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A Rapid Systematic Review and Meta-Analysis of the Efficacy of Slaughter and Processing Interventions to Control Non-Typhoidal *Salmonella* in Beef and Pork

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Abstract

Pork is one of the major food sources of human salmonellosis worldwide, while beef products have been implicated in numerous foodborne outbreaks. As a result, effective interventions to reduce *Salmonella* contamination during beef and pork processing are of interest to both regulators and industry. We conducted a rapid systematic review and meta-analysis of literature investigating the efficacy of slaughter and processing interventions to control *Salmonella* in beef and pork. Review steps included: a comprehensive search strategy; relevance screening of abstracts; relevance confirmation of articles; data extraction; risk-of-bias assessment; meta-analysis (where appropriate); and a weight-of-evidence assessment. A total of 191 relevant experimental studies were identified. Two controlled trials indicated that hot water and steam treatments are effective at reducing the prevalence of *Salmonella* on beef carcasses (relative risk [RR] = 0.11, 95% confidence interval [CI]: 0.02, 0.58), while four trials found that pre-chill organic acid washes are effective at reducing *Salmonella* on pork carcasses (RR = 0.32, 95% CI: 0.13, 0.78), with high confidence in the estimates of effect. Four quasi-experimental studies found that post-exsanguination chemical washes were effective to reduce the prevalence of *Salmonella* on cattle hides, with low confidence in the specific estimate of effect; moderate confidence was found for the effect estimates of scalding (RR = 0.20, 95% CI: 0.14, 0.29) and singeing (RR = 0.34, 95% CI: 0.22, 0.52) of pork carcasses. The overall evidence supported enhanced reductions of *Salmonella* through a multiple-hurdle approach. In conclusion, various slaughter and processing interventions can contribute to reducing *Salmonella* on beef and pork carcasses, depending on the context of application; an appropriate combination should be selected, validated, and verified by establishment operators within their local conditions.

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Keywords

knowledge synthesis; review; salmonellae; meat; interventions

Non-typhoidal *Salmonella* spp. (hereafter referred to as *Salmonella*) are one of the most common causes of gastroenteritis worldwide, resulting in an estimated 155,000 deaths and 80 million cases of foodborne illness annually (64). Moreover, non-typhoidal salmonellosis can lead to possible chronic sequelae such as reactive arthritis and irritable bowel syndrome (58). As a result, the disease has a substantial economic burden on society due to a number of direct (e.g. healthcare) and indirect (e.g. lost productivity) costs (55, 67). *Salmonella* can also have negative impacts on the agri-food industry and trade due to contamination events, associated food recalls, and loss of market access (30).

Along with poultry and eggs, pork products are frequently identified globally as one of the most common sources of non-typhoidal salmonellosis in humans (77). While beef products are less often associated with the burden of non-typhoidal salmonellosis, they have been implicated in several large outbreaks of *Salmonella* infections in the USA during the past 15 years, sometimes due to multi-drug resistant strains (61). In addition, recent outbreaks of non-typhoidal salmonellosis have occurred in several European countries due to the consumption of various beef products contaminated with multi-drug resistant strains (42, 70, 79).

Both pigs and cattle, along with other food-producing animals, are known reservoirs for *Salmonella*, which they frequently shed as asymptomatic carriers (18). *Salmonella* can spread between animals on the farm, during transport, and in lairage environments, contributing to the incoming level of contamination on animals at slaughter (4, 6, 18, 100). *Salmonella* contamination of pork and beef hides, internal organs, and viscera, as well as the abattoir environment and workers, can lead to cross-contamination of carcasses during slaughter and processing (5, 18, 32, 65, 82, 100). Processing establishments worldwide typically implement a variety and combination of decontamination interventions, along with good hygiene practices (GHP) and hazard analysis critical control point (HACCP) principles, to reduce the occurrence and levels of *Salmonella* and other microbial pathogens on beef and pork carcasses and meat products. The specific interventions used in the industry varies by country and setting according to the options approved for use in that region, in-plant efficacy, incoming pathogen load, and other factors such as installation and operation costs, feasibility of implementation, environmental impacts, occupational health and safety, and consumer acceptability and preferences (1, 4, 9, 100).

In 2011, the Codex Alimentarius Commission published international guidelines on the control of *Salmonella* and *Campylobacter* in chicken meat (21). In November, 2013, the Codex Committee on Food Hygiene (CCFH) agreed to develop similar guidelines for control of *Salmonella* in fresh beef and pork due to a recognized need for global guidance in this area (22). The CCFH later requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) Joint Expert Meeting on Microbiological Risk Assessment (JEMRA) to provide scientific advice to inform the new guidelines by conducting a systematic literature review and convening an expert meeting.

This article reports on the process and results from the systematic review of the global literature on interventions to control *Salmonella* in beef and pork during slaughter and processing. This review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (71).

Materials and Methods

Review approach and team

The review was conducted following recommended procedures for systematic reviews as outlined in a pre-specified protocol (52, 80), with some steps streamlined using a rapid approach given the broad review scope and limited timeframe for completion (protocol available from corresponding author upon request). The following specific steps were modified under the rapid approach: the search was targeted; detailed analyses were prioritized; and only one reviewer conducted the relevance confirmation and data extraction steps.

Review question and eligibility criteria

The initial review question was: “What is the efficacy of all possible interventions to control *Salmonella* in fresh pork and beef, from primary production to consumption?” Any interventions applied throughout the food chain continuum were initially considered relevant; however, post-processing interventions were later excluded from the scope as the search was not optimized to appropriately capture literature on interventions at this stage. This article only reports on relevant articles that investigated slaughter and processing interventions; studies investigating pre-harvest interventions will be reported in a separated publication. For a summary of all results, readers can refer to the full technical report (39).

The population of interest included all pigs and cattle produced for domestic meat consumption, including their carcasses and trimmings. Any outcome measure was considered relevant (including environmental samples). Manufactured (e.g. cured, dried, and fermented) products such as sausages and salamis were excluded. All available evidence published in English, French, or Spanish was considered for inclusion, including previously published systematic reviews, risk assessments and stochastic models, and primary research of any study design, as long as there was a measure of intervention efficacy reported in relation to a comparison group.

Search strategy

Comprehensive search algorithms were developed for beef and pork by extracting key words from a selection of known relevant articles, and by reviewing search strategies of previously published reviews and risk assessments. Key terms were combined using the Boolean operator OR into categories for Pathogen (*Salmonella* terms), Population (beef and pork terms), and Intervention (intervention terms), and the categories were combined using the AND operator. Algorithms were pre-tested in Scopus and final searches were implemented in the bibliographic databases Scopus and CAB Abstracts on February 11, 2015. For the beef search, no publication year or other restrictions were imposed. For the pork search, only literature from 2009-2015 was searched, as a citation list of potentially relevant articles

covering any intervention against *Salmonella* in the pork chain was available from a previously conducted scoping review that included a broad search of literature published up to 2009 (102). Search verification included: reviewing relevant conference proceedings; conducting targeted searches in Google to identify grey literature (e.g. government and industry reports); and reviewing the reference lists of a sample of relevant articles. In addition, any documents received from a complementary FAO/WHO public “call for data” were reviewed for potential inclusion.

Relevance screening, confirmation, and prioritization

Each reference was assessed for relevance to the review scope at the title and abstract level using an *a priori* developed form. After this stage, we decided to exclude all research published prior to 1990, as evidence on interventions published prior to this period was not considered reflective of current industry conditions and practices. Relevant references were procured as full articles and confirmed for relevance using another pre-specified form. This form was used to characterize articles according to the document type, study design, and commodity (beef or pork), point in chain, and intervention categories investigated. Results from this stage were used to prioritize more detailed data synthesis according to the availability and applicability of evidence. All experimental study designs (controlled trials, challenge trials, and quasi-experimental studies) were considered for detailed data extraction and risk-of-bias assessment.

Data extraction and risk-of-bias assessment

These steps were conducted using pre-specified tools. The data extraction tool included targeted questions about intervention and population descriptions, outcomes measured, and intervention efficacy results. The risk-of-bias tool was adapted from the Cochrane Collaboration’s recommended tools for randomized and non-randomized study designs (20, 52).

Review management

Identified references were uploaded to RefWorks (Thomson ResearchSoft, Philadelphia, PA), de-duplicated, and imported into the systematic review software DistillerSR (Evidence Partners, Ottawa, ON) for relevance screening, relevance confirmation, and risk-of-bias assessment. Data extraction was conducted in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). Review forms were pre-tested before use on a selection of relevant references and articles (30 for relevance screening and confirmation, and five for data extraction and risk-of-bias assessment). Reviewing for relevance screening proceeded only when consistent agreement was achieved between reviewers ($\kappa > 0.8$). For other steps, pre-tests were used to ensure consistent interpretation and clarity of the forms. Two independent reviewers (I.Y. and B.W.) conducted relevance screening and risk-of-bias assessment, while only one conducted relevance confirmation and data extraction. Any disagreements between reviewers were resolved through discussion. A copy of all forms used in the review and more details on the search strategy (e.g. search algorithms) are available in the full technical report (39).

Data analysis

For the purposes of this article, we focused quantitative analysis on studies conducted under commercial conditions (i.e. controlled trials and quasi-experiments). Challenge trials are summarized narratively only; quantitative log reduction summaries of these studies are available in the technical report (39). Results were stratified into comparable subgroups for meta-analysis (14, 52). Data were first stratified by point in the processing chain, then study design, then into specific intervention categories (Table 1) that were created based on insights from previous reviews (9, 100), and finally by different outcome measures (e.g. sample types). Random-effects meta-analysis was conducted in each subgroup when sufficiently reported data were available from ≥ 2 trials. The unit of analysis in all cases was individual trials reported within studies. All studies reported prevalence data, which were summarized using the relative risk (RR) measure (14, 52). Meta-analysis models were calculated using the restricted maximum-likelihood method in R Version 3.1.3 (78, 99).

RR estimates were used to determine the effect of each intervention on changing a given baseline prevalence of *Salmonella* as a more intuitive measure of intervention efficacy (52). Within each intervention subgroup, we used the median prevalence of *Salmonella* in the comparison groups of included studies as a baseline measure of prevalence. This value was multiplied by the respective RR measure to obtain the corresponding change in prevalence of *Salmonella* due to the intervention (52).

Heterogeneity was assessed using I^2 , which represents the proportion of variation in effect estimates across studies that is due to heterogeneity rather than sampling error (53). For each meta-analysis subgroup, heterogeneity was considered high and average estimates of effect were not shown when $I^2 \geq 60\%$ (52, 53). In these cases, a median and range of effect estimates from individual studies in the intervention subgroup was shown instead, as presenting pooled meta-analysis estimates in the presence of high heterogeneity can be misleading (54). Meta-analysis effect estimates were considered significant if the 95% confidence intervals (CI) excluded the null. We intended to assess possible publication bias in meta-analysis subgroups using Begg's rank correlation and Egger's regression tests if there were ≥ 10 trials and if heterogeneity was not high (52); however, none of the subgroups met these criteria.

GRADE assessment

The Cochrane Collaboration's Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach was used to assess the confidence in the estimated measures of intervention effect for each subgroup in terms of how well they might be expected to represent intervention effects in practice or in future studies conducted under similar conditions (48, 52). The GRADE approach classifies the confidence in findings from each subgroup into one of four levels: very low, low, moderate, or high. The approach was adapted to the needs of the topic and applicable study designs (52, 86). Due to inherent differences in strength of evidence for intervention efficacy by study design (48, 86), controlled trials started at high and quasi-experiments at a moderate rating. Five downgrading and three upgrading criteria were then assessed for each subgroup, which could lead to reducing or increasing, respectively, the GRADE levels (Table 2).

Results

Descriptive results

A flow chart of the review process and results is shown in Figure 1. From a total of 221 relevant articles that reported on the efficacy of slaughter and processing interventions against *Salmonella*, more (n=138 vs. 87) investigated beef than pork, with four articles covering both commodities. The key characteristics of the articles are shown in Table 3. Most studies (81%) investigating interventions in the beef chain were conducted in North America, while studies investigating interventions for pork had a more global distribution (Table 3). The most common study design for beef studies was challenge trials (80%), while pork studies mostly comprised challenge trials, quasi-experiments, and risk assessments and stochastic models (Table 3). Nearly 50% of studies investigating interventions in pork were conducted under commercial conditions, compared to only 20% for beef (Table 3). The most common category of interventions investigated was various washes, rinses, and sprays (66%) for beef, and processing steps and GHPs (54%) for pork (Table 3). As shown in Figure 1, only the experimental primary research studies (n=191) are summarized further in this article. A citation list of all relevant articles is available in technical report (39). More detailed characteristics, risk of bias and GRADE assessments, and results for each relevant study are available as supplementary materials: <http://tinyurl.com/zba5b6x>.

Controlled trial and quasi-experiment results for beef

The synthesized results from controlled trial and quasi-experimental studies measuring the effect of slaughter and processing interventions to control *Salmonella* in beef are shown in Table 4. Only three controlled trials were identified, with interventions supported mostly by only one study each (Table 4). However, two studies found that pre-chill hot water washes and steam pasteurization were effective to reduce the prevalence of *Salmonella* on beef carcasses (RR = 0.11, 95% CI: 0.02, 0.58), with high GRADE confidence in the estimate of effect (Figure 2).

Quasi-experimental studies found that washes containing chemicals other than organic acids (e.g. chlorine, sodium hydroxide, hydrogen bromide) significantly reduced the prevalence of *Salmonella* on cattle hides when applied post-exsanguination, but with low GRADE confidence in the specific effect estimate due to significant heterogeneity among studies (Table 4). A similar effect was found in one of the studies for an organic acid hide wash applied post-exsanguination (Table 4). Several studies that measured changes in *Salmonella* prevalence on carcasses sampled between pre-evisceration and chilling, after a series of in-plant interventions were applied, found large reductions in prevalence (Table 4), but with very low GRADE confidence in the effect estimates due to heterogeneity across studies and potential for other confounding factors to have contributed to the findings. Inconsistent results were identified in quasi-experimental studies for the effect of live animal hide washes, pre- and post-chill washes, chilling, and HACCP (Table 4).

Controlled trial and quasi-experiment results for pork

The synthesized results from controlled trial and quasi-experimental studies measuring the effect of slaughter and processing interventions to control *Salmonella* in pork are shown in

Table 5. Three controlled trial studies found that pre-chill organic acid washes were effective to reduce the prevalence of *Salmonella* on pork carcasses (RR = 0.32, 95% CI: 0.13, 0.78), with high GRADE confidence in the estimate (Figure 3). High confidence was also found for the effect estimate of hot water washes and steam pasteurization, which showed a trend for reduction, but was non-significant (Table 5). Other interventions investigated in controlled trials showed inconsistent results (e.g. spray vs. air chilling) or were supported by only one study (e.g. other chemical washes) (Table 5).

Quasi-experimental studies found that both scalding (RR = 0.20, 95% CI: 0.14, 0.29) and singeing (RR = 0.34, 95% CI: 0.33, 0.52) provided significant reductions in *Salmonella* prevalence, with moderate GRADE confidence in the effect estimates (Table 5 and Figures 4-5). Two studies found that cleaning and disinfection in the processing plant significantly reduced *Salmonella* prevalence, but with low GRADE confidence in the effect estimate because the studies only measured environmental samples (Table 5). Studies that measured changes in *Salmonella* prevalence on carcasses sampled between pre-evisceration and post-chilling, after a series of in-plant interventions were applied, found large reductions in prevalence, but inconsistent results were found in studies that measured the initial samples post-evisceration (Table 5). Inconsistent results were identified in quasi-experimental studies for the effects of pre-chill washes, chilling, and HACCP (Table 5).

Challenge trial results

A total of 143 challenge trial studies conducted under laboratory or pilot plant conditions were identified that evaluated a range of interventions along the processing chain for beef (n=111) and pork (n=33). Overall, the challenge trials found wide-ranging reductions in *Salmonella* concentrations for most investigated interventions in both commodities (ranging from almost no reduction to multiple logs). In general, challenge trials found that multiple combinations of interventions implemented concurrently or successively were the most consistently effective to reduce *Salmonella* concentrations on beef and pork compared with no treatment and ambient water washes.

Risk of bias

The overall results of the risk-of-bias assessment for the relevant studies are shown stratified by study design in Table 6. The main risks of bias for controlled trials related to a lack of reporting of random treatment allocation and associated risks that confounding factors other than the intervention could have contributed to the outcomes (Table 6). A limited number of challenge trials also had similar risks of bias, along with risks related to incomplete outcome data and selective reporting. The primary risk of bias for quasi-experiments related to the potential for confounding factors to have influenced the outcomes (Table 6).

Discussion

Efficacy of slaughter and processing interventions

Salmonella contamination of incoming cattle hides is a major source of beef carcass cross-contamination in processing establishments (6, 10, 82). A molecular source-tracking study in the USA found that cross-contamination of hides in the lairage environment accounted for

a larger proportion of cattle hide and carcass contamination with *Salmonella* than the initial level of contamination on cattle leaving the feedlot (6). Hide decontamination interventions can be applied to either the live animals or carcasses post-exsanguination to reduce the incoming *Salmonella* load. Quasi-experimental studies from the USA consistently found that post-exsanguination hide washes containing chemicals such as lactic acid, sodium hydroxide, hydrogen bromide, or chlorine were effective at reducing the prevalence of *Salmonella* (6, 7, 15, 88, 90), although the specific magnitude of reduction was variable. In contrast, ambient water washes without chemicals were not effective, and in some cases increased contamination, potentially because they might mobilize bacteria on the hide, which can be encapsulated in dirt, mud, and feces (69, 88). Similarly, hide washes applied to live animals showed inconsistent results, and additionally may cause animal stress or irritations (69).

Finisher pigs can harbor a wide variety of *Salmonella* serotypes in various tissues, which can lead to cross-contamination of carcasses during processing if GHPs are not followed (32, 34, 62). Scalding and singeing are process steps that provide opportunities to reduce *Salmonella* contamination of pork carcasses early along the slaughter line. The effect of both steps for reducing *Salmonella* prevalence was demonstrated in several quasi-experimental studies (13, 25, 26, 51, 76), as well as in observational studies (28, 62, 66). However, the efficacy of scalding and singeing depends on the procedures used, including achieving appropriate time-temperature combinations, and GHPs such as frequently changing the scald tank water and ensuring the singe flame reaches the entire carcass (34). Additionally, it is important to maintain GHPs in steps following scalding and singeing (e.g. polishing, evisceration, and splitting), including training and awareness of plant personnel, in order to prevent carcass recontamination (13, 75).

Beef and pork carcass decontamination treatments such as hot water, steam pasteurization, and organic acid washes are used by processing establishments worldwide, in accordance with local regulatory approvals (1, 44, 47, 100). For example, lactic acid was recently approved as a decontamination intervention for beef carcasses, halves, and quarters in the European Union, where previously only heat-based treatments were allowed (38).

Controlled trial studies indicated that pre-chill hot water washes and steam pasteurization were effective at reducing *Salmonella* contamination of beef carcasses, with a similar trend for pork carcasses, although the overall effect was not statistically significant. Controlled trials also found that organic acid washes were effective at reducing the prevalence of *Salmonella* on pork carcasses. In contrast, despite their wide use in practice and extensive evidence of efficacy under laboratory and pilot plant conditions (33), no evidence collected under commercial conditions was identified for their effect on beef carcasses. Inconsistent evidence from commercial settings has also been noted for the efficacy of organic acid washes for reducing populations of indicator organisms on beef carcasses, which could be due to several factors, such as potential dilution of chemical concentrations if applied on wet carcasses and difficulties in achieving uniform coverage of application across the whole carcass surface (44). The efficacy of carcass decontamination treatments may vary depending on the specific process parameters used (e.g. water temperature and pressure, treatment time, chemical concentration) as well as other factors (e.g. levels of carcass

contamination and bacterial attachment to the meat surface) (44, 100). They are typically applied using automated spray cabinets, which may be cost-prohibitive for smaller establishments. It has been suggested that operators of smaller establishments could consider the use of handheld spray devices as an alternative to automated cabinets (2, 97).

Additionally, decontamination interventions may cause a discoloration (e.g. cooked appearance) of the meat surface depending on the application parameters, but this change is usually temporary and may be reversible following chilling (2, 50, 100).

It has been suggested that the process of carcass chilling might contribute to a reduction in the prevalence of microbial pathogens such as *Salmonella* on beef and pork carcasses (45, 47); for example, through the combined effects of cold shock and desiccation (35). However, we identified inconsistent and variable evidence for chilling to reduce the prevalence of *Salmonella* on beef and pork carcasses, though there was more evidence suggesting a potential reduction effect in pork than in beef (Tables 4-5). The variability in the apparent effect of chilling for reducing *Salmonella* contamination could be due to a number of factors, such as potential issues with recovery and isolation of *Salmonella* from chilled carcasses, carcass cross-contamination in the chilling room, variable sampling and laboratory methods across studies, and differences in chilling methods and procedures (35, 45, 68, 84). The difference in evidence of effect for beef and pork could be due to additional processing steps in the latter, such as singeing, which might reduce attachment of *Salmonella* to carcass surfaces. Further research is needed to investigate if different chilling methods (e.g. spray or blast vs. conventional air chilling) might provide additional opportunities to reduce *Salmonella* contamination of beef and pork carcasses during processing.

Various additional interventions, such as carcass washes with chemicals other than organic acids, post-fabrication treatments, packaging-based treatments, and emerging technologies (e.g. natural extracts, non-thermal plasma), were investigated in laboratory-based challenge trials and show promise for reducing *Salmonella* contamination of beef and/or pork under those conditions (39). However, further research is needed to investigate their feasibility and effectiveness in commercial settings.

The overall body of evidence synthesized in this review for both beef and pork suggests that the implementation of multiple interventions concurrently or successively along the processing chain (i.e. a “multiple-hurdle” strategy) results in enhanced reductions in *Salmonella* compared to single interventions alone (39). However, given the wide variability of the studies synthesized in this review, the effectiveness of each intervention and combination thereof should be validated and verified within the context and conditions of each specific processing establishment. Additionally, pre-harvest control measures may also be needed as part of a holistic food-chain approach to controlling *Salmonella* in beef and pork (34, 63, 95). For example, research has shown that deep-tissue lymph nodes might be a more important source of *Salmonella* contamination in ground beef compared to fecal and hide contamination (59, 63). Processing interventions such as carcass decontamination washes are unlikely to affect *Salmonella* in these beef tissues, which are generally not removed prior to the grinding step (46, 59, 63). Similarly, research has shown that pig carcasses from farms with higher herd seropositivity for *Salmonella* are more likely to be

contaminated at the abattoir than those originating from lower prevalence herds (1, 32, 62). Therefore, appropriate pre-harvest interventions may be warranted for both commodities in order to reduce the incoming burden of *Salmonella* in different tissues and the subsequent potential for cross-contamination of beef and pork carcasses during processing.

Review strengths and limitations

Randomized controlled trials conducted under commercial conditions provide the strongest evidence to measure the efficacy of an intervention, because the random allocation of study units to treatment and control groups balances unmeasured confounding variables and helps to ensure that measured changes in the outcome (e.g. *Salmonella*) are due to the intervention and not to other factors (86). In our GRADE assessment of intervention efficacy measures, quasi-experiments were automatically downgraded by one level due to inherent study design differences: specifically, these studies do not have a concurrent comparison group, and any observed intervention effects between pre- and post-intervention samples cannot be assumed to be due to the intervention (86). Some risks of bias were identified for studies of both designs related to a lack of reporting of key design features (e.g. randomization allocation) and outcomes; future research in this field should report findings in accordance with internationally recommended reporting guidelines for experimental studies of interventions (29, 87). Due to their highly controlled conditions, we did not find many risks of bias in challenge trials that we believe could have affected their outcomes. However, they were summarized only narratively in this article, because they are conducted using specific strains of *Salmonella* that are inoculated, often at very high doses, to portions or pieces of meat under laboratory or pilot plant settings that do not accurately reflect real-world conditions, and the strains used may not be representative of those naturally found on pork and beef in different settings.

This review used a structured and transparent approach to identify, assess, and synthesize available evidence on the efficacy of interventions to control *Salmonella* during beef and pork processing, which provides more credible and reliable evidence to decision-makers than traditional narrative reviews (52, 80). One limitation of this review was that the search was streamlined using a rapid approach (only two bibliographic databases were used), and given the broad scope, some important search terms could have been missed. Therefore, it is possible that we could have missed some potentially relevant literature that might have resulted in additional evidence, though a search verification strategy was implemented to attempt to mitigate this bias. Another related limitation is that only literature published in English, French, and Spanish was considered for inclusion, which could have resulted in underrepresentation of evidence from some geographic regions. Only one reviewer conducted the relevance confirmation and data extraction steps of the review, which could have led to possible misclassification of articles or data extraction errors, respectively.

The original review question aimed to include evidence on post-processing interventions, but these were later excluded from the scope of the review as the search was not optimized to reliably capture such studies. Post-processing interventions, such as temperature control, cooking, and prevention of cross-contamination, are critical steps in the food chain for ensuring the safety of beef and pork and for reducing the risk of salmonellosis among

consumers (11, 35, 93). The slaughter and processing interventions summarized and reported in this review should be considered as important strategies to address in-plant *Salmonella* contamination of beef and pork, but appropriate post-processing controls are also needed as part of a whole food chain approach to prevent subsequent cross-contamination and *Salmonella* growth and survival prior to consumption.

Meta-analysis provides a useful analytical tool to combine the results of multiple primary research studies into a weighted, average estimate of intervention effect (52). However, a limitation of the analysis in this review is that study results were synthesized across relatively broad intervention categories. This was conducted for pragmatic reasons in order to facilitate summarization and presentation of intervention efficacy trends from a large body of literature. However, as a consequence, details such as intervention application parameters (e.g. dose, treatment duration) and differences in study sampling and laboratory methods were not investigated as possible sources of variation in intervention effects across studies. It is likely that these and other study factors could contribute to the heterogeneity in effects observed for many intervention categories, but it was beyond the scope of the review to investigate them in detail. Future research synthesis research focusing on more specific commodities, points in the food chain, and interventions could allow more in-depth investigation of such factors.

The meta-analysis estimates of intervention effect, along with the GRADE ratings of confidence in these effects, provide a guide for interpreting how likely one could expect a similar effect in future studies or applications. However, given that the intervention effects reported in the literature reflect the specific conditions and parameters of those studies, such interventions should always be validated under local plant settings and conditions. The GRADE weight-of-evidence approach used in this review was adapted from the healthcare sector. Further research is needed on how best to define and evaluate the specific parameters of the GRADE assessment criteria and how these should be ideally applied within the context of food safety research.

We identified very little research conducted under commercial conditions, and of those studies that were identified, most were quasi-experiments rather than controlled trials. This is likely due to pragmatic issues with evaluating various interventions in commercial processing establishments, such as infrequent in-plant *Salmonella* contamination of carcasses when operating under good hygiene and manufacturing practices. For example, we identified several additional controlled trials that measured *Salmonella* but did not isolate any positive samples (39), which precluded evaluation of intervention effect on this pathogen. We decided not to include intervention research on potential surrogate organisms (e.g. non-pathogenic *Escherichia coli*) within the scope of the review because it was not known to what extent these results might reflect and correspond similarly to the control of *Salmonella*. For example, the behavior of surrogates such as *E. coli* can differ when compared with different *Salmonella* serotypes, intervention processes, and meat products (73).

Nevertheless, we recognize that individual establishment operators will likely need to use surrogate organisms to validate and verify the effectiveness of interventions in their specific

settings and local conditions. Various studies have found that aerobic plate counts (APC) and Enterobacteriaceae could be considered as suitable surrogate measures for evaluating the in-plant effectiveness of interventions to reduce *Salmonella* contamination of beef and pork (3, 32, 84, 103). For example, in a study that measured >5000 samples from three processing plants in the USA over an 18-month period, Ruby et al. (84) found that both APC and Enterobacteriaceae counts were significantly associated with the presence of *Salmonella* on beef carcasses. Similarly, in a study that measured pork carcass swab samples from 152 processing establishments across the USA, Williams et al. (103) found that log reductions in APC between pre-evisceration and post-chill samples were associated with reductions in *Salmonella*. In contrast, while some studies have indicated that some non-pathogenic strains of *E. coli* might also be useful for this purpose in specific contexts (12, 19, 73), they are not likely to be suitable for monitoring reductions after chilling given the large proportion of samples that tend to be negative after this step (103).

Conclusion

Various slaughter and processing interventions were shown to be effective to reduce the prevalence of *Salmonella* in beef and pork under commercial conditions in different contexts. Several other interventions were investigated only under laboratory or pilot plant conditions and showed promise in those settings, but require further research on their commercial applicability. As the studies summarized in this review were conducted under varying conditions and often found wide-ranging effects, the specific selection and implementation of different interventions in practice should be guided by the local conditions and situation. Additionally, any interventions should be validated and verified for their effectiveness in each establishment setting. If *Salmonella* levels are low, then monitoring should be conducted using applicable surrogate organisms, though their limitations should be recognized. GHPs and HACCP-based principles provide the basis upon which slaughter and processing interventions should be implemented, as the positive effects of an intervention can be negated if cross-contamination occurs subsequently during processing and if contamination occurs further along the chain. In addition, consideration should be given to a holistic and integrated food-chain approach to *Salmonella* control, with slaughter and processing interventions implemented alongside appropriate pre-harvest and post-processing control measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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* Note that four of the relevant articles reported intervention efficacy data for both pork and beef outcomes.

Figure 1.
Systematic review flow chart.

Figure 2.

Forest plot of the results of controlled trials measuring the efficacy of pre-chill hot water washes and steam pasteurization to reduce the prevalence of *Salmonella* on beef carcasses.

RE=random-effects meta-analysis.

Figure 3. Forest plot of the results of controlled trials measuring the efficacy of pre-chill organic acid washes to reduce the prevalence of *Salmonella* on pork carcasses. RE=random-effects meta-analysis.

Figure 4.

Forest plot of the results of quasi-experiments measuring the efficacy of scalding to reduce the prevalence of *Salmonella* on pork carcasses. RE=random-effects meta-analysis.

Figure 5.

Forest plot of the results of quasi-experiments measuring the efficacy of singeing to reduce the prevalence of *Salmonella* on pork carcasses. RE=random-effects meta-analysis.

Table 1

Categories of processing interventions used to summarize and report efficacy data in this review.

Intervention categories ^a	Definition
Dry heat	Non-hydrating thermal interventions such as forced-air heating.
Electricity	Application of electricity through, for example, electric stunners.
Multiple intervention combinations	Combination of two or more intervention types.
Non-thermal	Non-chemical and non-heat-based interventions that aim to reduce microbial contamination while preserving product quality and nutrients that can be affected by thermal treatments (100). Examples include irradiation, plasma gases, and high pressure processing.
Packaging-based interventions	Includes a range of interventions that can be applied to prevent spoilage and inhibit microbial growth during final product distribution and storage.
Standard processing steps/GHPs	
<i>Bunging</i>	Closing off the rectum during removal of intact viscera at slaughter to minimize the spread of cross-contamination on a carcass (92).
<i>Chilling</i>	Step at the end of the slaughter process and before fabrication of rapidly reducing the carcass temperature to prevent microbial growth and preserve product quality.
<i>Cleaning/disinfection</i>	The removal of dirt and organic substances from and sanitation of meat processing plant equipment and environments.
<i>HACCP</i>	An internationally recognized system that identifies, evaluates, and controls hazards that are significant for food safety.
<i>Scalding</i>	Step during pork processing of immersing pigs in hot water tanks with the primary purpose of softening hairs on the pig skin to make them easier to remove.
<i>Singeing</i>	Step during pork processing of removing remaining hairs from a pig carcass.
<i>Steam vacuuming</i>	Spot application of steam and/or hot water to loosen soil and kill bacteria, followed by a vacuum to remove contaminants.
<i>Trimming</i>	Physical removal of visible contamination from carcasses.
Washes/rinses/sprays	
<i>Hot water/steam pasteurization</i>	Heat treatment to destroy microbial cells.
<i>Multiple wash combinations</i>	Combinations of two or more types of washes, rinses, or sprays.
<i>Natural extracts</i>	Plant-based and other (e.g. dairy) extracts.
<i>Organic acids</i>	Antimicrobials such as lactic, acetic, and citric acid that affect microbial growth through disruptions to nutrient transport and energy generation and that can cause injury to microbial cells through their low pH (100).
<i>Other chemicals</i>	Chemicals that destroy bacteria through various actions, such as oxidation and disruption of cellular functions, or that prevent bacterial attachment to meat (100). Examples include chlorine, trisodium phosphate, acidified sodium chlorite, and ozone.
<i>Protective bacterial cultures</i>	Lactic acid bacteria to control pathogens through the production of antimicrobial compounds.
<i>Water</i>	Ambient or cold water to physically remove contamination.

^aNote that categories are only shown here if they were represented by at least one study identified in the review and reported results on efficacy against *Salmonella*.

Table 2

GRADE approach and criteria applied to sub-groups of data where quantitative intervention efficacy results were summarized.

Criteria	GRADE rating change		Explanation
Downgrading criteria			
1	Individual study risk-of-bias rating	a) = -1 b) = -2	Rating from risk-of-bias tool
	a) <u>Serious</u> = >50% of trials had an unclear or high overall risk-of-bias rating		
	b) <u>Very serious</u> = the risk of bias may have a strong influence on the estimate of effect and study limitations are present in the majority of studies		
2.	Inconsistency of findings among studies	a) = -1 b) = -2	Heterogeneity in the results is measured by I^2 (only where meta-analysis was possible).
	a) <u>Serious</u> = some inconsistency is noted (point estimates vary widely across studies and/or confidence intervals show minimal overlap, or significant heterogeneity is noted in meta-analysis).		
	b) <u>Very serious</u> = studies show widely different directions of effect (some positive, some negative).		
3.	Imprecision of effect estimates	a) = -1	For <u>prevalence outcomes</u> , assume at least a 10% reduction in risk (for common outcomes, ~750-800 samples, for rare outcomes, ~200-300). Actual sample size determined by inputting median control group prevalence in a sample size calculator. For <u>concentration outcomes</u> , total sample size is >400. For rare events, may be less. Actual
	a) The total number of samples is less than that required by a conventional sample size calculation for a single adequately powered controlled trial		

Criteria			GRADE rating change	Explanation
				sample size determined by inputting median group means and SDs in sample size calculator.
4.	Indirectness of individual study parameter as representative of target parameter		a) = -1 b) = -2	Indirectness indicates studies do not directly measure the target parameter of interest to the review question. E.g. studies only measuring surrogate/intermediate outcomes (e.g. environmental samples) or those that deliberately contaminate carcasses with fecal material may be rated down.
	a)	Serious = >50% of trials indirectly measure the intervention, population, comparison, or outcome		
	b)	Very serious = >50% of trials measure two or more of the above parameters indirectly		
5.	Publication bias		a) = -1	If meta-analysis is possible, publication bias detected in dataset via statistical tests and visual examination. If meta-analysis is not possible, publication bias suspected by other means (e.g. lack of small studies showing no treatment effect).
	a)	Detected or suspected in data subset		
<i>Upgrading criteria</i>				
1.	Large magnitude of effect		a) = +1	Large effect considered at least a 2-fold reduction in risk.
	a)	Large effect in the absence of plausible confounders and major threats to validity		
2.	Direction of plausible bias		a) = +1	E.g. the actual effect of the intervention is likely to be even greater than the data suggest.
	a)	Results may have been underestimated due to the study design (e.g. population sampled)		
3.	Dose-response gradient		a) = +1	
	a)	Majority of trials identified a dose-response relationship		

Table 3

Key characteristics of 221 relevant articles that reported data on the efficacy of processing interventions to control *Salmonella* in fresh pork or beef.

Article characteristic	Beef (n=138)		Pork (n=87)	
	No.	%	No.	%
Region:				
North America	112	81.2	26	29.9
Europe	8	5.8	40	46.0
Asia/Middle East	8	5.8	14	16.1
Africa	5	3.6	0	0.0
Central and South America/Caribbean	3	2.2	5	5.7
Australia/South Pacific	2	1.4	2	2.3
Document type:				
Journal article	129	93.5	74	85.1
Conference proceedings paper/abstract	4	2.9	9	10.3
Report	4	2.9	3	3.4
Thesis	1	0.7	1	1.1
Study design:				
Challenge trial	111	80.4	33	37.9
Quasi-experiment	20	14.5	23	26.4
Controlled trial	3	2.2	7	8.0
Observational study	2	1.4	7	8.0
Risk assessment/stochastic model	2	1.4	17	19.5
Systematic review/meta-analysis	1	0.7	4	4.6
Study conditions:				
Laboratory	70	50.7	25	28.7
Pilot plant	39	28.3	4	4.6
Commercial	27	19.6	41	47.1
Not reported	0	0.0	1	1.1
Intervention type:				
Decontamination washes/rinses/sprays	91	65.9	36	41.4
Packaging-based interventions	31	22.5	8	9.2
Processing steps/GHPs	17	12.3	47	54.0
Other	11	8.0	10	11.5

Table 4

Synthesized results of controlled trials and quasi-experiments that investigated the efficacy of processing interventions to control *Salmonella* in beef.

Intervention / stage in chain (comparison group)	Study design	No. trials / studies	Relative risk estimate of effect: meta-analysis/single study (95% CI) or median (range) ^d	I ²	Estimated change in prevalence (%) in the study population (95% CI or range) ^b	GRADE ^c	References
Water wash							
Hide pre-slaughter (pre vs. post)	QE	2/1	MA = 1.22 (1.01, 1.47)	0%	46.7 to 56.8 (47.0, 68.6)	Very low	(69)
Hide post-exsanguination (pre vs. post)	QE	2/2	Median = 0.79 (0.28, 1.29)	99%	78.1 to 61.3 (21.5, 100)	Very low	(6, 88)
Pre-chill (vs. no treatment)	CT	1/1	Study = 0.30 (0.17, 0.53)	-	30.3 to 9.0 (5.1, 16.1)	Very low	(81)
Pre-chill (pre vs. post)	QE	4/4	MA = 0.59 (0.31, 1.13)	0%	7.2 to 4.2 (2.2, 8.1)	Low	(31, 49, 72, 96)
Hot water/steam							
Pre-chill (vs. no treatment)	CT	2/2	MA = 0.11 (0.02, 0.58)	0%	15.4 to 1.7 (0.3, 8.9)	High	(81, 97)
Pre-chill (pre vs. post)	QE	2/2	MA = 0.18 (0.02, 1.54)	0%	7.0 to 1.3 (0.2, 10.8)	Moderate	(74, 104)
Organic acid wash							
Hide pre-slaughter (pre vs. post)	QE	1/1	Study = 1.04 (0.78, 1.39)	-	50.0 to 52.2 (39.2, 69.5)	Very low	(69)
Hide post-exsanguination (pre vs. post)	QE	1/1	Study = 0.68 (0.49, 0.93)	-	74.0 to 50.0 (36.2, 69.0)	Low	(88)
Post-chill (pre vs. post)	QE	1/1	Study = 0.45 (0.16, 1.30)	-	1.0 to 0.5 (0.2, 1.3)	Very low	(84)
Other chemical wash							
Hide pre-slaughter (pre vs. post)	QE	1/1	Study = 0.93 (0.72, 1.19)	-	60.0 to 55.6 (43.3, 71.3)	Very low	(69)
Hide post-exsanguination (pre vs. post)	QE	5/4	Median = 0.70 (0.24, 0.87)	98%	62.3 to 43.6 (15.1, 54.3)	Low	(7, 15, 88, 90)
Pre-chill (vs. no treatment)	CT	1/1	Study = 1.18 (0.82, 1.71)	-	30.3 to 35.8 (24.8, 51.7)	Very low	(81)
Trimming							
Pre-chill (vs. no treatment)	CT	1/1	Study = 0.26 (0.14, 0.48)	-	30.3 to 7.7 (4.2, 14.4)	Very low	(81)
Multiple interventions							
Pre-chill (vs. no treatment)	CT	1/1	Study = 0.05 (0.01, 0.19)	-	30.3 to 1.4 (0.3, 5.6)	Very low	(81)
Pre-chill (pre vs. post)	QE	1/1	Study = 0.08 (0.06, 0.12)	-	28.1 to 2.3 (1.6, 3.5)	Low	(84)
Pre-evisceration to pre-chill (pre vs. post)	QE	7/7	Median = 0.02 (0.006, 0.62)	97%	28.7 to 0.5 (0.2, 17.9)	Very low	(8, 10, 16, 59, 82, 84, 91)
Processing steps/GHPs							
Bunging (before vs. after pre-evisceration wash)	CT	1/1	Study = 0.09 (0.005, 1.61)	-	8.3 to 0.8 (0.04, 13.4)	Low	(92)
Chilling (pre vs. post)	QE	5/5	MA = 1.50 (0.61, 3.74)	17%	2.0 to 3.0 (1.2, 7.4)	Low	(31, 40, 49, 91, 96)
HACCP (pre vs. post)	QE	5/5 ^d	MA = 1.13 (0.68, 1.87)	0%	1.2 to 1.4 (0.8, 2.2)	Moderate	(101)

CI=confidence interval; CT=controlled trial; GHP=good hygiene practice; MA=meta-analysis; QE=quasi-experiment.

^aMeta-analysis estimates from random-effects models are shown if I^2 was <60%. If I^2 was 60%, a median and range of effects are shown. If only one trial from a single study was available, the estimate and 95% CI from the study are shown.

^bThe first value is the median prevalence from the control group samples in the respective studies (baseline prevalence without intervention). The second value indicates the expected reduction (or increase) in prevalence due to the intervention, as calculated from the respective estimate of effect (see methods section for additional details). The values in brackets represent a 95% CI for the estimated change for meta-analysis and individual study estimates, or a range of values for intervention estimates with significant heterogeneity.

^cGRADE rating descriptions: very low=the true effect is likely to be substantially different from the measured estimate; low=the true effect may be substantially different from the measured estimate; moderate=the true effect is likely to be close to the measured estimate, but there is a possibility that it is substantially different; high=there is strong confidence that the true effect lies close to that of the measured estimate.

^dIndividual studies were summarized in a previous systematic review-meta-analysis.

Table 5

Synthesized results of controlled trials and quasi-experiments that investigated the efficacy of processing interventions to control *Salmonella* in pork.

Intervention / stage in chain (comparison group)	Study design	No. trials / studies	Relative risk estimate of effect: meta-analysis/single study (95% CI) or median (range) ^a	I ²	Estimated change in prevalence (%) in the study population (95% CI or range) ^b	GRADE ^c	References
Water wash (high pressure)							
Pre-chill (pre vs. post)	QE	1/1	Study = 0.18 (0.05, 0.65)	-	91.7 to 16.7 (4.6, 59.7)	Very low	(17)
Hot water/steam							
Pre-chill (vs. no treatment)	CT	4/3	MA = 0.29 (0.08, 1.08)	46%	4.3 to 1.2 (0.3, 4.6)	High	(36, 50, 97)
Organic acid wash							
Pre-chill (vs. no treatment)	CT	4/3	MA = 0.32 (0.13, 0.78)	0%	7.7 to 2.5 (1.0, 6.0)	High	(36, 37, 41)
Pre-chill (vs. water wash)	CT	2/1	Median = 0.26 (0.08, 0.44)	95%	100 to 26.0 (7.5, 44.4)	Very low	(98)
Pre-chill (pre vs. post)	QE	1/1	Study = 0.48 (0.25, 0.93)	-	13.9 to 6.7 (3.5, 12.9)	Low	(60)
Other chemical wash							
Pre-chill (vs. no treatment)	CT	1/1	Study = 0.44 (0.20, 0.98)	-	16.0 to 7.0 (3.1, 15.6)	Low	(50)
Unspecified wash							
Pre-chill (pre vs. post)	QE	2/2	MA = 0.60 (0.11, 3.17)	31%	5.1 to 3.1 (0.6, 16.1)	Low	(51, 65)
Steam vacuum							
Pre-chill (vs. trimming)	CT	1/1	Study = 3.0 (0.13, 71.74)	-	2.2 to 6.7 (0.3, 100)	Low	(83)
Multiple interventions							
Pre-chill (vs. no treatment)	CT	2/1	MA = 0.54 (0.09, 3.22)	0%	4.2 to 2.3 (0.4, 13.4)	Low	(36)
Pre-chill (pre vs. post)	QE	2/2	MA = 0.95 (0.52, 1.74)	0%	8.6 to 8.2 (4.5, 15.0)	Moderate	(24, 25)
Post-exsanguination to post-chill (pre vs. post)	QE	1/1	Study = 0.01 (0.002, 0.08)	-	73.0 to 0.8 (0.1, 5.8)	Very low	(94)
Pre-evisceration to post-chill (pre vs. post)	QE	4/4	Median = 0.08 (0.03, 0.19)	92%	14.9 to 0.9 (0.6, 2.9)	Very low	(57, 85, 89, 103)
Post-evisceration to post-chill (pre vs. post)	QE	3/3	Median = 0.71 (0.17, 2.94)	94%	9.4 to 9.1 (1.5, 18.8)	Very low	(3, 27, 56)
Processing steps/GHPs							
Scalding (pre vs. post)	QE	4/4	MA = 0.20 (0.14, 0.29)	0%	34.8 to 7.0 (4.9, 10.0)	Moderate	(13, 26, 51, 76)
Singeing (pre vs. post)	QE	4/4	MA = 0.34 (0.22, 0.52)	0%	18.1 to 6.1 (4.0, 9.4)	Moderate	(25, 26, 51, 76)
Chilling (spray vs. air)	CT	1/1	Study = 0.70 (0.37, 1.33)	-	13.3 to 9.3 (4.9, 17.8)	Low	(37)
Chilling (pre vs. post)	QE	13/13 ^d	Median = 0.44 (0.16, 2.50)	85%	10.3 to 4.6 (1.7, 25.7)	Very low	(45)
HACCP (pre vs. post)	QE	3/3 ^d	Median = 0.94 (0.59, 1.26)	89%	6.9 to 6.5 (4.0, 8.7)	Low	(101)
Cleaning and disinfection (pre vs. post)	QE	2/2	MA = 0.33 (0.20, 0.52)	0%	22.8 to 7.5 (4.7, 12.0)	Low	(23, 43)

CI=confidence interval; CT=controlled trial; GHP=good hygiene practice; MA=meta-analysis; QE=quasi-experiment.

^aMeta-analysis estimates from random-effects models are shown if I^2 was <60%. If I^2 was 60%, a median and range of effects are shown. If only one trial from a single study was available, the estimate and 95% CI from the study are shown.

^bThe first value is the median prevalence from the control group samples in the respective studies (baseline prevalence without intervention). The second value indicates the expected reduction (or increase) in prevalence due to the intervention, as calculated from the respective estimate of effect (see methods section for additional details). The values in brackets represent a 95% CI for the estimated change for meta-analysis and individual study estimates, or a range of values for interventions estimate with significant heterogeneity.

^cGRADE rating descriptions: very low=the true effect is likely to be substantially different from the measured estimate; low=the true effect may be substantially different from the measured estimate; moderate=the true effect is likely to be close to the measured estimate, but there is a possibility that it is substantially different; high=there is strong confidence that the true effect lies close to that of the measured estimate.

^dIndividual studies were summarized in a previous systematic review-meta-analysis.

Table 6

Risk-of-bias assessment summary for 191 experimental primary research articles that investigated the efficacy of processing interventions to control *Salmonella* in beef or pork. Some studies reported on more than one experimental design.

Criteria	No. of studies low/unclear/high risk		
	CT (n=9)	QE (n=43)	ChT (n=143)
Random sequence generation	2/6/1	N/A ^a	139/2/2
Allocation concealment	9/0/0	N/A ^a	143/0/0
Blinding of participants and personnel	9/0/0	N/A ^a	143/0/0
Independence of intervention effect from confounding bias	2/6/1	16/24/3	137/6/0
Incomplete outcome data	6/2/1	37/6/0	138/5/0
Selective reporting	9/0/0	43/0/0	135/8/0
Other biases	7/2/0	43/0/0	140/3/0
Overall risk-of-bias:	2/5/2	16/24/3	134/10/1

CT=controlled trials; ChT=challenge trials; QE=quasi-experiments.

^aN/A=These criteria were not assessed for this study design.