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Original article

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 $(1.31 \log_{10} CFU/carcass)$ reduction in odds; $P \le 0.01$ and chilling (121.50% reduction in relative risk; $P \le 0.01$) had significant reduction on Salmonella concentration and prevalence, respectively. Chemical additives reduced the concentration $(0.98 \log_{10} CFU/carcass; P < 0.01)$ 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 $(0.90 \log_{10}$ CFU/carcass; $P \le 0.01$) to spraying $(0.72 \log_{10} CFU/carcass; P \le 0.01)$. 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 typhoid fever being a symptom in major salmonellosis (Wattiau et al., 2011). Epidemiological investigations attribute the handling and consumption of contaminated 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 & Ahmad, 2019).

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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

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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 Mendeley 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, τ^2 , and Higgins' and Thompson's \tilde{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, τ^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, τ^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 $(1.82 \log_{10} \text{CFU/carcass})$ 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 (τ^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

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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.

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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} CFU/carcass in Salmonella concentration (95% CI: -0.98 to 0.72, $P = 0.77$). Between-study heterogeneity within these trials was high (τ^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 log₁₀ CFU/carcass; $P < 0.01$), hard scalding $(0.52 \text{ log}_{10} CFU/carcass; P = 0.02)$ 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

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

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

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.

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

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 crosscontamination 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

(b) L'Abbe Plot

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 $(0.76 \text{ log}_{10} \text{CFU/carcass}; P < 0.01)$ 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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Relative Risk

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, τ^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 $(121.50\%; P \le 0.01)$ with the least during post-chill $(43.10\%; P \le 0.01)$. 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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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 $(95\% \text{ CI: } 48.77; 85.93, P < 0.01)$ 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 $NaClO₂$ (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).

Table 5 The effects of specific physical decontamination techniques on the relative risk of Salmonella prevalence along broiler chicken primary processing

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 E De State in the international was complexed in the state of the complexed in the conditional conditional marked was complexed was complexed was complexed was the complexed was to a most of a most of a most of the complex

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

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.

CI, 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 Sal*monella* 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 $(0.15 \log_{10} CFU/carcass)$, 95% CI: 0.04; 0.26; $P < 0.01$). In addition, the inoculum type $(-0.02 \log_{10} CFU/carcass, 95\% CI: -0.03; -0.01;$ $P < 0.01$) and the exposed part (-0.29 log₁₀ 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

Table 7 Potential effect modifiers and multivariable meta-regression model on trials on Salmonella concentration and prevalence								
reduction	Salmonella concentration (log odd's ratio)				Salmonella prevalence (relative risk)			
Potential effect modifiers	n	Pooled effect $(log_{10} OR)$	95% CI lower; upper	P-value	n	Pooled effect (RR)	95% CI lower; upper	P-value
Intercept Sampling point	102 (19)	1.75	$-0.61; 4.11$	0.15	59 (22)	0.03	0.00; 0.39	< 0.01
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)			
Techniaue								
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 \rightarrow 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 conducted								
North America	74 (15) 27(3)	-0.17	$-0.53; 0.19$	0.35	53 (19) 2(1)	1.25	0.92; 1.70	0.15
Europe 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								
ΝA	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.

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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).

Ethics approval was not required for this research.

Peer review

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Data availability statement

Data available in article supplementary material.

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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.

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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.

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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.