2018; Stanaway et al., 2019). Salmonellosis is one of the most common food-borne diseases worldwide with nontyphoid Salmonella strains associated with minor salmonellosis and

nated swine and poultry meat products among the significant transmission routes for human salmonellosis (Ferrari *et al.*, 2019). The presence of *Salmonella* in poultry carcasses is associated with contamination and cross-contamination with intestinal contents along the slaughter process. Re-contamination from the slaughter equipment and processing water causes an estimated 12–34% increase in the likelihood of an outbreak (Akil

typhoid fever being a symptom in major salmonellosis

(Wattiau et al., 2011). Epidemiological investigations

attribute the handling and consumption of contami-

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& Ahmad, 2019).

Notwithstanding, the advancements in microbial risk management plans in Salmonella decontamination, potential re-contamination with faecal matter and cross-contamination among flocks from different farms persists along the chicken slaughter process (Rajan et al., 2017; Hardie et al., 2019). Persistence has been attributed to biofilms production and tolerance to various factors such as heat, acids and antibiotics, as well as and cross-tolerance to these factors, all of which reduce the efficacy of chemical and physical Salmonella decontamination interventions (Gruzdev et al., 2011; Akil & Ahmad, 2019). Codex guidelines recommend certain physical and chemical decontaminants in a Hazard Analysis Critical Control Points (HACCP)based system to control Salmonella during slaughter (Codex Alimentarius Commission, 2011). Food safety agencies are meant to adopt or adapt codex guidelines in regional and national risk assessment frameworks for Salmonella in broiler chickens.

Fragmented evidence exists on the efficacy of existing *Salmonella* decontamination methods at specific processing points, from scalding through defeathering and washing to chilling. Extraneous variables such as weather and seasons, decontaminant application technique and the carcass parts exposed, sampling, and microbial confirmation technique during analysis have been implicated as confounding factors in the reported data on the effectiveness of *Salmonella* decontamination (Bucher *et al.*, 2012a, 2012b; Bourassa *et al.*, 2015; Lin *et al.*, 2021).

Collectively, these factors create a unified effect size that can be estimated by collating existing data. Systematic review and meta-analysis provide an excellent platform to aggregate evidence from many studies into unified effect size estimates with better statistical power for risk assessment model parameterisation (Brockwell & Gordon, 2001). Reviews, meta-analyses and risk models on Salmonella decontamination during primary processing of poultry have been done to assess the effectiveness of decontaminant application techniques (FAO and WHO, 2009; Codex Alimentarius Commission, 2011; Bucher et al., 2012b), comparison of immersion and spray intervention techniques during chilling (Bucher et al., 2012a), assess the levels of efficacy of decontaminants (Kerr et al., 2013), and to evaluate variations along slaughter operations (Golden & Mishra, 2020). Variability in decontamination of Salmonella has been reported along the swine slaughter process but not the poultry slaughter process (Duarte et al., 2016).

This study aims to aggregate evidence from eligible studies into unified summary estimates for *Salmonella* decontamination interventions during broiler chicken slaughter process from scalding to post-chilling using systematic review and meta-analysis and validate the impact of modifier variables using meta-regression. The collation of data from multiple studies into a unified

effect size will provide a robust quantitative estimate of changes in *Salmonella* concentration and prevalence by applying decontamination hurdles along the slaughter process. This work includes a systematic review to identify antimicrobial activities commonly used in slaughterhouse decontamination interventions and a combination of meta-analysis and meta-regression that provide a solid base to support routine food safety decision-making.

#### Methodology

#### Protocol and research question

The systematic review was conducted on published articles on *Salmonella* decontamination interventions during slaughter. The review process was based on the PRISMA-P protocol (Liberati *et al.*, 2009; Moher *et al.*, 2015). The research question was "What is the efficacy of all possible interventions to control *Salmonella* in chicken carcasses along the slaughter process from scalding to post-chilling?"

#### Literature search strategy

The literature search was conducted in August 2022 using five electronic databases: Dimensions, Web of Science, PubMed, African Index Medicus Database and Google Scholar. The algorithm used was: ((Salmonella) AND (Broiler OR Chicken OR (Gallus))) AND ((Slaughter\* OR Abattoir)). Web-searching and handsearching were also done using Google and CAB abstracts search engines to find additional relevant publications and search verification as recommended (Richards, 2008; Paez, 2017). The citations were imported into the Mendeley (Version 1.19.4) reference manager for deduplication of the citation hits.

#### Criteria for relevance and eligibility screening

A two-level approach was used to perform the independent screening (protocol tools in Data S1). The first screening level involved the selection of articles based on the titles and abstracts to identify studies investigating changes in Salmonella concentration and prevalence in broiler chickens along the slaughter process. Google translate was used to translate the titles and abstracts for publications in languages other than English. At this stage, studies were excluded if (i) interventions were performed before scalding or during storage post-chill, (ii) available data in the manuscript were not on Salmonella, (iii) chicken sampled were not broilers, and (iv) environmental samples, such as processing water and surface swabs, were analysed. The second screening level selected articles based on the methodological soundness of the study designs. Studies were excluded if (i) complete data (including standard deviation/error) for the control and treatment groups were not extractable, (ii) the intervention was described in a manner that replication would not be possible, or (iii) intervention conducted on organs and not carcasses.

### Assessing risk of bias and data extraction of included studies

A checklist was developed using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) guidelines to assess the risk of bias from the eligible studies as recommended (Schünemann et al., 2011). Parameters used to evaluate risk of bias were: (i) study design adequacy, (ii) sample size justification, (iii) sampling process, (iv) study setup, (v) appropriateness of control group, (vi) statistical analysis, (vii) understated results, and (viii) presentation of estimates and variability. Microsoft® Access was used to extract data on article description, intervention points, intervention details, sampling points and protocols, isolation and confirmation media, prevalence and counts. Data analysis was done using R (version 3.6.0) using metafor (version 2.0-0) and meta (version 6.1-0) packages (Viechtbauer, 2015).

#### Review management

Two independent reviewers performed the relevance and eligibility screening, risk of bias assessment and data extraction, while a third reviewer confirmed the completeness of these processes. Pre-tested checklists were used to guide screening and evaluate bias with consensus applied in case of disagreements in the review process. Deduplication was done using Mendelev reference management software (version 1.19.8).

#### Data processing and analysis

The effect size for the meta-analysis was measured using standardised mean difference, the odds ratio for Salmonella concentration and relative risks for prevalence as recommended (Sterne et al., 2005; Higgins et al., 2019). Heterogeneity due to differences in study designs was accounted for using the risk of bias assessment. Statistical heterogeneity was assessed using Cochran's Q test,  $\tau^2$ , and Higgins' and Thompson's  $\hat{I}^2$ value (Schwarzer et al., 2015; Veroniki et al., 2016). A weighted-random-effect model was adopted to calculate effect size where between-study variability,  $I^2$ , was high. The Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC) and AIC-adjusted (AICc) for small sample size detailed the model selection as recommended (Brewer et al., 2016). The "method of moments" (DerSimonian and Laird) and

Restrictive Maximum Likelihood (REML) methods were used to estimate variability,  $\tau^2$  (tau-squared), in concentration studies and prevalence studies, respectively (DerSimonian & Kacker, 2007; Viechtbauer, 2007). The 'Mantel–Haenszel' model was used for homogenous data in estimating the pooled effect (Deeks *et al.*, 2008).

Funnel plots asymmetry was used to indicate the presence of publication bias with detailed assessment done using Egger's regression test and Begg's rank correlation test as recommended (Macaskill et al., 2001; Rothstein et al., 2005; Lau et al., 2006; Sutton & Higgins, 2008). Publication bias was assumed to be present where either Egger's or Begg's tests were significant (P < 0.05). For studies on prevalence, the Bubble Plot, radial and L'Abbe plots were used for detailed assessment of publication bias due to small study effects. Fourteen potential modifier variables that could confound the effect sizes were identified a priori based on their perceived impact on (i) study characteristics, (ii) risk of bias and (iii) study design (Hardie et al., 2019) and a mixed-effect model was used to run a meta-regression as recommended (Higgins & Thompson, 2004; Jain et al., 2019).

#### **Results and discussion**

#### Literature search

The systematic review process is summarised as shown in Fig. 1. The literature search identified 3809 articles, from which 2232 were eliminated after deduplication, 1483 were excluded during the title and abstract screening, fifty-one were excluded as they failed to meet the eligibility criteria while screening articles' methodology and results and a further seven had a high risk of bias due to study design inadequacy. After the systematic review process, thirty-six articles were selected. Data were extracted from 161 trials, 102 trials on *Salmonella* concentration were extracted from nineteen articles, and fifty-nine trials on *Salmonella* prevalence were extracted from twenty-two articles.

## Meta-analysis on studies reporting Salmonella concentration as an outcome

The publication bias within the Salmonella concentration studies is summarised using Galbraith radial plot (Fig. 2a), contour-enhanced funnel plots (Fig. 2b), and quantile–quantile plot (Fig. 2c). There was minimal publication bias observed from the symmetrical distribution of studies within the funnel, radial and QQ plots and further confirmed by Egger's regression and Begg's rank tests as recommended (Veroniki et al., 2016). Despite the low-level risk of bias observed, inadequate generation of allocation sequence, lack of concealment and blinding, poor description of sampling procedures

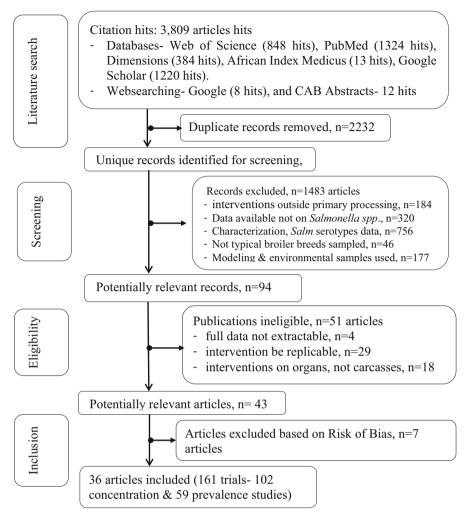


Figure 1 Flow of studies for the systematic-review meta-analysis study for Salmonella in broilers during primary processing of broiler chicken.

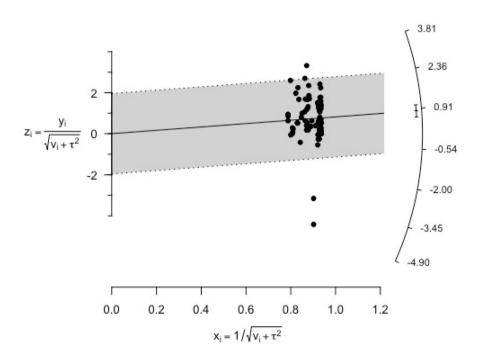
and use of artificially *Salmonella* inoculated chicken carcasses would potentially increase heterogeneity within the studies as previously reported (Gichure *et al.*, 2022). The lack of standard guidelines on reporting the effect of *Salmonella* decontamination on chicken carcasses have been reported as a critical setback when pooling results from several studies in food safety assessment (EFSA, 2010; Kahan *et al.*, 2015).

The forest plots visualising the pooled effects of *Salmonella* concentration at different points along the chicken slaughter process from different authors with statistical heterogeneity presented Cochran's Q test,  $\tau^2$ , and Higgins' and Thompson's  $I^2$  have been shown in Fig. 3. A net decrease of 0.82  $\log_{10}$  CFU/carcass in *Salmonella* concentration was observed (95% CI: 0.60–1.04, P < 0.01), with the highest reduction during carcass wash (1.31  $\log_{10}$  CFU/carcass) and an increase (1.82  $\log_{10}$  CFU/carcass) during scalding and defeathering. A high between-study heterogeneity

 $(\tau^2 = 1.14)$  accounted for 99.49% of the variability. Egger's regression test (P = 0.26) and Begg's rank test (P < 0.01) further confirmed that publication bias was insignificant.

The pooled effects on the odds of *Salmonella* with the application of specific chemical additives during the broiler slaughter process are presented in Table 1. A net reduction of 0.98  $\log_{10}$  CFU/carcass in *Salmonella* concentration (95% CI: 0.80–1.17, P < 0.01) was observed from 87 trials. Between-study heterogeneity was high ( $\tau^2 = 0.83$ ), accounting for 97.29% variability. Publication bias was minimal, as illustrated by the symmetrical funnel plot and further confirmed by the insignificant Egger's regression test (P = 0.17) and Begg's rank correlation test (P = 0.96). Adjusting the pH from acidic to basic and *vice versa* of electrolyzed water, acetic acid and trisodium phosphate during processing using chlorine reduced the odds of *Salmonella* concentration by more than 2 log cycles, as previously

#### (a) Radial Plot



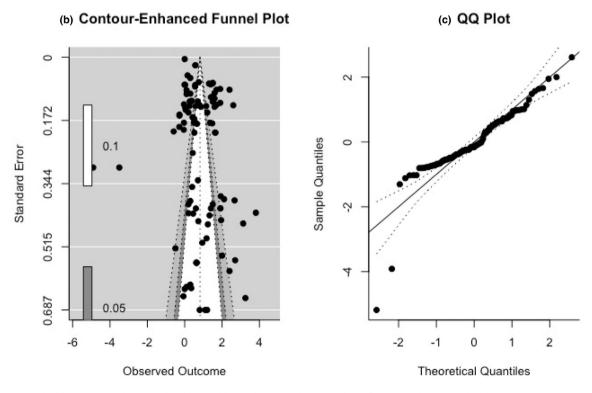


Figure 2 Graphical illustration of publication bias within studies reporting the effect of decontamination techniques on *Salmonella* concentration during broiler chicken primary processing a: Radial Plot; b: Contour-Enhanced Funnel Plot; c: Quantile-Quantile Plot.

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Figure 3 Forest plots to visualise the pooled effect sizes within the Salmonella concentration studies at different points along the chicken processing with corresponding statistical heterogeneity at each sampling point.

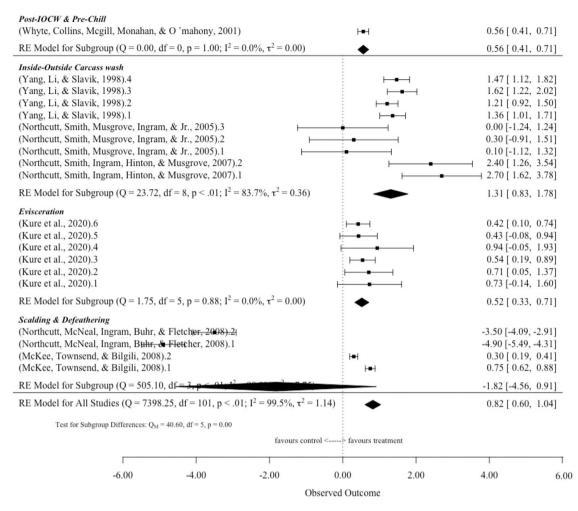


Figure 3 Continued.

reported (Bucher et al., 2012a). The effect sizes reported on reducing Salmonella concentration at different points along the slaughter process for chemical and physical approaches were within reported ranges (FAO and WHO, 2009). The results on Salmonella concentration were within similar to reported findings on trisodium phosphate (12%), acidified sodium chlorite (1200 ppm), peroxy acids (220–700 ppm); acidified sodium sulphate (pH 1.1), and cetylpyridinium chloride (4000 ppm) with differences arising from the concentration used and the sampled chicken parts (Li et al., 1997; Alonso-Hernando et al., 2012; Scott et al., 2015).

The pooled effects on the odds of *Salmonella* with the application of specific physical decontaminants along the broiler slaughter process are presented in Table 2. Data extracted from 15 trials on physical decontamination revealed an inconclusive pooled increase of  $0.13 \log_{10} \text{CFU/carcass}$  in *Salmonella* concentration (95% CI: -0.98 to 0.72, P = 0.77).

Between-study heterogeneity within these trials was high ( $\tau^2 = 1.66$ ), accounting for 99.94% of the variability. Publication bias was considerable, as observed from asymmetry within the funnel plot. However, Egger's regression test (P = 0.56) and Begg's rank correlation test (P = 0.15) gave conflicting estimates on publication bias. A shift from immersion to air chilling (0.57  $\log_{10}$  CFU/carcass; P < 0.01), hard scalding  $(0.52 \log_{10} \text{CFU/carcass}; P = 0.02)$  and steam pasteurisation (0.52  $\log_{10}$  CFU/carcass; P < 0.01) had the greatest reduction in the odds of Salmonella concentration. The pooled reduction in Salmonella caused by hot water and steam was lower than the reported average of 0.9-2.1 and 2.3-3.8 log units, respectively. This points to reduced efficacy of heat treatment in pilot plant set-up when compared to when it is applied in the laboratory. The pH of the processing water affects the heat resistance of microorganisms on poultry carcasses with reduction in Salmonella concentrations impaired at pH above nine or below three

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Table 1 The pooled effect of chemical decontamination interventions on Salmonella concentration along broiler chicken slaughter operations

Intervention	Concentration	n trials (studies)	Pooled effect (log <sub>10</sub> OR)	(95% CI) UCL; LCL	<i>P</i> - value	Heterogeneity $(\tau^2)$ ; variability $(f')$	Publication bias
Overall chemical	NA	87 (13)	0.98	0.80; 1.17	<0.01	0.83; 97.29%	InT
	Chill and Post chill	77 (9)	0.96	0.76; 1.16	< 0.01	0.84; 97.50%	InT
	IOCW	9 (3)	1.20	0.74; 1.67	< 0.01	0.41; 90.61%	InT
	Evisceration	1 (1)	0.30	0.19; 0.41	< 0.01	FE	InT
Electrolysed water (↑pH spray followed by ↓pH dip)	EO water spray (pH 11.6, -795 mV ORP), followed by immersion in EO water (pH 2.4 -2.7, 1150 mV ORP, 50 ppm free CL) at Chilling and Post chill	2 (1)	2.88	2.32; 3.44	<0.01	FE	InT
Acetic acid & NaCIO hurdle	2% Acetic acid spray, followed by 50 ppm NaCIO immersion during chilling	2 (1)	2.60	1.50; 3.71	<0.01	0.39; 61.19%	InT
TSP hurdles & NaClO hurdle	10% TSP spray, followed by 50 ppm NaCIO immersion post-chill	2 (1)	2.36	1.78; 2.93	<0.01	FE	InT
Binary Ionisation Technology	BIT spray (30 mL/min, 15 000 V), 36–60 s IOCW	3 (1)	1.93	1.39; 2.46	<0.01	FE	InT
TSP hurdles (ASC)	0.1% ASC dip followed by a 10.0% TSP dip, and <i>vice versa</i> post-chill	8 (1)	1.62	1.52; 1.73	<0.01	0.01; 61.77%	Egger's $P = 0.71$ Begg's $P = 0.40$
Cetylpyridinium chloride	0.5% Cetylpyridinium chloride (CPC) spray at IOCW	1 (1)	1.62	1.22; 2.02	<0.01	FE	InT
Sodium bisulphate & trisodium phosphate	5% SBS spray (17 s, 35 °C) IOCW	1 (1)	1.47	1.12; 1.82	<0.01	FE	InT
hurdle	10% TSP immersion and spray chill and post-chill	10 (4)	1.34	0.79; 1.90	<0.01	0.74; 98.02%	InT
Lactic acid	2% lactic acid IOCW spray, 17 s, temperature 35 °C	1 (1)	1.21	0.92; 1.50	<0.01	FE	InT
Acidified NaClO <sub>2</sub>	0.1% ASC- Acidified using citric acid post-chill	5 (2)	1.18	0.63; 1.72	<0.01	0.38; 97.28%	InT
Peracetic acid	0.0025–0.1% PA immersion chill and post chill	9 (3)	0.75	0.59; 0.91	<0.01	0.01; 15.43%	Egger's $P = 0.08$ Begg's $P = 0.7$
Acetic acid	2% Acetic Acid during chilling (immersion and spray)	4 (1)	0.74	-0.07; 1.55	0.07	0.56; 91.02%	InT
Electrolyzed water	pH 2.4–2.7, 1150–1180 mV ORP, 50 ppm free CL at IOCW, Chilling and Post-Chill	5 (2)	0.69	-0.46; 1.84	0.24	1.56; 94.84%	InT
Portable water	Sterile/distilled water spray and immersion chill and post-chill	9 (3)	0.47	0.09; 0.85	0.01	0.26; 91.96%	InT
Chlorine	40–50 ppm at IOCW, Chilling and Post-Chill	12 (3)	0.46	0.26; 0.66	<0.01	0.08; 84.01%	InT
Lysozyme	0.1–0.5% lysozyme post-chill	2 (1)	0.25	-0.30; 0.80	0.37	FE	InT
Monochloramine	50 ppm monochloramine chilling	1 (1)	0.02	-0.13; 0.17	0.79	FE	InT
Sodium hypochlorite	20–50 ppm NaCIO spray and immersion IOCW, Chill and Post-chill	6 (3)	-0.01	-0.13; 0.12	0.91	FE	InT
Ozonated water	10 mg/L ozonated water chilling and post-chill	4 (1)	-0.06	-0.29; 0.17	0.61	FE	InT

Heterogeneity is high, hence use of Random effect unless specified FE (Fixed Effect model).

Publication bias was tested using Egger's regression asymmetry test and Begg's (continuity corrected) adjusted rank correlation test. InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing. CI, confidence interval; LB, lower bound; UB, upper bound.

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Table 2 The pooled effect of physical decontamination interventions on Salmonella concentration along broiler chicken slaughter operations

Intervention	Concentration	n trials (studies)	Pooled effect (log <sub>10</sub> OR)	(95% CI) UCL; LCL	<i>P</i> ₋ value	Heterogeneity $(\tau^2)$ ; variability $(P)$	Publication bias ( <i>P</i> -value)
Overall physical	NA	15 (6)	-0.13	-0.98; 0.72	0.77	1.66; 99.94%	InT
	Chill and Post chill	5 (3)	0.32	0.06; 0.59	0.02	0.28; 99.38%	InT
	IOCW	1 (1)	0.30	0.19; 0.41	< 0.01	FE	InT
	Evisceration	9 (3)	-0.43	-1.85; 0.98	0.55	4.62; 99.12%	InT
Immersion → air chilling	Immersion 0.6 °C, 2 rpm, 50– 80 min → air velocity 3.5 m/s, temp 0 °C, RH 72%, 120–150 min	2 (2)	0.57	0.53; 0.61	<0.01	FE	InT
Hard scalding	Soft scald (pH of 11.0, 50 °C for 90 s) $\rightarrow$ Hard scald (pH of 11.0, 56.6 °C for 45 s)	2 (1)	0.52	0.08; 0.96	0.02	FE	InT
Steam pasteurisation	Steam at 95–120 °C, 3–5 s Post IOCW	6 (1)	0.52	0.33; 0.71	<0.01	FE	InT
Air chilling before typical immersion chill	0.5–1.1 °C immersion, 5 mg/kg free chlorine, time 80 min before immersion at air velocity 3.6 m/min, temperature 0 °C, RH 72%, time 120 min	2 (1)	0.28	-0.27; 0.84	0.32	0.16; 99.85%	InT
Visible faecal & ingesta removal	Washing off faecal material during immersion chilling	1 (1)	0.10	-0.10; 0.30	0.32	FE	InT
Forced Cloacal Faecal Expulsion	Washing → squeeze only pre-scald	2 (1)	-4.20	-4.62; -3.78	<0.01	FE	InT

Heterogeneity is high, hence use of Random effect unless specified FE (Fixed Effect model).

 $In T = insufficient \ number \ of \ trials \ to \ perform \ a \ publication \ bias \ test \ (<10 \ trials) \ or \ high \ heterogeneity \ precluded \ publication \ bias \ testing.$ 

CI, confidence interval; LB, lower bound; UB, upper bound.

Table 3 The pooled effect of decontamination interventions technique applied through immersion or spray on Salmonella concentration along broiler chicken slaughter operations

Mode of application	Processing step and type of decontaminant	n trials (studies)	Pooled effect (log <sub>10</sub> OR)	(95% CI) UCL; LCL	<i>P</i> ₋ value	Heterogeneity ( $\tau^2$ ); variability ( $\ell$ )	Publication bias ( <i>P</i> -value)
Immersion	Overall	60 (11)	0.90	0.72; 1.08	<0.01	0.44; 97.08%	InT
	Chill & Post-chill	57 (9)	0.92	0.73; 1.11	< 0.01	0.46; 96.85%	InT
	IOCW	1 (1)	0.56	0.41; 0.71	< 0.01	FE	InT
	Scald & evisceration	2 (1)	0.52	0.08; 0.96	0.08	0.05; 91.63%	InT
	Chemical	57 (9)	0.93	0.74; 1.12	< 0.01	0.45; 96.83	InT
	Physical	3 (2)	0.39	0.01; 0.76	0.04	0.10; 95.23%	InT
Spray	Overall	30 (6)	0.72	0.41; 1.03	< 0.01	0.85; 95.38%	InT
	Chill & Post-chill	15 (2)	0.48	0.02; 0.95	0.02	0.78; 95.80%	InT
	IOCW	9 (3)	1.31	0.83; 1.78	< 0.01	0.36; 83.70%	InT
	Scald & evisceration	6 (1)	0.52	0.33; 0.71	< 0.01	FE	InT
	Chemical	24 (5)	0.76	0.37; 1.16	< 0.01	0.85; 95.38	InT
	Physical	6 (1)	0.52	0.33; 0.71	<0.01	FE	InT

Heterogeneity is high, hence use of Random effect unless specified FE (Fixed Effect model).

InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing. CI, confidence interval; LB, lower bound; UB, upper bound.

during scalding (Buncic & Sofos, 2012). Physical decontamination techniques increased *Salmonella* prevalence and concentration due to potential cross-contamination within batches and re-contamination with gastral-intestinal content during slaughter when pressure was applied to the carcasses.

A comparison of the pooled effects sizes of *Salmonella* concentration with the application of decontaminants either through immersion or spraying at different processing steps during broiler slaughter is presented in Table 3. Application of decontaminants through immersion was superior (0.90  $\log_{10}$  CFU/carcass; P < 0.01) to

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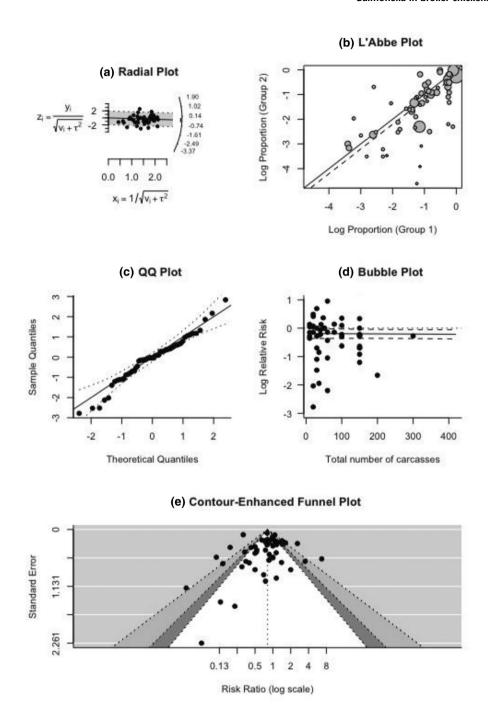


Figure 4 Graphical illustration of publication bias within studies reporting the effect of decontamination techniques on *Salmonella* prevalence during broiler chicken primary processing a: Radial Plot; b: L'abbe Plot; c: Quantile-Quantile Plot; d: Bubble Plot; e: Contour-Enhanced Funnel Plot.

spraying (0.72  $\log_{10}$  CFU/carcass; P < 0.01). Betweenstudy heterogeneity on immersion trials was lower ( $\tau^2 = 0.44$ ) than spraying trials ( $\tau^2 = 0.85$ ) which was attributed to majority of the trials on immersion being done during chilling and post-chill. Chemical additives were more effective when applied through immersion (0.93  $\log_{10}$  CFU/carcass; P < 0.01) when compared to spraying (0.76  $\log_{10}$  CFU/carcass; P < 0.01) which points to greater residual activity of chemical additives when applied through immersion. Immersion was most effective when used during chilling and post-chill while spraying was most effective during carcass wash.

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Figure 5 Forest plots to visualise the pooled effect sizes within the Salmonella prevalence studies at different points along the chicken processing with corresponding statistical heterogeneity at each sampling point.

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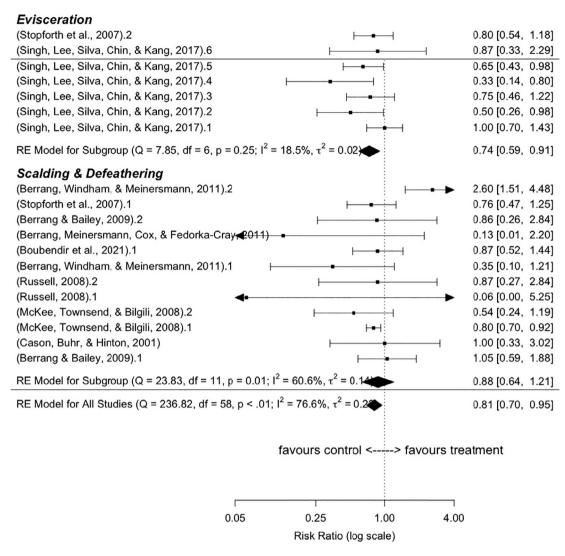


Figure 5 Continued.

# Meta-analysis on studies reporting Salmonella prevalence as an outcome

The heterogeneity within trials on the prevalence of *Salmonella* is graphically presented using A: Radial Plot; B: L'abbe Plot; C: Quantile-Quantile Plot; D: Bubble Plot; E: Contour-Enhanced Funnel Plot. There was minimal publication bias from the outliers observed in the radial and QQ plots. The L'abbe plot revealed that most of the trials in the control and the treatment groups reported similar precision, with the log risk in several treatment groups (Group 1) being lower than the risk in the control group (Group 2). This was further confirmed by the minimal bias observed due to the sample size in the bubble plot. The funnel plot was asymmetrical, revealing potential publication bias accounted for heterogeneity (Fig. 4).

The forest plot visualising the pooled effect on the prevalence of Salmonella at different points along the chicken slaughter process with statistical heterogeneity using Cochran's Q test,  $\tau^2$  and Higgins' and Thompson's  $I^2$  is shown in Fig. 5. A pooled reduction in the relative risk of 81.43% (95% CI: 69.63; 95.23, P < 0.01) of Salmonella prevalence was observed, with the greatest reduction reported during chilling (121.50%; P < 0.01) with the least during post-chill (43.10%; P < 0.01). Possible recontamination and cross-contamination during scalding, defeathering, evisceration, pre-chill and post-chill were evident from the pooled relative risk values as reported previously (Nde et al., 2007; Stopforth et al., 2007). There was moderate between-study heterogeneity ( $\tau^2 = 0.22$ ) which accounted for 76.60% of the variance, which was collaborated by significant Egger's regression test (P = 0.02) and Begg's rank test (P = 0.02).

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Table 4 The effects of chemical decontaminants on the relative risk of Salmonella in broiler chicken primary processing

Intervention	Concentration	n trials (studies)	Pooled effect (% RR)	(95% CI) UCL; LCL	<i>P</i> - value	Heterogeneity $(\tau^2)$ ; variability $(f^2)$	Publication bias ( <i>P</i> -value)
Chemical	Overall	30 (13)	64.74	48.77; 85.93	<0.01	0.39; 80.13%	InT
	Chill and Post chill	12 (4)	55.66	31.95; 96.99	0.04	0.68; 82.78%	InT
	IOCW	9 (5)	72.85	47.86; 110.91	0.14	0.26; 79.43%	Egger's $P = 0.54$ ; Begg's $P = 0.48$
	Scald, defeather and evisceration	9 (5)	66.70	37.87; 117.47	0.16	0.44; 73.78%	Egger's $P = 0.04$ ; Begg's $P = 0.61$
Chlorine + high pH	High chlorine dip (83.3 ppm) after high pH (9.89) scald	1 (1)	259.76	150.66; 447.85	<0.01	FE	InT
Sodium hydroxide	High pH 8.5 (using NaOH)	1 (1)	142.11	91.81; 219.94	0.11	FE	InT
TSP + HCI	pH 7.0 adjusted TSP dip using HCI post-IOCW	1 (1)	110.00	69.33; 174.52	0.69	FE	InT
Portable water	Two tap water dips each (25 °C, 45 s) post evisceration	1 (1)	100.00	69.92; 143.02	1.00	FE	InT
BIT	10 000 V sprayed for 4-12 s	3 (1)	85.04	66.84; 108.19	0.19	FE	InT
Chlorine	20, 50 & 500 ppm CI during chilling and post chill	7 (2)	84.00	58.00; 121.66	0.36	FE	InT
Chlorine dioxide	50 ppm of CIO <sub>2</sub> at defeathering	3 (2)	81.15	15.04; 437.72	0.81	1.56; 75.85	Egger's $P = 0.44$ ; Begg's $P = 1.00$
Trisodium phosphate	8–12% TSP (pH neutralised to 7 using HCI)	3 (3)	62.03	42.27; 91.04	0.01	0.01; 8.91%	Egger's $P = 0.18$ ; Begg's $P = 0.33$
Acidic copper sulphate	pH 2.0, 2.0 mg/L CuSO <sub>4</sub> , 2 min counter-current flow scalder	2 (1)	57.41	8.78; 375.39	0.56	0.72; 20.88%	InT
NaOH + high temperature	Hard scald (56.6 °C for 45 s) at high pH of 11.1 using NaOH	1 (1)	53.57	24.18; 18.70	0.12	FE	InT
Calcium hydroxide	Lime slurry Ca(OH) <sub>2</sub> pH (9.89) scalding	1 (1)	35.04	10.11; 121.44	0.10	FE	InT
TSP + high temperature	8% TSP dip at 25 °C, 45 s followed by hot water dip at 71 °C 45 s	1 (1)	33.37	13.90; 80.13	0.01	FE	InT
Cetylpyridinium chloride	Cetylpyridinium chloride	1 (1)	22.79	1.14; 454.34	0.33	FE	InT
Peracetic acid + hydrogen peroxide	85 ppm $CH_3CO_3H$ and $H_2O_2$ mixture chilling	1 (1)	19.01	9.50; 38.03	<0.01	FE	InT
Acidified sodium chlorite	500–1200 NaClO <sub>2</sub> , pH 2.5–2.6, acidified using citric acid, IOCW and Post-chill	3 (3)	15.43	5.05; 47.14	<0.01	0.65; 71.54%	InT

Heterogeneity is high, hence use of Random effect unless specified FE (Fixed Effect model).

Publication bias was tested using Egger's regression asymmetry test and Begg's (continuity corrected) adjusted rank correlation test.

InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing.

CI, confidence interval; LB, lower bound; UB, upper bound.

The pooled effect on the relative risk of *Salmonella* prevalence with specific chemical additives during the broiler slaughter process is presented in Table 4. A net reduction in the relative risk of 64.74% in *Salmonella* prevalence (95% CI: 48.77; 85.93, P < 0.01) was observed from 30 trials. Moderate between-study heterogeneity was observed ( $\tau^2 = 0.39$ ), accounting for 80.13% variability. Sub-group analysis revealed that chemical additives were most effective during IOCW, with a 72.85% pooled reduction in prevalence. Immersion of carcasses in chlorinated water (83.3 ppm) after a high pH (9.89) scald reduced the relative risk of *Salmonella* by 259.76%. Increasing the pH of processing

water to 8.5 using sodium hydroxide reduced the relative risk by (142.11%) while reducing the pH of trisodium phosphate solution with hydrochloric acid reduced the prevalence by (110.00%). Similar trends in prevalence have been reported for acidified NaClO<sub>2</sub> (750 ppm), 8–12% trisodium phosphate, peracetic acid (400–1000 ppm), cetylpyridinium chloride (0.35–0.60%) and chlorine (50 ppm) (Codex Alimentarius Commission, 2011; González et al., 2019). Using pH regulators such as sodium hydroxide or hydrochloric acid during scalding reduced the relative risk of Salmonella prevalence by more than 100% (McKee et al., 2008).

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Intervention	Concentration	n trials (studies)	Pooled effect (% RR)	(95% CI) UCL; LCL	<i>P-</i> value	Heterogeneity $(\tau^2)$ ; variability $(P)$	Publication bias ( <i>P</i> -value)
Overall physical	Overall	29 (14)	95.34	84.28; 107.86	0.45	0.03; 38.53	Egger's P = 0.79; Begg's P = 0.84
	Chilling and Post chill	11 (7)	109.27	73.57; 162.29	0.66	0.32; 84.52%	InT
	IOCW	8 (3)	105.53	85.11; 130.83	0.62	0.00; 0.00%	InT
	Scald & Evisceration	10 (7)	79.84	71.38; 89.32	< 0.01	FE	InT
Immersion → immersion-air combi chilling	Four immersion chill tanks temperature (8 °C for 20 s, 5 °C for 40 s, 5 °C for 80 s, and 2 °C for 80 s) followed by air chill at air velocity 3.6 m/min, 0 °C and RH 72%, for 120 min	1 (1)	163.33	97.71; 273.02	0.06	FE	InT
Immersion → air chilling	Comparison of immersion (with or without cetylpyridinium chloride) to air chilling (with or without chlorine or peracetic acid)	5 (4)	154.57	79.28; 301.40	0.20	0.41; 77.88%	Egger's P = 0.02; Begg's P = 0.48
High pressure	High-pressure spray during IOCW	3 (1)	116.23	89.53; 150.89	0.26	0.01; 9.28%	Egger's P = 0.21; Begg's P = 1.00
Visible faecal/ ingesta	Physical removal of faecal contamination during immersion chilling	2 (2)	109.29	78.62; 151.92	0.60	0.02; 25.41%	InT
Air → immersion- air combi chilling	Air velocity 3.6 m/min, temperature 0 °C, RH of 72%, chilling time 120 min	1 (1)	107.69	86.94; 133.40	0.50	FE	InT
Brushing + Hot- water carcass rinse	1st brushing (with tap water dip 25 °C, 45 s) the 2nd brush (with hot water, 71 °C, 45 s) with intermittent manual brushing (5 s on/5 s off). Post evisceration	1 (1)	86.96	32.96; 229.38	0.78	FE	InT
Additional washers	Additional washers, with water sprays, with 0–50 ppm Cl pre-scald, defeathering, IOCW	9 (2)	82.54	65.79; 103.57	0.10	FE	InT
High temperature	Tap Water Dip (25 °C, 45 s) followed by Hot Water Dip (71 °C, 45 s) Post evisceration High-temperature scald 57 °C for 45 s	4 (4)	80.47	70.59; 91.74	<0.01	FE	InT
Brushing	1st brush (with tap water dip (25 °C, 45 s)), then 2nd brush (with tap water dip (25 °C, 45 s)) with intermittent manual brushing (5 s on/5 s off). Post evisceration	1 (1)	64.94	43.19; 97.63	0.04	FE	InT
Dry ice	Dry ice blast/immersion (liquid CO <sub>2</sub> ) for 15 s, post chilling	2 (1)	28.80	10.23; 81.05	0.02	0.31; 51.27%	InT

Heterogeneity is high, hence use of Random effect unless specified FE (Fixed Effect model).

Publication bias was tested using Egger's regression asymmetry test and Begg's (continuity corrected) adjusted rank correlation test. InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing. CI, confidence interval; LB, lower bound; UB, upper bound.

The pooled effects on the relative risk of *Salmonella* prevalence with the application of specific physical decontaminants during the broiler slaughter process is presented in Table 5. Data extracted from twenty-nine trials on physical decontamination

revealed an inconclusive pooled decrease in relative risk of 95.34% in *Salmonella* prevalence (95% CI: 84.28; 107.86, P = 0.45). Between-study heterogeneity within these trials was low ( $\tau^2 = 0.03$ ), accounting for 38.53% of the variability. Physical decontaminants

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**Table 6** The pooled effect of decontamination interventions technique applied through immersion or spray on *Salmonella* prevalence along broiler chicken slaughter operations

Mode of application	Processing step and type of decontaminant	n trials (studies)	Pooled effect (RR)	(95% CI) UCL; LCL	<i>P</i> - value	Heterogeneity $(\tau^2)$ ; variability $(f^2)$	Publication bias ( <i>P</i> -value)
Immersion	Overall	25 (13)	75.67	56.78; 100.86	0.06	0.37; 84.57%	InT
	Chill & Post-chill	8 (6)	52.40	20.74; 132.38	0.17	1.50; 92.00%	InT
	IOCW	5 (2)	104.51	79.42; 137.52	0.75	0.03; 26.60%	Egger's $P = 0.87$ ; Begg's $P = 1.00$
	Scald & evisceration	12 (5)	78.40	56.43; 108.94	0.08	0.19; 73.77%	Egger's $P = 0.12$ ; Begg's $P = 0.31$
	Chemical	19 (9)	67.14	44.47; 101.36	0.06	0.61; 83.49%	InT
	Physical	9 (7)	82.49	73.15; 93.01	<0.01	0.00; 4.05%	Egger's $P = 0.94$ ; Begg's $P = 0.61$
Spray	Overall	22 (7)	85.23	74.52; 97.47	0.02	FE	InT
	Chill & Post-chill	8 (3)	76.69	61.60; 95.48	0.02	FE	InT
	IOCW	10 (4)	95.70	75.10; 121.97	0.72	0.02; 14.34%	Egger's $P = 0.04$ ; Begg's $P = 0.16$
	Scald & evisceration	4 (3)	77.49	57.63; 104.20	0.09	FE	InT
	Chemical	12 (5)	69.31	54.40; 88.30	<0.01	0.02; 11.56%	Egger's $P < 0.01$ ; Begg's $P = 0.03$
	Physical	11 (4)	91.40	74.91; 111.52	0.38	0.02; 15.27%	Egger's $P = 0.11$ ; Begg's $P = 0.76$

Heterogeneity is high; hence, the use of Random effect unless specified FE (Fixed Effect model).

Publication bias was tested using Egger's regression asymmetry test and Begg's (continuity corrected) adjusted rank correlation test. InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing. Cl, confidence interval; LB, lower bound; UB, upper bound.

were most effective at chilling and post-chill, with a pooled reduction in the prevalence of 109.27%. Publication bias was considerable, as observed from asymmetry within the funnel plot. However, Egger's regression test (P = 0.79) and Begg's rank correlation test (P = 0.84) gave conflicting estimates on publication bias. Additional air chill at air velocity 3.6 m/min, 0 °C and RH 72%, for 120 min after four immersion chill tanks temperature (8 °C for 20 s, 5 °C for 40 s, 5 °C for 80 s and 2 °C for 80 s) reduced the relative risk of Salmonella prevalence by 163.33% while air-chilling without prior immersion chilling reduced the prevalence by 154.57%. Increasing the spray pressure during carcass wash, wiping off visible ingesta/faecal matter, hot-water carcass rinse, brushing, additional washers, and extra rinsing tanks, and use of dry ice reduced the relative risk of Salmonella prevalence which resonates with previous studies (Wang et al., 1997; Buncic & Sofos, 2012; Zhang et al., 2013; Singh et al., 2017).

A comparison of the relative risks of *Salmonella* prevalence with the application of decontaminants, either through immersion or spraying at different steps during broiler slaughter, is presented in Table 6. Applying decontaminants through spray was superior (85.23%; P = 0.02) in reducing the relative risk of *Salmonella* to spraying (75.67%; P = 0.06). Between-study

heterogeneity within the spray-based trials was negligible, and a fixed effect model was used to estimate the effect size. Immersion-based trials had minimal variability ( $\tau^2 = 0.37$ ), which accounted for 84.57% of the variability.

#### Meta-regression

The potential modifier variables on the effect sizes reported in the Salmonella concentration and prevalence mixed effect meta-regression model have been presented in Table 7. The multivariable meta-regression model revealed that the effect sizes were confounded by six of the fourteen variables identified a priori. For Salmonella concentration trials, the odds reduction was significantly modified by the decontamination technique (0.27  $log_{10}$  CFU/carcass, 95% CI: 0.09; 0.44; P < 0.01) and the kind of sample analysed (0.15 log<sub>10</sub> CFU/carcass, 95% CI: 0.04; 0.26; P < 0.01). In addition, the inoculum type  $(-0.02 \log_{10} \text{CFU/carcass}, 95\% \text{CI: } -0.03; -0.01;$ P < 0.01) and the exposed part (-0.29 log<sub>10</sub> CFU/carcass, 95% CI: -0.49; -0.1; P < 0.01) significantly increased the odds for Salmonella concentration. The relative risks within the prevalence trials were significantly increased by the intercept of 0.03% (95% CI: 0.00; 0.39; P < 0.01) and microbial confirmation of 1.14% (95% CI: 1.02; 1.27; P = 0.02). High between-

 Table 7
 Potential effect modifiers and multivariable meta-regression model on trials on Salmonella concentration and prevalence reduction

	Salmonella c	concentration (log ode	d's ratio)		Salmonella prevalence (relative risk)				
Potential effect modifiers	n	Pooled effect (log <sub>10</sub> OR)	95% Cl lower; upper	<i>P</i> -value	n	Pooled effect (RR)	95% Cl lower; upper	<i>P</i> -value	
Intercept	102 (19)	1.75	-0.61; 4.11	0.15	59 (22)	0.03	0.00; 0.39	<0.01	
Sampling point									
Scald & pluck	4 (2)	-0.11	-0.31; 0.09	0.30	12 (8)	0.93	0.86; 1.01	0.08	
Evisceration	6 (1)				7 (2)				
IOCW	10 (4)				17 (7)				
Chilling	37 (8)				13 (8)				
Post-chill	45 (5)				10 (4)				
Intervention type									
Physical decontamination	15 (6)	0.01	-0.02; 0.04	0.37	29 (14)	1.01	0.98; 1.04	0.54	
Chemical decontamination	87 (13)				30 (13)				
Technique									
Immersion	60 (11)	0.27	0.09; 0.44	<0.01	27 (14)	0.94	0.86; 1.04	0.24	
Spray	30 (6)				22 (7)				
Immersion → air chilling	2 (2)				5 (4)				
Other techniques	10 (3)				5 (3)				
Exposure time									
<1 min	58 (11)	0.08	-0.03; 0.19	0.14	18 (9)	1.02	0.98; 1.06	0.31	
More than 1 min	12 (4)				17 (8)				
Not described	32 (5)				24 (8)				
Country where the study cond	lucted								
North America	74 (15)	-0.17	-0.53; 0.19	0.35	53 (19)	1.25	0.92; 1.70	0.15	
Europe	27 (3)				2 (1)				
Others	1 (1)				4 (2)				
Inoculum type									
Specific		-0.02	-0.03; -0.01	<0.01		1.01	1.00; 1.02	0.21	
Exposed part									
Whole carcass	66 (16)	-0.29	-0.49; -0.1	<0.01	55 (21)	1.40	0.92; 2.14	0.11	
Carcass parts	36 (4)				4 (2)				
Type of analysed sample									
Whole carcass rinse	51 (13)	0.15	0.04; 0.26	<0.01	44 (18)	1.02	0.88; 1.17	0.84	
Carcass parts swabs	4 (1)				8 (2)				
Carcass parts rinse	47 (7)				7 (3)				
Isolation media									
Specific	NA	0.09	0.00; 0.18	0.06	NA	1.00	0.95; 1.05	0.99	
Publication year									
1998–2003	35 (3)	-0.03	-0.15; 0.09	0.67	4 (4)	1.01	0.94; 1.08	0.73	
2004–2010	40 (11)				31 (9)				
2011–2016	17 (3)				13 (6)				
2017–2022	10 (2)				11 (3)				
Microbial confirmation									
Serology and morphology	34 (2)	-0.05	-0.35; 0.25	0.74	6 (2)	1.14	1.02; 1.27	0.02	
Biochemical & serology	6 (3)				16 (9)				
Biochemical	4 (2)				4 (2)				
Morphology only	48 (10)				8 (4)				
Other	10 (2)				25 (6)				
Sample size- treatment group									
<10	84 (12)	-0.02	-0.06; 0.03	0.44	11 (4)	1.40	0.55; 3.56	0.47	
11–30	13 (5)				20 (9)				
More than 100	5 (2)				28 (9)				
Sample size- control group									
<10	80 (11)	0.01	-0.03; 0.05	0.59	11 (4)	0.71	0.28; 1.80	0.47	
11–30	13 (5)				20 (9)				
More than 30	9 (3)				28 (9)				
Overall risk of bias									
NA	NA	-0.57	-1.35; 0.21	0.15	NA	1.23	0.70; 2.18	0.46	

Heterogeneity is high; hence, use of Random effect unless specified FE (Fixed Effect model).

Publication bias was tested using Egger's regression asymmetry test and Begg's (continuity corrected) adjusted rank correlation test. InT = insufficient number of trials to perform a publication bias test (<10 trials) or high heterogeneity precluded publication bias testing. CI, confidence interval; LB, lower bound; UB, upper bound.

study heterogeneity was observed within the trials on *Salmonella* concentration ( $\tau^2 = 0.80$ ), accounting for 98.33% of the variability. There was considerable publication bias from Egger's regression test (P = 0.01) and Begg's rank correlation test (P < 0.01). A moderate between-study heterogeneity ( $\tau^2 = 0.49$ ) accounting for 69.71% of the variability in the trials on *Salmonella* prevalence. Similarly, considerable publication bias was observed in the studies on *Salmonella* prevalence from the asymmetry in the funnel plot, which was confirmed by Egger's regression test (P = 0.12) and Begg's rank correlation test (P = 0.02).

The type of analysed sample and microbial enrichment has been reported to modify the prevalence of Salmonella in immersion-based and spray-based interventions (Bourassa et al., 2015). It is worth noting that chemical decontaminants may impart a carryover effect to the rinsate, and there is a lack of standard methods to assess this during the assessment of residual bactericidal activity (Gamble et al., 2017). For this reason, a mixed-model factoring in pre-enrichment was conducted, and the results obtained proved contrary to the a priori assumptions. The amount of rinsing water and neutralising buffered peptone water has been reported to aid recovery of sub-lethally injured Salmonella cells, which may impact the reported effects sizes (Bourassa et al., 2019). Pre-enrichment and sensitivity of selective media for isolating microorganisms may also have contributed to heterogeneity within the reported concentration and prevalence trials (Chon et al., 2012; Cox et al., 2020).

#### Conclusion

This systematic review presents pooled effect sizes in the concentration and prevalence of Salmonella with the application of microbial intervention strategies during the slaughter process of broiler chicken. The study revealed that using pH regulators in electrolysed water during chilling, spraying with either acetic acid or trisodium phosphate after immersion in NaClO during chilling reduced the concentration of Salmonella by more than two log cycles. Sodium hypochlorite was used to generate free chlorine, increasing Salmonella decontamination of organic acids such as acetic acid, citric acid and lactic acid or inorganic oxidising agents such as trisodium phosphate, hydrogen peroxide and peroxy acids. Immersion of chicken carcasses in 83 ppm chlorine after a high pH scald, and use of sodium hydroxide, adjusting the pH of trisodium phosphate solution using hydrogen chloride and additional immersion treatment using portable water reduced the relative risk of Salmonella prevalence by more than 100%. Applying chemical additives through immersion was superior in reduction of concentration but not in prevalence compared to spraying.

Combinations of immersion and spraying with physical or chemical additives increased the rate of decline in prevalence and concentration. Heterogeneity was high within trials on physical methods; therefore, the pooled effect sizes were inconclusive due to variations in processing aids and possible cross-contamination between carcasses and contamination from faecal and ingesta.

Publication bias within the studies was minimal, with between-study variability accounting for over 80% of the variability in most sub-groups. Inadequate allocation sequence generation and lack of concealment and blinding raised concerns when collating and comparing different studies. This raises the need to consider confounding variables in the standardisation of internationally recognised standard protocols when evaluating Salmonella decontamination by factoring (i) decontamination technique, (ii) exposed part, (iii) type of analysed sample and (iv) microbial confirmation. This study provides a basis to build on further discussion for future developments and applications in microbial decontamination along primary broiler processing. It sheds light on the extent of Salmonella decontamination during primary processing and estimates poultry safety using pooled effect size.

#### **Conflict of interest**

None.

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#### **Author contributions**

Josphat Njenga Gichure: Data curation (lead); formal analysis (lead); visualization (supporting); writing – original draft (lead). Ranil Coorey: Conceptualization (equal); funding acquisition (lead); project administration (lead); resources (lead); supervision (equal); writing - review and editing (equal). Patrick Murigu Kamau Njage: Funding acquisition (supporting); writing - review and editing (supporting). Joseph M. Wambui: Formal analysis (supporting); writing – review and editing (supporting). Gary A. Dykes: Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); visualization (equal); writing – review and editing (equal). Elna M. Buys: Conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); visualization (equal); writing – review and editing (equal).

#### **Ethical statement**

Ethics approval was not required for this research.

#### Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peerreview/10.1111/ijfs.16759.

#### **Data availability statement**

Data available in article supplementary material.

#### References

- Akil, L. & Ahmad, H.A. (2019). Quantitative risk assessment model of human salmonellosis resulting from consumption of broiler chicken. *Diseases*, 7, 19.
- Alonso-Hernando, A., Capita, R. & Alonso-Calleja, C. (2012). Behaviour of co-inoculated pathogenic and spoilage bacteria on poultry following several decontamination treatments. *International Journal of Food Microbiology*, **159**, 152–159.
- This articles evaluated the effects of immersion for 15 min in 12% trisodium phosphate, 1200 ppm acidified sodium chlorite, 2% citric acid, 220 ppm peroxyacids and 50 ppm chlorine dioxide. The authors provide practical examples on several chemical decontamination interventions, making this article a useful guide to understand the ranges of decontamination.
- Als, D., Radhakrishnan, A., Arora, P. et al. (2018). Global trends in typhoidal salmonellosis: a systematic review. American Journal of Tropical Medicine and Hygiene, 99, 10–19.
- Ao, T.T., Feasey, N.A., Gordon, M.A., Keddy, K.H., Angulo, F.J. & Crump, J.A. (2015). Global burden of invasive nontyphoidal Salmonella disease, 2010. Emerging Infectious Diseases, 21, 941–949.
- Bourassa, D.V., Holmes, J.M., Cason, J.A., Cox, N.A., Rigsby, L.L. & Buhr, R.J. (2015). Prevalence and serogroup diversity of *Salmonella* for broiler neck skin, whole carcass rinse, and whole carcass enrichment sampling methodologies following air or immersion chilling. *Journal of Food Protection*, **78**, 1938–1944.
- This article evaluated the extent of decontamination when different parts of the carcass are sampled. In addition, the article evaluates differences if decontaminant is added either during air or immersion chilling. The authors provide practical examples on the effects of sampling and decontamination application techniques, making this article a useful guide to understand potential modifier variables.
- Bourassa, D.V., Lapidus, J.L., Kennedy-Smith, A.E. & Morey, A. (2019). Efficacy of neutralizing buffered peptone water for recovery of *Salmonella*, Campylobacter, and Enterobacteriaceae from broiler carcasses at various points along a commercial immersion chilling process with peroxyacetic acid. *Poultry Science*, **98**, 393–397.
- Brewer, M.J., Butler, A. & Cooksley, S.L. (2016). The relative performance of AIC, AICC and BIC in the presence of unobserved heterogeneity. *Methods in Ecology and Evolution*, **7**, 679–692.
- This article reviewed literature on model selection, particularly the use of the Akaike Information Criterion (AIC). It provides a framework for study model selection to address heterogeneity. The research output from this study builds on our methodology, making it a valuable resource for understanding heterogeneity.
- Brockwell, S.E. & Gordon, I.R. (2001). A comparison of statistical methods for meta-analysis. Statistics in Medicine, 20, 825–840.
- Bucher, O., Farrar, A.M., Totton, S.C. et al. (2012a). A systematic review-meta-analysis of chilling interventions and a meta-regression

- of various processing interventions for Salmonella contamination of chicken. Preventive Veterinary Medicine, 103, 1–15.
- This study evaluated the efficacy of chilling on reducing *Salmonella* prevalence or concentration on broiler carcasses using systematic review-meta-analysis. The research further explored sources of heterogeneity among the identified studies using a meta-regression. The research applies meta-analysis and meta-regression to predict changes in contamination during the chilling process.
- Bucher, O., Rajić, A., Waddell, L.A., Greig, J. & McEwen, S.A. (2012b). Do any spray or dip treatments, applied on broiler chicken carcasses or carcass parts, reduce *Salmonella* spp. prevalence and/or concentration during primary processing? A systematic review-meta-analysis. *Food Control*, 27, 351–361.
- Buncic, S. & Sofos, J. (2012). Interventions to control Salmonella contamination during poultry, cattle and pig slaughter. Food Research International, 45, 641–655.
- This article reviewed different treatments applied on poultry carcasses or parts including water, steam and chemical solutions (e.g. lactic or acetic acid, chlorine-based compounds, cetylpyridinium chloride and trisodium phosphate). The article also expounds on the effect of organic material on the extent of decontamination. This makes the article a useful guide for understanding the effect of decontamination interventions on *Salmonella*.
- Chon, J.W., Hyeon, J.Y., Park, J.H., Song, K.Y. & Seo, K.H. (2012). Comparison of 2 types of broths and 3 selective agars for the detection of *Campylobacter* species in whole-chicken carcassrinse samples. *Poultry Science*, **91**, 2382–2385.
- Codex Alimentarius Commission. (2011). Guidelines for the control of *Campylobacter* and *Salmonella* in chicken. CAC/GL 78-2011, assessment.
- Cox, N.A., Berrang, M.E., House, S.L., Hinton, A., Jr., Eric Line, J. & Wiggins, L.T. (2020). Detection of multiple naturally occurring *Salmonella* serotypes from commercial broiler carcasses with conventional methods. *Journal of Food Safety*, 40, 1–4.
- Deeks, J., Higgins, J.P. & Altman, D.G. (2008). Analysing data and undertaking meta-analyses. In: *Cochrane Handbook for Systematic Reviews of Interventions: cochrane Book Series* (edited by J.P.T. Higgins & S. Green). Pp. 243–296. Oxford, UK: Wiley Blackwell.
- DerSimonian, R. & Kacker, R. (2007). Random-effects model for meta-analysis of clinical trials: an update. *Contemporary Clinical Trials*, 28, 105–114.
- Duarte, A.S.R., Nauta, M.J. & Aabo, S. (2016). Variation in the effect of carcass decontamination impacts the risk for consumers. *Food Control*, **59**, 12–19.
- EFSA. (2010). Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal*, **8**, 1637. https://doi.org/10.2903/j.efsa.2010.1637
- FAO and WHO. (2009). Salmonella and Campylobacter in Chicken Meat, Microbiological Risk Assessment Series 19. Rome, Italy: FAO.
- Ferrari, R.G., Rosario, D.K.A., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E.A. & Conte-Junior, C.A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. *Applied and Environmental Microbiology*, **85**, 1–21.
- Gamble, G.R., Berrang, M.E., Buhr, R.J. *et al.* (2017). Neutralization of bactericidal activity related to antimicrobial carryover in broiler carcass rinse samples. *Journal of Food Protection*, **80**, 685–691.
- Gichure, J.N., Kamau Njage, P.M., Wambui, J.M., Dykes, G.A., Buys, E.M. & Coorey, R. (2022). Systematic-review and meta-analysis on effect of decontamination interventions on prevalence and concentration of *Campylobacter* spp. during primary processing of broiler chickens. *Food Microbiology*, **102**, 103923.
- Golden, C.E. & Mishra, A. (2020). Prevalence of *Salmonella* and *Campylobacter* spp. in alternative and conventionally produced chicken in the United States: a systematic review and meta-analysis. *Journal of Food Protection*, **83**, 1181–1197.
- González, R.J., Sampedro, F., Feirtag, J.M., Sánchez-Plata, M.X. & Hedberg, C.W. (2019). Prioritization of chicken meat processing

- interventions on the basis of reducing the *Salmonella* residual relative risk. *Journal of Food Protection*, **82**, 1575–1582.
- Gruzdev, N., Pinto, R. & Sela, S. (2011). Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Applied and Environmental Microbiology*, 77, 1667–1673.
- Hardie, K.M., Guerin, M.T., Ellis, A. & Leclair, D. (2019). Associations of processing level variables with *Salmonella* prevalence and concentration on broiler chicken carcasses and parts in Canada. *Preventive Veterinary Medicine*, **168**, 39–51.
- Higgins, J.P., Li, T. & Deeks, J.J. (2019). Choosing effect measures and computing estimates of effect. In: *Cochrane Handbook for Systematic Reviews of Interventions* (edited by J.P.T. Higgins, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page & V.A. Welch). Pp. 143–176. Oxford, UK: John Wiley & Sons Ltd.
- Higgins, J.P.T. & Thompson, S.G. (2004). Controlling the risk of spurious findings from meta-regression. *Statistics in Medicine*, **23**, 1663–1682.
- Jain, S., Sharma, S.K. & Jain, K. (2019). Meta-analysis of fixed, random and mixed effects models. *International Journal of Mathematical, Engineering and Management Sciences*, 4, 199–218.
- Kahan, B.C., Rehal, S. & Cro, S. (2015). Risk of selection bias in randomised trials. *Trials*, 16, 1–7.
- Kerr, A.K., Farrar, A.M., Waddell, L.A. et al. (2013). A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on Salmonella spp. prevalence and concentration in broiler chickens. Preventive Veterinary Medicine, 111, 112–125.
- Lau, J., Ioannidis, J.P., Terrin, N., Schmid, C.H. & Olkin, I. (2006). The case of the misleading funnel plot. *British Medical Journal*, 333, 597–600.
- Li, Y., Slavik, M.F., Walker, J.T. & Xiong, H. (1997). Pre-chill spray of chicken carcasses to reduce *Salmonella* typhimurium. *Journal of Food Science*, **62**, 605–607.
- Liberati, A., Altman, D.G., Tetzlaff, J. et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology*, **62**, e1–e34.
- Lin, C.H., Adams, P.J., Huang, J.F., Sun, Y.F., Lin, J.H. & Robertson, I.D. (2021). Prevalence and risk factors for *Salmonella* spp. contamination of slaughtered chickens in Taiwan. *Preventive Veterinary Medicine*, **196**, 105476.
- Macaskill, P., Walter, S.D. & Irwig, L. (2001). A comparison of methods to detect publication bias for meta-analysis of continuous data. *Statistics in Medicine*, **20**, 641–654.
- McKee, S.R., Townsend, J.C. & Bilgili, S.F. (2008). Use of a scald additive to reduce levels of *Salmonella* typhimurium during poultry processing. *Poultry Science*, **87**, 1672–1677.
- Moher, D., Shamseer, L., Clarke, M. et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews, 4, 1–9.
- Nde, C.W., McEvoy, J.M., Sherwood, J.S. & Logue, C.M. (2007). Cross contamination of Turkey carcasses by *Salmonella* species during defeathering. *Poultry Science*, 86, 162–167.
- Paez, A. (2017). Gray literature: an important resource in systematic reviews. *Journal of Evidence-Based Medicine*, **10**, 233–240.
- Rajan, K., Shi, Z. & Ricke, S.C. (2017). Current aspects of *Salmonella* contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. *Critical Reviews in Microbiology*, **43**, 370–392.
- Richards, D. (2008). Handsearching still a valuable element of the systematic review: commentary. *Evidence-Based Dentistry*, **9**, 85.
- Rothstein, H.R., Sutton, A.J. & Borenstein, M. (2005). Publication bias in meta-analysis: prevention, assessment and adjustments. Available at: https://doi.org/10.1002/0470870168
- Schünemann, H.J., Vist, G.E., Higgins, J.P.T. et al. (2011). Interpreting results and drawing conclusions. In: Cochrane Handbook for

- Systematic Reviews of Interventions (edited by J.P.T. Higgins, J. Thomas, J. Chandler, M. Cumpston, T. Li, M.J. Page & V.A. Welch). Pp. 359–387. Oxford, UK: Wiley Blackwell.
- Schwarzer, G., Carpenter, J.R. & Rucker, G. (2015). *UseRMetaAnalysis*. Edited by R. Gentleman, K. Hornik, and G. Parmigiani. Oxford, UK: Springer International Publishing. https://doi.org/10.1007/978-3-319-21416-0
- Scott, B.R., Yang, X., Geornaras, I. et al. (2015). Antimicrobial efficacy of a sulfuric acid and sodium sulfate blend, peroxyacetic acid, and cetylpyridinium chloride against Salmonella on inoculated chicken wings. Journal of Food Protection, 78, 1967–1972.
- Singh, P., Lee, H.C., Silva, M.F., Chin, K.B. & Kang, I. (2017). Trisodium phosphate dip, hot water dip, and combination dip with/without brushing on broiler carcass decontamination. *Food Control*, 77, 199–209.
- Stanaway, J.D., Parisi, A., Sarkar, K. *et al.* (2019). The global burden of non-typhoidal *Salmonella* invasive disease: a systematic analysis for the global burden of disease study 2017. *The Lancet Infectious Diseases*, **19**, 1312–1324.
- Sterne, J.A.C., Becker, B.J. & Egger, M. (2005). Statistical methods for assessing publication bias the funnel plot. In: *Publication Bias in Meta-Analysis: prevention, Assessment and Adjustments* (edited by H.R. Rothstein, A.J. Sutton & M. Borenstein). Pp. 75–98. West Sussex, England: John Wiley& Sons.
- Stopforth, J.D., O'Connor, R., Lopes, M., Kottapalli, B., Hill, W.E. & Samadpour, M. (2007). Validation of individual and multiple-sequential interventions for reduction of microbial populations during processing of poultry carcasses and parts. *Journal of Food Protection*, **70**, 1393–1401.
- Sutton, A.J. & Higgins, J.P.T. (2008). Recent developments in metaanalysis. Statistics in Medicine, 27, 625–650.
- Veroniki, A.A., Jackson, D., Viechtbauer, W. et al. (2016). Methods to estimate the between-study variance and its uncertainty in meta-analysis. Research Synthesis Methods, 7, 55–79.
- Viechtbauer, W. (2007). Bias and efficiency of meta-analytic variance estimators in the random-effects model. *Journal of Educational and Behavioral Statistics*, **30**, 261–293.
- Viechtbauer, W. (2015). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1–48.
- Wang, W.C., Li, Y., Slavik, M.F. & Xiong, H. (1997). Trisodium phosphate and cetylpyridinium chloride spraying on chicken skin to reduce attached *Salmonella* typhimurium. *Journal of Food Protection*, **60**, 992–994.
- Wattiau, P., Boland, C. & Bertrand, S. (2011). Methodologies for Salmonella enterica subsp. enterica subtyping: gold standards and alternatives. Applied and Environmental Microbiology, 77, 7877– 7885
- Zhang, L., Singh, P., Lee, H.C. & Kang, I. (2013). Effect of hot water spray on broiler carcasses for reduction of loosely attached, intermediately attached, and tightly attached pathogenic (*Salmonella* and *Campylobacter*) and mesophilic aerobic bacteria. *Poultry Science*, 92, 804–810.

#### **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Supplementary data on the systematic review on decontamination interventions on the prevalence and concentration of Salmonella in broiler chickens during primary processing.