UNIVERSITY OF LIVERPOOL

Final Report

Meta-analysis of the efficacy of interventions applied during primary processing to reduce microbiological contamination in beef

FSA Project FS430388

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Abbreviations

ACC	Aerobic Colony Counts
ASC	Acidified sodium chlorite
B/A	Before-and-after trial
Beefxide	A proprietary mixture of lactic and citric acids in water
CFU	Colony Forming Units
ChT	Challenge trial (with artificially inoculated microorganisms)
Citrilow	A proprietary mixture of citric and hydrochloric acids
CPC	Cetylpyridinium Chloride
CT	Controlled trial
EBC	*Enterobacteriaceae* Counts
EFSA	European Food Safety Authority
FBO	Food Business Operator
FreshBloom™	A proprietary mixture of citric acid, ascorbic acid and erythorbic acid spray
FSA	Food Standards Agency
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
Inspexx© 200	A proprietary mixture of organic acids and hydrogen peroxide (acetic acid, octanoic acid, peroxyacetic acid, peroxyoctanoic acid, and hydroxyethylidene-1,1-diphosphoric acid)
LTTC	Less than thoroughly cooked burgers
PAA	Peroxyacetic acid (peracetic acid and hydrogen peroxide)
QAC	Quaternary ammonium compounds (sanitisers)
RoB	Risk-of-bias assessment
STEC	Shiga toxin-producing *Escherichia coli*
TSP	Trisodium phosphate
TW20	Tween 20 (Polyoxyethylene (20) sorbitan monolaurate)
VFC	Visible faecal contamination

Executive summary

The sale and consumption of burgers served less than thoroughly cooked and pink in the middle is a steadily increasing trend in the UK. Considering that the consumption of these products can be associated with an increased risk of exposure to *Escherichia coli* O157, other Shiga toxin-producing *E. coli* (STEC) and *Salmonella* spp., the Food Standards Agency (FSA) commissioned a critical literature review to understand this problem and ways of addressing it. The review assessed the significance of various interventions to reduce the microbiological load on beef in primary production identified in a previous literature review ("A critical literature review to assess the significance of intervention methods to reduce the microbiology load on beef through primary production", FSA project FS301044). The review used a systematic approach, and provided quantitative data on interventions' effectiveness against main hazards found in beef. The review concluded that the most promising interventions to reduce microbial load on beef were cattle hide interventions, carcass pasteurisation treatments and organic acid washes, as well as the sequential use of these. However, the review was critical and observational in its nature and did not address differences in study designs and the consequences on the intervention efficacies between multiple identified studies. As a result, it could not provide a more robust estimate of interventions' effectiveness that would deliver more reliable conclusions for risk management decisions. A systematic literature review coupled with meta-analysis is one of the methods used to address high heterogeneity between experimental methods and results within a body of literature. This tool is used in food safety to measure intervention effectiveness with reduced bias and increased transparency.

There were two objectives of this study:

1. To perform a meta-analysis of the efficacy of interventions applied during primary processing to reduce microbiological contamination in beef

2. To make recommendations on the effectiveness (the quantifiable level of bacterial reduction) of specific interventions for beef that will inform the risk management decisions for further work

The data on interventions in beef primary processing that were analysed in this study were identified in a previous critical review (FSA project FS301044). The study covered a large body of literature (the period of the last 25 years), and included interventions from the pre-slaughter stage (lairage interventions), cattle hide interventions, beef carcass interventions and post-fabrication interventions for beef trim. A meta-analysis tool to combine the results of multiple primary research studies into a weighted, average estimate of intervention effect was performed on data investigating reduction effect on pathogenic microorganisms (*Salmonella* spp. and pathogenic *E. coli*) and indicator microorganisms (aerobic bacteria, *Enterobacteriaceae* and generic *E. coli*).

Following a rigorous methodological approach, significant number of studies were excluded from meta-analysis due to their insufficient methodological quality and lack of adequate reporting of intervention protocols, units of outcome measurement and results. Therefore, there were insufficient data available for meta-analysis of some interventions, such as lairage and cattle hide interventions (cattle handling in lairage, cattle hide clipping, bacteriophage and chemical treatments), beef carcass interventions (standard procedures for carcasses, organic acid and other carcass chemical washes) and interventions for beef primals, subprimals and trim (chemical washes and novel interventions). There were some interventions for which limited data were available and meta-analysis was performed, such as cattle hide interventions (some chemical washes and shellac hide coating) and beef carcass interventions (knife trimming, steam vacuuming, lactic acid and other organic acid washes and multiple interventions). Some of these interventions, such as shellac cattle hide coating, carcass lactic acid wash and steam vacuuming, were showing promising reduction but due to small number of trials, their efficacy is inconclusive. Interventions for which there were sufficient data for meta-analysis include hide cleanliness assessment and water wash, beef carcass water wash, hot water wash, steam pasteurisation and chilling (dry, water spray and spray chilling with chemicals) and some chemical washes of fabricated beef.

Hide cleanliness assessment, scoring system widely used in UK abattoirs, showed consistent reduction on resulting carcasses for ACC, EBC and generic *E. coli* of up to 1 log when clean cattle are compared to dirty cattle. The results indicate the uselfulness of this scoring system in separating clean and dirty animals and thus proactively reducing potential carcass contamination during subsequent dehiding process. Hide washing did not have any effect in reducing *E. coli* O157 and non-O157 prevalence and aerobic colony counts on hides, questioning the usefulness and practicality of hide water wash as a standalone intervention. Other cattle hide interventions investigated under commercial abattoir conditions, such as shellac hide coating and chemical washes with cetylpyridinium chloride, sodium hydroxide and proprietary sanitiser, showed significant reduction in transfer of ACC and EBC to carcasses of up to 1 log. This result indicates the usefulness of cattle hide interventions to proactively reduce potential carcass contamination during subsequent dehiding process.

Final beef carcass wash using cold or warm water largely showed no evidence of a reduction in levels or prevalence of microorganisms on carcasses before chilling. Several commercial trials found that water washes did not change generic *E. coli* prevalence on washed beef carcasses and did not reduce aerobic colony counts on beef carcasses. Overall, across all five microorganisms, steam and hot water carcass pasteurisation had the largest potential impact on decreasing the prevalence and concentration of contaminated beef carcasses. Some of the most reliable and consistent results were generated for carcass pasteurisation treatments, as a standalone interventions or when followed on with acid wash or as a part of multiple hurdle system. When they are followed by dry chilling, the residual action on the carcasses over 24-72 hours of chilling is even more noticeable.

Controlled trials performed under commercial abattoir conditions found that lactic acid 4% washes significantly reduced generic *E. coli* prevalence on beef carcass sides post-chilling, and led to significant reduction in aerobic colony counts of >3 logs. However, there was a discrepancy in the results when comparing these reductions with those achieved when lactic acid sprays were used prior to chilling, which were considerably smaller. Nevertheless, when hot water or steam pasteurisation treatments were applied to carcases prior to lactic acid spray wash, the reduction of microorganisms increased. Trials with low heterogeneity performed under commercial abattoir conditions found that carcass pasteurisation with hot water or steam and subsequent lactic acid or peroxyacetic acid spray washes significantly reduced *E. coli* prevalence on beef carcass sides and showed reduction in aerobic colony counts of 1.4 logs.

Dry chilling following multiple slaughter line interventions under commercial abattoir conditions led to a significant reduction of *E. coli* prevalence on beef carcass sides and reductions of generic *E. coli* counts of 0.6 log and aerobic colony counts of around 2 logs, likely due to residual effect of acid interventions used before chilling. Dry chilling investigated under commercial abattoir conditions with no interventions applied in the pre-chill stage on the slaughter line, lead to significant reduction in aerobic colony counts of around 1 log. Water spray chilling showed inconsistent effect when investigated in commercial abattoir conditions.

Multiple interventions were investigated in several commercial trials, usually associated with high heterogeneity due to inherent differences between trials and multiple hurdle systems used. Multiple pasteurisation and acid interventions led to a significant reduction in generic *E. coli* prevalence on beef carcass sides, and aerobic colony counts and generic *E. coli* counts of around 2 logs.

There was limited amount of data available for meta-analysis of interventions effects for beef trim. Data on the interventions efficacy against pathogenic bacteria (*Salmonella* spp. and pathogenic *E. coli*) were mostly available from challenge trials conducted under laboratory or pilot plant conditions. Their efficacies were investigated using artificially inoculated bacteria and consequently the effects are likely exaggerated and would not reflect real life conditions that exist in abattoirs. Nevertheless, the results are useful to provide some indication of the relative efficacy of specific interventions.

Overall, there was a lack of large controlled trials conducted under commercial conditions, with sound study design and adequate reporting of intervention protocols. This was particularly the case with cattle hide interventions and multiple beef carcass interventions at slaughter, prior to dehiding to pre-fabrication stage.

1. Background and aims/objectives

The sale and consumption of burgers served less than thoroughly cooked (LTTC) and pink in the middle is a steadily increasing trend in the UK (FSA, 2015). Considering that the consumption of these products can be associated with an increased risk of exposure to *Escherichia coli* O157, other Shiga toxin-producing *E. coli* (STEC) and *Salmonella* spp., the Food Standards Agency (FSA) commissioned a critical literature review to understand this problem and ways of addressing it. The review assessed the significance of various interventions[1] to reduce the microbiological load in a minced beef production chain ("A critical literature review to assess the significance of intervention methods to reduce the microbiology load on beef through primary production", FSA project FS301044[2]), (Antic, 2019). The review used a systematic approach, and provided quantitative data on interventions' effectiveness against main hazards found in beef. The review concluded that the most promising interventions to reduce microbial load on beef were cattle hide interventions, carcass pasteurisation treatments and organic acid washes, as well as the sequential use of these.

However, the review was critical and observational in its nature and did not address differences in study designs and the consequences on the intervention efficacies between multiple identified studies. As a result, it could not provide a more robust estimate of interventions' effectiveness that would deliver more reliable conclusions for risk management decisions. A systematic literature review coupled with meta-analysis is one of the methods used to address high heterogeneity between experimental methods and results within a body of literature. This tool is used in food safety to measure intervention effectiveness with reduced bias and increased transparency.

The main aim of this study was to perform a meta-analysis of the efficacy of interventions applied during primary processing to reduce microbiological contamination in beef using data generated in previous project FS301044. There were two objectives of this study:

1. To perform a meta-analysis of the efficacy of interventions applied during primary processing to reduce microbiological contamination in beef
2. To make recommendations on the effectiveness (the quantifiable level of bacterial reduction) of specific interventions for beef that will inform the risk management decisions for further work

[1] **Interventions** are actions taken during beef processing to reduce microbial contamination of carcasses: for example, surface trimming or lactic acid wash

[2] **A critical literature review to assess the significance of intervention methods to reduce the microbiological load on beef**

2. Methods

2.1 Interventions selection and scope

The interventions in beef primary processing that were analysed in this report were identified in previous systematic review (FSA project FS301044) and included interventions from the pre-slaughter stage (lairage interventions) to post-abattoir stage (interventions for beef primals, subprimals and trim inclusive). More details about beef production processes at abattoir and post abattoir level and interventions are provided in Appendices G and H.

The population of interest included cattle, including their carcasses at processing, beef primal and subprimal cuts[3] and finished products (beef trim and ground/minced beef[4]). Also, population of interest included potential sources of beef contamination during processing (i.e. cattle hides, lairage environment surfaces and knives). Any interventions applied from cattle received in abattoir up to and inclusive interventions for beef primals, subprimals and trim were considered relevant. Relevant outcome measures for interventions were the effectiveness of each intervention in reducing log levels of indicator bacteria (aerobic colony counts, *Enterobacteriaceae* counts, and generic *E. coli* counts) and log levels of foodborne pathogens (primarily *E. coli* O157 and other non-O157 STEC serogroups and *Salmonella* spp.).

2.2 Search strategy and information sources

In addition to the search performed in project FS301044 on the 14th of September 2018 (covering the period 1996-2018), additional search was performed on the 4th of June 2020. The aim was to provide an update of previous systematic review and determine if any potentially eligible studies were published since the last database search. Hence, the updated search identified all relevant published data in the last 25 years (1996-2020). The search algorithm was modified to include relevant search terms for the interventions for relevant processing stages (Appendix A). The searches were implemented in the bibliographic databases Scopus and CAB Direct. No language restriction was imposed.

[3] A **primal cut** or cut of meat is a piece of meat initially separated from the carcass during fabrication. Examples of primals include the round, loin, rib, and chuck for beef. Each primal cut is then reduced into subprimal cuts. Individual portions derived from subprimal cuts are referred to as fabricated cuts.

[4] **Minced beef**: Boned beef that has been minced into fragments and contains less than 1% salt. In the case of beef minced meat produced from chilled meat, the requirements specified in the hygiene regulations are that it must be prepared: i) within no more than six days of animal slaughter or ii) within no more than 15 days from the date of slaughter of the animals in the case of boned, vacuum-packed beef and veal EC (2004) 'Commission Regulation (EC) No 853/2004 of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs', *Official Journal of the European Union L*, 47, 55-205.

Relevance screening and confirmation were performed according to the protocols described in the FS301044 report.

2.3 Risk-of-bias assessment

The Risk-of-bias (RoB) assessments was conducted for 259 primary research[5] articles prioritized in project FS301044 and 7 articles found during new updated search (266 in total). The RoB assessment was performed using pre-specified tool that was adapted to suit the needs of the topic and study designs, from the Cochrane Collaboration's recommended tools for randomized and non-randomized study designs (RoB 2, Appendix B) (McGuinness & Higgins, 2020; Sterne et al. 2019). Two reviewers conducted RoB assessment independently and any disagreements between them was resolved by the third reviewer. The tool is structured into five domains through which bias might be introduced into the results:

(1) bias arising from the randomization process;

(2) bias due to deviations from intended interventions;

(3) bias due to missing outcome data;

(4) bias in measurement of the outcome;

(5) bias in selection of the reported result.

The possible risk-of-bias judgements are: (1) Low risk of bias; (2) Some concerns; and (3) High risk of bias. More details and protocol are provided in Appendix B.

2.4 Data extraction

Only studies assessed to be at 'low' risk of bias were considered for detailed data extraction. The detailed protocol is presented in Appendix B. The data extraction tool included targeted questions about intervention (stage, category, specific and detailed description) and population terms, outcomes measured, comparison group(s) and intervention efficacy results. Data were first stratified by study design and conditions, and then into specific intervention categories that were defined based on the previous review, and finally by different outcome measures (*Salmonella* spp., STEC and indicator microorganisms).

All experimental and observational study designs[6] were considered for detailed data extraction (these include controlled trials, challenge trials and before-and-after trials, and

[5] **Primary research** is defined as original research during which authors generated and reported their own data.

[6] **Experimental study**: Each subject is assigned to a treated group or a control group before the start of the treatment. Lab trials are executed under highly controlled conditions. Field/commercial (abattoir) trials are executed under less controlled and more "real" conditions.

other observational studies). Therefore, all study designs measuring intervention efficacy through concentration (e.g. colony forming units 'CFU'/sample) and/or prevalence (presence or absence) of indicator or pathogenic microorganisms were considered.

Intervention application conditions were described as commercial (large or small) abattoirs and pilot plants (experiments using industrial equipment in non-industrial conditions), as well as research conducted under laboratory conditions as long as it was applied on specific target population (i.e. cattle hides, carcass meat, beef trim, ground/minced beef, tools/knives). The interventions were categorised into the three main stages of minced beef production chain: i) abattoir pre-slaughter (lairage interventions); ii) abattoir processing (slaughter and post-slaughter); and iii) post-abattoir processing. Also, they were presented as per ten broad intervention categories: i) lairage interventions (Lairage cleaning, Cattle handling in lairage and Hide cleanliness assessment); ii) abattoir processing (Cattle hide interventions, Knives sanitation, Standard processing procedures/GHP, Carcass interventions, Chilling and spray chilling and Multiple interventions); iii) post-abattoir processing (Post fabrication interventions for trim/ground beef). Where inadequate amount of data were presented in articles (for example with no measure of variability or when only reductions were reported), and no other extractable data were present in the text, articles were excluded from further analysis.

2.5 Random-effect meta-analysis and reporting

Data were first stratified by study design and conditions, and then grouped into specific interventions and finally by different outcome measures (*Salmonella* spp., STEC, aerobic colony counts, *Enterobacteriaceae* counts and generic *E. coli*). In regard to STEC, for the purpose of comparisons, all STEC serogroups were analysed and reported together where it was practical. This was also a practical necessity in the case of challenge trials where cocktails comprising *E. coli* O157 and non-O157 serogroups were used.

If there were less than three trials in a comparison group, their results were reported descriptively and in tabulated form. Comparison groups with three or more trials were eligible for meta-analysis. Data from mean difference studies were transformed to the lowest comparable $\log_{10}$/CFU unit (i.e. CFU/cm²). If this was not possible, non-transformable studies were excluded from the group and reported descriptively. Mean CFU/cm², CFU/100

Observational study: Assignment of subjects into a treated group versus a control group is outside the control of the investigator.
Controlled trial: Subjects are allocated to intervention/comparison groups and evaluated for outcomes (natural pathogen exposure).
Challenge trial: Similar to controlled, but subjects are artificially challenged or exposed to the disease agent and then allocated to the intervention groups for evaluation of the outcome (artificial pathogen exposure).
Before-and-after trial: Observations (for intervention outcome) are made on a population before and after receiving an intervention.

cm^2 (or other) and respective standard deviations (SD) or SE (usually presented on the log scale), were extracted from the studies measuring the concentration outcomes and the standardized mean difference (SD) were calculated. When only a pooled standard error of the mean (SEM) was reported, a pooled SD was calculated.

In the groups analysed under meta-analysis, random-effects models were used to calculate pooled summary statistics. These were either pooled risk ratios (RR), for prevalence outcomes, or pooled log mean difference, for concentration outcomes. Random-effects models were used because they assume that there was variance in effect size between studies, as studies were not conducted on the same populations. Using random-effects models will lead to more conservative models and resultant summary statistics. These models reflect the assumption that the effects measured in each study are not identical, but follow the same distribution. For example, the studies may be performed in different abattoirs, or that hot water washes are used for different lengths of time or at different temperatures. The resultant summary statistics therefore represent the weighted average of the effect generated by each specific intervention. Thus, they can be used to describe trends and patterns in groups of papers where there is a high degree of heterogeneity.

A Mantel-Haenszel method was used for the random-effects models based on prevalence studies, whilst an inverse-variance method was used for mean difference studies. An RR of 1.0 indicates that there is no difference in risk between the groups being compared. An RR > 1.0 indicates an increase in risk among the treated compared to the untreated, whereas a RR <1.0 indicates a decrease in risk in the treated group. As for other summary statistics, confidence intervals were also calculated for RR. Weights in the random-effects meta-analysis were based on the size of each study (ie number of observations).

To summarise the meta-analyses and their associated summary statistics and heterogeneity measures, Forest plots were created. Variability between studies is described as heterogeneity, this can be due to differences in actual samples themselves, the study design and methodology, the study location, and the levels of bias within the study. This results in the intervention effects being more different between studies than expected through chance. Heterogeneity can statistically tested for each meta-analysis to show how similar the studies are too each. The more homogenous the studies, the higher the likelihood that the summary effect size calculated reflects the true nature of the intervention. Heterogeneity was assessed using I^2, which measures the percentage of variability in the effect size, which is not result of sampling error. If I^2 values are <25% there are low levels of heterogeneity, if they are 25-50% there are moderate levels of heterogeneity, and if they are >75% there are substantial levels of heterogeneity. A test for heterogeneity was performed (Cochran's Q-Statistic), which evaluates the null hypothesis that all studies evaluate the same effect. Resultant p-values are presented; values less than 0.05 showed that studies are significantly heterogeneous. The between study-variance (τ^2) was calculated using the Sidik-Jonkman method, this methodology is recommended when the between-

study variance is large, and when a small number of studies are being compared. By examining these three measures, the degree of homogeneity, and therefore comparability, of the studies can be drawn.

The resultant Forest plots are split into three groups. Those that were homogenous ($p>0.05$ on the test for heterogeneity), those that were moderately heterogeneous ($p<0.05$, $I^2<=60\%$), and those that were highly heterogeneous ($p<0.05$, $I^2>60\%$). Only the meta-analyses with results in the first group are presented in the following sections grouped as per different Intervention Category, and then specific interventions. The remaining forest plots and the results from studies with no direct comparisons can be found in the Appendix D.

All statistical analyses were carried out using R language (version 3.2.0) (R Core Team 2015), using the packages Tidyverse, meta, and metafor. Results were deemed significant where $p < 0.05$.

2.6 References

Antic, D. (2019). A critical literature review to assess the significance of intervention methods to reduce the microbiological load on beef through primary production. FSA Project FS301044 report.

FSA (2015). Development of the framework for controls relating to foods where risks per serving are significant, and its further application to burgers served rare in catering outlets. Report 15/09/04. Food Standards Agency, London, UK.

McGuinness, L. A., & Higgins, J. P. T. (2020). Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Research Synthesis Methods, n/a(n/a)

Sterne, J. A., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., . . . Eldridge, S. M. (2019). RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ, 366, 14898.

3. Results

3.1 Risk-of-bias assessment

Key characteristics of 266 relevant articles for beef interventions at processing that entered first stage, risk-of-bias assessment, are shown in Table 1.

Table 1. Key characteristics of relevant primary research articles on beef interventions in minced beef production chain

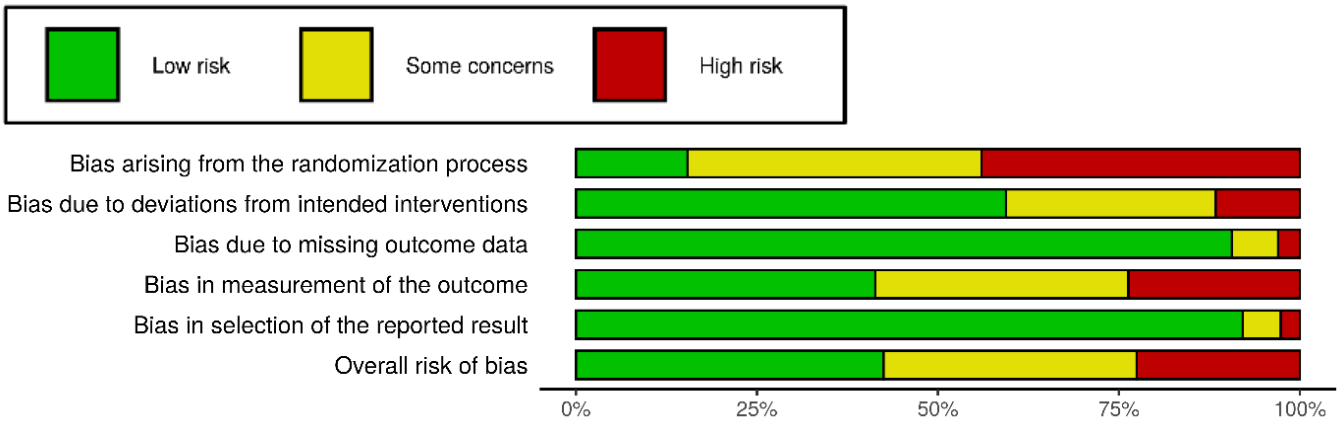
Article characteristic	**Number of articles***	**%**
Region		
North America	187	70.3%
Europe	54	20.3%
Australia/South Pacific	9	3.4%
Asia/Middle East	6	2.2%
Central and South America/Caribbean	8	3.0%
Africa	2	0.8%
Document type		
Journal article	254	95.5%
Thesis	7	2.6%
Conference paper	2	0.8%
Government or research report	3	1.1%
Study design		
Challenge trial	143	53.8%
Before-and-after trial	87	32.7%
Controlled trial	36	13.5%
Observational study	18	6.8%
Study conditions		
Laboratory conditions	124	46.6%
Commercial abattoir conditions	115	43.2%
Research/pilot plant	39	14.7%
Intervention stage/category		
Lairage cleaning	4	1.5%
Cattle handling in lairage	7	2.6%
Hide cleanliness assessment	5	1.9%

Article characteristic	**Number of articles***	**%**
Cattle hide interventions (pre- exsanguination)	8	3.0%
Cattle hide interventions (post- exsanguination)	34	12.8%
Cleaning/disinfection of tools/knives	10	3.8%
Standard processing procedures/GHP	13	4.9%
Carcass interventions (pre- and post- evisceration, pre-chill)	92	34.6%
Chilling and spray chilling	38	14.3%
Post chill and pre-fabrication carcass treatments	10	3.8%
Multiple interventions	20	7.5%
Post fabrication interventions (trim/ground beef)	51	19.2%
Outcomes investigated		
Pathogenic *E. coli*	143	53.8%
Salmonella	111	41.8%
Aerobic colony counts	138	51.9%
Generic *E. coli* counts	99	37.2%
Enterobacteriaceae counts	47	17.7%
Risk-of-bias results		
Low‡	113	42.5%
Some concerns	93	35%
High	60	22.5%

* The total number of articles per category not necessarily equals to 266 as one article often reports on the study conducted in more than one study condition, intervention stage/category, using different study designs and investigating different outcomes.
‡ In addition, seven articles judged to be at 'low' RoB only for some parts of the study

The results from the Risk-of-bias (RoB) assessment process for 266 articles are presented in Figure 1, in the form of weighted bar plots of the distribution of risk-of-bias judgements within each bias domain. More detailed RoB results for 14 distinctive groups of intervention categories and study designs combinations are presented in Appendix C.

Figure 1. Distribution of RoB judgements within each bias domain for all 266 studies investigating beef interventions



In total, 266 relevant articles entered RoB assessment, with 113 judged to be at 'low' RoB, and seven judged to be at 'low' RoB only for some parts of the study (i.e. where some limited data could be extracted for further analysis).

However, out of 120 articles fully or partially judged to be at 'low' RoB, further analysis showed that only 68 articles (56.7%) had extractable data that could be used for meta-analysis, while 52 articles (43.3%) were excluded from meta-analyses, with reasons provided in Table 2.

Table 2. Reasons for further exclusion of articles with low risk of bias from meta-analysis

Reason for exclusion	**Number of articles***	**%**
Study design		
Challenge trial	39	70.9
Before-and-after trial	7	12.7
Controlled trial	8	14.5
Observational study	1	1.8
Study conditions		
Laboratory conditions	28	51.9
Commercial abattoir conditions	8	14.8
Research/pilot plant	18	33.3
Intervention stage/category		
Lairage cleaning	0	0
Cattle handling in lairage	0	0
Hide cleanliness assessment	0	0
Cattle hide interventions (pre- exsanguination)	0	0
Cattle hide interventions (post- exsanguination)	4	7.1
Cleaning/disinfection of tools/knives	4	7.1
Standard processing procedures/GHP	1	1.8
Carcass interventions (pre- and post- evisceration, pre-chill)	25	44.6
Chilling and spray chilling	5	8.9
Post chill and pre-fabrication carcass treatments	2	3.6
Multiple interventions	1	1.8
Post fabrication interventions (trim/ground beef)	14	25
Outcomes investigated		
Pathogenic *E. coli*	34	29.3
Salmonella	27	23.3
Aerobic colony counts	27	23.3
Generic *E. coli* counts	20	17.2
Enterobacteriaceae counts	8	6.9
Reasons for exclusion		
No measure of variability	25	37.3
Graphical data only	12	17.9
Only reductions reported	19	28.4
Insufficient data reported	8	11.9
Other[ł]	3	4.5

* The total number of articles per category not necessarily equals to 120 as one article often reports on the study conducted in more than one study condition, intervention stage/category, using different study designs and investigating different outcomes.

ł Reasons include one each of other unit of outcome measurement (BOIF (bacterial output/bacterial input) factor); only two replications used in pilot plant trial; and values too low for calculation

3.2 Random-effects meta-analysis

The results from the data analysis of 68 articles are presented in several possible ways, per different Intervention Category (IC), and then specific interventions:

- Results from the studies where there were less than three trials in a comparison group, are reported descriptively in the text and shown in tabulated form and forest plots in Appendix D
    - If there was only one trial per comparison group, the result is shown in tables
    - If there were two trials per comparison group, the forest plot is generated for comparison purposes, but no summary effect of intervention was calculated (in total, 36 forest plots)
- Results from studies in comparison groups with three or more trials in a comparison group were analysed under meta-analysis, and random-effects models were used to calculate pooled summary effects ('the diamond', which represents the point estimate and confidence intervals of all the studies combined using the random effects model). These were either pooled risk ratios (RR), for prevalence outcomes, or pooled log mean difference, for concentration outcomes
    - If the results from the studies were homogenous ($p>0.05$ on the test for heterogeneity), the resulting forest plots are presented in the text in following sections (in total, 33 forest plots)
    - If the results from the studies were moderately heterogeneous ($p<0.05$, $I^2<=60\%$), or highly heterogeneous ($p<0.05$, $I^2>60\%$), the resulting forest plots can be found in the Appendix D (in total, 69 forest plots).

In addition, the key findings are outlined below and presented in tabulated form in this chapter (Table 3).

The only lairage intervention for which there was sufficient data for meta-analysis was cattle hide cleanliness assessment. This procedure is a visual scoring and categorisation of animals according to the cleanliness of their hides, which can lead to subsequent actions (or other interventions) in case animals are too dirty. The summary effects from meta-analysis show consistent reduction for all indicator microorganisms (aerobic colony counts, *Enterobacteriaceae* counts and generic *E. coli*) when clean cattle are compared to dirty cattle, on hides and resulting carcasses. Mean reductions on carcass surfaces were 0.9 $\log_{10}$ CFU/cm² for aerobic colony counts, 0.71 $\log_{10}$ CFU/cm² for *Enterobacteriaceae* counts, and 0.75 $\log_{10}$ CFU/cm² generic *E. coli*.

There was also limited data for meta-analysis of cattle hide interventions. Hide washing did not have any effect in reducing *E. coli* O157 and non-O157 prevalence (RR 0.85) and aerobic colony counts on hides (statistically not significant reduction of 0.6 $\log_{10}$ CFU/100 cm²), questioning the usefulness and practicality of hide water wash as a standalone intervention.

Studies investigating cattle hide shellac coating showed promising reduction effects in reducing aerobic colony counts and *Enterobacteriaceae* on resulting beef carcass under commercial abattoir conditions. The reduction in transfer was 1.07 $\log_{10}$ CFU/cm^2 for aerobic colony counts and 0.59 $\log_{10}$ CFU/cm^2 for *Enterobacteriaceae* counts. However, due to small number of trials and high heterogeneity between studies, the effect was not statistically significant and more research is needed. Overall cattle hide interventions, such as shellac hide coating and chemical washes with cetylpyridinium chloride, sodium hydroxide and proprietary sanitiser, under commercial abattoir conditions, showed significant reduction in transfer of aerobic colony counts and *Enterobacteriaceae* counts to carcasses of 1.09 $\log_{10}$ CFU/cm² and 0.81 $\log_{10}$ CFU/cm², respectively.

Final beef carcass wash using cold or warm water largely showed no evidence of a reduction in levels or prevalence of microorganisms on carcasses before chilling. Several commercial trials found that water washes did not change generic *E. coli* prevalence on washed beef carcasses (RR 0.88) and did not reduce aerobic colony counts on beef carcasses (0.05 $\log_{10}$ CFU/cm²). Hot water wash led to a significant reduction in generic *E. coli* on beef carcasses, 0.59 $\log_{10}$ CFU/cm² in trials performed under commercial abattoir conditions. Similarly, in commercial studies, hot water washes reduced the prevalence of generic *E. coli* (RR 0.32) and aerobic colony counts (1.58 $\log_{10}$ CFU/cm²).

Steam pasteurisation was the intervention where significant amount of data was generated from both commercial and laboratory condition trials, mostly with low heterogeneity between studies. Studies performed under commercial abattoir conditions found that steam pasteurisation significantly reduced generic *E. coli* and *Enterobacteriaceae* prevalence on beef carcass sides (RR 0.15 and 0.17, respectively). On the other hand, only one controlled trial study in commercial abattoir conditions showed no evidence of a reduction in aerobic colony counts (0.32 $\log_{10}$ CFU/1000cm²) and generic *E. coli* counts (0.22 $\log_{10}$ CFU/1000cm²), but three trials showed a reduction in *Enterobacteriaceae* counts (0.89 $\log_{10}$ CFU/1000cm²). Trials with high heterogeneity performed under commercial abattoir conditions showed reduction of aerobic colony counts of 1.14 $\log_{10}$ CFU/cm², generic *E. coli* counts of 0.54 $\log_{10}$ CFU/cm² and *Enterobacteriaceae* counts of 1.04 $\log_{10}$ CFU/cm².

Three controlled trials performed under commercial abattoir conditions found that lactic acid 4% washes significantly reduced generic *E. coli* prevalence on beef carcass sides post-chilling (RR 0.02), and led to significant reduction in aerobic colony counts of 3.16 $\log_{10}$ CFU/100cm², with low heterogeneity between trials. Similarly, abattoir before-and-after trials showed reductions of aerobic colony counts of 0.62 $\log_{10}$ CFU/cm², with high heterogeneity. However, lactic acid pre-chill wash showed no evidence of a reduction in generic *E. coli* prevalence (RR 0.93) and numbers (0.63 $\log_{10}$ CFU/cm²) also with high heterogeneity between trials.

When hot water or steam pasteurisation treatments were applied to carcases prior to lactic acid spray wash, the reduction of microorganisms increased. Trials with low heterogeneity

performed under commercial abattoir conditions found that carcass pasteurisation with hot water or steam and subsequent lactic acid or peroxyacetic acid spray washes significantly reduced *E. coli* prevalence on beef carcass sides (RR 0.01). Similarly, summary effects from studies with high heterogeneity under commercial conditions, showed reduction in aerobic colony counts of 1.41 $\log_{10}$ CFU/cm².

Dry chilling following multiple slaughter line interventions under commercial abattoir conditions led to a significant reduction of *E. coli* prevalence on beef carcass sides (RR 0.07) with low heterogeneity. In studies showing high heterogeneity, reductions of generic *E. coli* counts (0.60 $\log_{10}$ CFU/cm²) and aerobic colony counts (2.09 $\log_{10}$ CFU/cm²) were found. The increased reduction in these trials was also likely due to residual effect of acid interventions used before chilling. Dry chilling investigated under commercial abattoir condition with no interventions applied in the pre-chill stage on the slaughter line, lead to significant reduction in aerobic colony counts of 1.11 $\log_{10}$ CFU/cm². Water spray chilling showed inconsistent effect when investigated in commercial abattoir conditions. Trials with low heterogeneity performed under commercial abattoir conditions, found that water spray chilling, when compared to conventional dry chilling, led to a small but significant 0.38 $\log_{10}$ CFU/cm² reduction in aerobic colony counts on beef carcass sides. However, there was no evidence of a reduction of generic *E. coli* prevalence (RR 0.67) and aerobic colony counts when comparing to before treatment (0.44 $\log_{10}$ CFU/cm²).

Multiple interventions were investigated in several commercial trials, usually associated with high heterogeneity due to inherent differences between trials and multiple hurdle systems used. Multiple pasteurisation and acid interventions led to a significant reduction in generic *E. coli* prevalence on beef carcass sides (RR 0.30), aerobic colony counts (1.92 $\log_{10}$ CFU/cm²) and generic *E. coli* counts (2.41 $\log_{10}$ CFU/cm²).

There was limited amount of data available for meta-analysis of interventions effects for beef trim. Challenge trials performed under laboratory conditions found that sodium metasilicate dipping led to a significant 1.04 $\log_{10}$ CFU/cm² reduction in *E. coli* O157:H7 and non-O157 numbers on beef trim when compared to water dipping. Similarly, laboratory challenge trials found that lactic acid and sodium metasilicate dipping led to a significant 1.30 $\log_{10}$ CFU/cm² and 1.28 $\log_{10}$ CFU/cm² reduction in *Salmonella* spp. numbers on beef trim when compared to water dipping, respectively.

Table 3. A summary of overall meta-analysis estimates of interventions' effects

Intervention	**Outcome (micro-organism)[a]**	**Study design and conditions (No. of studies/trials)[‡]**	**RR (95% CI) or MD (95% CI)**	**Heterogeneity, I^2 (%)**
Hide cleanliness assessment*	ACC	Obs/Comm (4/20)	MD -0.90 (-1.26, -0.54)	High (88.4%)

Intervention	Outcome (micro-organism)[a]	Study design and conditions (No. of studies/trials)[‡]	RR (95% CI) or MD (95% CI)	Heterogeneity, I^2 (%)
Hide cleanliness assessment*	EBC	Obs/Comm (2/10)	MD -0.71 (-1.05, -0.36)	High (74%)
Hide cleanliness assessment*	Generic *E. coli*	Obs/Comm (1/6)	MD -0.75 (-0.65, -0.85)	Low (0%)
Hide cleanliness assessment	ACC	Obs/Comm (2/10)	MD -1.68 (-2.36, -1.01)	High (95.9%)
Hide cleanliness assessment	EBC	Obs/Comm (2/10)	MD -1.33 (-1.87, -0.79)	High (92.3%)
Hide cleanliness assessment	Generic *E. coli*	Obs/Comm (1/6)	MD -1.51 (-1.94, -1.08)	High (75.6%)
Overall hide interventions effect on carcass*	ACC	CT/Comm (4/6)	MD -1.09 (-1.53, -0.65)	High (100%)
Overall hide interventions effect on carcass*	EBC	CT/Comm (4/6)	MD -0.81 (-1.35, -0.28)	High (93%)
Hide water wash	ACC	BA/Comm (3/4)	MD -0.60 (-1.22, 0.02)	High (99.7%)
Hide water wash	EBC	BA/Comm (2/3)	MD -1.77 (-5.50, -1.96)	High (99.9%)
Hide water wash	*E. coli* O157 and non-O157	BA/Comm (4/6)	RR 0.85 (0.66, 1.09)	High (85%)
Hide chlorine wash	*E. coli* O157 and non-O157	BA/Comm (1/5)	RR 0.96 (0.60, 1.54)	Low (0%)
Hide chlorine wash	*Salmonella* spp.	ChT/Lab (1/3)	MD -0.85 (-1.78, 0.08)	Low (0%)
Hide ethanol wash	*Salmonella* spp.	ChT/Lab (1/3)	MD -5.16 (-5.73, -4.59)	Low (0%)
Hide lactic acid wash	*Salmonella* spp.	ChT/Lab (1/3)	MD -3.25 (-8.13, -1.62)	High (83.8%)
Hide acetic acid wash	*Salmonella* spp.	ChT/Lab (1/3)	MD -3.60 (-6.87, -0.33)	High (68%)
Hide sanitiser wash*	ACC	CT/Lab (1/3)	MD -1.09 (-3.79, -1.61)	High (91.7%)
Hide sanitiser wash*	EBC	CT/Lab (1/3)	MD -1.86 (-2.93, -0.80)	Medium (52.7%)
Hide sanitiser wash*	Generic *E. coli*	CT/Lab (1/3)	MD -1.51 (-1.89, -1.13)	Low (0%)
Shellac hide coating*	ACC	CT/Comm (2/3)	MD -1.09 (-2.43, 0.29)	High (85.7%)
Shellac hide coating*	EBC	CT/Comm (2/3)	MD -0.59 (-2.22, 1.05)	High (85.1%)
Shellac in ethanol hide coating*	ACC	CT/Lab (2/5)	MD -2.47 (-3.49, -1.45)	High (83.4%)
Shellac in ethanol hide coating*	EBC	CT/Lab (2/5)	MD -2.12 (-3.12, -1.13)	High (88.2%)

Intervention	Outcome (micro-organism)[a]	Study design and conditions (No. of studies/trials)[‡]	RR (95% CI) or MD (95% CI)	Heterogeneity, I^2 (%)
Shellac in ethanol hide coating*	Generic *E. coli*	CT/Lab (1/3)	MD -1.37 (-2.20, -0.54)	Low (0%)
Aqueous shellac hide coating*	ACC	CT/Lab (1/7)	MD -2.02 (-2.70, -1.35)	High (87.7%)
Aqueous shellac hide coating*	EBC	CT/Lab (1/7)	MD -1.68 (-1.99, -1.38)	Low (0%)
Carcass water wash	ACC	BA/Comm (2/6)	MD 0.05 (-0.37, 0.47)	High (80%)
Carcass water wash	Generic *E. coli*	BA/Comm (1/5)	RR 0.88 (0.44, 1.79)	Low (14.7%)
Carcass water wash	*E. coli*	ChT/Lab (2/3)	MD -2.33 (-3.13, -1.53)	High (72.1%)
Carcass water wash	*E. coli* O157:H7	ChT/Lab (2/5)	MD -1.19 (-1.51, -0.88)	High (100%)
Carcass knife trimming	ACC	CT/Comm (1/7)	MD -1.47 (-1.86, -1.09)	High (70.9%)
Carcass hot water wash	ACC	BA/Comm (5/11)	MD -1.58 (-1.95, -1.21)	High (100%)
Carcass hot water wash	ACC	ChT/Comm (2/3)	MD -1.26 (-6.08, 3.55)	High (99.6%)
Carcass hot water wash	Generic *E. coli*	BA/Comm (2/8)	RR 0.32 (0.17, 0.58)	High (69%)
Carcass hot water wash	Generic *E. coli*	BA/Comm (2/6)	MD -0.59 (-0.76, -0.42)	Low (0%)
Carcass hot water wash	*E. coli*	ChT/Lab (2/5)	MD -3.29 (-3.93, -2.64)	High (80.1%)
Carcass hot water wash	*E. coli* O157:H7	ChT/Lab (2/4)	MD -4.21 (-5.25, -3.17)	High (100%)
Carcass steam vacuuming (no contamination)	ACC	CT/Comm (1/7)	MD -0.61 (-0.89, -0.32)	Medium (60%)
Carcass steam vacuuming (visible contamination)	ACC	CT/Comm (1/7)	MD -1.84 (-2.17, -1.50)	Medium (60.6%)
Carcass steam vacuuming	ACC	BA/Comm (2/7)	MD -0.51 (-0.70, -0.32)	Medium (58.9%)
Carcass steam vacuuming	Generic *E. coli*	BA/Comm (1/3)	MD -0.45 (-1.17, 0.27)	Low (9.7%)
Carcass steam pasteurisation	ACC	BA/Comm (4/11)	MD -1.14 (-1.35, -0.93)	High (100%)
Carcass steam pasteurisation	ACC	CT/Comm (1/3)	MD -0.32 (-0.84, 0.21)	Medium (59.4%)

Intervention	Outcome (micro-organism)[a]	Study design and conditions (No. of studies/trials)[‡]	RR (95% CI) or MD (95% CI)	Heterogeneity, I^2 (%)
Carcass steam pasteurisation	EBC	BA/Comm (1/5)	MD -1.04 (-1.48, -0.60)	High (84.4%)
Carcass steam pasteurisation	EBC	BA/Comm (2/10)	RR 0.17 (0.07, 0.43)	Low (23.8%)
Carcass steam pasteurisation	EBC	CT/Comm (1/3)	MD -0.89 (-1.10, -0.67)	Low (0%)
Carcass steam pasteurisation	Generic *E. coli*	BA/Comm (4/12)	RR 0.15 (0.09, 0.26)	Low (0%)
Carcass steam pasteurisation	Generic *E. coli*	BA/Comm (2/6)	MD -0.54 (-0.73, -0.34)	High (91.7%)
Carcass steam pasteurisation	Generic *E. coli*	CT/Comm (1/3)	MD -0.22 (-0.80, 0.35)	Medium (67.4%)
Carcass lactic acid wash	ACC	BA/Comm (3/9)	MD -0.62 (-1.08, -0.17)	High (100%)
Carcass lactic acid wash	Generic *E. coli*	BA/Comm (1/5)	RR 0.93 (0.42, 2.07)	High (69.1%)
Carcass lactic acid wash	Generic *E. coli*	BA/Comm (1/3)	MD -0.63 (-1.89, 0.62)	High (97.1%)
Carcass lactic acid wash	*E. coli*	ChT/Lab (1/3)	MD -1.03 (-1.97, -0.09)	High (76.4%)
Carcass lactic acid wash	*E. coli* O157 and non-O157	ChT/Lab (1/3)	MD -0.72 (-1.16, -0.28)	Low (0%)
Carcass lactic and citric acid wash	*E. coli*	ChT/Lab (1/3)	MD -0.30 (-0.39, -0.21)	Low (0%)
Carcass lactic and citric acid wash	*E. coli* O157 and non-O157	ChT/Lab (2/6)	MD -0.53 (-1.10, 0.05)	High (94%)
Carcass lactic acid wash (post-chill)	ACC	CT/Comm (1/3)	MD -3.16 (-3.56, -2.75)	Low (0%)
Carcass lactic acid wash (post-chill)	Generic *E. coli*	CT/Comm (1/3)	RR 0.02 (0, 0.14)	Low (0%)
Carcass various acid spray washes	ACC	BA/Comm (1/5)	MD -0.42 (-0.81, -0.03)	High (86.9%)
Carcass various acid spray washes	Generic *E. coli*	BA/Comm (1/5)	MD -0.36 (-0.66, -0.06)	High (78.8%)
Carcass sulfuric acid and sodium sulfate spray wash	*Salmonella* spp.	ChT/Lab (1/3)	MD -1.50 (-3.05, 0.05)	High (94.9%)
Carcass pasteurisation and acid spray wash	ACC	BA/Comm (3/5)	MD -1.41 (-2.10, -0.72)	High (97.2%)

Intervention	Outcome (micro-organism)[a]	Study design and conditions (No. of studies/trials)[‡]	RR (95% CI) or MD (95% CI)	Heterogeneity, I^2 (%)
Carcass pasteurisation and acid spray wash	Generic *E. coli*	BA/Comm (1/3)	RR 0.01 (0, 0.03)	Low (0%)
Carcass dry chilling following multiple interventions	ACC	BA/Comm (1/8)	MD -2.09 (-2.78, -1.40)	High (94.8%)
Carcass dry chilling following multiple interventions	Generic *E. coli*	BA/Comm (1/8)	MD -0.60 (-1.13, -0.08)	High (98.7%)
Carcass dry chilling following multiple interventions	Generic *E. coli*	BA/Comm (2/9)	RR 0.07 (0.03, 0.16)	Low (6.6%)
Carcass dry chilling up to 24 h	ACC	BA/Comm (2/9)	MD -1.11 (-1.58, -0.63)	High (93.5%)
Carcass dry chilling up to 24 h	*E. coli* O157:H7	ChT/Lab (4/14)	MD -1.04 (-1.37, -0.70)	Low (39.2%)
Carcass dry chilling up to 48 h	*E. coli* O157:H7	ChT/Lab (3/12)	MD -1.29 (-1.65, -0.94)	Low (37.9%)
Carcass dry chilling up to 72 h	*E. coli* O157:H7	ChT/Lab (2/11)	MD -1.54 (-1.99, -1.09)	Low (31.3%)
Carcass dry chilling up to 24 h	*Salmonella* spp.	ChT/Lab (3/8)	MD -0.24 (-0.56, 0.08)	High (62.3%)
Carcass dry chilling up to 48 h	*Salmonella* spp.	ChT/Lab (2/8)	MD -0.63 (-1.11, -0.15)	High (81.4%)
Carcass dry chilling up to 72 h	*Salmonella* spp.	ChT/Lab (1/6)	MD -0.53 (-1.16, -0.11)	High (86%)
Carcass dry chilling following chemical washes	*E. coli* O157:H7	ChT/Lab (2/14)	MD -2.86 (-3.33, -2.39)	High (82.4%)
Carcass dry chilling following chemical washes	*Salmonella* spp.	ChT/Lab (1/8)	MD -3.48 (-4.04, -2.92)	High (70.8%)
Carcass dry aging up to 14 days	*E. coli*	ChT/Lab (1/4)	MD -3.66 (-4.22, -2.89)	Low (0%)
Carcass dry aging up to 14 days	*E. coli* O157:H7	ChT/Lab (2/6)	MD -3.91 (-4.83, -3.00)	High (90.7%)
Carcass water spray chilling	ACC	BA/Comm (6/17)	MD -0.44 (-1.06, 0.19)	High (96.2%)
Carcass water spray chilling (vs. dry chill)	ACC	CT/Comm (1/8)	MD -0.38 (-0.59, -0.16)	Low (0%)

Intervention	Outcome (micro-organism)[a]	Study design and conditions (No. of studies/trials)[‡]	RR (95% CI) or MD (95% CI)	Heterogeneity, I^2 (%)
Carcass water spray chilling	Generic *E. coli*	BA/Comm (4/8)	RR 0.67 (0.29, 1.54)	High (63.9%)
Carcass water spray chilling	*E. coli*	ChT/Lab (1/4)	MD -0.58 (-0.94, -0.23)	Low (53.5%)
Carcass water spray chilling	*E. coli* O157:H7	ChT/Lab (3/7)	MD -1.46 (-2.06, -0.86)	High (95.3%)
Carcass water spray chilling (vs. dry chill)	*E. coli* O157:H7	ChT/Lab (1/3)	MD -0.49 (-1.14, 0.16)	Low (30.6%)
Carcass water spray chilling	*Salmonella* spp.	ChT/Lab (2/7)	MD -1.44 (-1.86, -1.02)	High (88.9%)
Carcass water spray chilling (vs. dry chill)	*Salmonella* spp.	ChT/Lab (1/3)	MD -0.31 (-0.44, -0.17)	Low (0%)
Carcass spray chilling with chemicals	*E. coli*	ChT/Lab (1/8)	MD -2.40 (-3.85, -0.94)	High (99.8%)
Carcass spray chilling with chemicals (vs. water spray chilling)	*E. coli*	ChT/Lab (1/8)	MD -1.85 (-3.12, -0.58)	High (99.4%)
Carcass spray chilling with chemicals	*Salmonella* spp.	ChT/Lab (1/8)	MD -2.28 (-3.62, -0.94)	High (99.8%)
Carcass spray chilling with chemicals (vs. water spray chilling)	*Salmonella* spp.	ChT/Lab (1/8)	MD -0.96 (-1.94, 0.03)	High (99.8%)
Carcass spray chilling with chemicals	*E. coli* O157:H7	ChT/Lab (2/16)	MD -2.85 (-3.57, -2.13)	High (98.4%)
Carcass spray chilling with chemicals (vs. water spray chilling)	*E. coli* O157:H7	ChT/Lab (2/16)	MD -1.93 (-2.65, -1.21)	High (99.2%)
Carcass multiple pasteurisation and acid interventions	ACC	BA/Comm (4/13)	MD -1.92 (-2.33, -1.52)	High (100%)
Carcass multiple pasteurisation and acid interventions	Generic *E. coli*	BA/Comm (3/9)	MD -2.41 (-3.32, -1.49)	High (97.5%)
Carcass multiple pasteurisation and acid interventions	Generic *E. coli*	BA/Comm (3/12)	RR 0.30 (0.16, 0.59)	High (92.4%)
Beef trim lactic acid dipping (vs. water dipping)	*E. coli* O157 and non-O157	ChT/Lab (1/8)	MD -0.88 (-1.26, -0.51)	High (68.2%)

Intervention	**Outcome (micro-organism)[a]**	**Study design and conditions (No. of studies/trials)[‡]**	**RR (95% CI) or MD (95% CI)**	**Heterogeneity, I^2 (%)**
Beef trim lactic acid dipping (vs. water dipping)	*Salmonella* spp.	ChT/Lab (1/4)	MD -1.30 (-2.42, -0.18)	Low (50.2%)
Beef trim sodium metasilicate dipping (vs. water dipping)	*E. coli* O157 and non-O157	ChT/Lab (1/8)	MD -1.04 (-1.27, -0.81)	Low (16.2%)
Beef trim sodium metasilicate dipping (vs. water dipping)	*Salmonella* spp.	ChT/Lab (1/4)	MD -1.28 (-2.43, -0.14)	Low (52.3%)
Beef trim peroxyacetic acid dipping	*E. coli* O157:H7	ChT/Lab (2/6)	MD -1.06 (-1.49, -0.62)	High (86.2%)
Beef trim peroxyacetic acid dipping	*Salmonella* spp.	ChT/Lab (2/6)	MD -0.85 (-1.12, -0.58)	High (66.4%)
Beef trim various chemicals dipping	*E. coli* O157:H7	ChT/Lab (1/3)	MD -1.12 (-2.53, -0.30)	High (98%)
Beef trim various chemicals dipping	*Salmonella* spp.	ChT/Lab (1/3)	MD -1.42 (-3.65, -0.81)	High (96.2%)

[‡] CT-controlled trial; BA-before-and-after trial; ChT-challenge trial; Obs-observational study; Comm-commercial abattoir conditions; Lab-laboratory conditions

* Reduction in hide-to-carcass/beef cuts transfer ('carcass effect')

[a] ACC-aerobic colony counts; EBC-*Enterobacteriaceae* counts

3.3 Interventions description

Cattle hide cleanliness assessment: refers to the scoring and categorisation of hide cleanliness before cattle slaughter (usually in the lairage[7]) according to the established objective system, and actions taken in case animals are too dirty to be processed hygienically.

Cattle hide clipping: refers to clipping or shaving hair from the hide surface to physically remove contamination from hides.

Cattle hide microbial immobilisation treatments ('cattle hide shellac coating'): refers to a spray treatment of cattle hides with natural resin shellac, to form a protective coating as a barrier to microorganisms and the reduction in their transfer to beef carcasses.

Cattle hide chemical dehairing: process of applying successive water and chemical washes (sodium sulphide followed by a neutralizing solution of hydrogen peroxide) in a cabinet to remove hair, improve visible cleanliness, and reduce microbial loads on animal hides.

Water wash: refers to an ambient or cold-temperature wash to physically remove contamination from hides or carcasses. Warm water wash (usually <60 °C) have a similar effect in removing bacteria (depending on the pressure used), and when applied for a short time does not have a microbicidal effect.

Thermal treatments: refers to various heat treatment washes to destroy microbial cells. Examples include scalding bob-veal hide-on carcasses, hot water wash and treatments with steam.

Scalding: usually used for bob-veal hide-on carcasses (water usually >60°C).

Hot water wash: refers to washing carcasses with water at temperatures >74 °C, up to 85 °C.

Steam vacuuming: spot application of steam and/or hot water (usually >82 °C) to loosen contamination and kill bacteria, followed by a vacuuming.

Steam pasteurisation: Steam (water at temperatures over 74 °C, usually >82 °C, up to 105 °C) applied to a whole beef carcass in a closed cabinet. Method involves: i) removal of water from carcass side surfaces, which remains after post-evisceration washing, using air blowers or vacuum; ii) surface ''pasteurisation'' with pressurized steam (6.5–10 s); and iii) a cold-water spray to cool down carcass surfaces before they are moved to chillers.

[7] Lairage refers to holding facilities (pens, yards and other holding areas) used for accommodating animals in order to give them necessary attention (such as water, feed, rest) before they are moved on or used for specific purposes, including slaughter.

Organic acid washes: refers to washes with antimicrobials such as lactic, acetic and citric acids that affect microbial growth through disruptions to nutrient transport and energy generation and can cause injury to microbial cells through their low pH.

Washes containing other chemicals and oxidizers: includes washes containing other miscellaneous products that destroy bacteria through various actions, such as oxidation and disruption of cellular functions, or that prevent bacterial attachment to meat. Examples include: i) Oxidisers (electrolyzed oxidized (EO) water, ozonated water, peroxyacetic acid, hypobromous acid, acidified sodium chlorite and hydrogen peroxide); ii) Surfactants (sodium dodecyl sulfate, octenidine hydrochloride); iii) Quaternary ammonium compounds (QAC) (different proprietary sanitisers); iv) Other chemicals (chlorine solutions, cetylpyridinium chloride, sodium hydroxide, sodium metasilicate, trisodium phosphate, alcohols, phosphoric acid, caprylic acid, B-resorcylic acid, chloroform and carvacrol).

Standard processing procedures and good hygiene practices (GHP): includes a range of different practices that are pre-requisites to hazard-based interventions, are qualitative in nature and based on empirical knowledge and experience, and may have a pathogen-reduction effect.

Bung bagging (bunging): Closing off the rectum by cutting around the anus, placing a bag over the rectum and securing it in place with an elastic band or similar during evisceration, to minimize the spread of contamination on a carcass.

Trimming: Physical removal of visible contamination from carcasses with knife.

Dry chilling: refers to chilling following all dressing procedures on the slaughter line without the use of any additional spray (acid or water) and up to 72 hours (3 days).

Spray chilling: intermittent spraying beef carcass with water during the first several hours of the entire cooling process.

Dry aging: refers to multiday refrigeration of carcasses (>72 hours, often up to 14 days or more).

Multiple interventions: refers to an application of interventions based on the 'multiple hurdle approach', where chemical and/or physical interventions (usually more than two) are applied in sequence or simultaneously, inflicting concurrent and variable injuries to bacterial cells. Sequential application of interventions may involve use of interventions on cattle hides, followed by skinned carcass knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam, organic acid rinsing, chilling, and chemical spraying before carcass fabrication.

3.4 Lairage interventions

3.4.1 Hide cleanliness assessment

Four observational studies that investigated the relationship between cattle hide cleanliness and microbiological status of derived beef carcasses progressed to meta-analysis stage (McEvoy et al. 2000; Hauge et al. 2012; Blagojevic et al. 2012; Serraino et al. 2012). Scoring of hide cleanliness before cattle slaughter is a measure commonly implemented in only several countries, mainly in Europe, such as the UK, Ireland and Norway. UK and Irish scoring system is based on a similar five-category scale, whereas Norwegian is based on somewhat simplified three-category scale (i.e. clean, moderately dirty and dirty animals):

- categories 1 and 2 (UK/Irish system) and category 0 (Norwegian system) are similarly described as 'visually clean hide with minor quantities of faecal material or mud';
- category 3 (UK/Irish system) and category 1 (Norwegian system) are moderately dirty hides, with (dry) dirt covering cutting lines (UK/Irish system) or 20-40% of areas on the thighs covered by dry dirt, and/or up to 50% of mid-line cut on the abdomen and brisket covered by dry dirt (Norwegian system); and
- categories 4 and 5 (UK/Irish system) and category 2 (Norwegian system) are very dirty hides, with dump or wet dirt covering large areas of hide (UK/Irish system) or more than 40% of the thighs and legs covered in dry dirt and/or more than 50% of mid-line on the abdomen and brisket covered in dry dirt, etc (Norwegian system).

For the comparison purposes, the results from these four studies were grouped in two categories, 'clean' cattle (categories 1 and 2 based on the UK and Irish scoring system, and category 0 based on Norwegian scoring system), and categories 3, 4 and 5 (UK and Irish system) and 1 and 2 (Norwegian system) of 'dirty' cattle. The summary effects from random-effect meta-analysis model show consistent reduction for all indicator microorganisms (aerobic colony counts (ACC), *Enterobacteriaceae* counts (EBC) and generic *E. coli*) when clean cattle are compared to dirty cattle, on hides and resulting carcasses. Least-squares mean reductions ($\log_{10}$ CFU/cm²) on carcass surfaces were 0.9 (95% confidence interval (CI): 0.54 to 1.26) for ACC and 0.71 (95% CI: 0.36 to 1.05) for EBC, but with high heterogeneity between studies (Appendix D). Three trials found that utilising UK hide cleanliness scoring system led to a significant 0.75 $\log_{10}$ CFU/cm² reduction in *E.coli* (95% CI: 0.65 to 0.85), shown in Figure 2 below. Similarly, the microbial load on clean hides was lower than on dirty hides, but with high heterogeneity between studies (Appendix D).

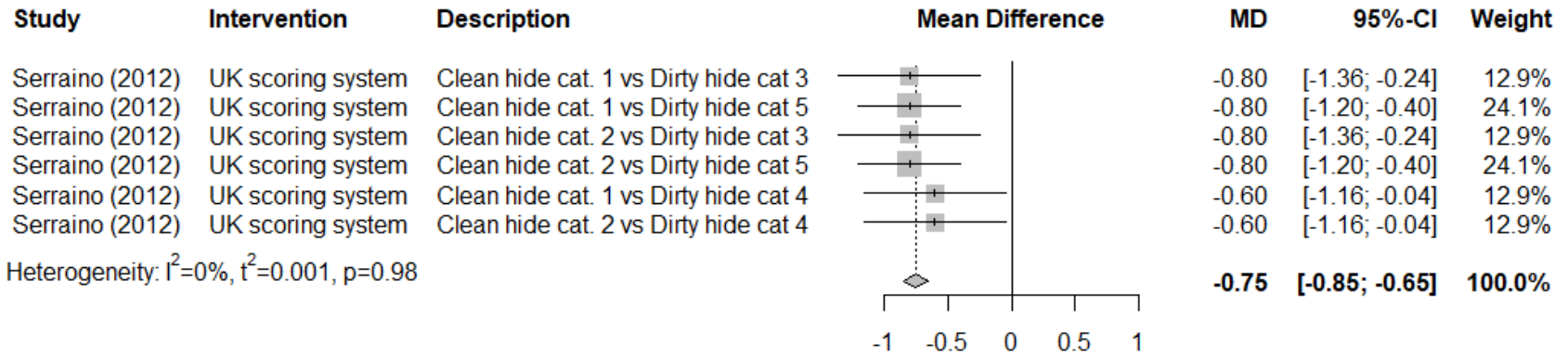


Figure 2.

Forest plot of the results of observational trials investigating hide cleanliness assessment, to determine the effect in reducing generic *E. coli* counts (log_{10} CFU) on beef carcasses produced from clean animals compared to dirty anima

3.5 Cattle hide interventions

The review identified nine controlled and challenge trials and nine before-and-after trials studies conducted under commercial abattoir conditions and 22 laboratory studies (predominantly with challenge trial study design), describing cattle hide interventions. Majority of studies (particularly before-and-after and lab trials) were judged to be at high RoB or to raise some concerns (Appendix C), so did not progress to the meta-analysis stage. The remaining 11 studies (Appendix E) that reported extractable data were analysed and only the results for the interventions where it was possible to calculate the summary effects (and from those of low heterogeneity) are presented in this section. The remaining forest plots can be found in Appendix D.

There were insufficient number of hide interventions studies for most interventions, to draw some firm conclusions about their efficacy. This was particularly the case with hide clipping, bacteriophage treatment, some chemical washes, and some harsh treatments like chemical dehairing or thermal treatments. On the other hand, some evidence was generated for interventions such as hide water wash, hot water wash and scalding (for bob-veal skin-on carcasses), washes containing chemicals (sodium-hydroxide, sanitisers and cetylpyridinium chloride) and microbial immobilisation treatments ('hide coating' with shellac).

3.5.1 Hide washing and clipping

The meta-regression effect revealed that hide water wash may have some limited protective effect in reducing *E. coli* O157 and non-O157 prevalence on hides, although high heterogeneity found in the summary effects indicates that the intervention results differ substantially (RR 0.85; 95% CI: 0.66 to 1.09) (Appendix D).

The least-squares mean reduction effect in reducing levels of aerobic bacteria on hides was low, 0.6 $\log_{10}$ CFU/100 cm² and showing no effect, questioning the usefulness and practicality of hide water wash as a standalone intervention (Appendix D). The results for other microorganisms are presented in forest plots in Appendix D and too few studies have been identified to calculate the meta-analysis effect.

With respect to hide clipping, four studies identified in previous review investigating this intervention were judged to be at high RoB or to raise some concerns (Appendix C), so were not meta-analysed.

3.5.2 Chemical dehairing and thermal interventions

Several harsh hide treatments have been described in literature, mostly in lab conditions. Given the fact that the hide is damaged during the process, these harsh interventions are

more suitable for bob veal calves which usually stay with the skin-on, or in situations where hides are not used for the leather production.

The three studies identified in previous review investigating chemical dehairing were judged to be at high RoB or to raise some concerns (Appendix C), so were not meta-analysed.

Only one challenge trial study investigated different single or multiple thermal treatments for bob veal calves which stay with the hide-on throughout the dressing process was identified (Hasty et al. 2018). The meta-analysis summary effect was not calculated and forest plots can be found in Appendix D.

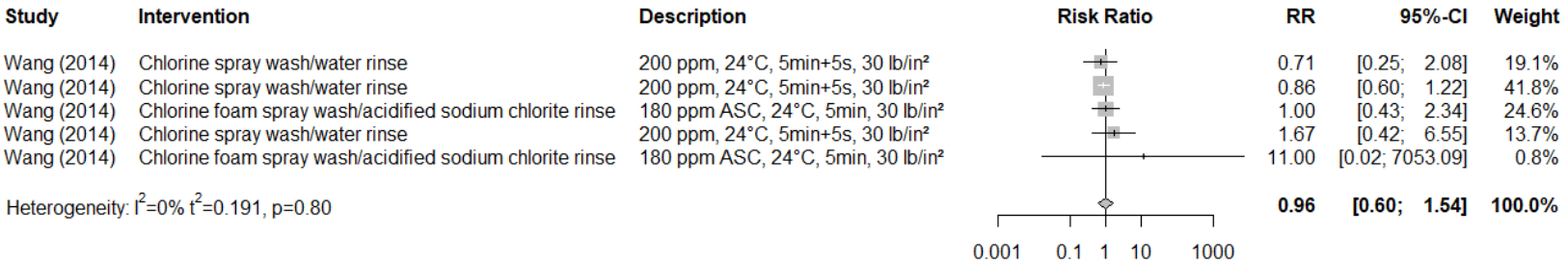
3.5.3 Hide washing with organic acids

Only two studies with different study design were identified investigating hide washing with organic acids, the forest plots are shown in Appendix D due to high heterogeneity (Mies et al. 2004; Scanga et al. 2011).

3.5.4 Hide washing with oxidisers/other chemicals

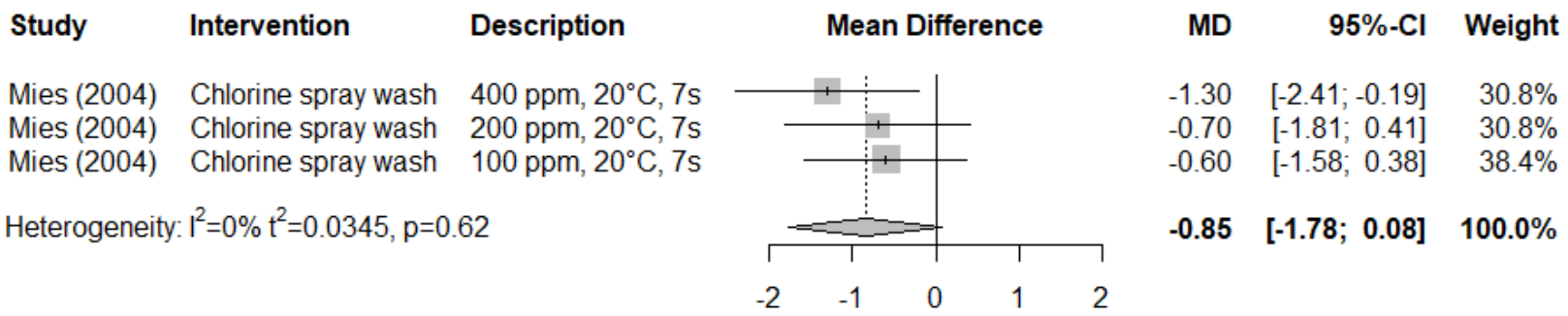
Several studies were available that investigated hide washes with chemicals, with sufficient data to calculate meta-regression summary effect. Five before-and-after trials under commercial abattoir conditions found that chlorine foam or spray washes did not change *E. coli* O157 and non-O157 prevalence on cattle hides (RR 0.96, 95% CI: 0.60 to 1.54, I^2=0%), shown in Figure 3.

Figure 3. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide chlorine wash in reducing *E. coli* O157 and non-O157 prevalence on hides



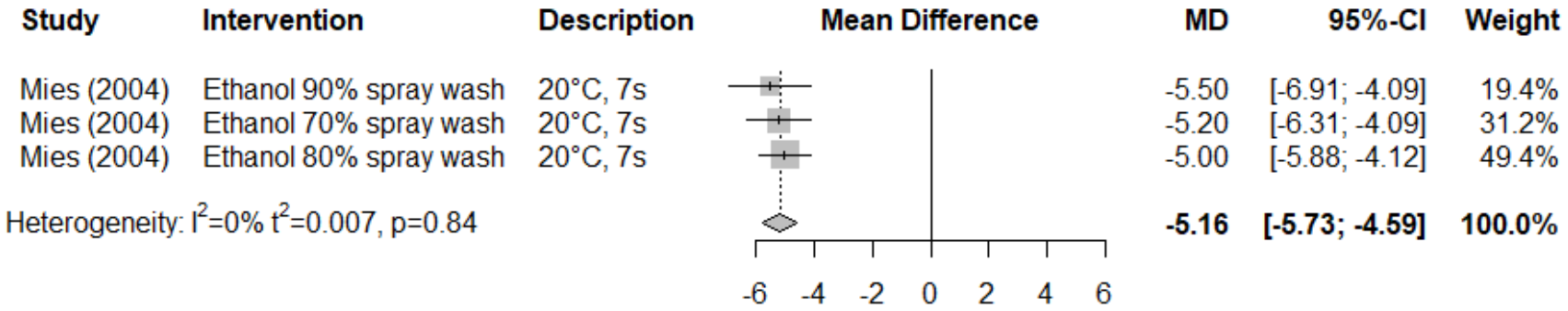
One challenge trial study investigated efficacy of chlorine wash and ethanol spray on *Salmonella* spp. reduction on cattle hides. Three trials found that chlorine spray washes did not lead to a reduction in *S*. Typhimurium (MD -0.85, 95% CI: -1.78 to 0.08, I^2=0%), Figure 4.

Figure 4. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of cattle hide chlorine wash in reducing Salmonella spp. (log_{10} CFU) on hides



On the other hand, three challenge lab trials found that ethanol spray washes led to a significant 5.16 log_{10} CFU/cm² reduction in *S.* Typhimurium (95% CI: -5.73 to -4.59), Figure 5.

Figure 5. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of cattle hide ethanol wash in reducing Salmonella spp. (log_{10} CFU) on hides



One controlled trial conducted under laboratory conditions showed that spraying QAC sanitiser on cattle hides sections, can reduce transfer of *Enterobacteriaceae* and generic *E. coli* on beef cuts. Three trials found that proprietary QAC sanitiser spray wash with vacuum led to a significant log_{10} 1.86 CFU/cm² reduction in *Enterobacteriaceae* (MD -1.86, 95% CI: -2.93 to -0.80, I^2=52.7%), Figure 6. Similarly, three trials found that proprietary QAC sanitiser spray wash with vacuum led to a significant 1.51 log_{10} CFU/cm² reduction in generic *E.coli* counts (MD -1.51, 95% CI: -1.89 to -1.13, I^2=0%), Figure 7.

Figure 6. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of cattle hide proprietary QAC sanitiser wash in reducing Enterobacteriaceae counts (log_{10} CFU) transfer on beef cuts

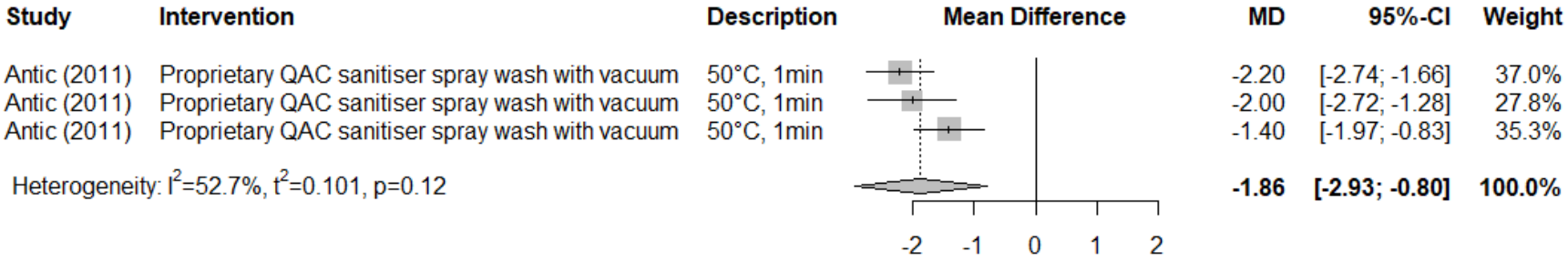
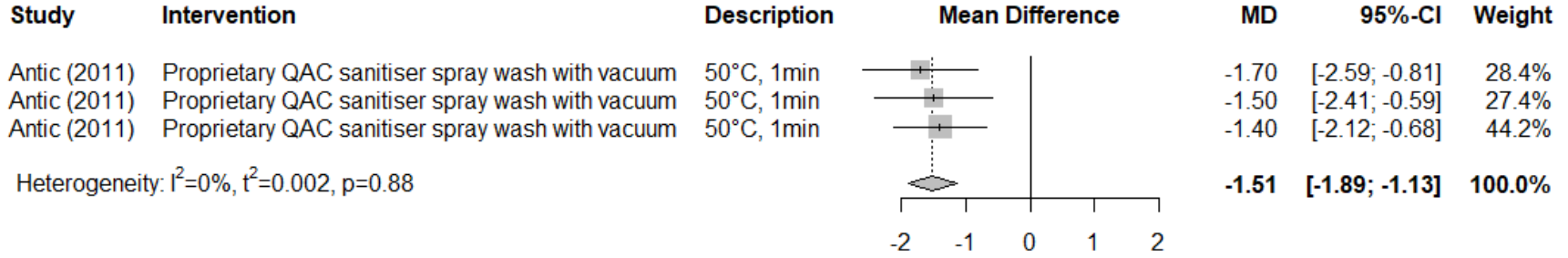


Figure 7. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of cattle hide proprietary QAC sanitiser wash in reducing generic *E. coli* counts (log_{10} CFU) transfer on beef cuts



The other results for the effect of hide washes with chemicals (such as sodium-hydroxide, sanitisers, chlorine and cetylpyridinium chloride) are presented in Appendix D.

3.5.5 Microbial immobilisation treatments

Three studies that investigated this novel cattle hide intervention, generated some data from which some summary effects were calculated (Antic et al. 2010, 2011, 2018). The least-squares mean reduction effect (log_{10} CFU/cm²) in reducing levels of aerobic bacteria and *Enterobacteriaceae* on resulting beef carcass under commercial abattoir conditions (reduction-in-transfer) was 1.07 log_{10} CFU/cm² (95% CI: 0.29 to 2.43) for ACC and 0.59 log_{10} CFU/cm² (95% CI: 1.05 to 2.22) for EBC. Forest plots are presented in Appendix D. The high heterogeneity found in the summary effects indicates that the intervention results differ substantially and more research is needed.

In laboratory trials, shellac (aqueous or ethanol solution) hide coating had a protective effect in reducing transfer of *E. coli* and *Enterobacteriaceae* from hides to beef cuts (Figures 8 and 9). Three trials found that Shellac (23%) in ethanol spray led to a significant 1.37 log_{10} CFU/cm² reduction in generic *E. coli* (MD -1.37, 95% CI: -2.20 to -0.54, I^2=0%), while seven trials found that aqueous shellac spray led to a significant 1.68 log_{10} CFU/cm² reduction in *Enterobacteriaceae* counts transfer on beef cuts (MD -1.68, 95% CI: -1.99 to -1.38, I^2=0%).

Other forest plots where summary effect was not calculated or where high heterogeneity was found are presented in Appendix D.

Figure 8. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of shellac in ethanol hide coating in reducing generic *E. coli* (log_{10} CFU) transfer on beef cuts

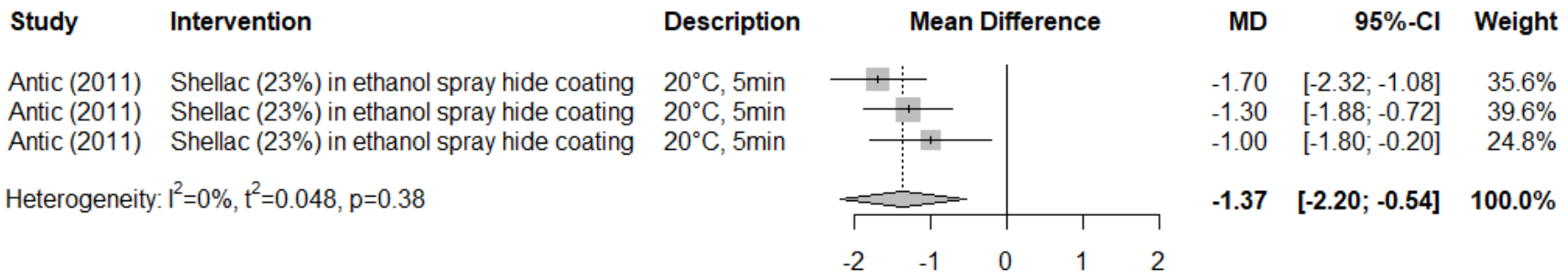
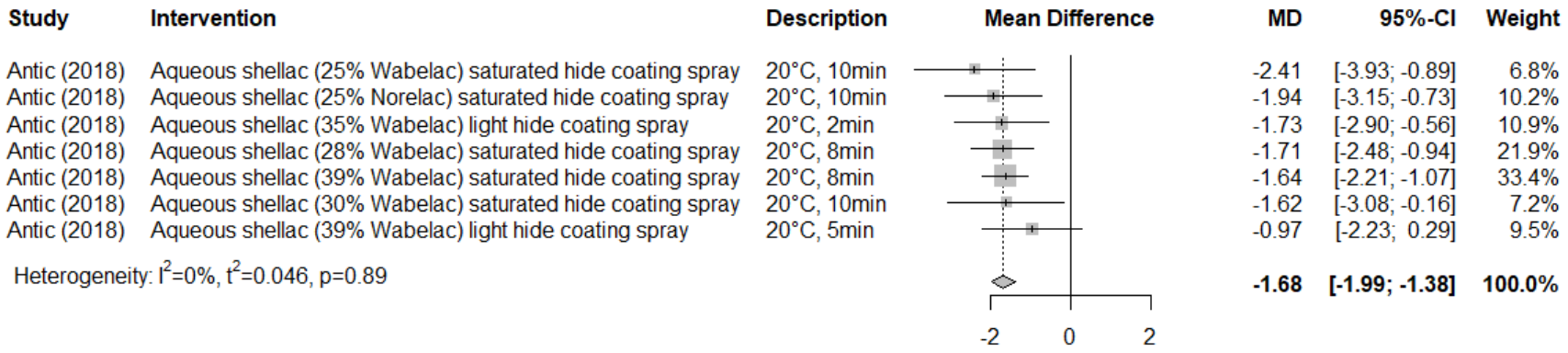


Figure 9. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of aqueous shellac hide coating in reducing Enterobacteriaceae counts (log_{10} CFU) transfer on beef cuts



3.6 Beef carcass interventions

3.6.1 Standard processing procedures and GHP

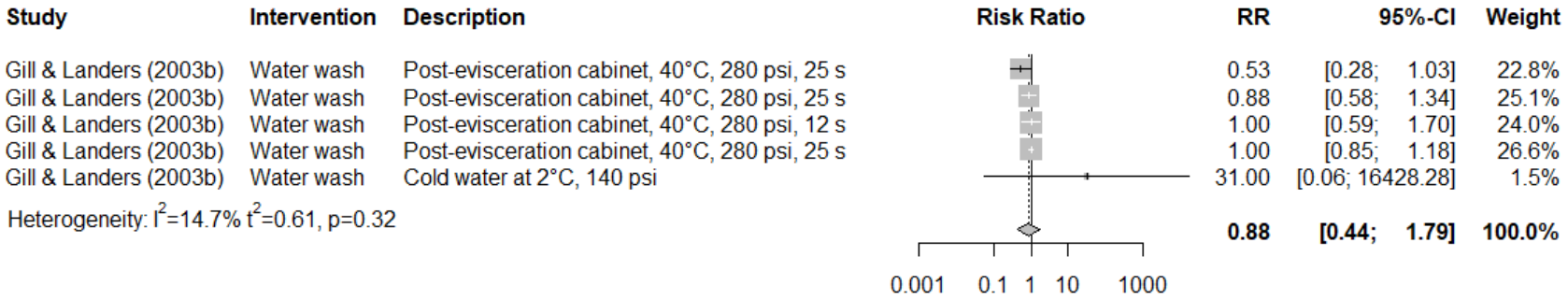
Only three studies in this intervention category progressed to data analysis, one investigating the efficacy of improved hygiene during hide removal, one alternative knife sanitation with warm water and one bung bagging (McEvoy et al. 2000; Eustace et al. 2007; Stopforth et al. 2006). There was no sufficient number of trials for meta-analysis and the results are presented in Appendix D.

3.6.2 Pre-chill carcass treatments

Water wash

Five trials found that water washes did not change generic *E. coli* prevalence on washed beef carcasses (RR 0.88, 95% CI: 0.44 to 1.79, I^2=14.7%).

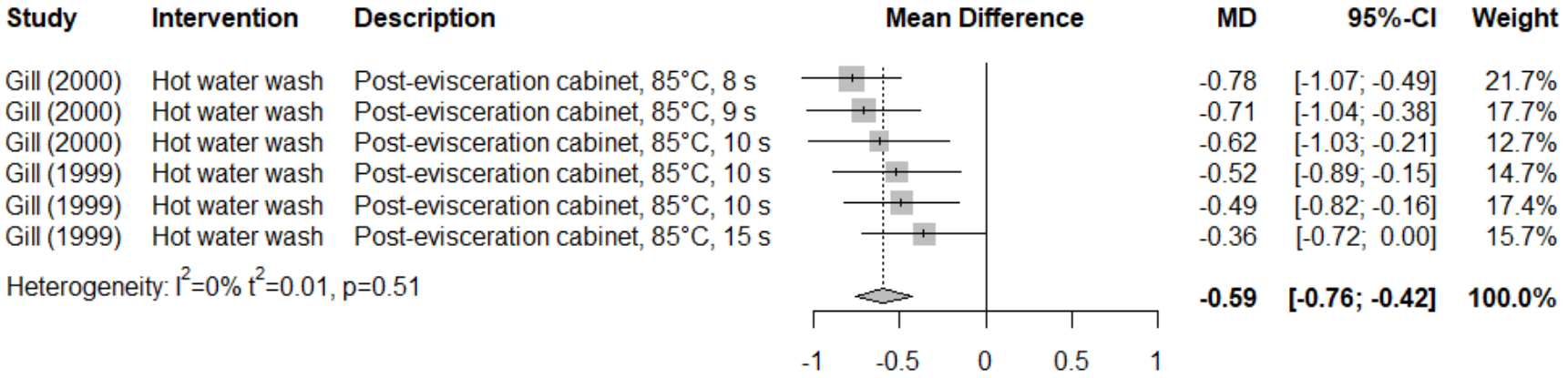
Figure 10. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water wash in reducing generic E. coli prevalence on beef carcasses



Hot water wash

Six trials found that hot water washes led to a significant 0.59 log_{10} CFU/100cm² reduction in generic *E. coli* on beef carcasses (MD -0.59, 95% CI: -0.76 to -0.42, I^2=0%).

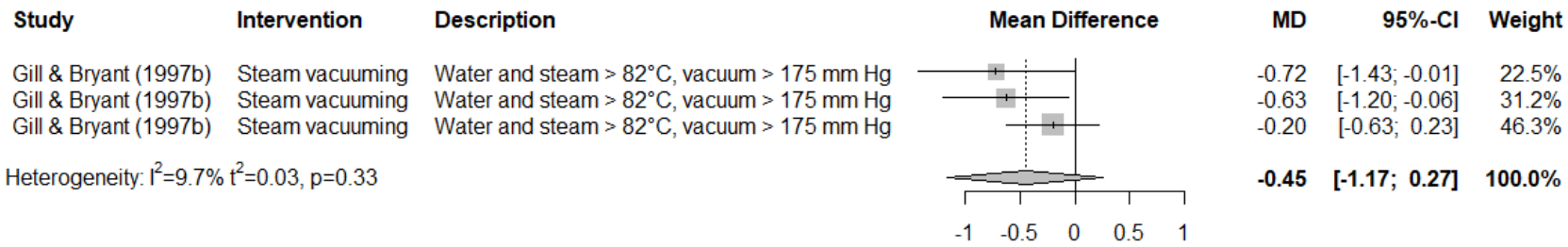
Figure 11. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of hot water wash in reducing generic *E. coli* counts on beef carcasses



Steam vacuuming

Three trials found that steam vacuuming did not lead to a significant reduction in generic *E. coli* numbers on beef carcasses (MD -0.45, 95% CI: -1.17 to 0.27, I^2=9.7%).

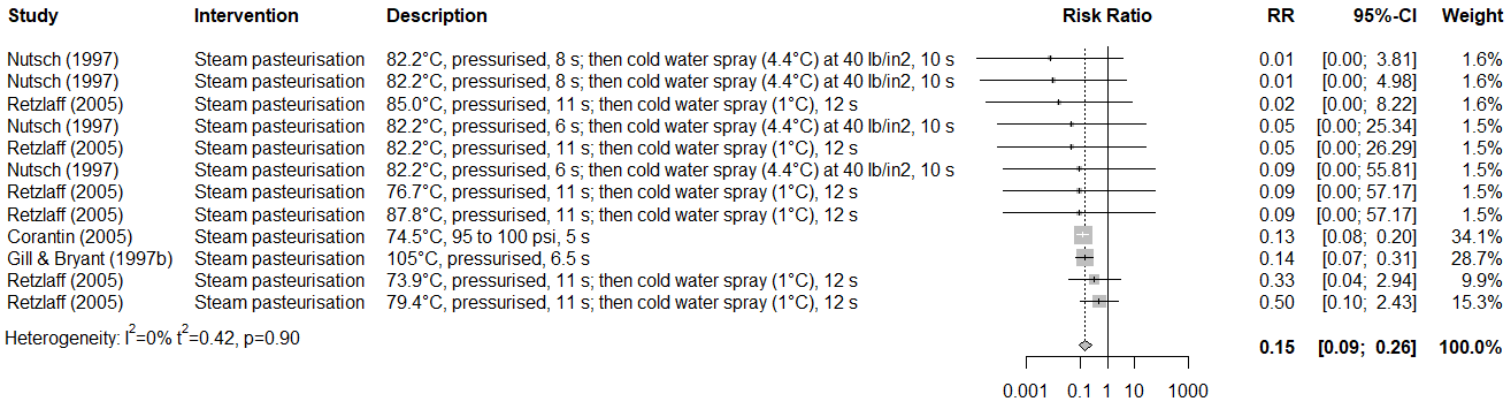
Figure 12. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam vacuuming in reducing generic *E. coli* counts on beef carcasses



Steam pasteurisation

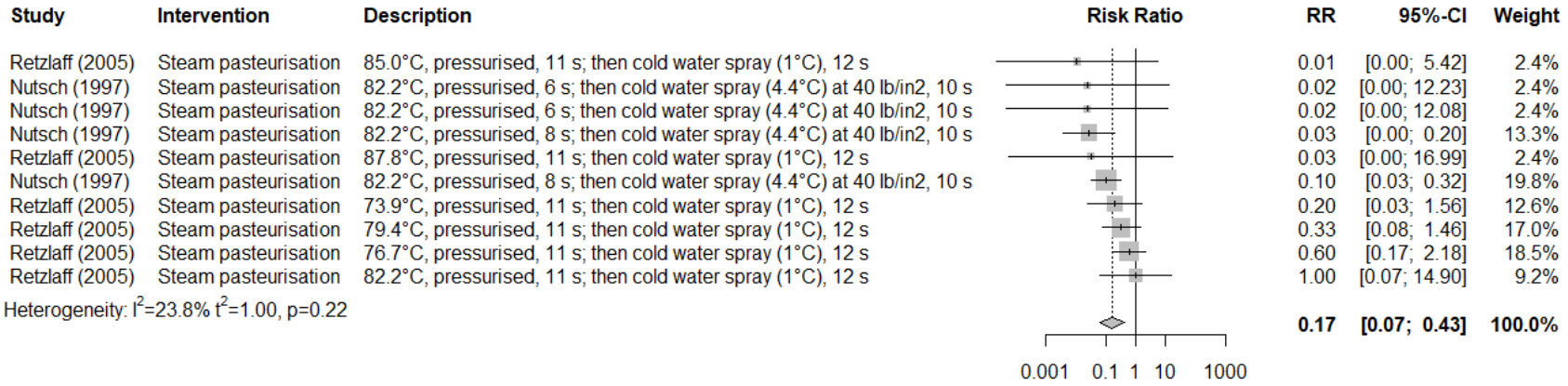
Twelve before-and-after trials from four studies performed under commercial abattoir conditions found that steam pasteurisation significantly reduced generic *E. coli* prevalence on beef carcass sides (RR 0.15, 95% CI: 0.09 to 0.26, I^2=0%).

Figure 13. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing generic E. coli prevalence on beef carcasses



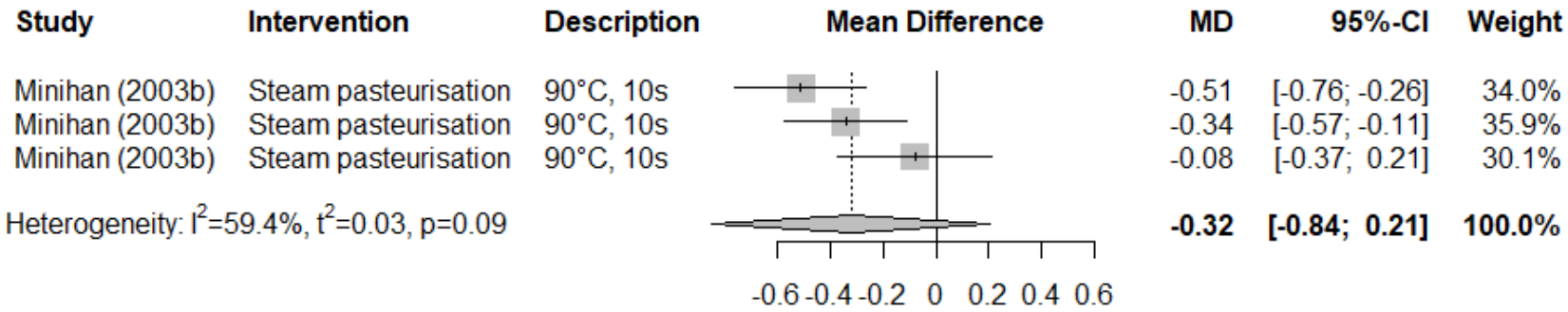
Ten before-and-after trials performed under commercial abattoir conditions found that steam pasteurisation significantly reduced *Enterobacteriaceae* prevalence on beef carcass sides (RR 0.17, 95% CI: 0.07 to 0.43, I^2=23.8%).

Figure 14. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing Enterobacteriaceae prevalence on beef carcasses



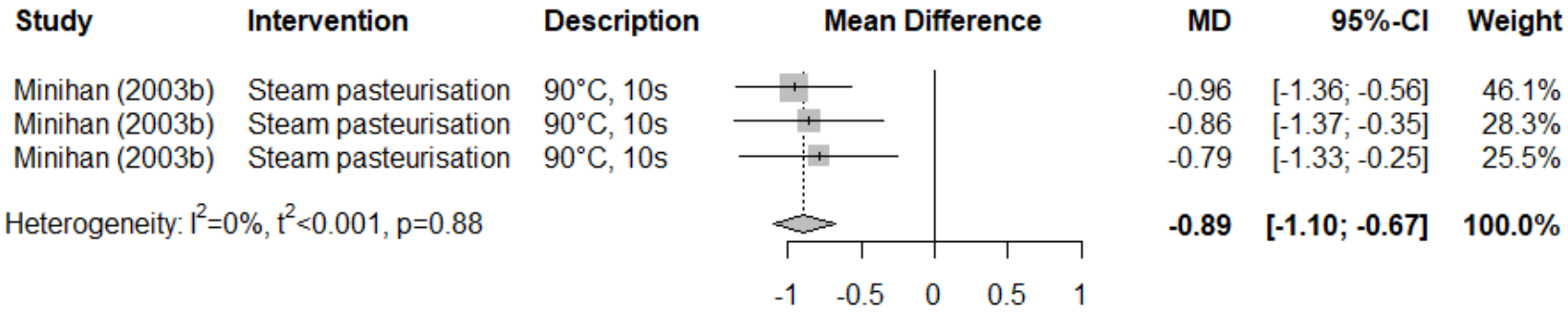
Three controlled trials performed under commercial abattoir conditions found that steam pasteurisation did not lead to a reduction in ACC on beef carcass sides (MD -0.32, 95% CI: -0.84 to 0.21, I^2=59.4%).

Figure 15. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing aerobic colony counts on beef carcasses



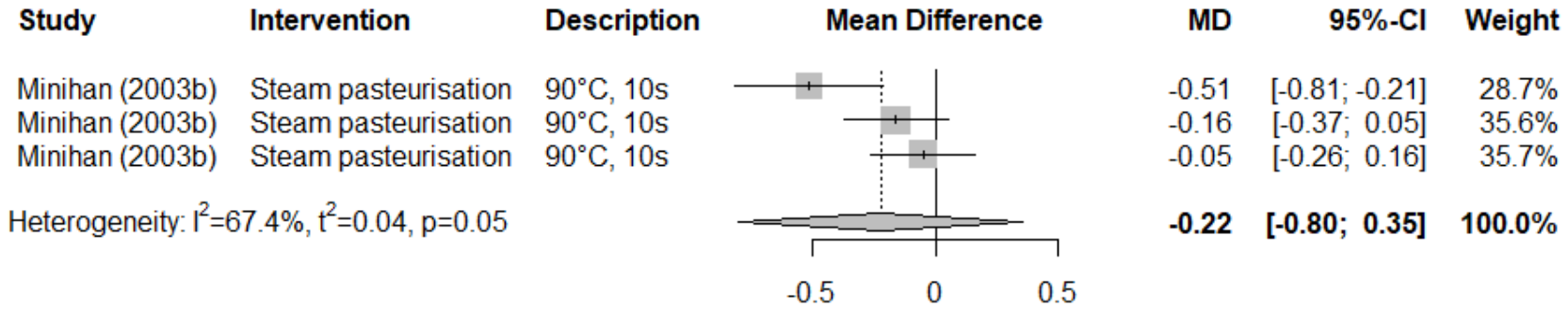
Three controlled trials performed under commercial abattoir conditions found that steam pasteurisation led to a significant 0.89 $\log_{10}$ CFU/1000cm² reduction in *Enterobacteriaceae* counts on beef carcass sides (MD -0.89, 95% CI: -1.10 to -0.67, I^2=0%).

Figure 16. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing Enterobacteriaceae counts on beef carcasses



Three controlled trials performed under commercial abattoir conditions found that steam pasteurisation did not lead to a reduction in generic *E. coli* counts on beef carcass sides (MD -0.22, 95% CI: -0.80 to 0.35, I^2=67.4%).

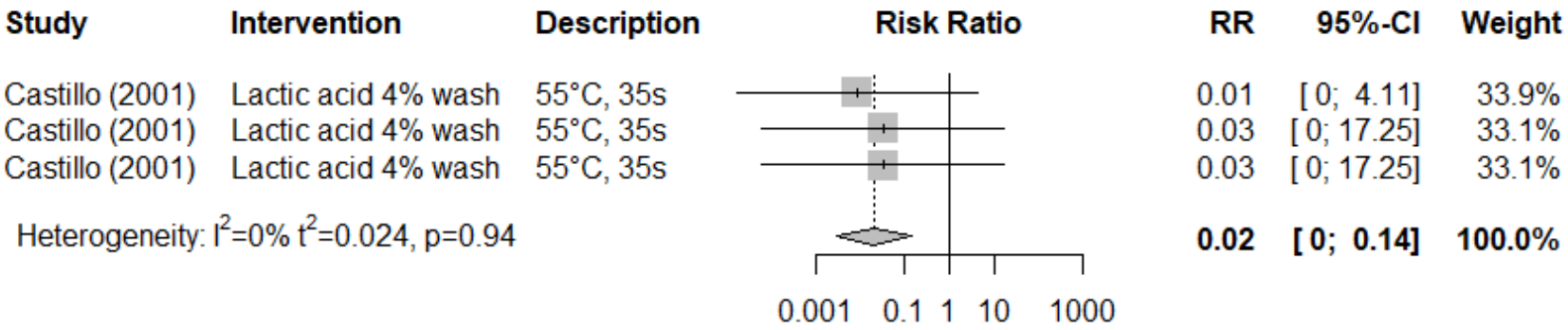
Figure 17. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing generic *E. coli* counts on beef carcasses



Lactic acid wash

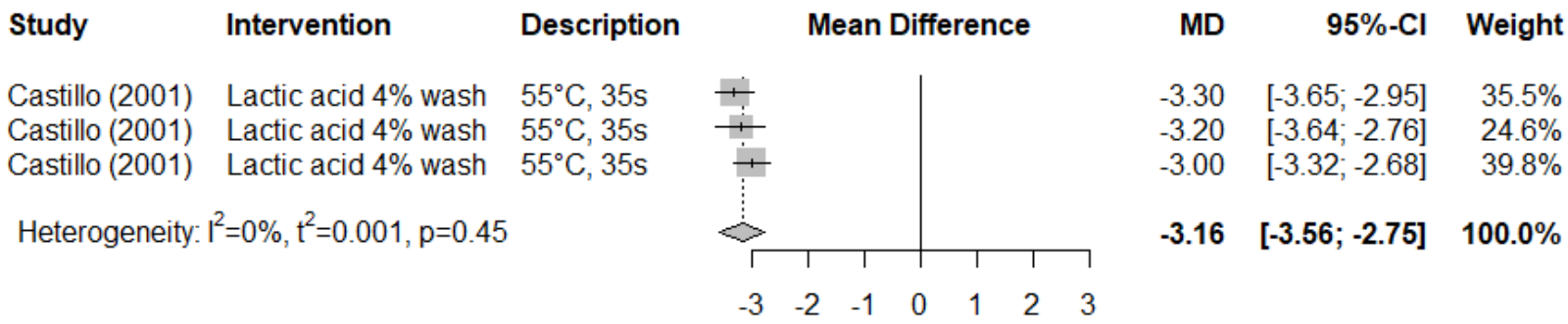
Three controlled trials performed under commercial abattoir conditions found that lactic acid 4% washes significantly reduced generic *E. coli* prevalence on beef carcass sides post-chilling (RR 0.02, 95% CI: 0 to 0.14, I^2=0%).

Figure 18. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of lactic acid 4% spray wash in reducing generic *E. coli* prevalence on beef carcasses post-chilling



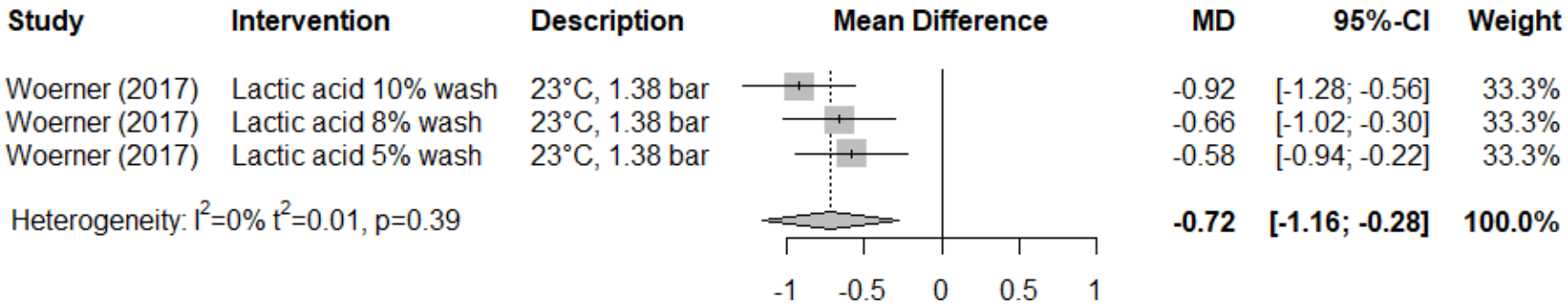
Three controlled trials performed under commercial abattoir conditions found that lactic acid washes led to a significant 3.16 $\log_{10}$ CFU/100cm² reduction in aerobic colony counts on beef carcass sides post-chilling (MD -3.16, 95% CI: -3.56 to -2.75, I^2=0%).

Figure 19. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of lactic acid 4% spray wash in reducing aerobic colony counts on beef carcasses post-chilling



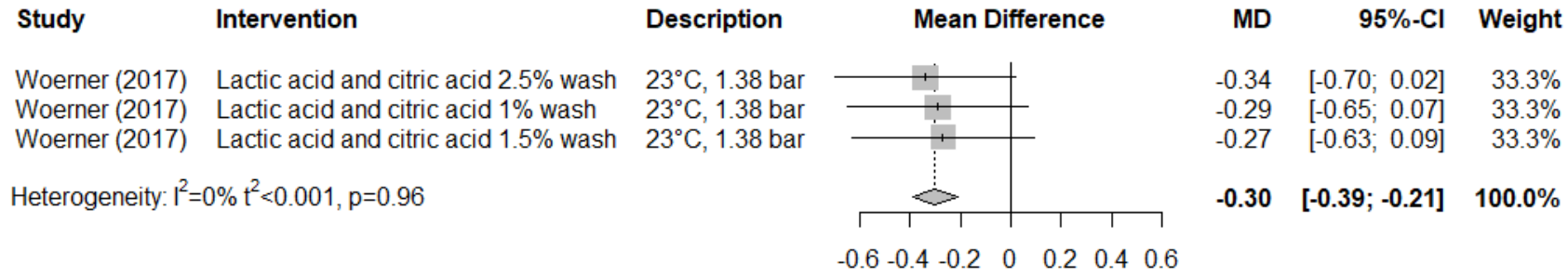
Three challenge trials performed under laboratory conditions found that lactic acid washes led to a significant 0.72 log_{10} CFU/cm² reduction in STEC on beef (MD -0.72, 95% CI: -1.16 to -0.28, I^2=0%). The authors investigated an effect on inoculated STEC cocktail comprising *E. coli* O157 and six non-O157.

Figure 20. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid wash in reducing *E. coli* O157 and non-O157 numbers on beef



Three challenge trials performed under laboratory conditions found that lactic acid and citric acid washes led to a significant 0.30 log_{10} CFU/cm² reduction in generic *E. coli* counts on beef (MD -0.30, 95% CI: -0.39 to -0.21, I^2=0%).

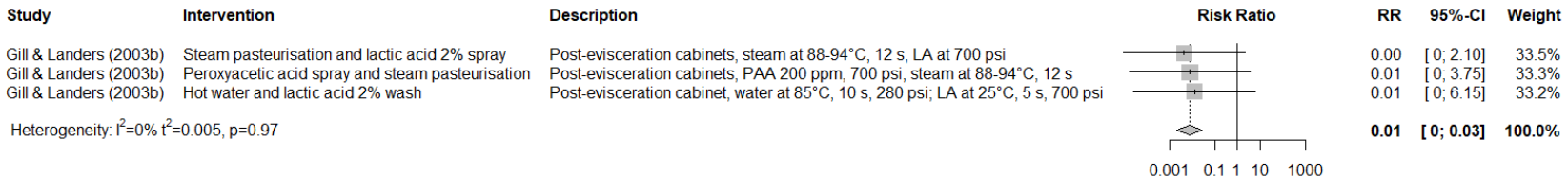
Figure 21. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid and citric acid washes in reducing *E. coli* numbers on beef



Pasteurisation and acid wash

Three before-and-after trials performed under commercial abattoir conditions found that carcass pasteurisation with hot water or steam and subsequent lactic acid or peroxyacetic acid spray washes significantly reduced generic *E. coli* prevalence on beef carcass sides (RR 0.01, 95% CI: 0 to 0.03, I^2=0%).

Figure 22. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of pasteurisation and subsequent acid spray washes in reducing generic E. coli prevalence on beef carcasses

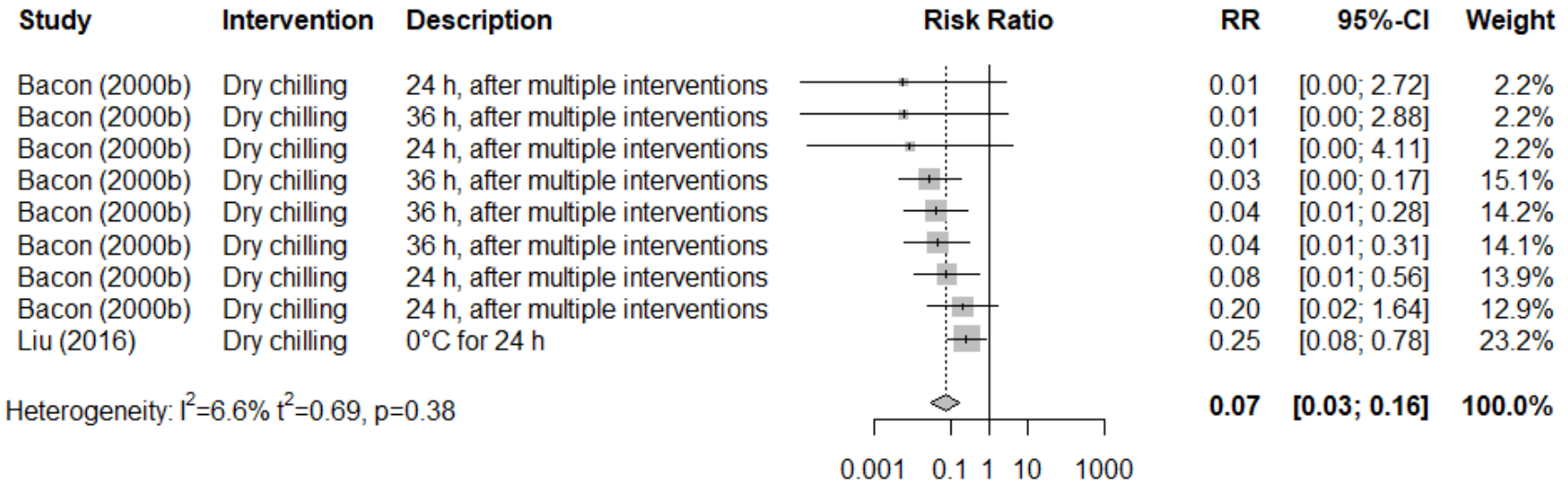


3.6.3 Chilling

Dry chilling

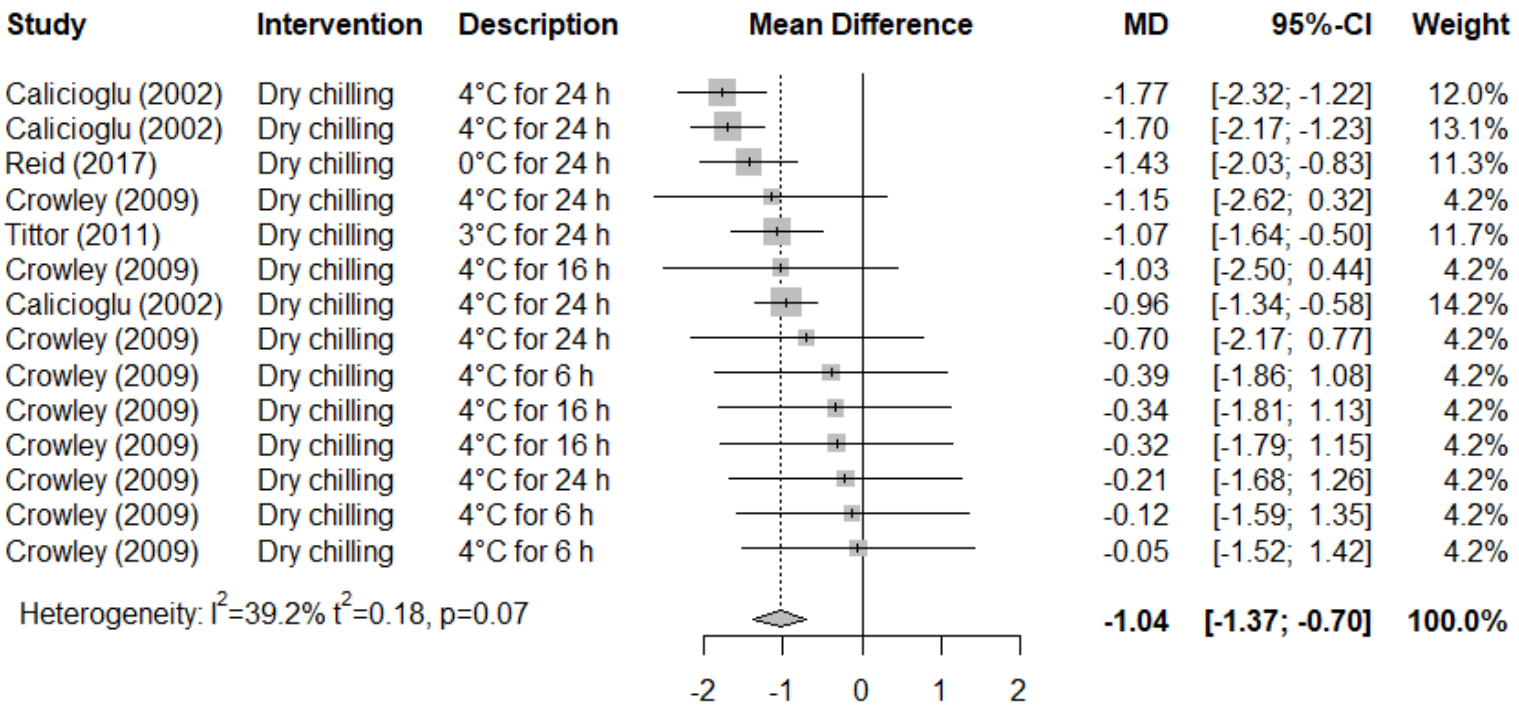
Nine before-and-after trials performed under commercial abattoir conditions found that dry chilling following multiple slaughter line interventions significantly reduced generic *E. coli* prevalence on beef carcass sides (RR 0.07, 95% CI: 0.03 to 0.16, I^2=6.6%).

Figure 23. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of dry chilling following multiple slaughter line interventions in reducing generic E. coli prevalence on beef carcasses



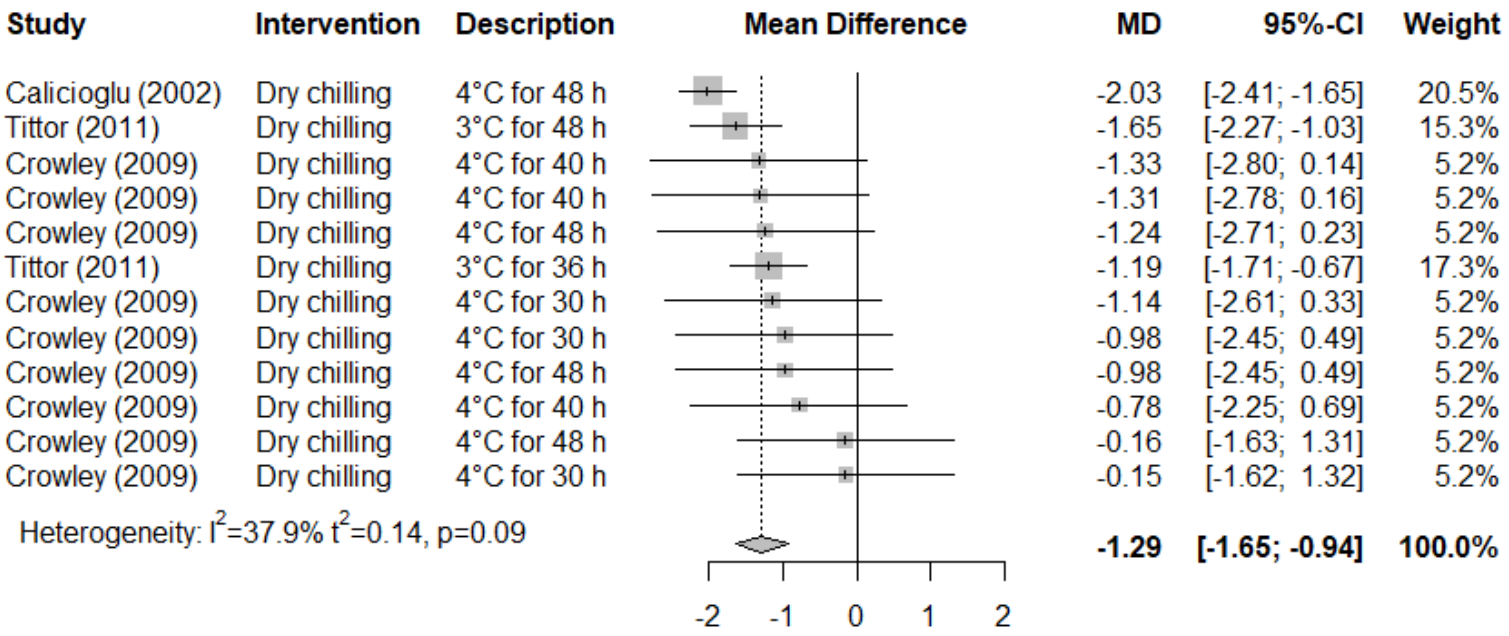
Fourteen challenge trials performed under laboratory conditions found that dry chilling up to 24 hours led to a significant 1.04 $\log_{10}$ CFU/cm² reduction in *E. coli* O157:H7 on beef (MD -1.04, 95% CI: -1.37 to -0.70, I^2=39.2%).

Figure 24. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 24 hours in reducing E. coli O157:H7 numbers on beef



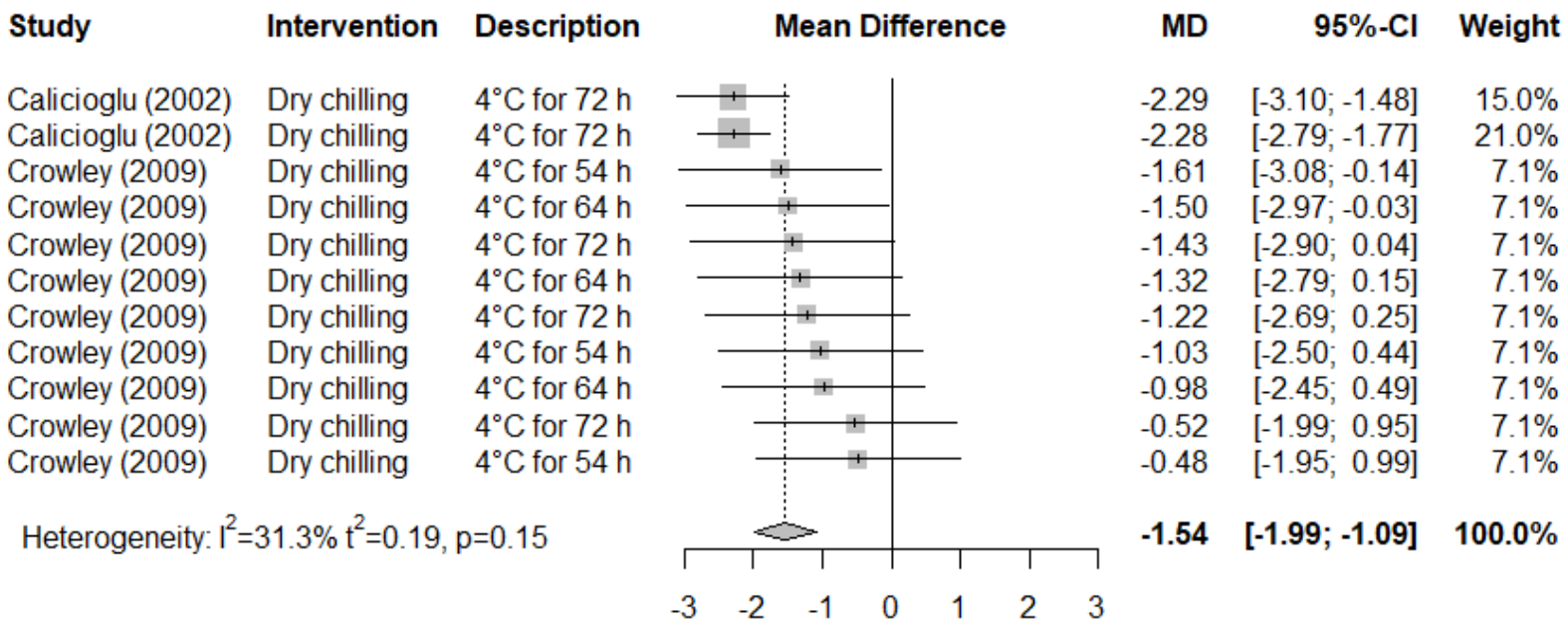
Twelve challenge trials performed under laboratory conditions found that dry chilling up to 48 hours led to a significant 1.29 log_{10} CFU/cm² reduction in *E. coli* O157:H7 on beef (MD -1.29, 95% CI:-1.65 to -0.94, I^2=37.9%).

Figure 25. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 48 hours in reducing E. coli O157:H7 numbers on beef



Eleven challenge trials performed under laboratory conditions found that dry chilling up to 72 hours led to a significant 1.54 $\log_{10}$ CFU/cm² reduction in *E. coli* O157:H7 on beef (MD -1.54, 95% CI: -1.99 to -1.09, I^2=31.3%).

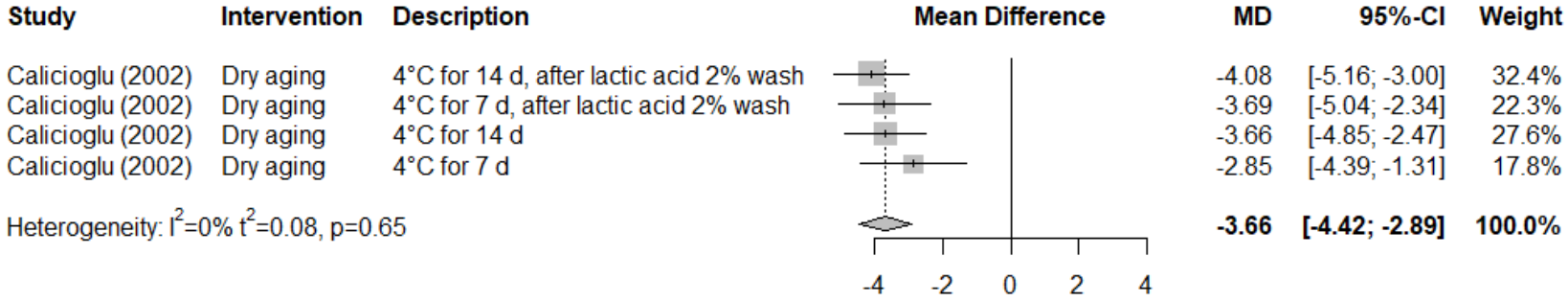
Figure 26. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 72 hours in reducing *E. coli* O157:H7 numbers on beef



Dry aging

Four challenge trials performed under laboratory conditions found that dry aging up to 14 days led to a significant 3.66 $\log_{10}$ CFU/cm² reduction in generic *E. coli* on beef (MD -3.66, 95% CI: -4.22 to -2.89, I^2=0%).

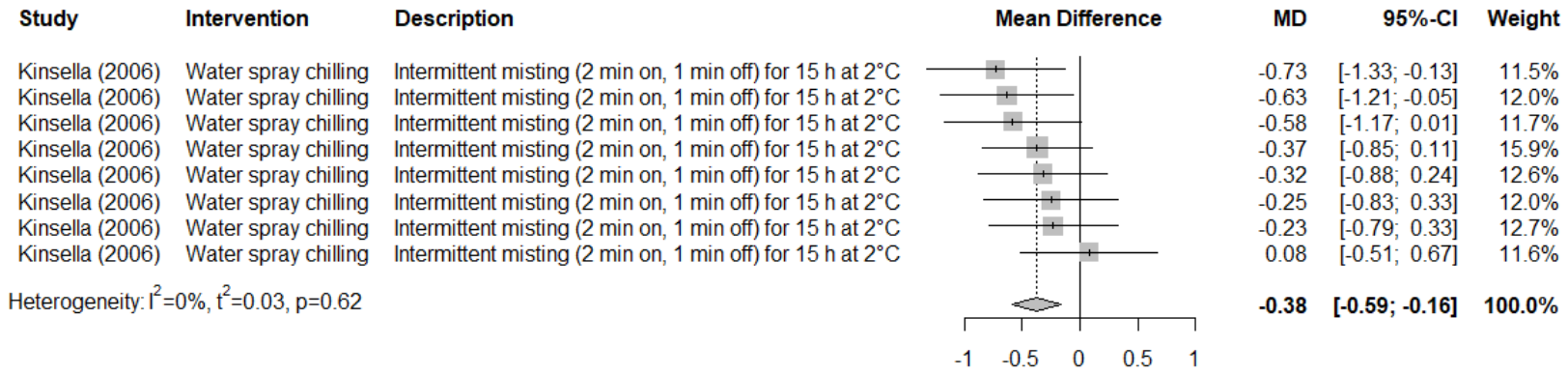
Figure 27. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry aging up to 14 days in reducing generic *E. coli* numbers on beef



Water spray chilling

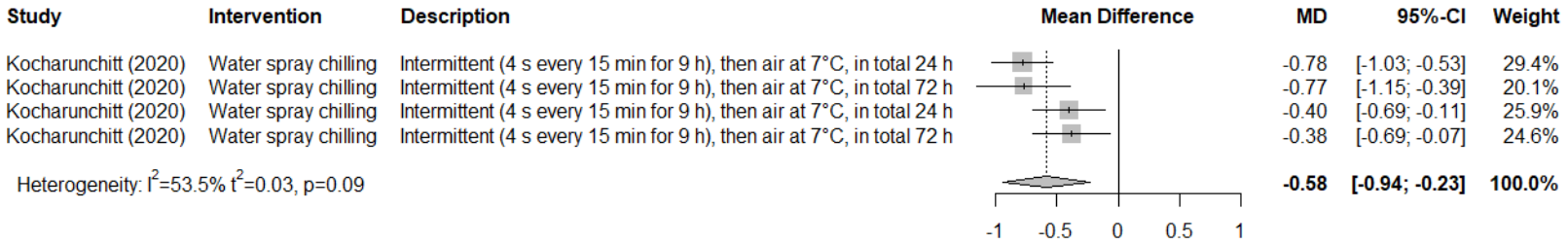
Eight controlled trials performed under commercial abattoir conditions found that water spray chilling, when compared to conventional dry chilling, led to a significant 0.38 $\log_{10}$ CFU/cm² reduction in aerobic colony counts on beef carcass sides (MD -0.38, 95% CI: -0.59 to -0.16, I^2=0%).

Figure 28. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of water spray chilling vs. dry chilling in reducing aerobic colony counts on beef carcasses



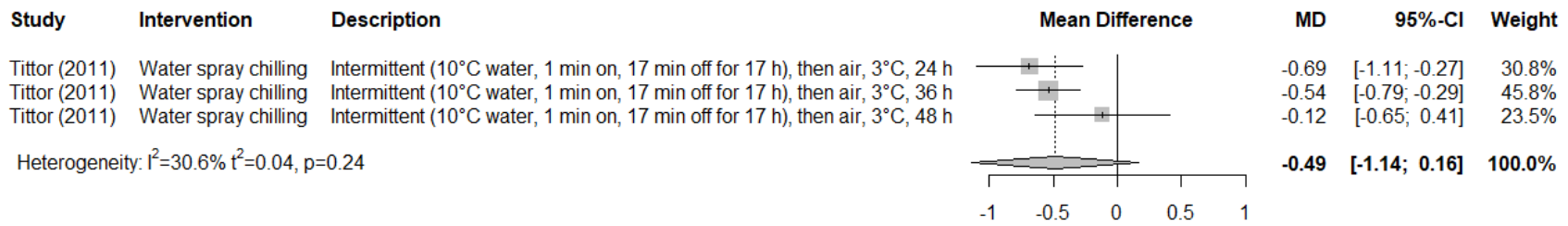
Four challenge trials performed under laboratory conditions found that water spray chilling led to a significant 0.58 log_{10} CFU/cm² reduction in generic *E. coli* on beef (MD -0.58, 95% CI: -0.94 to -0.23, I^2=53.5%).

Figure 29. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling in reducing E. coli numbers on beef



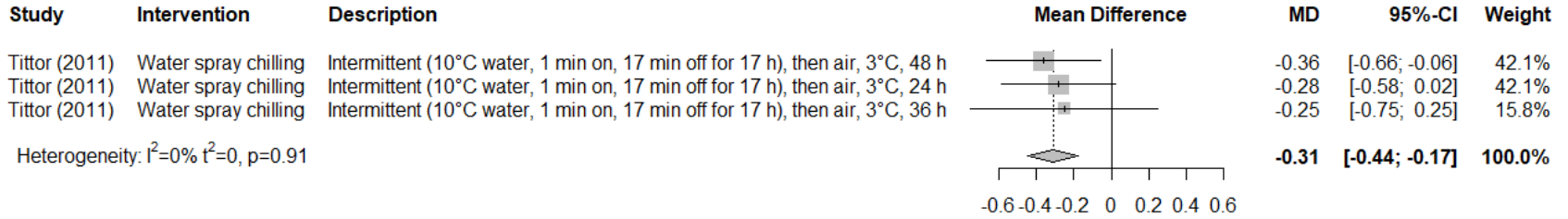
Three challenge trials performed under laboratory conditions found that water spray chilling, when compared to dry chilling, did not lead to a reduction in *E. coli* O157:H7 on beef (MD -0.49, 95% CI: -1.14 to 0.16, I^2=30.6%).

Figure 30. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling vs. dry chilling in reducing E. coli O157:H7 numbers on beef



Three challenge trials performed under laboratory conditions found that water spray chilling, when compared to dry chilling, led to a significant 0.31 $\log_{10}$ CFU/cm² reduction in *Salmonella* spp. on beef (MD -0.31, 95% CI: -0.44 to -0.17, I^2=0%).

Figure 31. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling vs. dry chilling in reducing *Salmonella* spp. numbers on beef



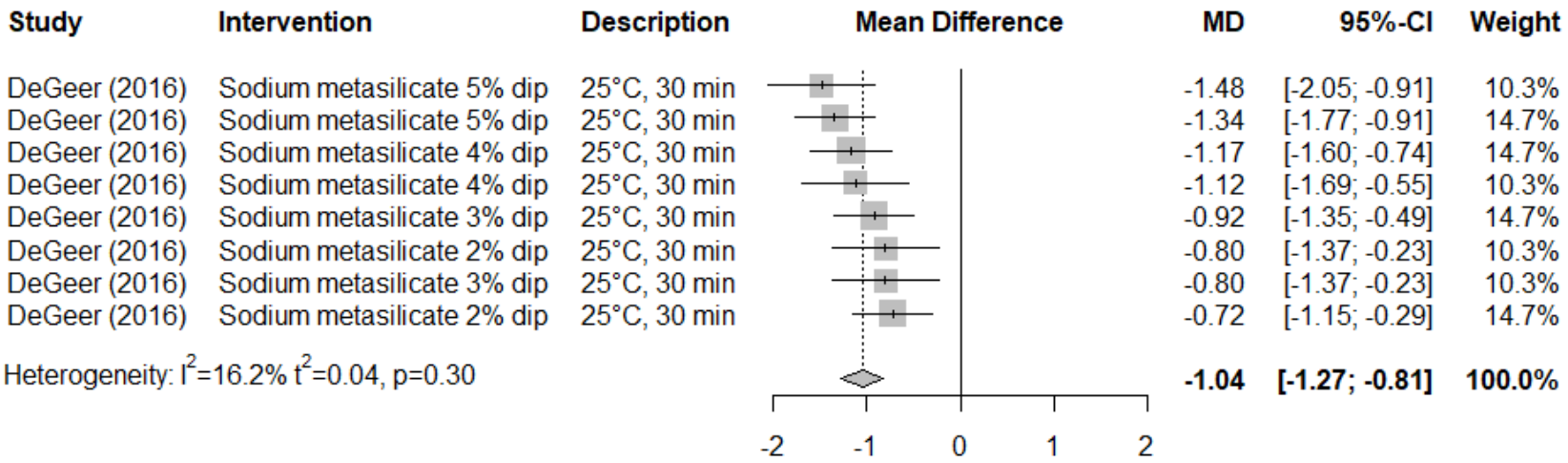
3.7 Post- carcass fabrication interventions

3.7.1 Interventions for beef primals, subprimals and trim

Chemical interventions for beef trim

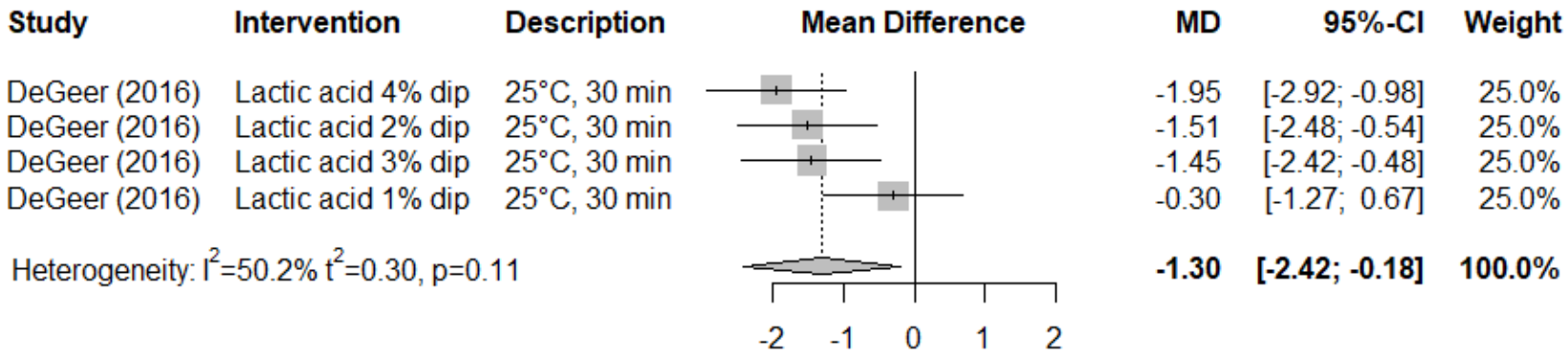
Eight challenge trials performed under laboratory conditions found that sodium metasilicate dipping led to a significant 1.04 log_{10} CFU/cm² reduction in *E. coli* O157:H7 and non-O157 numbers on beef trim (MD -1.04, 95% CI: -1.27 to -0.81, I^2=16.2%).

Figure 32. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of sodium metasilicate dipping vs. water dipping in reducing E. coli *O157:H7 and non-O157 numbers on beef trim*



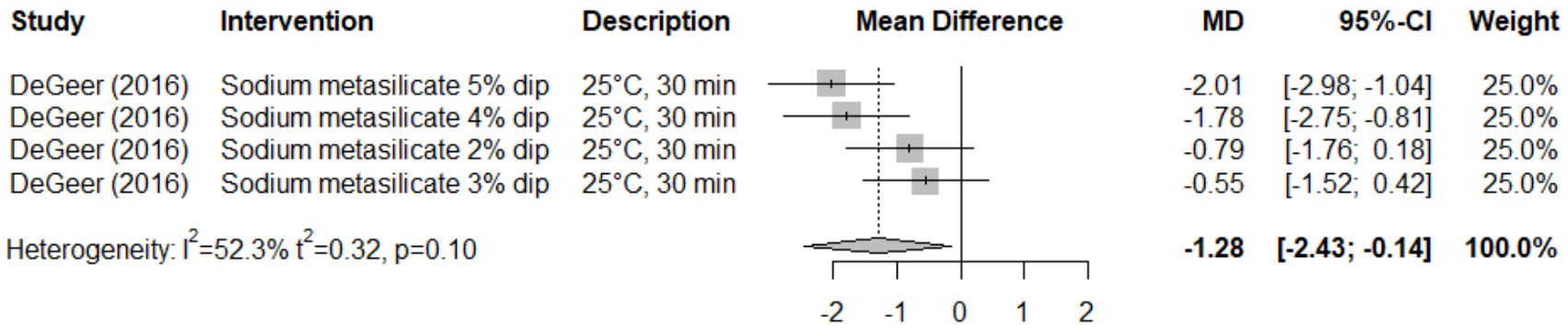
Four challenge trials performed under laboratory conditions found that lactic acid dipping led to a significant 1.30 log_{10} CFU/cm² reduction in *Salmonella* spp. numbers on beef trim (MD -1.30, 95% CI: -2.42 to -0.18, I^2=50.2%).

Figure 33. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid dipping vs. water dipping in reducing Salmonella spp. numbers on beef trim



Four challenge trials performed under laboratory conditions found that sodium metasilicate dipping led to a significant 1.28 log_{10} CFU/cm² reduction in *Salmonella* spp. numbers on beef trim (MD -1.28, 95% CI: -2.43 to -0.14, I^2=52.3%).

Figure 34. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of sodium metasilicate dipping vs. water dipping in reducing Salmonella spp. numbers on beef trim



4. Discussion

This study analysed data on interventions from the database generated in previous critical review project FS301044, with new updated search to capture any potentially eligible studies published since the last database search in 2018. Therefore, a large body of literature was used to create the whole dataset which covered period of 25 years (1996-2020) of literature publication. The primary aim of this study was to identify and recommend the beef interventions that have a significant reduction effect on microorganisms of concern, using statistical power of a meta-analysis tool. For the purpose of meta-analysis, data were used from studies on lairage interventions, cattle hide and beef carcass interventions, and interventions for fabricated beef. A risk of bias assessment was used to objectively quantify the robustness of the data in these articles. In total, 266 papers were evaluated with 113 papers judged to be at 'low' risk of bias progressed to detailed data extraction (seven more partially 'low' so some data could be used). The reasons for articles being judged to be at 'unclear' or 'high' risk of bias were mainly due to the bias arising from the randomization process and due to confounders not appropriately identified and accounted for (mainly in commercial abattoir trials). Weighted bar plots of the distribution of risk of bias judgements, within each bias domain, are presented in details for all studies in Figure 1 and per different intervention categories/study settings and designs, in Appendix C. The significant dropout rate at this point implies the necessity of a proper study design when conducting intervention trials. Furthermore, out of 120 articles fully or partially judged to be at 'low' RoB, further analysis showed that only 68 articles had extractable data that could be used for meta-analysis, while 52 articles were excluded from meta-analyses, with reasons provided in Table 2. Most of these studies did not report measures of variability, which was essential for meta-analysis, or were data presented in difficult to extract graph format. In line with the problems with methodological study design in more than a half of articles reviewed, the data reporting was a significant obstacle in obtaining more useful data for analysis purpose.

Extracted data were first stratified by study design, and then into specific intervention categories and specific interventions that were defined in the previous project FS301044, and finally by five different outcome measures (*Salmonella* spp., pathogenic *E. coli* and indicator microorganisms – aerobic bacteria, *Enterobacteriaceae* and generic *E. coli*). Random-effects meta-analysis was conducted in each subgroup where three and more trials were available. The effectiveness is expressed as the quantifiable level of bacterial reduction of specific interventions (concentration outcomes, mean changes in log bacterial counts (e.g. $\log_{10}$ CFU/cm²)) or difference in risk prevalence between the groups being compared (risk ratio (RR)). A median and range of effect estimates from individual studies in the meta-analysis subgroup are presented. Heterogeneity was assessed using I^2, which measures the percentage of variability in the effect size, which is not result of sampling error. Finally, evidence from each data subgroup was compiled and summarized by beef processing stage in a practical and interpretable format.

This meta-analysis study generated a total of 138 forest plots. Among them, where three or more trials were available in a comparison group, they were analysed under meta-analysis, and random-effects models were used to calculate pooled summary effects (pooled risk ratios (RR), for prevalence outcomes, or pooled log mean difference for concentration outcomes). This resulted in a total of 102 forest plots and summary effects, and among them, 33 forest plots with low heterogeneity (therefore better confidence in the results, in Results section) and 69 forest plots with medium or high heterogeneity (therefore less confidence in the results, in Appendix D). The remaining 36 forest plots where there were two trials per comparison group, were generated for comparison purposes, but no summary effect of intervention was calculated (Appendix D). The rest of the results where only one trial was identified is presented in tabulated form (Appendix D).

For a better overview of these summary effects, bar charts were created and are shown below (Figures 35-40). They are created for all five outcome microorganisms investigated, i.e. ACC, EBC, generic *E. coli* (both concentration and prevalence studies), pathogenic *E. coli* (*E. coli* O157 and non-O157) and *Salmonella* spp. While bar charts for indicator organisms show results from commercial abattoir conditions and trials, there were insufficient data for STEC and *Salmonella* spp. to replicate this. Therefore, the bar charts for these two pathogens were created using summary effects from challenge trials conducted under laboratory or pilot plant conditions (using artificially inoculated microorganisms), so the efficacies are likely exaggerated and would not reflect real life conditions that exist in abattoirs. Nevertheless, they are useful to provide some indication of relative efficacy of specific interventions. These bar charts also indicate confidence intervals, number of studies/trials compared in each group and most importantly, level of heterogeneity for each group. Therefore, they should only be used for illustration and basic comparative purposes. They do not display the nuances of each of the meta-analysis, and careful examination should be made of each one before any management or policy decisions are made.

Most of data were generated from highly heterogeneous studies and trials (highlighted in red). Meta-analyses highlighted in green are generated from summary effects and studies with low heterogeneity, hence there is more confidence in the produced results. During interpretation attention must also be paid to the confidence intervals displayed as they indicate how significant the results are.

For example, in log mean reduction results, if confidence intervals cross 0, such as in case of shellac hide coating intervention for ACC, it indicates that the intervention had no statistically significant effect in reducing ACC as a group, even though the reduction in transfer effect was substantial, -1.07 $\log_{10}$ CFU/cm² (95% CI:-2.43 to 0.29). This is due to high heterogeneity of the two studies and three trials investigated, and broad confidence intervals (Figures 35 and 80). When comparing to dry chilling that had similar intervention effect in reducing ACC, of -1.11 $\log_{10}$ CFU/cm² (95% CI: -0.63 to -1.58) and was statistically significant (Figures 35 and 119) the likely difference in confidence intervals was due to

number of studies/trials compared (i.e. 2/3 for shellac and 2/9 for dry chilling). This indicates need for further research for all interventions showing promising reduction effects but for which insufficient amount of data is available (e.g. the same applies to organic acid washes and steam vacuuming for generic *E. coli* where multiple trials were analysed from only one study, Figure 37).

Meta-analysis is a useful analytical tool for combining the results of multiple primary research studies into a weighted, average estimate of intervention effect. The limitation of this analysis could be that, even though every effort was made to stratify data in the most similar subgroups, sometimes there may be groups with larger differences between data. This approach was used for pragmatic reasons to combine a sufficient number of trials for meta-analysis, wherever it was possible, from a limited pool of data. For example, hot water washing trials investigating different water temperatures, application methods, duration, etc, were analysed together, and for chemical interventions, those with different concentrations (for example lactic acid 2-5%). As a consequence, details about intervention application parameters (e.g., concentration, temperature, duration, etc) and differences between study sampling and laboratory methods were not investigated as possible sources of variation in intervention effects across studies. 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 these factors in detail. However, the created forest plots contain sufficient information and description about analysed interventions, so they should be carefuly examined for more details.

Overall, we have identified a lack of large controlled trials conducted under commercial conditions, with sound study design and adequate reporting of intervention protocols. This was particularly case with cattle hide interventions and multiple beef carcass interventions at slaughter, prior to dehiding to pre-fabrication stage. Inadequte reporting of protocols, addressing confounders, units of outcome measurement (e.g. presenting data per cm^2 instead 100 cm^2 in cases where very low bacterial numbers were detected) and results (e.g. lack of measures of variability) were common and reduced further already sparse pool of scientific knowledge in this area.

In terms of the interventions identified in the previous project FS301044 that progressed to meta-analysis, they are listed below.

Interventions for which there were insufficient data for meta-analysis or that did not progress further due to being judged at ‘unclear’ or ‘high’ risk of bias:

- Lairage interventions: i) lairage cleaning; ii) cattle handling in lairage; iii) pre-slaughter cattle hide interventions (washing, clipping, bacteriophage spray)
- Cattle hide interventions: i) chemical dehairing; ii) most chemical washes

- Beef carcass interventions: i) standard processing procedures and GHP (knives sanitation, hide removal, bung bagging); ii) most organic acid washes (apart from lactic acid wash) and other chemical washes (for example peroxyacetic acid, trisodium phosphate, acidified sodium chlorate, etc)
- Interventions for beef primals, subprimals and trim: i) most chemical washes (apart from lactic and peroxiacetic acid and sodium metasilicate); ii) some novel physical (electron beam (E-beam) and ultraviolet (UV) light irradiation) or biological interventions (bacteriophage spray, nisin, lactoferrin, use of lactic acid bacteria, etc)

Interventions for which there were limited data for meta-analysis:

- Cattle hide interventions: i) some chemical washes (organic acids, chlorine, sanitiser); ii) shellac hide coating
- Beef carcass interventions: i) knife trimming; ii) steam vacuuming; iii) lactic acid wash; iv) other organic acid washes; v) multiple interventions

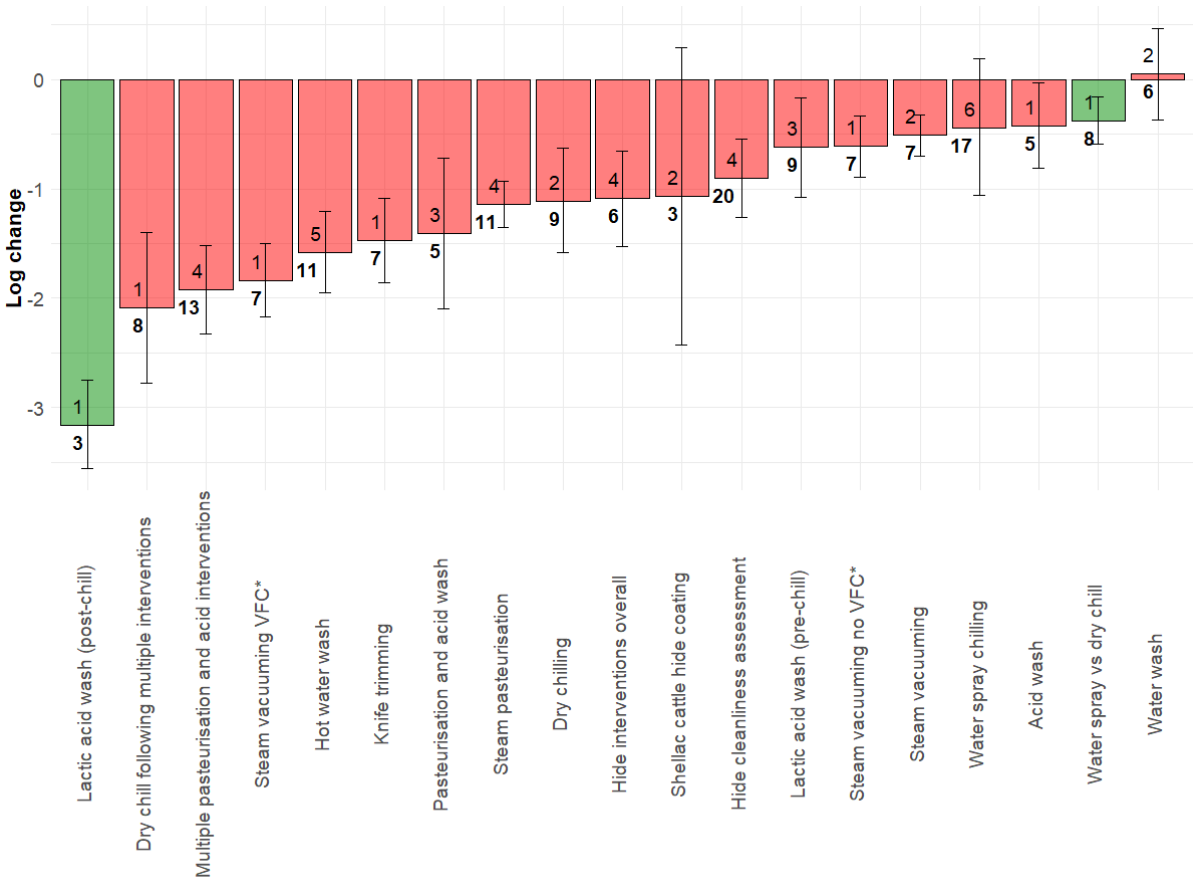
Interventions for which there were sufficient data for meta-analysis:

- Lairage interventions: i) hide cleanliness assessment
- Cattle hide interventions: i) water wash
- Beef carcass interventions: i) water wash; ii) hot water wash; iii) steam pasteurisation; iv) chilling (dry, water spray and spray chilling with chemicals)
- Interventions for beef primals, subprimals and trim: i) some chemical washes (lactic and peroxiacetic acid and sodium metasilicate)

4.1 The efficacy of interventions on aerobic colony counts

The beef interventions efficacies were investigated in majority of studies using indicator microorganisms, particularly aerobic colony counts. In total, 19 summary effects results are presented for ACC in Figure 35.

Figure 35. A comparison of meta-analyses of cattle hide and beef carcass processing interventions on aerobic colony counts (pooled log change) on beef carcasses under commercial abattoir conditions



Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

Cattle hide interventions report reduction in hide-to-carcass transfer ('carcass effect'), if not explicitly stated 'hide' all interventions relate to skinned beef carcass treatment

The only lairage intervention for which there were sufficient data for meta-analysis was cattle hide cleanliness assessment. This procedure is a visual scoring and categorisation of animals according to the cleanliness of their hides, which can lead to subsequent actions (or

other interventions) in case animals are too dirty. This scoring system is widely used in UK abattoirs. While this procedure is not a 'true' intervention, but is rather a visual scoring and categorisation of animals according to their hides cleanliness, the subsequent actions (or interventions) conducted in case animals are too dirty to be processed hygienically are informed by it. The summary effects from random-effect meta-analysis model showed consistent reduction for ACC when clean cattle are compared to dirty cattle, on hides and resulting carcasses. Mean reduction (log_{10} CFU/cm²) on carcass surfaces was -0.9 (95% CI: -0.54 to -1.26), but with high heterogeneity between studies (Figure 55). The results indicate the uselfulness of this scoring system in separating clean and dirty animals and thus proactively reducing potential carcass contamination during subsequent dehiding process.

Regarding cattle hide interventions, the results presented in Figure 35 present only those where the efficacy was investigated as a reduction in hide-to-carcass microbial transfer ('carcass effect'). The only one for which summary effect could be calculated (i.e. had at least three reported trials) was shellac hide coating. The reduction in transfer was -1.07 log_{10} CFU/cm² (95% CI: -2.43 to 0.29) for ACC (Figure 80). However, due to small number of trials and high heterogeneity between studies, the effect was not statistically significant overall, implying that more research is needed. Other similar controlled trials (conducted under abattoir conditions and reporting efficacy as a reduction in hide-to-carcass microbial transfer) included cetylpyridinium chloride wash, sodium hydroxide wash and proprietary sanitiser wash (Figures 35 and 60). When plotted together, the summary effect showed significant reduction in transfer of ACC to carcasses of -1.09 log_{10} CFU/cm² (95% CI: -0.65 to -1.53). Expectedly, high heterogeneity was shown due to inherent differences between these hide interventions and studies. Nevertheless, the meta-analysis clearly shows that cattle hide interventions, when applied under commercial abattoir conditions, are able to deliver around 1 log reduction in transfer of bacteria to carcasses and are efficacious.

Other cattle hide interventions analysed reported data on the intervention efficacy in reducing microorganisms on hides. Hide washing was ineffective in reducing ACC, with a reduction of -0.6 log_{10} CFU/100 cm² (95% CI: -1.22 to 0.22), Figure 65. This raises questions around the usefulness and practicality of hide water washing as a standalone intervention to reduce microbial contamination found on hides.

Some carcass interventions had a little or no effect at all, such as final carcass water wash. Six commercial trials generally found that water washes at best did not reduce ACC on beef carcasses, and at worst it can even increase contamination (0.05 log_{10} CFU/cm², 95% CI: -0.37 to 0.47), Figure 88. Knife trimming showed significant effect in reducing ACC on beef carcasses, with -1.47 log_{10} CFU/cm² reduction (95% CI: -1.09 to -1.86), but the results came from just one study with seven trials conducted under commercial abattoir conditions and with high heterogeneity between trials (Figure 93). Hot water wash led to a significant reduction in ACC on beef carcasses of -1.58 log_{10} CFU/cm² (95% CI: -1.21 to -1.95), Figure 95.

Steam vacuuming investigated in controlled commercial trials lead to a significant reduction in ACC on carcass areas with no visible faecal contamination, with medium heterogeneity between trials, of -0.61 log_{10} CFU/cm² reduction (95% CI: -0.32 to -0.89), Figure 101. Higher reduction effect on ACC was shown when steam vacuuming was used on areas with visible faecal contamination (-1.84 log_{10} CFU/cm², 95% CI: -1.50 to -2.17), with high heterogeneity between trials (Figure 102). Similarly, in before-and-after trials in commercial condition, steam vacuuming showed a reduction effect on ACC (-0.51 log_{10} CFU/cm², 95% CI: -0.32 to -0.70), with medium heterogeneity between trials and studies (Figure 103). This intervention is regularly used in most abattoirs and apparently it can be highly efficacious when properly applied, delivering statistically significant reduction effect. However, reduction effects highly depend on the skill and diligence of the user to spot visible contamination and efficiently remove it, therefore interventions' parameters are difficult to optimise to achieve consistent effect in reducing microbial hazards.

Furthermore, apart from the lactic acid wash, other organic acids and chemicals have not been widely researched to produce some data for meta-analysis. Lactic acid washes (2-5%) had largely significant reduction effect, from -0.62 log_{10} CFU/cm² (95% CI: -0.17 to -1.08) pre-chill (Figure 108), to -3.16 log_{10} CFU/100cm² (95% CI: -2.75 to -3.56) post-chill (Figure 19). The discrepancy is likely due to the small number of trials and only one study involved (Figure 35).

Water spray chilling showed inconsistent effect when investigated in commercial abattoir conditions. Eight controlled trials with low heterogeneity performed under commercial abattoir conditions, found that water spray chilling, when compared to conventional dry chilling, led to a small but significant -0.38 log_{10} CFU/cm² reduction in ACC on beef carcass sides (95% CI: -0.16 to -0.59, Figure 28), but no effect or it even increased ACC levels when comparing to before treatment in 17 before-and-after trials (-0.44 log_{10} CFU/cm², 95% CI: -1.06 to 0.19, Figure 131). This relatively small reduction effect raises questions around the usefulness and practicality of water spray chilling and indicates likely potential for further bacterial growth on carcass surfaces post-chill due to their increased moisture.

There was an increasing trend in interventions efficacies noted when using pasteurisation treatments, acid washes and dry chilling, individually or in a multiple sequential system. For example, dry chilling reduced ACC by 1.11 log_{10} CFU/cm² (95% CI: -0.63 to -1.58, Figure 119) < steam pasteurisation 1.14 log_{10} CFU/cm² (95% CI: -0.93 to -1.35, Figure 104) < pasteurisation and acid wash 1.41 log_{10} CFU/cm² (95% CI: -0.72 to -2.10, Figure 117) < multiple pasteurisation and acid interventions 1.92 log_{10} CFU/cm² (95% CI: -1.52 to -2.33, Figure 147) < dry chilling following multiple interventions 2.09 log_{10} CFU/cm² (95% CI: -1.40 to -2.78, Figure 120) (likely due to residual effect on carcasses).

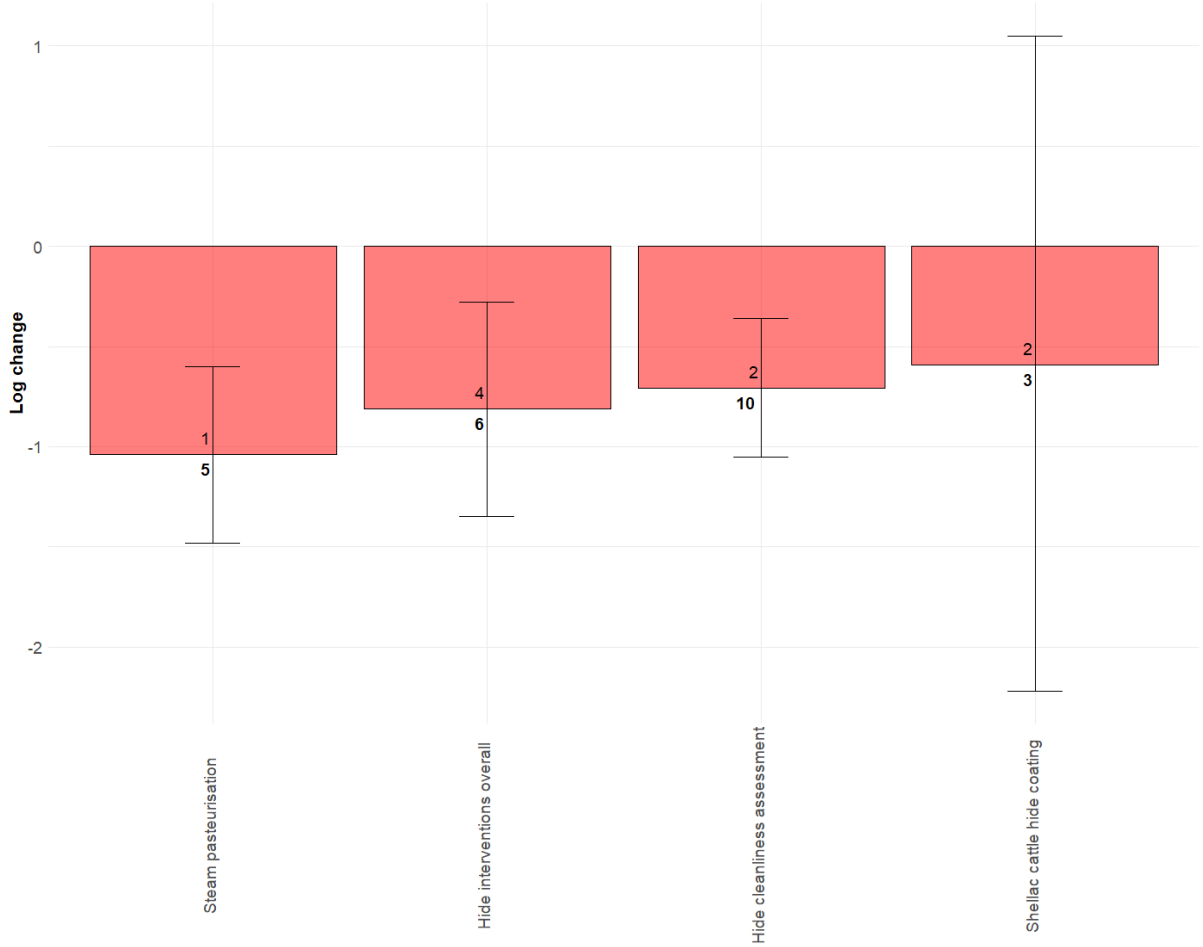
Where multiple interventions are applied, it is reasonable to expect that the overall improvement of the microbiological status of beef carcasses would be determined by a combination of microbial reductions achieved by all interventions, and be greater than the

individual effect of each intervention in isolation. Also, when carcass interventions are combined with cattle hide interventions in a sequential way, even greater reductions are achievable. The 'multiple-hurdle approach' in this case would rely on properly implemented prerequisite GHP-based measures in place, for example lairage cleaning, proper cattle handling in the lairage, hide cleanliness assessment, carcass knife trimming and steam vacuuming alongside careful hide removal and bunging/rodding. This can then extend to the hazard-based cattle hide interventions (chemical hide washes or microbial immobilisation treatment), beef carcass interventions at slaughter (pasteurisation treatments with hot water and/or steam and organic acid washes) and carcass interventions at chill/post-chill stage (organic acid washes of carcasses); concluding with interventions for beef cuts post-chill (organic acid washes), and also interventions in packaging stage (modified atmosphere and vacuum packaging of meat with added lactic acid).

4.2 The efficacy of interventions on *Enterobacteriaceae* counts

Only four summary effects were created for interventions efficacies on *Enterobacteriaceae* counts due to lack of data (Figure 36).

Figure 36**.** A comparison of meta-analyses of cattle hide and beef carcass processing interventions on *Enterobacteriaceae* counts (pooled log change) on beef carcasses under commercial abattoir conditions



Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

Cattle hide interventions report reduction in hide-to-carcass transfer (‘carcass effect’), if not explicitly stated ‘hide’ all interventions relate to skinned beef carcass treatment

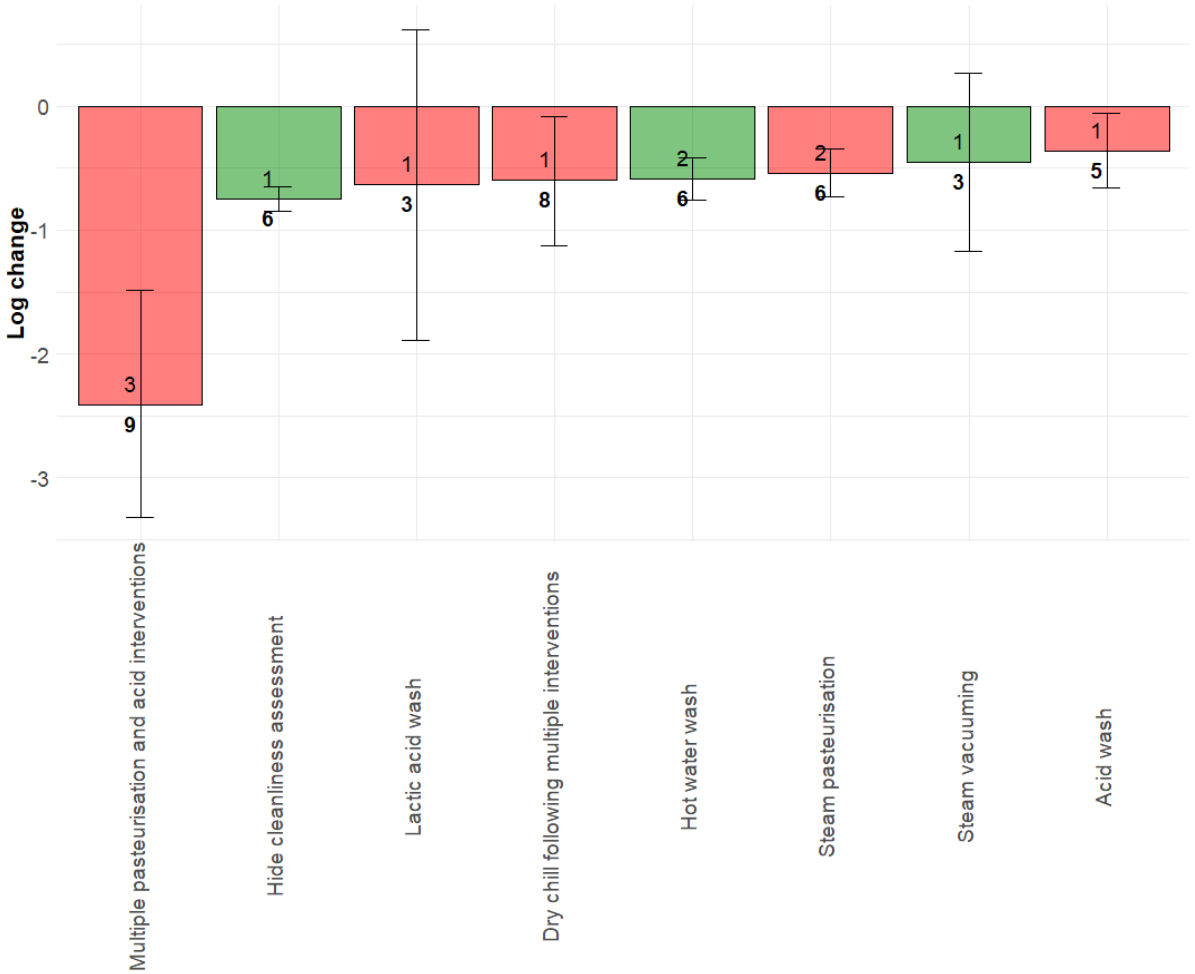
Mean reduction effect of hide cleanliness assessment for EBC (log_{10} CFU/cm²) on carcass surfaces was 0.71 (95% CI: -0.36 to -1.05), but with high heterogeneity between studies (Figure 56). Similarly to ACC, the effect was significant and indicates the usefulness of this scoring system in controlling beef carcass contamination. Shellac hide coating showed overall low efficacy, with reduction in transfer of 0.59 log_{10} CFU/cm² (95% CI: -2.22 to 1.05), Figure 81. However, due to small number of trials and high heterogeneity between studies, the effect was not statistically significant overall, and more research is needed. Overall hide interventions investigated in six controlled trials (conducted under abattoir conditions and reporting efficacy as a reduction in hide-to-carcass microbial transfer) showed reduction in EBC transfer to carcasses of 0.81 log_{10} CFU/cm² (95% CI: -0.28 to -1.35), Figure 61. Similarly to ACC, it indicates the usefulness of cattle hide treatments in controlling microbiological contamination that potentially can be transferred to carcasses during dehiding process.

Data on other carcass interventions effects on EBC were largely scarce. Regarding steam pasteurisation, only one controlled trial study in commercial abattoir conditions showed significant effect in reducing EBC in three trials (-0.89 log_{10} CFU/1000cm², 95% CI: -0.67 to -1.10, Figure 16). Similarly, in five before-and-after trials reduction of EBC was 1.04 log_{10} CFU/cm² (95% CI: -0.60 to -1.48, Figure 106). Ten before-and-after trials performed under commercial abattoir conditions found that steam pasteurisation significantly reduced *Enterobacteriaceae* prevalence on beef carcass sides (RR 0.17, 95% CI: 0.07-0.43), with low heterogeneity between studies (Figure 14).

4.3 The efficacy of interventions on generic *E. coli*

Eight summary effects on generic *E. coli* were created from data from studies investigating effect on *E. coli* concentration (Figure 37) and nine summary effects from prevalence studies (Figure 38).

Figure 37**.** A comparison of meta-analyses of cattle hide and beef carcass processing interventions on generic *E. coli* counts (pooled log change) on beef carcasses under commercial abattoir conditions



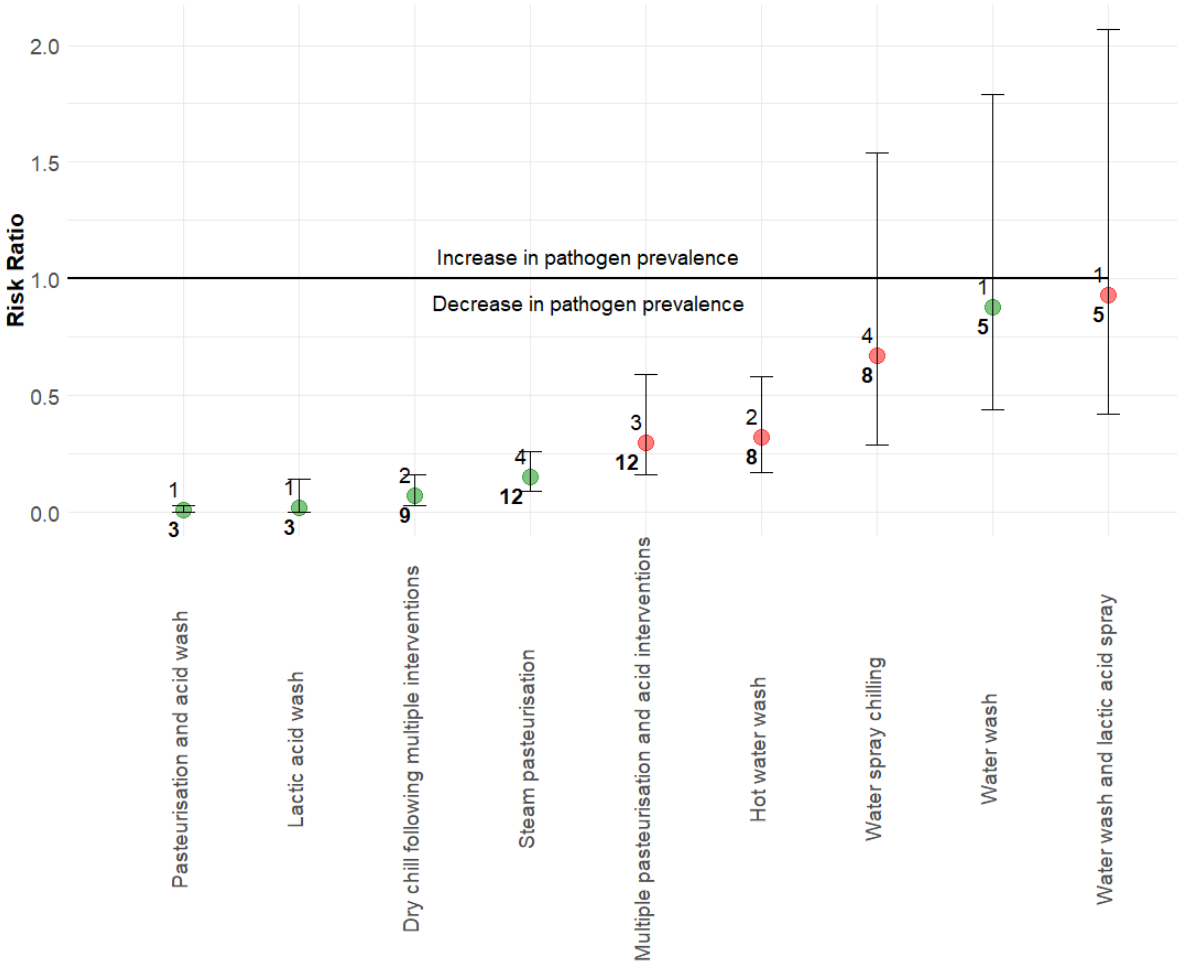
Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

Cattle hide interventions report reduction in hide-to-carcass transfer (‘carcass effect’), if not explicitly stated ‘hide’ all interventions relate to skinned beef carcass treatment

Figure 38. A comparison of meta-analyses of beef carcass processing interventions on generic *E. coli* prevalence (pooled risk ratios) on beef carcasses under commercial abattoir conditions



Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

Following similar pattern as with ACC and EBC, hide cleanliness assessement showed a significant 0.75 $\log_{10}$ CFU/cm² reduction effect in generic *E. coli* (95% CI: -0.65 to -0.85), with low heterogeneity between trials (Figure 2). Hot water wash led to a significant reduction in generic *E. coli* on beef carcasses, -0.59 $\log_{10}$ CFU/cm² (95% CI: -0.42 to -0.76) in trials performed under commercial abattoir conditions, with low heterogeneity between trials (Figure 11).

Similarly, multiple pasteurisation and acid interventions showed reduction in generic *E. coli* counts of 2.41 $\log_{10}$ CFU/cm² (95% CI: -1.49 to -3.32, Figure 148), however, associated with high heterogeneity due to inherent differences between trials and multiple 'hurdle' systems used. In the case of other interventions, relatively low reduction effects can be attributed to often low generic *E. coli* counts on beef carcasses under commercial abattoir conditions, which makes it difficult to investigate quantitative effect in reduction. Hence, the change in prevalence gives better overview (Figure 38).

Final carcass water wash had a little or no effect at all (Figure 10). Five commercial trials generally found that water washes did not change generic *E. coli* prevalence on washed beef carcasses (RR 0.88, 95% CI: 0.44 to 1.79). Furthermore, apart from the lactic acid wash, other organic acids and chemicals have not been widely researched to produce some data for meta-analysis. Post-chill carcass application of lactic acid (4%) reduced *E. coli* prevalence significantly (RR 0.02, 95% CI: 0 to 0.14, Figure 18), but from only one study and three trials. Hot water wash had overall positive effect in reducing prevalence of generic *E. coli* (RR 0.32, 95% CI: 0.17 to 0.58, Figure 94). Other pasteurisation treatments follow similar pattern. Twelve before-and-after trials from three studies performed under commercial abattoir conditions found that steam pasteurisation significantly reduced generic *E. coli* prevalence on beef carcass sides (RR 0.15, 95% CI: 0.09 to 0.26). Three before-and-after trials performed under commercial abattoir conditions found that carcass pasteurisation with hot water or steam and subsequent lactic acid or peroxyacetic acid spray washes significantly reduced *E. coli* prevalence on beef carcass sides (RR 0.01, 95% CI: 0 to 0.03) with low heterogeneity between trials (Figure 22).

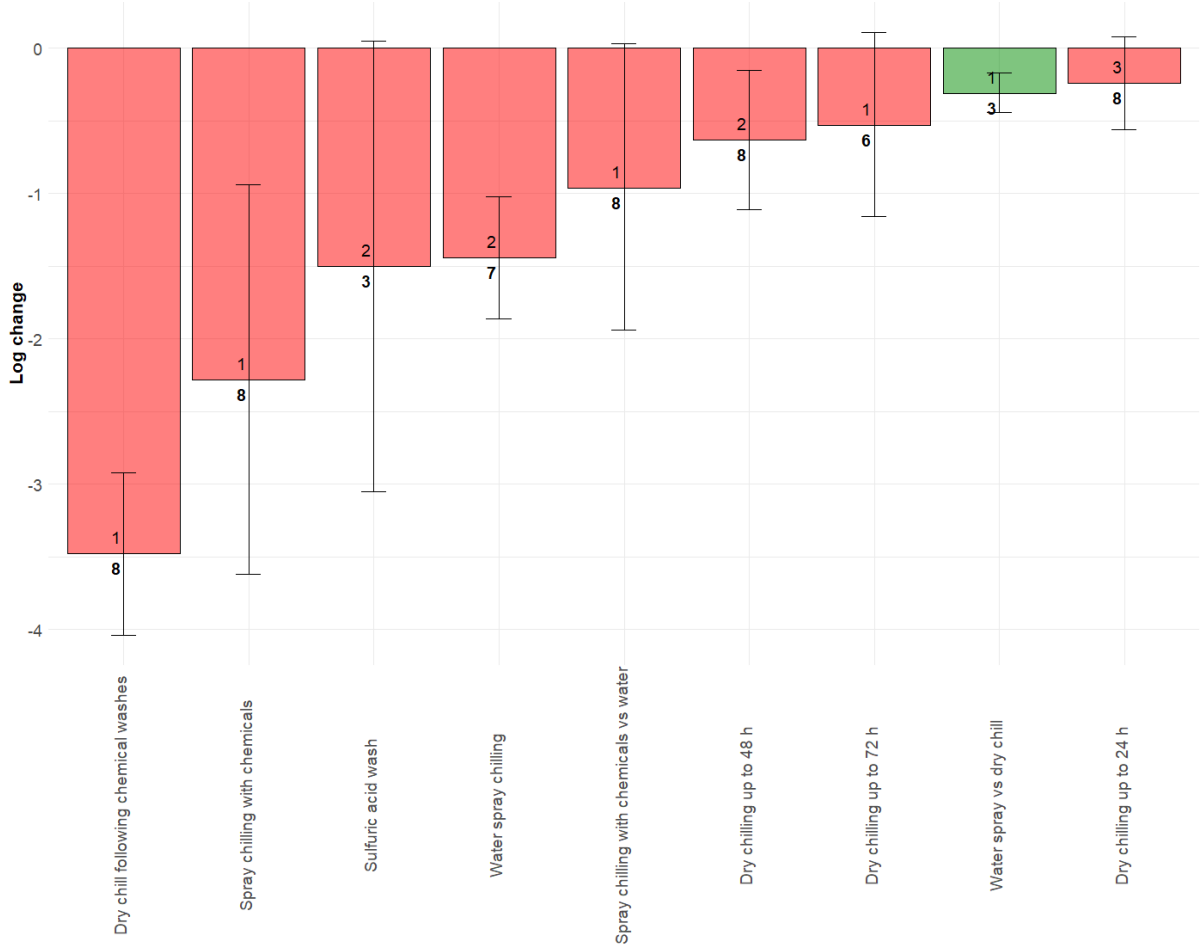
Water spray chilling showed inconsistent effect when investigated in commercial abattoir conditions. Eight before-and-after trials with high heterogeneity performed under commercial abattoir conditions, found that water spray chilling, at best showed no effect on reduction of generic *E. coli* prevalence and at worst even increased it (RR 0.67, 95% CI: 0.29 to 1.54, Figure 130).

Dry chilling following multiple slaughter line interventions was investigated in nine before-and-after trials performed under commercial abattoir conditions. They found significant reduction of *E. coli* prevalence on beef carcass sides (RR 0.07, 95% CI: 0.03 to 0.16) with low heterogeneity. Similarly to the efficacy on ACC, it seems that multiple interventions overall improve microbiological status of beef carcasses greater than the individual effect of each intervention in isolation.

4.4 The efficacy of interventions on *Salmonella* spp.

The following bar chart shows summary effects from challenge trials conducted under laboratory or pilot plant conditions on *Salmonella* spp., as there were insufficient data from commercial abattoir conditions and trials for the analysis. Therefore, the efficacies were investigated using artificially inoculated bacteria and consequently the effects are likely exaggerated and would not reflect real life conditions that exist in abattoirs. It can be noticed that comparable interventions showed 1-2 log higher reduction effect in the laboratory than in commercial conditions. Nevertheless, the results are useful to provide some indication of the relative efficacy of specific interventions.

Figure 39**.** A comparison of meta-analyses of beef carcass processing interventions on *Salmonella* spp. counts (pooled log change) on beef meat under laboratory conditions



Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

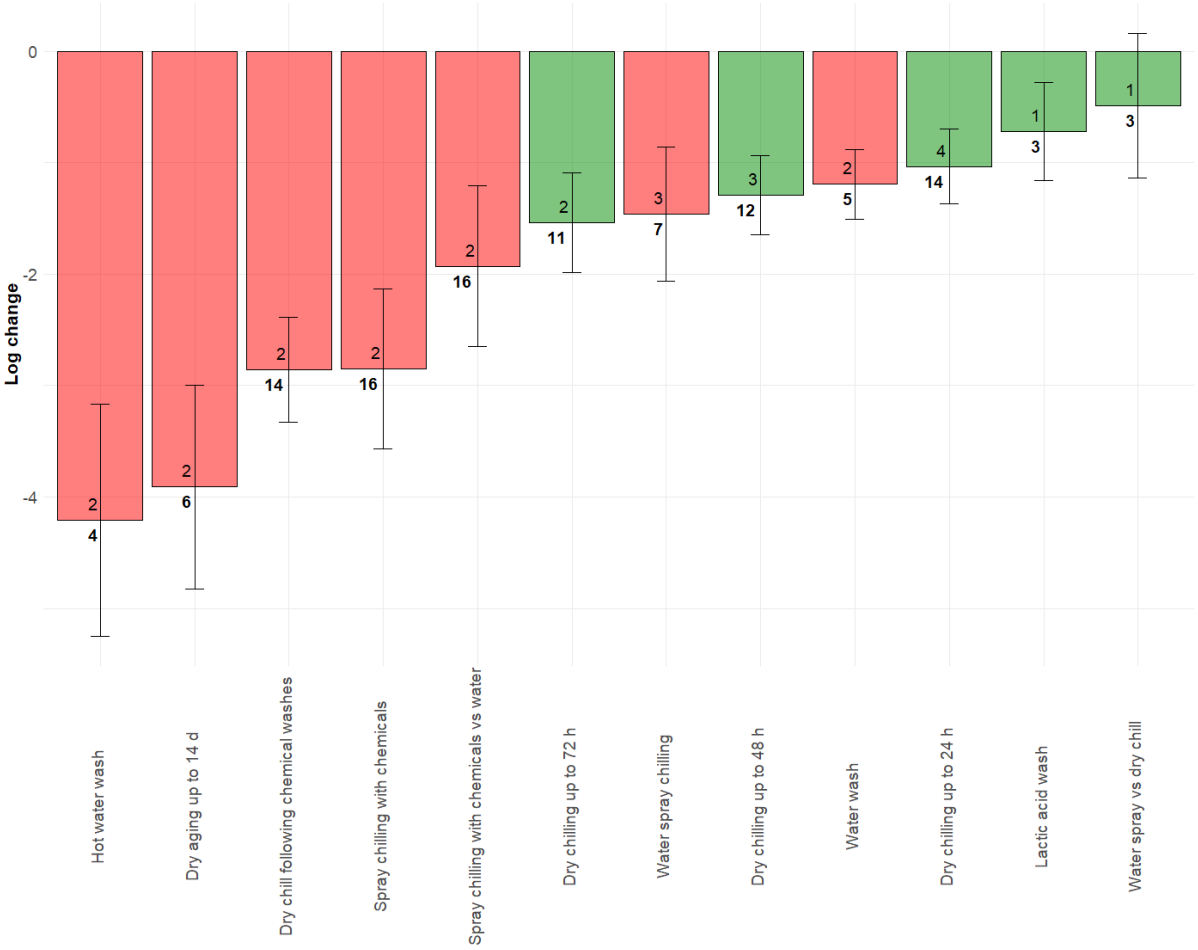
Dry chilling with no interventions used beforehand showed no effect in reducing *Salmonella* spp. numbers -0.24 $\log_{10}$ CFU/cm² (95% CI: -0.56 to 0.08, Figure 122) when the effect was measured after 24 hours, limited effect of -0.63 $\log_{10}$ CFU/cm² (95% CI: -0.15 to -1.11, Figure 123) up to 48 hours and no effect of -0.53 $\log_{10}$ CFU/cm² (95% CI: -1.16 to 0.11, Figure 124) up to 72 hours, with high heterogeneity between trials. Dry chilling following chemical washes led to significant reduction in *Salmonella* spp. numbers (-3.48 $\log_{10}$ CFU/cm², 95% CI: -2.92 to -4.04, Figure 125). Spray chilling using chemicals was investigated in several challenge trials performed under laboratory conditions, showing high heterogeneity between trials and mostly evidence of a reduction. The intervention reduced *Salmonella* spp. numbers by 2.28 $\log_{10}$ CFU/cm² (95% CI: -0.94 to -3.62, Figure 142) when comparing to before treatment, and 0.96 $\log_{10}$ CFU/cm² (95% CI: -0.03 to -1.94, Figure 143) when comparing to water spray chilling.

Four challenge trials performed under laboratory conditions found that lactic acid dipping led to a significant 1.30 $\log_{10}$ CFU/cm² reduction in *Salmonella* spp. numbers on beef trim (95% CI: -0.18 to -2.42, Figure 33) when compared to water dipping. Four challenge trials performed under laboratory conditions found that sodium metasilicate dipping led to a significant 1.28 $\log_{10}$ CFU/cm² reduction in *Salmonella* spp. numbers on beef trim (95% CI: -0.14 to -2.43, Figure 34) when compared to water dipping. These three comparisons were associated with low heterogeneity between trials.

4.5 The efficacy of interventions on pathogenic *E. coli*

The following bar chart shows summary effects from challenge trials conducted under laboratory or pilot plant conditions on pathogenic *E. coli*, as there were insufficient data from commercial abattoir conditions and trials for the analysis. Similarly to the results on *Salmonella* spp., the efficacies were investigated using artificially inoculated bacteria and consequently the effects are likely exaggerated and would not reflect real life conditions that exist in abattoirs. It can be noticed that comparable interventions showed 1-2 log higher reduction effect in the laboratory than in commercial conditions.

Figure 40. A comparison of meta-analyses of beef carcass processing interventions on pathogenic *E. coli* counts (pooled log change) on beef meat under laboratory conditions



Green: Homogenous trials

Red: Heterogeneous trials

Numbers in bar chart: Top number = Number of studies, Bottom number = Number of trials

Dry chilling with no interventions used beforehand led to a significant reduction in *E. coli* O157:H7 numbers of 1.04 $\log_{10}$ CFU/cm² (95% CI: -0.70 to -1.37, Figure 24) when the effect was measured after 24 hours, 1.29 $\log_{10}$ CFU/cm² (95% CI: -0.94 to -1.65, Figure 25) up to 48 hours and 1.54 $\log_{10}$ CFU/cm² (95% CI: -1.09 to -1.99, Figure 26) up to 72 hours, with low heterogeneity between trials.

Dry chilling following chemical washes led to significant reduction in *E. coli* O157:H7 numbers of 2.86 $\log_{10}$ CFU/cm² (95% CI: -2.39 to -3.33, Figure 126). Similar study design showed that dry aging up to 14 days led to reduction of *E. coli* O157:H7 numbers of 3.91 $\log_{10}$ CFU/cm² (95% CI: -3.00 to -4.83, Figure 129).

Spray chilling using chemicals was investigated in several challenge trials performed under laboratory conditions, showing high heterogeneity between trials and mostly evidence of a reduction. The intervention reduced generic *E. coli* O157:H7 by 2.85 $\log_{10}$ CFU/cm² (95% CI: -2.13 to -3.57, Figure 144) when comparing to before treatment, and 1.93 $\log_{10}$ CFU/cm² (95% CI: -1.21 to -2.66, Figure 145) when comparing to water spray chilling, with high heterogeneity between trials.

There was limited amount of data available for meta-analysis of interventions effects for beef trim. Eight challenge trials performed under laboratory conditions found that sodium metasilicate dipping led to a significant 1.04 $\log_{10}$ CFU/cm² reduction in *E. coli* O157:H7 and non-O157 numbers on beef trim (95% CI: -0.81 to -1.27, Figure 32) when compared to water dipping. Lactic acid dipping led to a significant 0.88 $\log_{10}$ CFU/cm² reduction in *E. coli* O157:H7 and non-O157 numbers on beef trim (95% CI: -0.51 to -1.26, Figure 151).

5. Conclusions

- This study analysed a large body of literature on beef interventions, covering the period of the last 25 years, using a meta-analysis tool to combine the results of multiple primary research studies into a weighted, average estimate of intervention effect. Data from studies on lairage interventions, cattle hide and beef carcass interventions, and interventions for fabricated beef investigating reduction effect on pathogenic microorganisms (*Salmonella* spp. and pathogenic *E. coli*) and indicator microorganisms (aerobic bacteria, *Enterobacteriaceae* and generic *E. coli*) were analysed and reported.

- Following a rigorous methodological approach, significant number of studies were excluded from meta-analysis due to their insufficient methodological quality and lack of adequate reporting of intervention protocols, units of outcome measurement and results. Therefore, there were insufficient data available for meta-analysis of some interventions, such as lairage and cattle hide interventions (cattle handling in lairage, cattle hide clipping, bacteriophage and chemical treatments), beef carcass interventions (standard procedures for carcasses and GHPs, organic acid and other carcass chemical washes) and interventions for beef primals, subprimals and trim (chemical washes and novel interventions).

- There were some interventions for which limited data were available and meta-analysis was performed, such as cattle hide interventions (some chemical washes and shellac hide coating) and beef carcass interventions (knife trimming, steam vacuuming, lactic acid and other organic acid washes and multiple interventions). Some of these interventions, such as shellac cattle hide coating, carcass lactic acid wash and steam vacuuming, were showing promising reduction but due to small number of trials, their efficacy is inconclusive. Therefore, more research is needed for all interventions where there is lack of data.

- Interventions for which there were sufficient data for meta-analysis include hide cleanliness assessment and water wash, beef carcass water wash, hot water wash, steam pasteurisation and chilling (dry, water spray and spray chilling with chemicals) and some chemical washes of fabricated beef.

- Hide cleanliness assessment, scoring system widely used in UK abattoirs, showed consistent reduction on resulting carcasses for ACC, EBC and generic *E. coli* of up to 1 log when clean cattle are compared to dirty cattle. The results indicate the uselfulness of this scoring system in separating clean and dirty animals and thus proactively reducing potential carcass contamination during subsequent dehiding process.

- Other cattle hide interventions investigated under commercial abattoir conditions, such as shellac hide coating and chemical washes with cetylpyridinium chloride, sodium hydroxide and proprietary sanitiser, showed significant reduction in transfer of ACC and EBC to carcasses of up to 1 log. This result indicates the usefulness of cattle hide interventions to proactively reduce potential carcass contamination during subsequent dehiding process.

- Overall across all five microorganisms, steam and hot water carcass pasteurisation had the largest potential impact on decreasing the prevalence and concentration of contaminated beef carcasses. Some of the most reliable and consistent results were generated for carcass pasteurisation treatments, as a standalone interventions or when followed on with acid wash or as a part of multiple hurdle system. When they are followed by dry chilling, the residual action on the carcasses over 24-72 hours of chilling is even more noticeable.

- Data on the interventions efficacy against pathogenic bacteria (*Salmonella* spp. and pathogenic *E. coli*) were mostly available from challenge trials conducted under laboratory or pilot plant conditions. Their efficacies were investigated using artificially inoculated bacteria and consequently the effects are likely exaggerated and would not reflect real life conditions that exist in abattoirs. Nevertheless, the results are useful to provide some indication of the relative efficacy of specific interventions.

- Overall, there was a lack of large controlled trials conducted under commercial conditions, with sound study design and adequate reporting of intervention protocols. This was particularly the case with cattle hide interventions and multiple beef carcass interventions at slaughter, prior to dehiding to pre-fabrication stage.

6. Recommendations and future work

On the basis of the work undertaken during this meta-analysis study, some key findings and recommendations are summarised below.

- Hide cleanliness assessment, scoring system widely used in UK abattoirs, showed consistent reduction on resulting carcasses for ACC, EBC and generic *E. coli* of up to 1 log when clean cattle are compared to dirty cattle. The results indicate that this procedure is useful and proactively reduce potential carcass contamination during subsequent dehiding process. Its continuous use can be recommended in UK abattoirs.

- There was a limited amount of data available for meta-analysis of cattle hide interventions and they merit more research efforts. Under commercial abattoir conditions, some hide interventions such as shellac hide coating and chemical washes with cetylpyridinium chloride, sodium hydroxide and proprietary sanitiser, showed significant reduction in transfer of ACC and EBC to carcasses of up to 1 log. On the basis of limited available data, they can be recommended for consideration as hazard-based interventions when applied post-exsanguination and before dehiding for reducing microbial contamination of resulting beef carcasses, but more research is needed to increase the confidence in results.

- Beef carcass interventions, such as pasteurisation treatments with hot water and/or steam, had the largest potential impact on decreasing the prevalence and concentration of contaminated beef carcasses across all five microorganisms. Both carcass pasteurisation treatments and organic (lactic) acid washes can be recommended for consideration as hazard-based interventions when applied after dehiding and pre-chill. They are already permitted for use in the UK and should be taken advantage of. It would be beneficial to conduct more research into their sequential use within multiple hurdle system.

- The results provide a high degree of certainty for studies with low heterogeneity. For studies with high heterogeneity, results can be used with caution. For interventions where limited number of trials was identified, more research is needed to increase the confidence in results and provide more robust understanding of their effectiveness for risk management decisions. There is a need for large controlled trials conducted under commercial conditions, particularly investigating multiple beef interventions at slaughter, prior to dehiding to pre-fabrication stage. Methodologies and data recording needs to be harmonised. These are the areas where further research is needed to fill the knowledge gaps.

- These data provided by this meta-analysis provides a firm basis for parameter estimates within quantitative microbial risk assessments (QMRAs), providing point

values within a specified distribution which can be used for variability or uncertainty distributions (depending on the study). Whilst this meta-analysis provides an initial exploration into the usefulness of measures applied during primary processing, a further quantitative microbial risk assessment (QMRA) would offer additional benefits. For example, a QMRA approach would provide estimates of the reduction in the population-level exposure to foodborne zoonoses at the point of consumption (or at other points along the farm-to-consumption pathway) through interventions at the abattoir. This enables a better understanding of the public health and economic benefits of interventions at the abattoir. Moreover, although meta-analysis provides an estimate of the direct pathogen reduction to each carcass, it cannot incorporate the complicated infection dynamics which occur at the abattoir. For example, interventions may have additional indirect benefits if they reduce the pathogen load which therefore subsequently reduces the risk of cross-contamination of machinery and previously clean carcasses. QMRA approaches enable a deeper exploration of both the direct and indirect effects of interventions, both at the abattoir and further down the foodchain.

Appendix A: Search strategy details

Full search algorithm used for the additional search of peer-reviewed literature since previous search performed in project FS301044 on the 14th of September 2018 (spanning 1996-2018).

Date	04/06/2020
Performed by	Dragan Antic
Database	Scopus
Search string:	*("escherichia coli" OR "e. coli" OR O157 OR shiga* OR STEC OR VTEC OR salmonella OR salmonellae OR aerob* OR enterobacteriaceae) AND (intervention* OR decontaminat* OR treatment* OR antimicrobial* OR inactiv* OR reduc* OR efficacy OR cleaning OR disinfect* OR slaughter* OR lairage* OR abattoir* OR hide* OR carcas* OR skin* OR hygien* OR dehid* OR eviscerat* OR bung* OR rodding OR wash* OR rins* OR spray* OR vacuum* OR steam OR trim* OR pasteuriz* OR pasteuris* OR "hot water" OR "organic acid*" OR "lactic acid" OR chill*) AND (beef OR cattle OR bovine OR cow OR cows OR calf OR calves OR veal)* in Article title OR in Abstract OR in Key words
Limits	2018-2020
Hits	1,168

Date	04/06/2020
Performed by	Dragan Antic
Database	CAB Direct
Search string:	*(“escherichia coli” OR “e. coli” OR O157 OR shiga* OR STEC OR VTEC OR salmonella OR salmonellae OR aerob* OR enterobacteriaceae) AND (intervention* OR decontaminat* OR treatment* OR antimicrobial* OR inactiv* OR reduc* OR efficacy OR cleaning OR disinfect* OR slaughter* OR lairage* OR abattoir* OR hide* OR carcas* OR skin* OR hygien* OR dehid* OR eviscerat* OR bung* OR rodding OR wash* OR rins* OR spray* OR vacuum* OR steam OR trim* OR pasteuriz* OR pasteuris* OR “hot water” OR “organic acid*” OR “lactic acid” OR chill*) AND (beef OR cattle OR bovine OR cow OR cows OR calf OR calves OR veal)* in Article title OR in Abstract
Limits	2018-2020
Hits	713

Appendix B: Risk of bias assessment and data extraction protocols

Risk of Bias form

The possible risk of bias judgements are: (1) Low risk of bias; (2) Some concerns; and (3) High risk of bias. Each paper was ranked as low, some concerns or high for each of the five bias domains (see table). Overall scores were calculated as follows:

- Papers which scored at least 4 / 5 domains with the same rank (low, some concerns or high), were given an overall score of that value.
- Papers which had fewer than 4/5 domains in the same rank was given the middle value between the highest and lowest rank (for example, a paper scoring three lows and two highs had an overall score of some concerns).

Bias domain: risk of bias arising from the randomization process

Signalling questions	**Risk-of-bias judgment**
1.1. Was the allocation sequence random?	**Low**: a random component was used in the sequence generation process. **High**: no random element was used in generating the allocation sequence or the sequence is predictable. **Unclear**: insufficient information provided to permit judgement
1.2. Was the allocation sequence concealed until samples were assigned to interventions?	**Low**: study instigators were blinded to group allocations during the study period. **High**: study instigators were not blinded to group allocations during the study period. **Unclear**: insufficient information provided to permit judgement
1.3. Did baseline differences between groups suggest a problem with the randomization process?	**Low**: no imbalances apparent between groups or observed imbalances are compatible with chance **High**: imbalances indicate problems with randomization process **Unclear**: insufficient information provided to permit judgement

Bias domain: risk of bias due to deviations from the intended interventions

Signalling questions	**Risk-of-bias judgement**

2.1. Were researchers aware of the assigned interventions?	**Low**: researchers unaware of assigned intervention **High**: researchers aware of assigned intervention **Unclear**: insufficient information provided to permit judgement
2.2. Were there deviations from the planned interventions/ methodologies?	**Low**: no deviations from trial protocol **High**: evidence, or strong reason to believe, that the trial context led to failure to implement the protocol interventions **Unclear**: insufficient information provided to permit judgement
2.3. Did these deviations likely affect the outcome?	**Low**: no reason to believe that deviations from trial protocol affected the trial outcome **High**: evidence, or strong reason to believe, that the deviations could affect the trial outcome **Unclear**: insufficient information provided to permit judgement
2.4. Are there any concerns that confounders have not been appropriately identified and accounted for?	**Low**: all relevant confounders have been managed throughout study design or analysis **High**: at least one relevant confounder has not been appropriately managed in either the study design or analysis **Unclear**: insufficient information provided to permit judgement

Bias domain: missing outcome data

Signalling questions	**Risk-of-bias judgement**
3.1. Was there missing data?	**Low**: no expected data was missing from analysis **High**: evidence, or strong reason to believe that appropriate data is missing **Unclear**: insufficient information provided to permit judgement
3.2. Would the level of missing data affect the outcome data?	**Low**: no reason to believe that missing data would have any effect on the outcome data **High**: evidence, or strong reason to believe that missing data could impact upon outcome data **Unclear**: insufficient information provided to permit judgement

Bias domain: risk of bias in measurement of the outcome

Signalling questions	Risk-of-bias judgement
4.1. Was the method of measuring the outcome appropriate?	**Low**: methods of outcome measurement are sensible and suitable to the study aim. **High**: methods of outcome measurement are unsuitable for the study aim **Unclear**: insufficient information provided to permit judgement
4.2. Could measurement of the outcome differ between groups?	**Low**: methods of outcome measurement are standardised throughout the study **High**: methods of outcome measurement are unstandardized or open to diagnostic detection bias **Unclear**: insufficient information provided to permit judgement
4.3. Were outcome assessors aware of the intervention groups?	**Low**: assessors were blinded to intervention status. **High**: assessors unblinded to intervention status **Unclear**: insufficient information provided to permit judgement
4.4. Could assessment of outcome be influenced by knowledge of intervention received?	**Low**: all study invigilators were blinded to intervention status. **High**: all study invigilators unblinded to intervention status **Unclear**: insufficient information provided to permit judgement
4.5. Is it likely that assessment of outcome was influenced by knowledge of intervention?	**Low**: knowledge of intervention status was unlikely to influence outcome assessment **High**: knowledge of intervention status was likely to influence outcome assessment **Unclear**: knowledge of intervention status could have influenced outcome assessment but there is no reason to believe that it did

Bias domain: risk of bias in selection of the reported result

Signalling questions	Risk-of-bias judgement

5.1. Did authors report all outcomes?	**Low**: all expected outcomes reported **High**: at least one expected outcome has not been reported **Unclear**: expected outcomes unclear
5.2. Did the outcomes match with the intended aim and plan of the study?	**Low**: outcomes appropriate for aims of study **High**: outcomes inappropriate for aims of study **Unclear**: aims of study unclear
5.3. Is there an appropriate justification why the outcome measure was selected?	**Low**: justification provided for selection of outcome measure **High**: no justification provided for selection of outcome measure **Unclear**: some ambiguity around justification for outcome measure

Data extraction form

Question	**Options**
Specify **intervention stage** in the minced beef production chain where intervention is applied	1. Abattoir pre-slaughter (lairage interventions) 2. Abattoir processing (slaughter and post-slaughter) – Pre-evisceration – Post-evisceration, pre-chill – Chilling – Post-chill 3. Post abattoir processing
Specify broad **intervention category** (and subcategory) being extracted	– Lairage cleaning – Cattle handling in lairage – Hide cleanliness assessment – Cattle hide interventions – Cleaning/disinfection of tools/knives – Standard processing procedures/GHP – Carcass interventions – Chilling and spray chilling – Multiple interventions – Post fabrication interventions (trim/ground beef)
Specify intervention	– _____
Intervention description (concentration, temperature,	– _____

Question	Options
application method, contact time, pressure)	
Specify target (intervention) **population/sample category** to which intervention is applied	1. Live animal 2. Cattle hide 3. Carcass 4. Beef trim 5. Ground/minced beef 6. Environment surfaces 7. Tools/knives/equipment
Specify target (intervention) **population/sample** more in details	– _____
Specify **outcome sample** category	1. Cattle hide 2. Carcass 3. Beef cuts 4. Ground/minced beef 5. Environment surfaces 6. Tools/knives/equipment
What **type of outcome sample** was measured?	1. Swab (sponge, other) 2. Excised meat sample 3. Ground
Specify **comparison** (control) group	1. No treatment 2. Pre treatment 3. Water wash 4. Other: _____
What **outcome group** did the study investigate?	1. Aerobic colony counts (ACC) 2. *Enterobacteriaceae* counts (EBC) 3. Generic *E. coli* counts 4. Pathogenic *E. coli* (STEC) 5. *Salmonella*
What **outcome strains** did the study investigate?	– _____
What outcome data were measured?	1. Concentration (log_{10} CFU): specify area of measurement: _____ 2. Prevalence (presence/absence)
Extract quantitative outcome data in text boxes for each relevant category	**Concentration outcomes** – Mean of control (Mc) – Standard deviation of control group (CDc) – Standard error of control group (SEMc) – Confidence interval of control group (CIc) – Number in control group (Nc) – Mean of intervention group (Me)

Question	Options
	– Standard deviation of intervention group (SDe) – Standard error of intervention group (SEMe) – Confidence interval of intervention group (CIe) – Number in intervention group (Ne) **Prevalence outcomes** – Number of events (i.e. positives) in control group (Ec) – Number of participants in control group (Nc) – Number of events (i.e. positives) in intervention group (Ee) – Number of participants in intervention group (Ne)

Appendix C: Risk of bias assessment results

The results from the Risk-of-bias (RoB) assessment process are presented below. The results are formatted according to the Robvis RoB 2 tool used to perform the assessments (McGuinness & Higgins, 2020; Sterne et al., 2019). The tool enables visualizing risk-of-bias assessments and creates:

1. "Traffic light" plots of the domain-level judgements for each individual result; and
2. Weighted bar plots of the distribution of risk-of-bias judgements within each bias domain.

The results are presented for 14 distinctive groups of intervention categories and study designs combinations. For the reason of brevity, only weighted bar plots of the distribution of risk-of-bias judgements, within each bias domain, are presented in this section.

Figure 41. Distribution of RoB judgements within each bias domain for studies investigating 'Lairage cleaning'

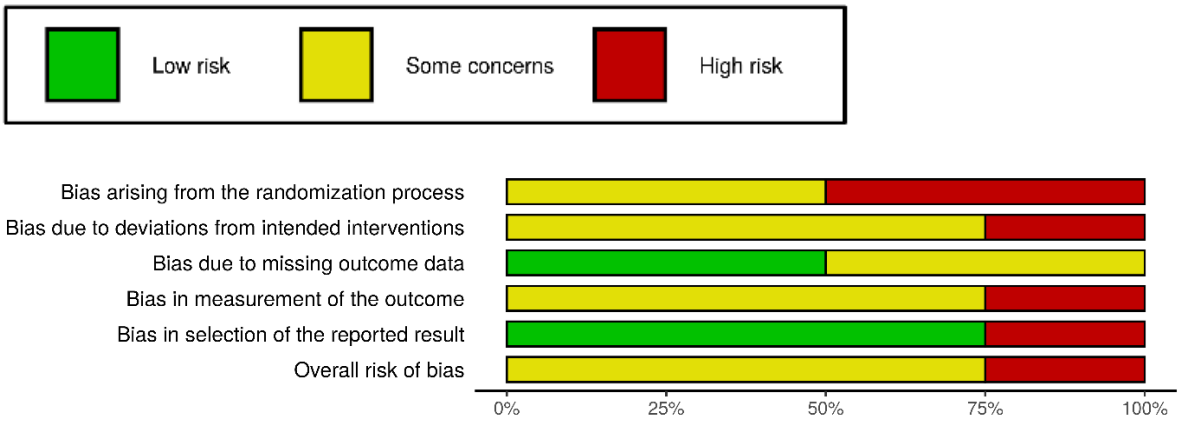


Figure 42. Distribution of RoB judgements within each bias domain for studies investigating 'Cattle handling in lairage'

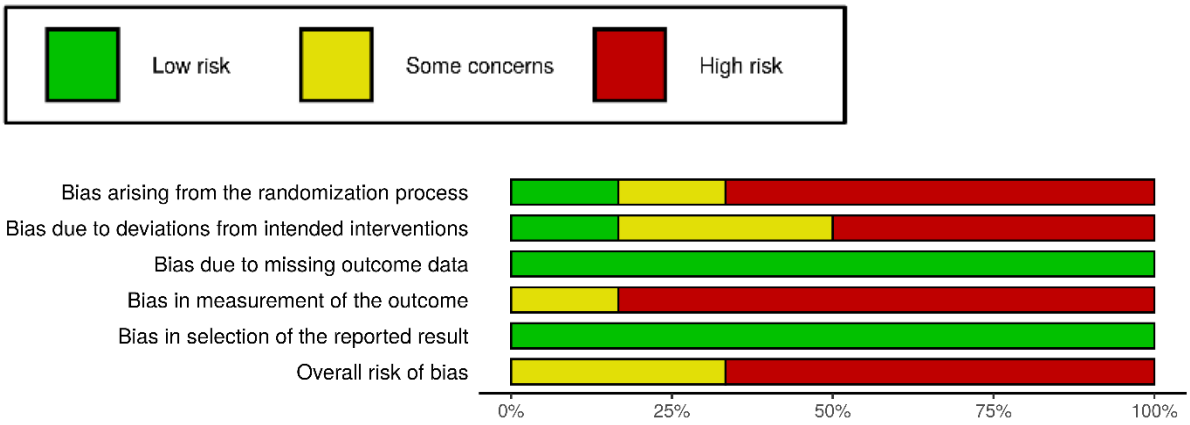


Figure 43. Distribution of RoB judgements within each bias domain for studies investigating 'Hide cleanliness assessment'

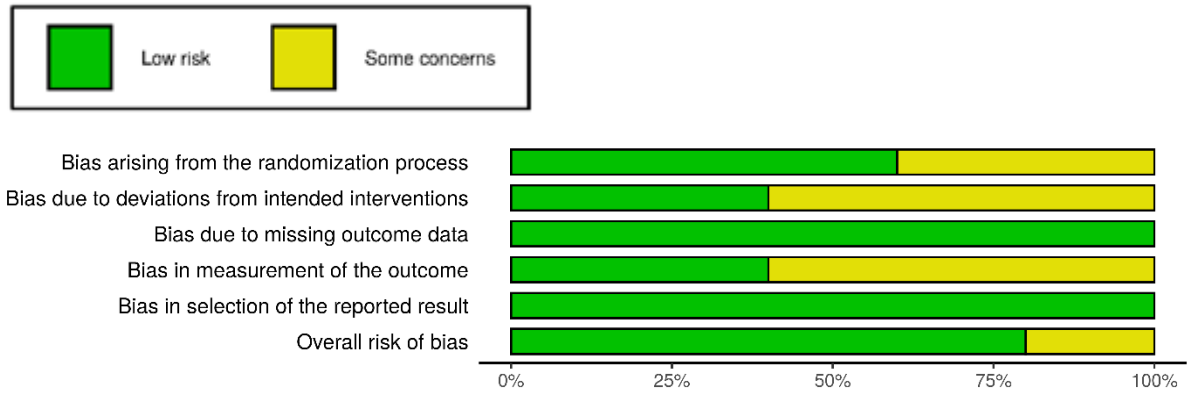


Figure 44. Distribution of RoB judgements within each bias domain for controlled trial studies performed under commercial abattoir conditions investigating 'Cattle hide interventions'

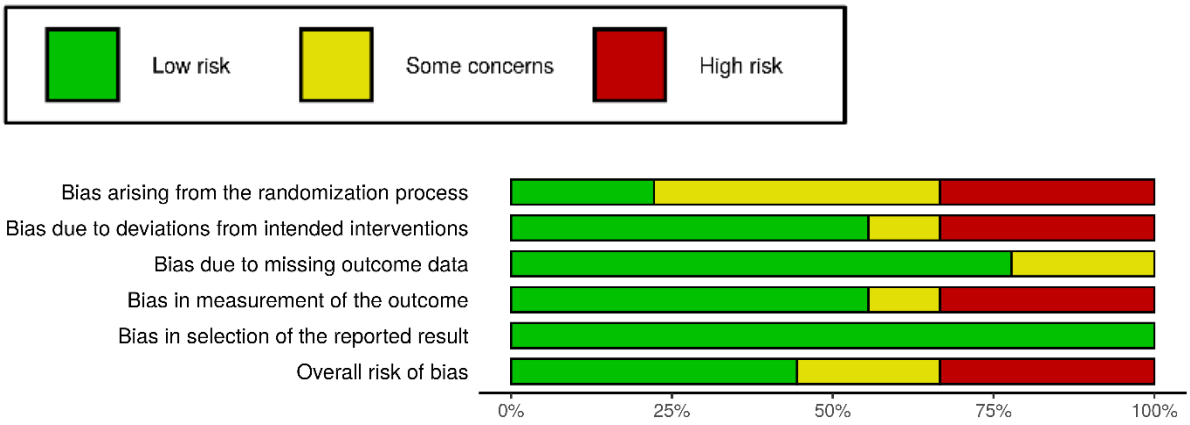


Figure 45. Distribution of RoB judgements within each bias domain for before-and-after trial studies performed under commercial abattoir conditions investigating ‘Cattle hide interventions’

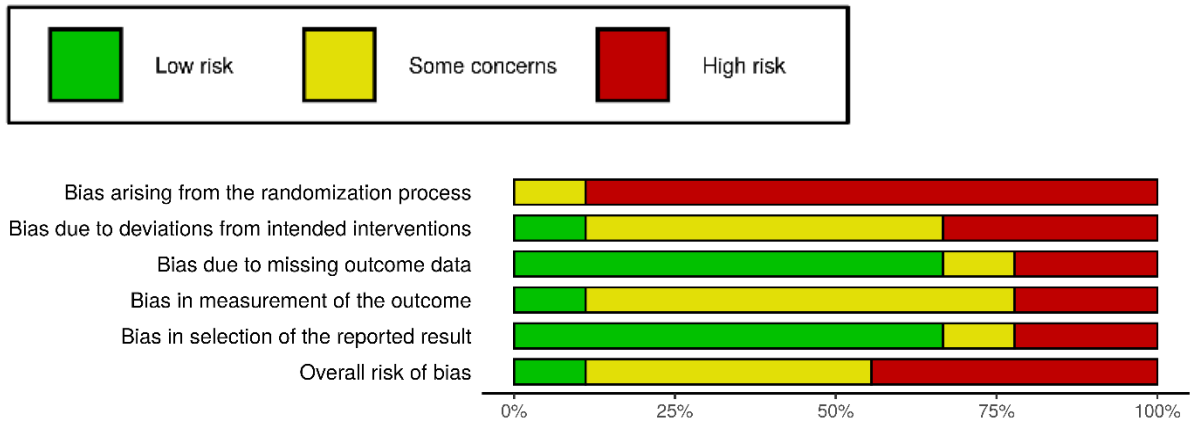


Figure 46. Distribution of RoB judgements within each bias domain for all studies performed under laboratory and pilot plant conditions investigating ‘Cattle hide interventions’

Figure 47. Distribution of RoB judgements within each bias domain for all studies performed under laboratory and pilot plant conditions investigating ‘Cattle hide interventions’

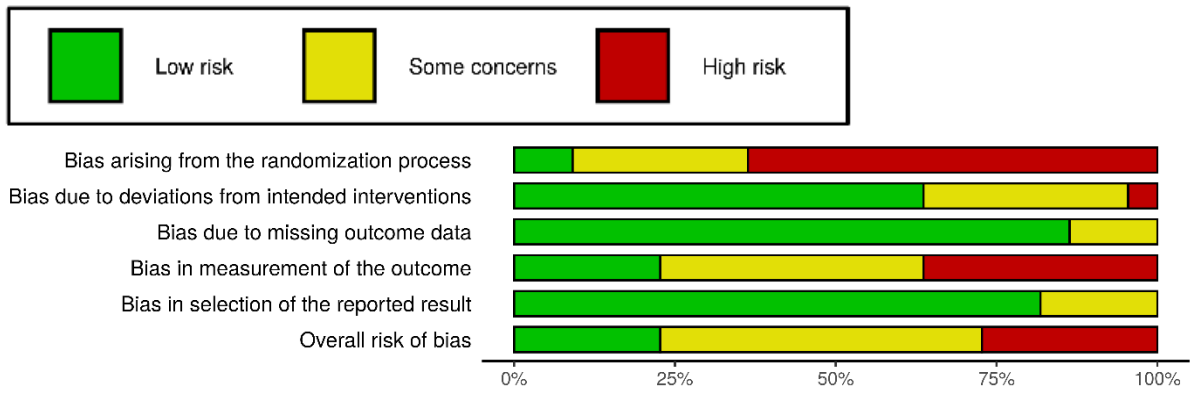


Figure 48. Distribution of RoB judgements within each bias domain for studies investigating ‘Knives sanitation’

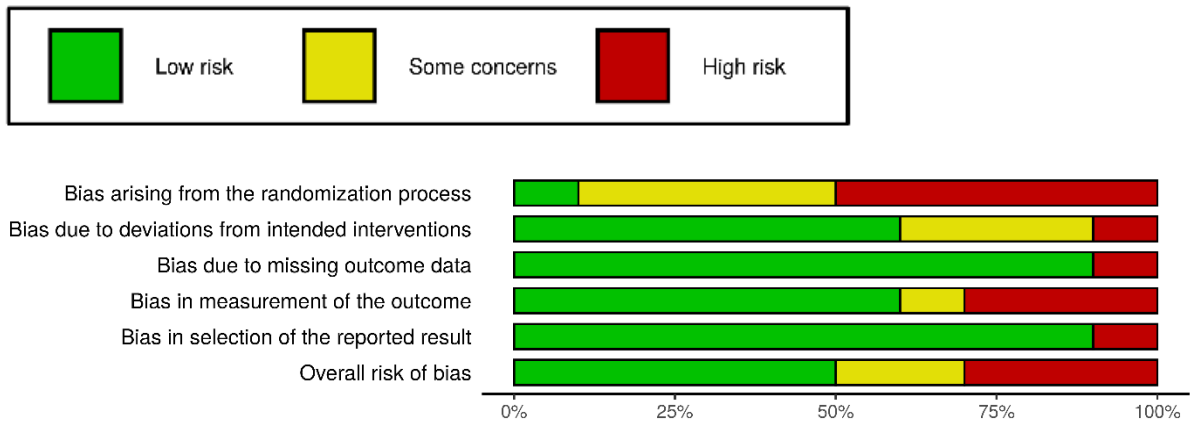


Figure 49. Distribution of RoB judgements within each bias domain for studies performed under commercial abattoir conditions investigating ‘Standard processing procedures and Good hygiene practices’

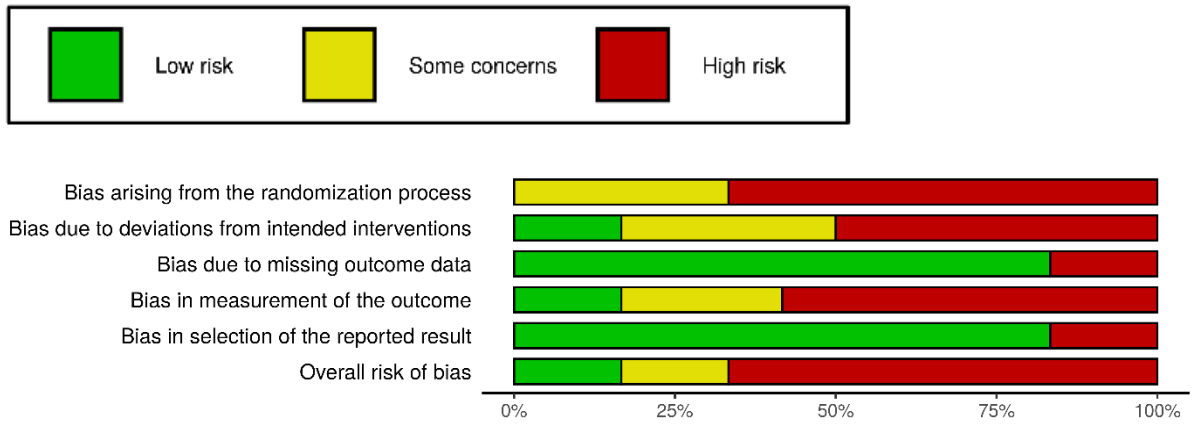


Figure 50. Distribution of RoB judgements within each bias domain for controlled and challenge trial studies performed under commercial abattoir conditions investigating ‘Carcass interventions’

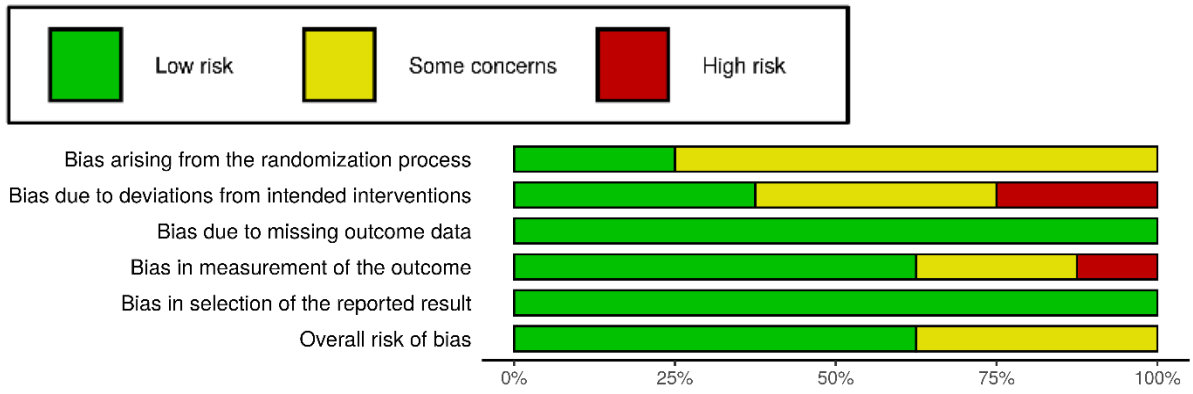
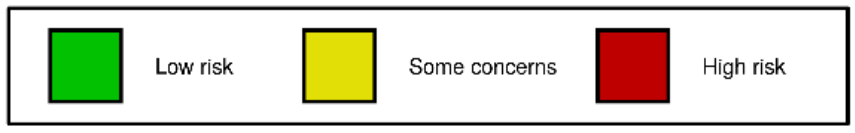


Figure 51. Distribution of RoB judgements within each bias domain for before-and-after trial studies performed under commercial abattoir conditions investigating ‘Carcass interventions’



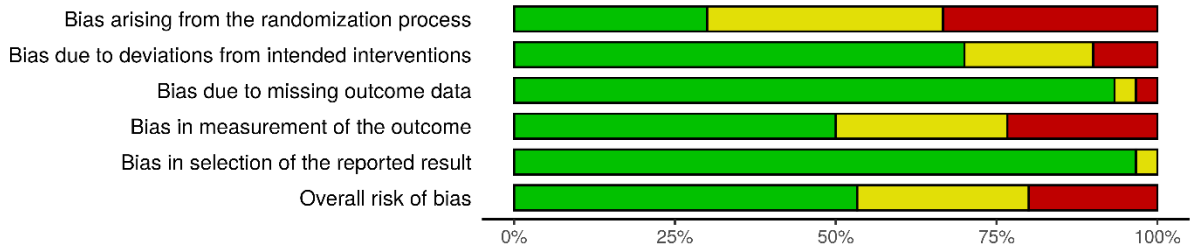


Figure 52. Distribution of RoB judgements within each bias domain for all studies performed under laboratory and pilot plant conditions investigating 'Carcass interventions and chilling'

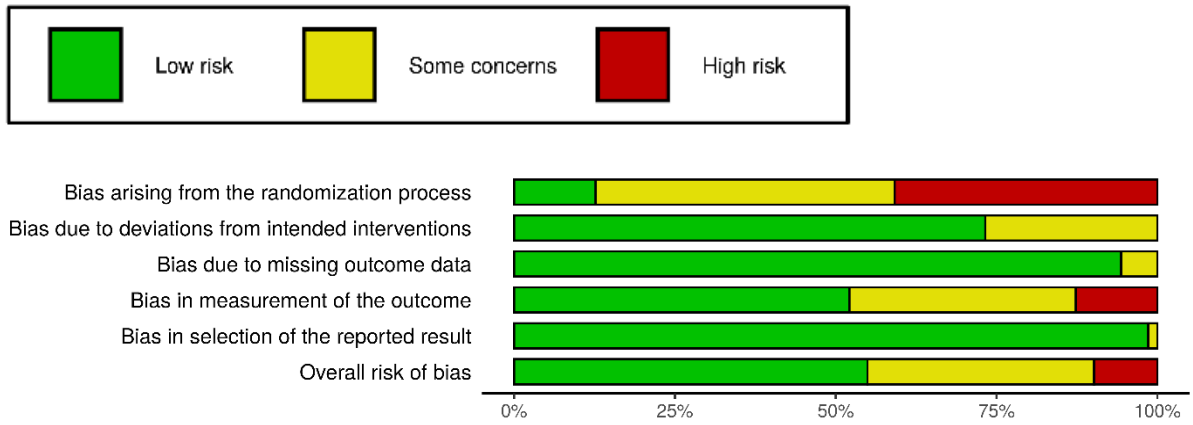


Figure 53. Distribution of RoB judgements within each bias domain for before-and-after trial studies performed under commercial abattoir conditions investigating ‘Chilling and spray chilling’

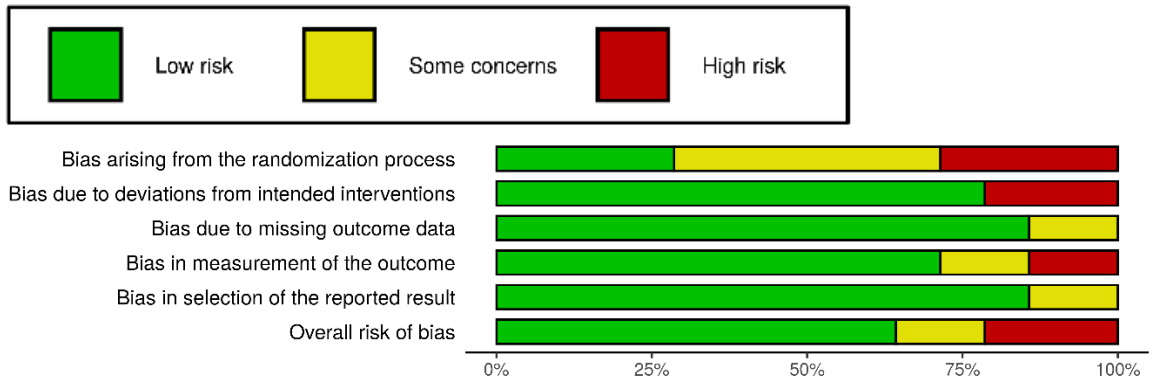


Figure 54. Distribution of RoB judgements within each bias domain for before-and-after trial studies performed under commercial abattoir conditions investigating ‘Multiple interventions’

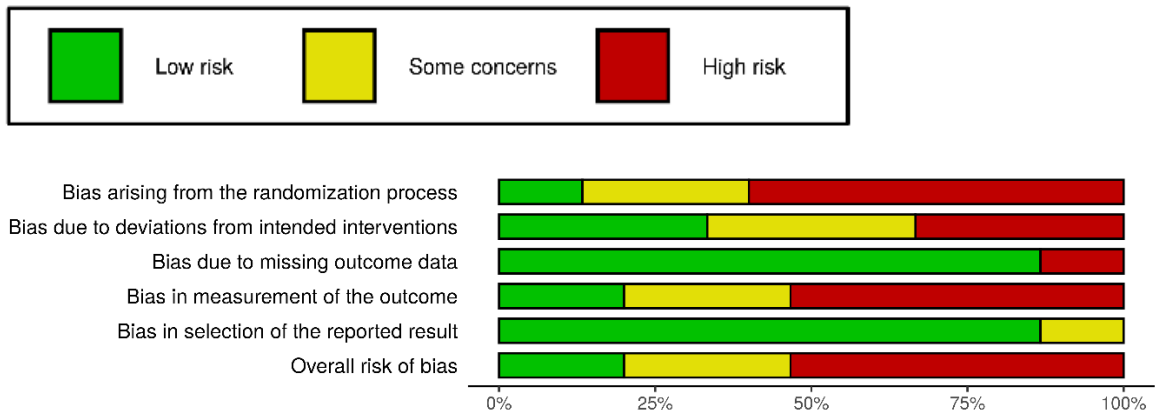
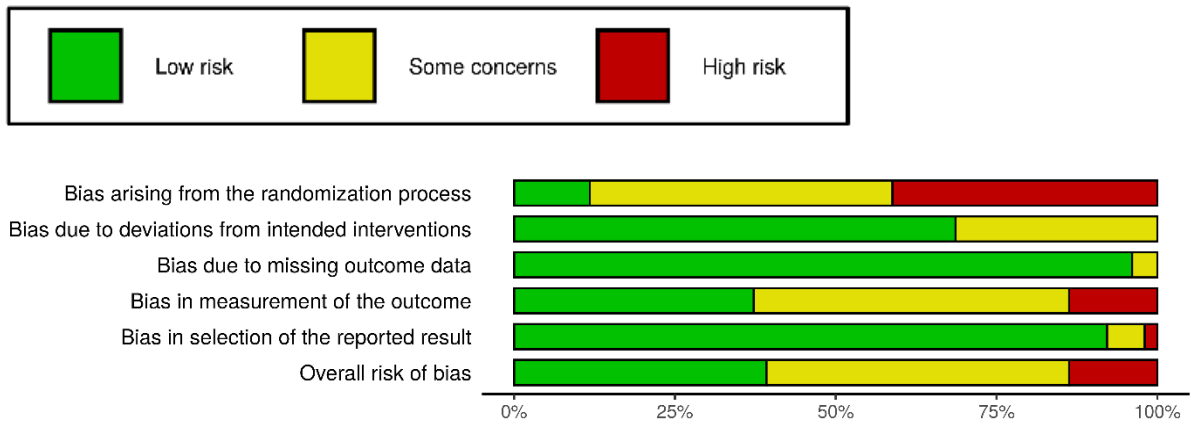


Figure 55. Distribution of RoB judgements within each bias domain for all studies performed under laboratory and pilot plant conditions investigating ‘Post fabrication interventions for trim/ground beef’



Appendix D: Intervention forest plots and results from studies with no direct comparisons

D1. Hide cleanliness assessment

Figure 56. Forest plot of the results of observational trials investigating hide cleanliness assessment, to determine the effect in reducing aerobic colony counts (log_{10} CFU) on beef carcasses produced from clean animals compared to dirty animals. High heterogeneity, positive effect (MD -0.90, 95% CI: -1.26- -0.54, I^2=88.4%).

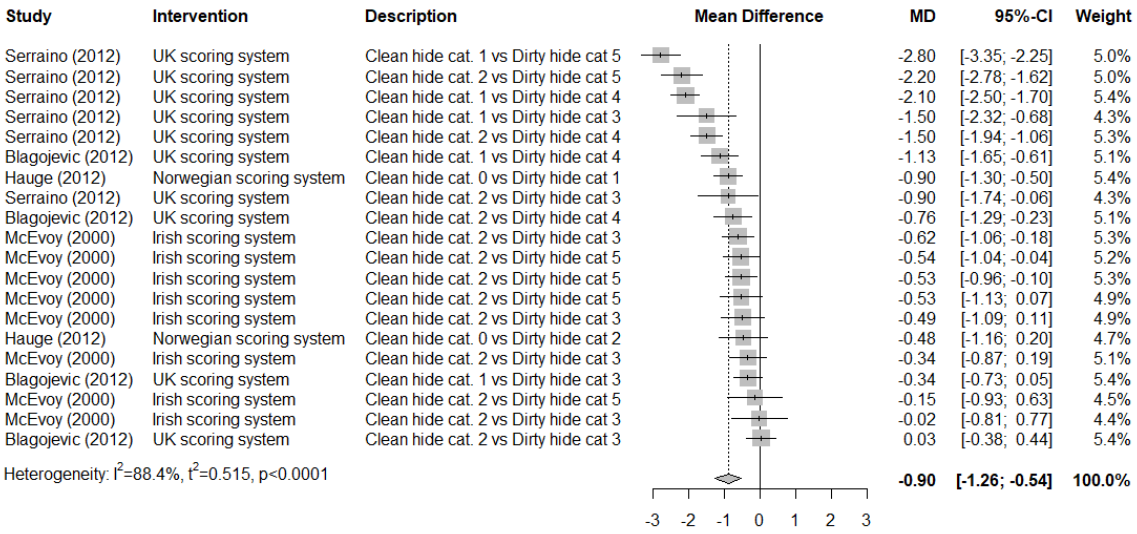


Figure 57. Forest plot of the results of observational trials investigating hide cleanliness assessment, to determine the effect in reducing Enterobacteriaceae counts (log_{10} CFU) on beef carcasses produced from clean animals compared to dirty animals. High heterogeneity, positive effect (MD -0.71, 95% CI: -1.05- -0.36, I^2=74.0%).

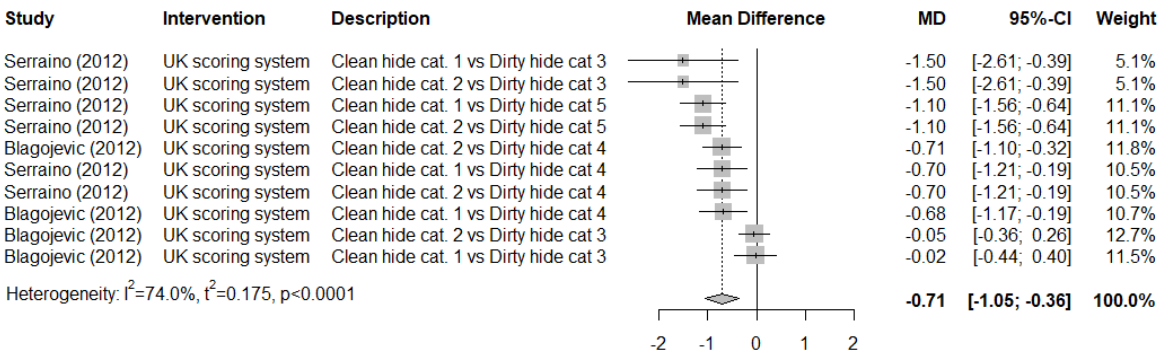


Figure 58. Forest plot of the results of observational trials investigating hide cleanliness assessment, showing the difference in aerobic colony counts (log_{10} CFU) on cattle hides between the clean and dirty cattle. High heterogeneity, positive effect (MD -1.68, 95% CI: -2.36- -1.01, I^2=95.9%).

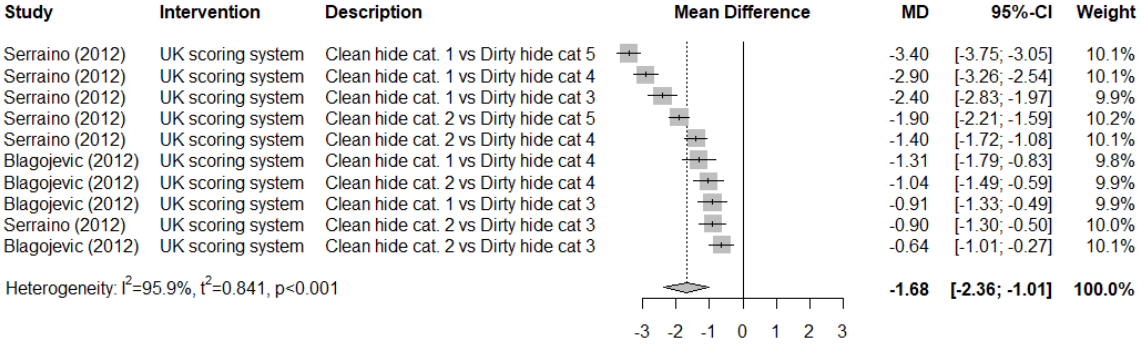


Figure 59. Forest plot of the results of observational trials investigating hide cleanliness assessment, showing the difference in Enterobacteriaceae counts (log_{10} CFU) on cattle hides between the clean and dirty cattle. High heterogeneity, positive effect (MD -1.33, 95% CI: -1.87- -0.79, I^2=92.3%).

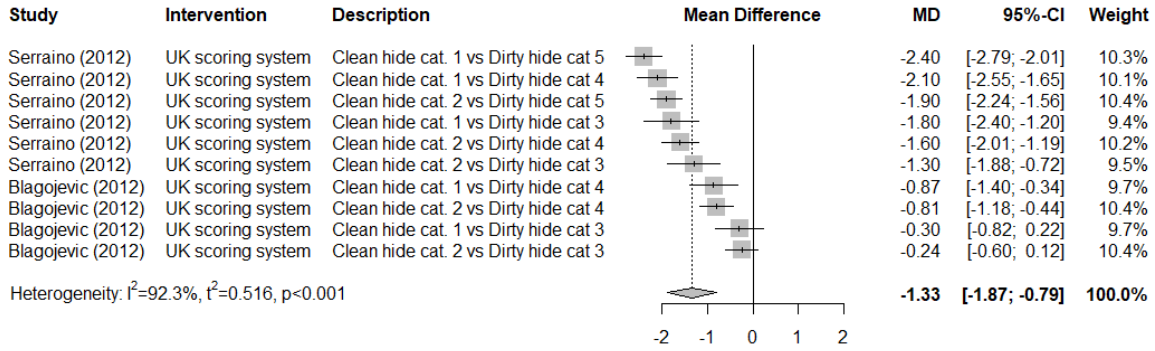
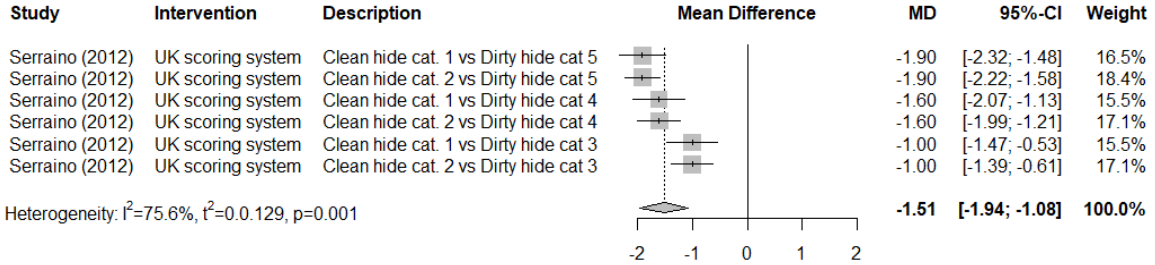


Figure 60. Forest plot of the results of observational trials investigating hide cleanliness assessment, showing the difference in generic E. coli counts (log_{10} CFU) on cattle hides between the clean and dirty cattle. High heterogeneity, positive effect (MD -1.51, 95% CI: -1.94- -1.08, I^2=75.6%).



D2. Cattle hide interventions

D2.1. Overall cattle hide interventions efficacy in reducing microbial transfer to beef carcasses

Figure 61. Forest plot of the results of six controlled trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide interventions in reducing aerobic colony counts (log_{10} CFU) on resulting beef carcasses. High heterogeneity, positive effect (MD -1.09, 95% CI: -1.53- -0.65, I^2=100%)

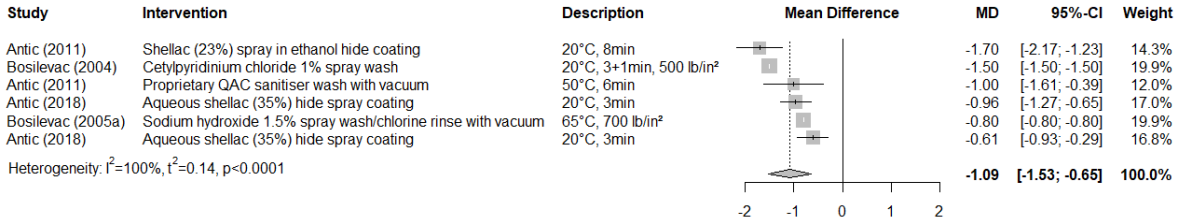


Figure 62. Forest plot of the results of six controlled trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide interventions in reducing Enterobacteriaceae counts (log_{10} CFU) on resulting beef carcasses. High heterogeneity, positive effect (MD -0.81, 95% CI: -1.35- -0.28, I^2=93.0%)

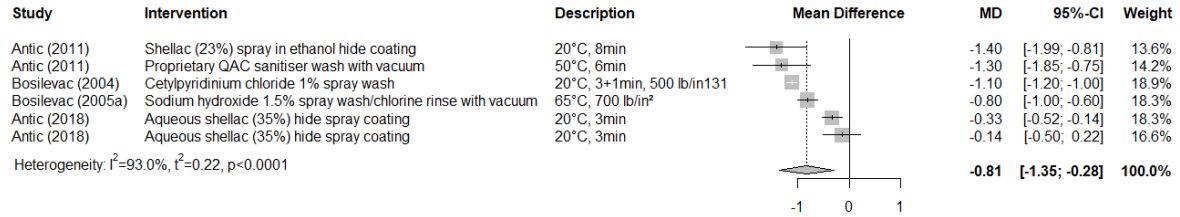
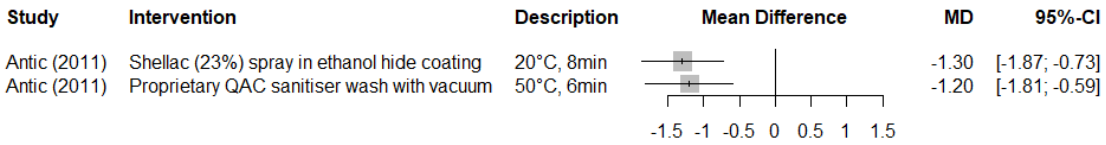


Figure 63. Forest plot of the results of two controlled trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide interventions in reducing generic *E. coli* counts (log_{10} CFU) on resulting beef carcasses


Study
Intervention
Description
Mean Difference
MD
95%-CI
Antic (2011)
Shellac (23%) spray in ethanol hide coating
20°C, 8min
-1.30
[-1.87; -0.73]
Antic (2011)
Proprietary QAC sanitiser wash with vacuum
50°C, 6min
-1.20
[-1.81; -0.59]
-1.5
-1
-0.5
0
0.5
1
1.5

D2.2. Water wash

Figure 64. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide water wash in reducing *E. coli* O157 and non-O157 prevalence on hides. High heterogeneity, no effect (RR 0.85, 95% CI: 0.66-1.09, I^2=85.0%)

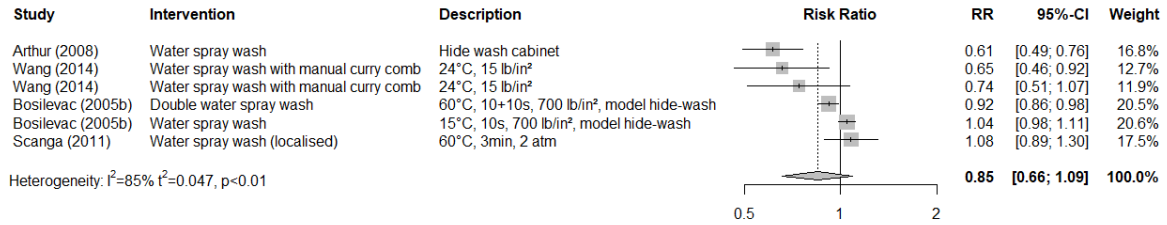


Figure 65. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide water wash in reducing *Salmonella* spp. prevalence on hides

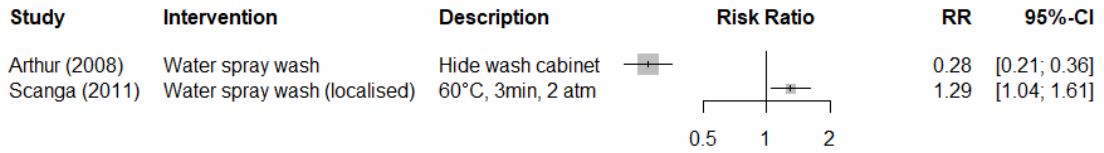


Figure 66. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide water wash in reducing aerobic colony counts (log_{10} CFU) on hides. High heterogeneity, no effect (MD -0.60, 95% CI: -1.22-0.02, I^2=99.7%)

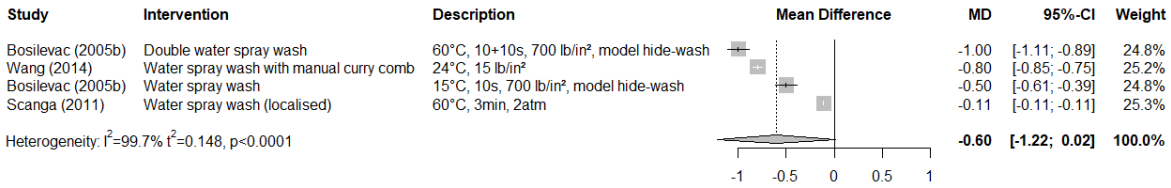


Figure 67. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide water wash in reducing Enterobacteriaceae counts (log_{10} CFU) on hides. High heterogeneity, no effect (MD -1.77, 95% CI: -5.50-1.96, I^2=99.9%)

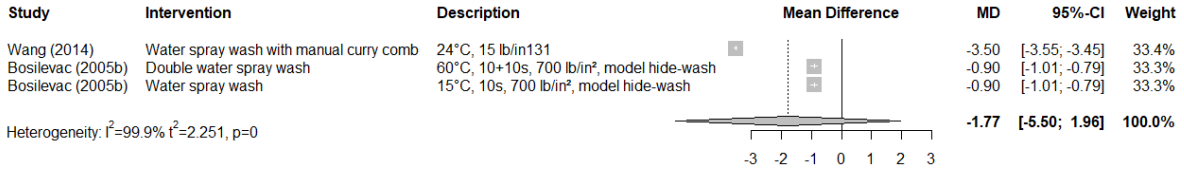
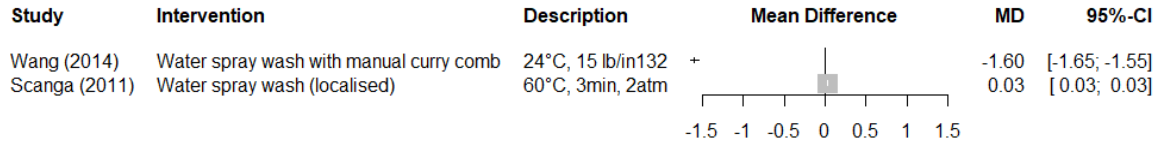


Figure 68. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide water wash in reducing generic E. coli counts (log_{10} CFU) on hides



D2.3. Bob veal hide treatments

Figure 69. Forest plot of the results of challenge trials performed under pilot plant conditions to investigate the efficacy of bob veal hide pre-evisceration scalding in reducing *E. coli* (log_{10} CFU) on hides

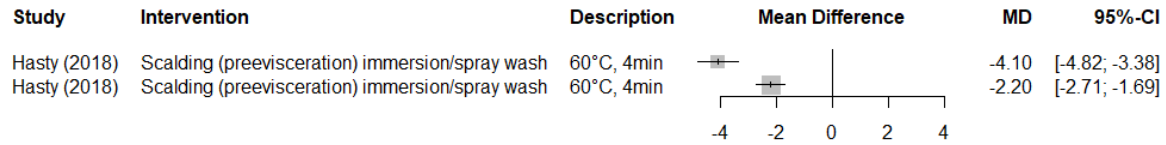


Figure 70. Forest plot of the results of challenge trials performed under pilot plant conditions to investigate the efficacy of bob veal hide pre-evisceration scalding with hot water rinse in reducing E. coli (log_{10} CFU) on hides

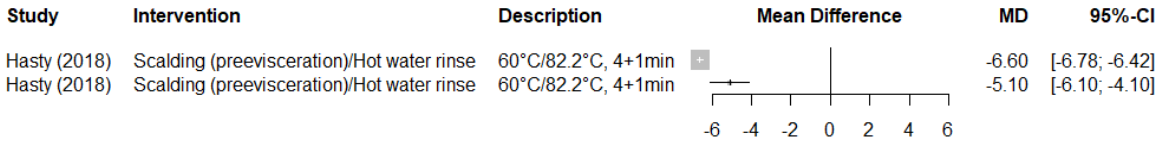


Figure 71. Forest plot of the results of challenge trials performed under pilot plant conditions to investigate the efficacy of bob veal hide pre-evisceration scalding with hot water rinse and final hot water wash, in reducing *E. coli* (log_{10} CFU) on hides

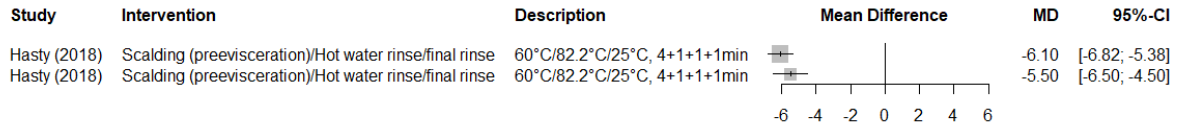
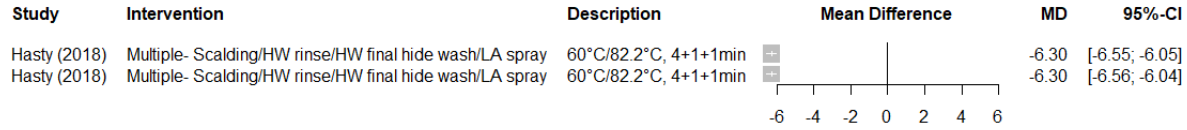
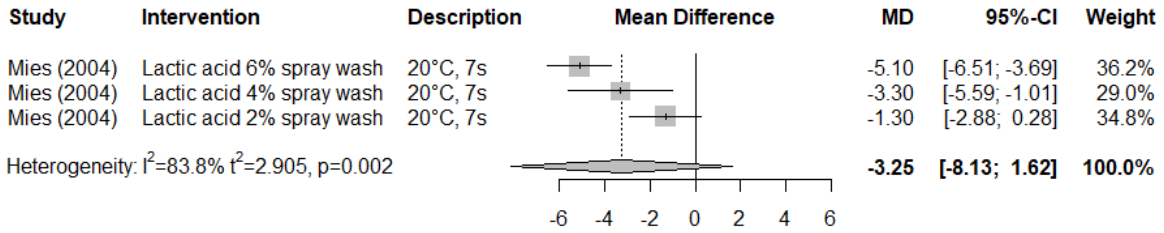


Figure 72. Forest plot of the results of challenge trials performed under pilot plant conditions to investigate the efficacy of multiple interventions for bob veal hide: scalding with hot water rinse, final hot water wash and lactic acid spray, in reducing *E. coli* (log_{10} CFU) on hides



D2.4. Organic acid washes

Figure 73. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of cattle hide lactic acid wash in reducing *Salmonella* spp. (log_{10} CFU) on hides. High heterogeneity, no effect (MD -3.25, 95% CI: -8.13-1.62, I^2=83.8%)



D2.5. Other chemical washes

Figure 74. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of cattle hide acetic acid wash in reducing *Salmonella* spp. (log_{10} CFU) on hides. High heterogeneity, positive effect (MD -3.60, 95% CI: -6.87- -0.33, I^2=68.0%)

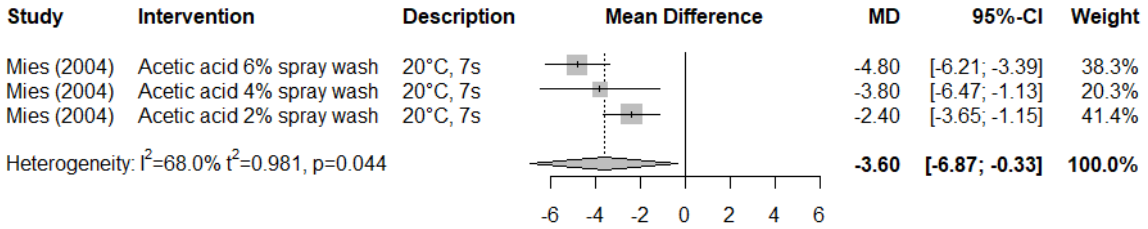


Figure 75. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide sodium hydroxide wash in reducing *E. coli* O157 prevalence on hides

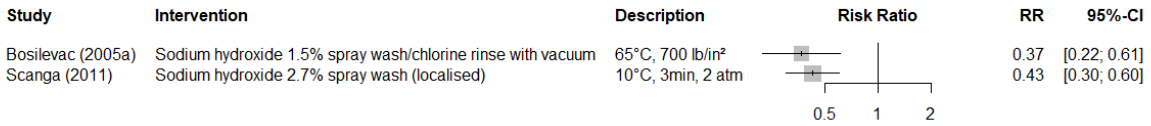


Figure 76. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide sodium hydroxide wash in reducing aerobic colony counts (log_{10} CFU) on hides

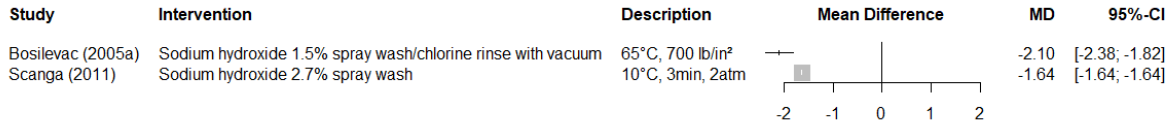


Figure 77. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide chlorine wash in reducing aerobic colony counts (log_{10} CFU) on hides

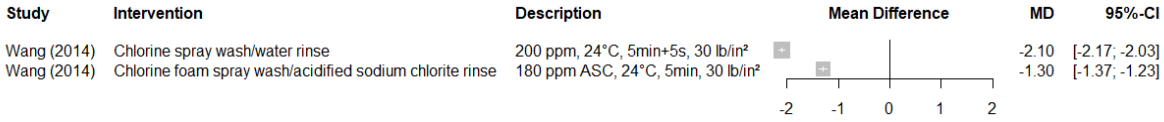


Figure 78. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide chlorine wash in reducing Enterobacteriaceae counts (log_{10} CFU) on hides

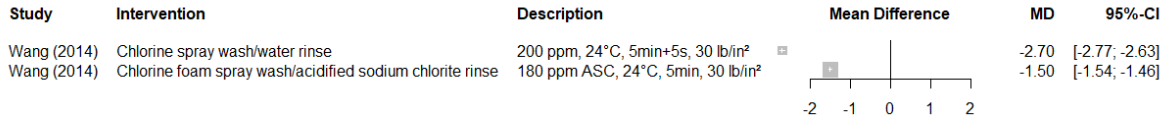


Figure 79. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of cattle hide chlorine wash in reducing generic *E. coli* counts (log_{10} CFU) on hides

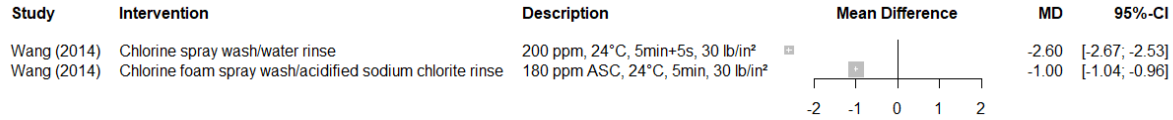
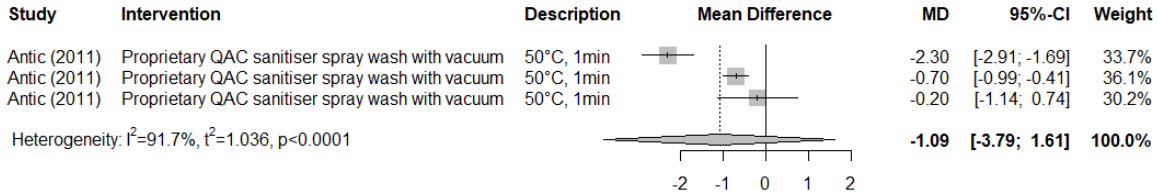


Figure 80. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of cattle hide proprietary QAC sanitiser wash in reducing aerobic colony counts (log_{10} CFU) transfer on beef cuts. High heterogeneity, no effect (MD -1.09, 95% CI: -3.79-1.61, I^2=91.7%)



D2.6. Shellac hide coating

Figure 81. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of shellac hide coating in reducing aerobic colony counts (log_{10} CFU) on resulting beef carcasses. High heterogeneity, no effect (MD -1.09, 95% CI: -2.43-0.29, I^2=85.7%)

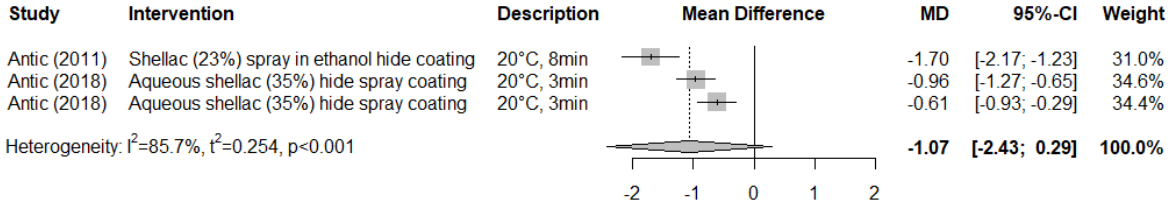


Figure 82. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of shellac hide coating in reducing *Enterobacteriaceae* counts (log_{10} CFU) on resulting beef carcasses. High heterogeneity, no effect (MD -0.59, 95% CI: -2.22-1.05, I^2=85.1%)

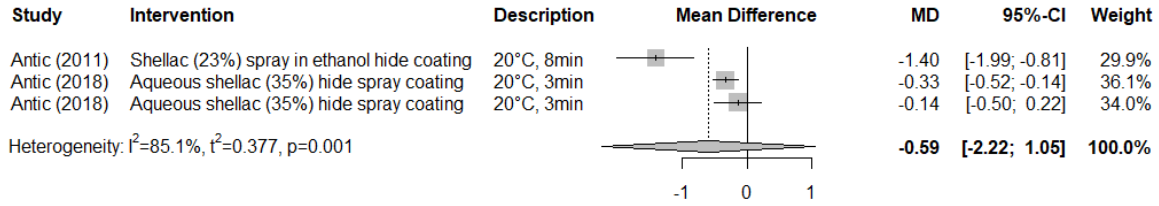


Figure 83. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of shellac in ethanol hide coating in reducing aerobic colony counts (log_{10} CFU) transfer on beef cuts. High heterogeneity, positive effect (MD -2.47, 95% CI: -3.49- -1.45, I^2=83.4%)

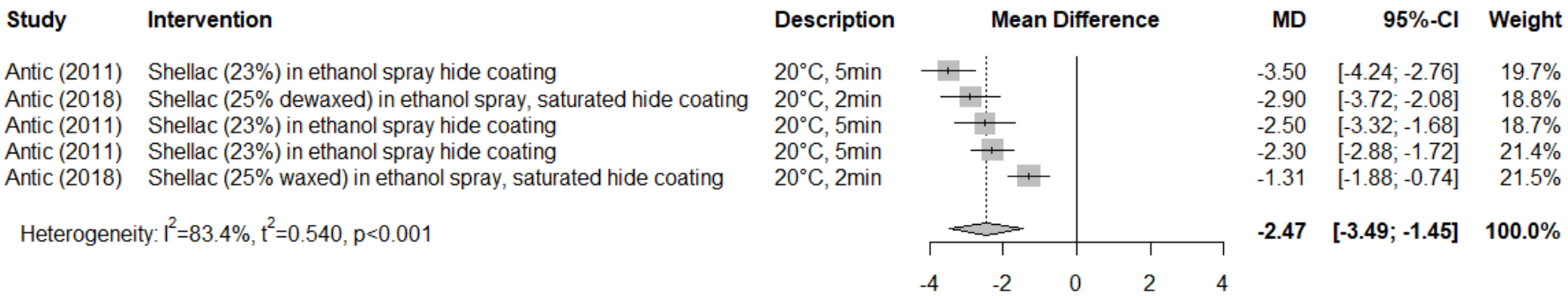


Figure 84. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of shellac in ethanol hide coating in reducing Enterobacteriaceae counts (log_{10} CFU) transfer on beef cuts. High heterogeneity, positive effect (MD -2.12, 95% CI: -3.12- -1.13, I^2=88.2%)

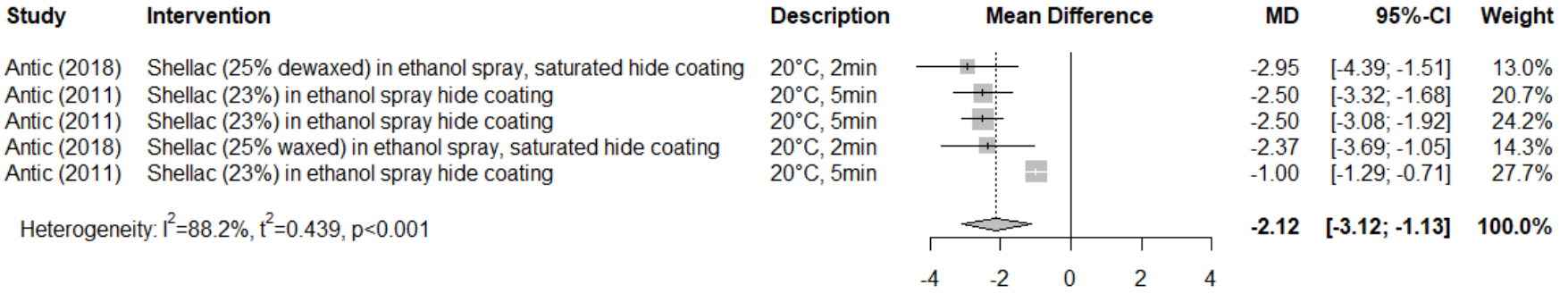


Figure 85. Forest plot of the results of controlled trials performed under laboratory conditions to investigate the efficacy of aqueous shellac hide coating in reducing aerobic colony counts (log_{10} CFU) transfer on beef cuts. High heterogeneity, positive effect (MD -2.02, 95% CI: -2.70- -1.35, I^2=87.7%)

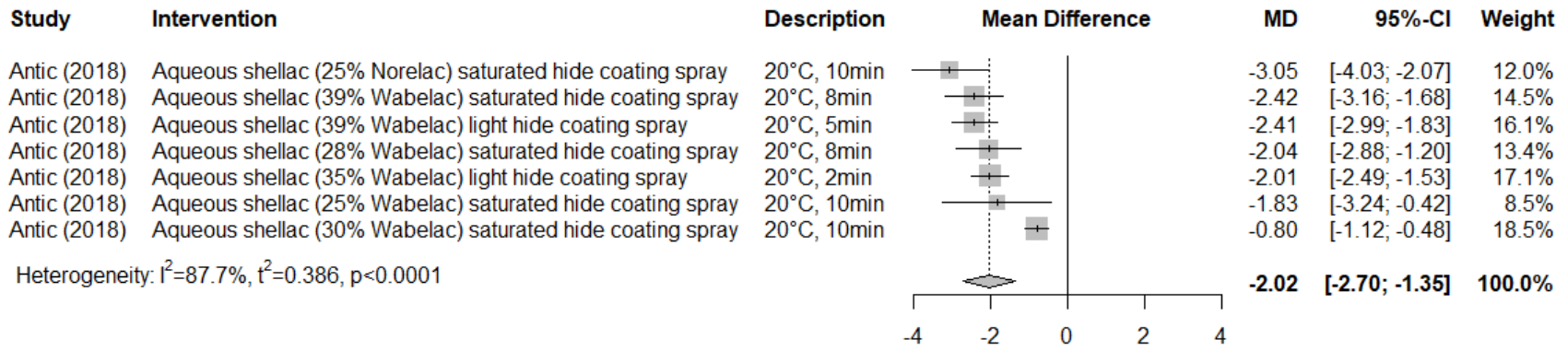


Figure 86. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of aqueous shellac hide coating in reducing *E. coli* O157 (log_{10} CFU) transfer on beef cuts

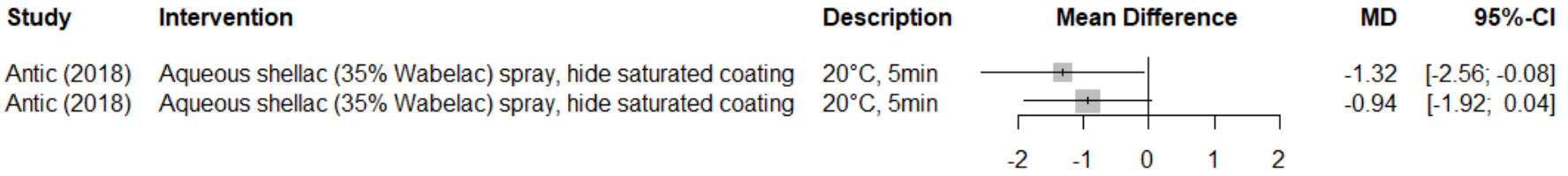


Table 4. Results on cattle hide intervention efficacies from studies with no direct comparisons investigating prevalence outcomes

Study design/ conditions‡	**Study**	**Intervention**	**Description**	**Micro-organism**	**RR (95%-CI)**	**p-value**
CT/Comm	Bosilevac (2004)	Cetylpyridinium chloride 1% spray wash (carcass effect)*	20°C, 3+1 min, 500 lb/in²	E. coli O157:H7	0.13 (0.05-0.35)	<0.001
CT/Comm	Bosilevac (2004)	Cetylpyridinium chloride 1% spray wash	20°C, 3+1 min, 500 lb/in²	E. coli O157:H7	0.61 (0.46-0.80)	<0.001
CT/Comm	Bosilevac (2005a)	Sodium hydroxide 1.5% spray wash/chlorine rinse with vacuum (carcass effect)*	65°C, 700 lb/in²	E. coli O157:H7	0.11 (0.05-0.28)	<0.001
BA/Comm	Scanga (2011)	Lactic acid 6% spray wash (localised)	30°C, 3min, 2 atm	Salmonella	0.68 (0.49-0.93)	0.02
BA/Comm	Scanga (2011)	Sodium hydroxide 2.7% spray wash (localised)	10°C, 3min, 2 atm	Salmonella	0.70 (0.47-1.04)	0.08
BA/Comm	Bosilevac (2005b)	Electrolyzed double water spray wash	70 ppm chlorine, 52°C, 10s, 700 lb/in² / water 60°C, 10s, 250 lb/in², model hide-wash	E. coli O157	0.43 (0.32-0.57)	<0.001
BA/Comm	Bosilevac (2005b)	Ozonated double water spray wash	2 ppm, 15°C, 10s, 700 lb/in²/ water 5s, 35 lb/in², model hide-wash	E. coli O157	0.35 (0.26-0.48)	<0.001
BA/Comm	Scanga (2011)	Acetic acid 5% spray wash (localised)	30°C, 3min, 2 atm	E. coli O157:H7	0.39 (0.25-0.62)	<0.001
BA/Comm	Scanga (2011)	Lactic acid 6% spray wash (localised)	30°C, 3min, 2 atm	E. coli O157:H7	0.67 (0.51-0.88)	<0.01
CT/Lab	Antic (2010)	Shellac (23%) in ethanol spray hide coating	20°C, 6min	E. coli O157	0.27 (0.08-0.88)	0.03

‡ CT-controlled trial; BA-before-and-after trial; Comm-commercial abattoir conditions; Lab-laboratory conditions

* Reduction in hide-to-carcass transfer

Table 5. Results on cattle hide intervention efficacies from studies with no direct comparisons investigating concentration outcomes

Study design/ conditions‡	**Study**	**Intervention**	**Description**	**Micro-organism[a]**	**Log_{10} mean difference (95%-CI)**	**p-value**
CT/Comm	Bosilevac (2004)	Cetylpyridinium chloride 1% spray wash*	20°C, 3+1min, 500 lb/in²	ACC	-1.50 (-1.61 - -1.39) log_{10} CFU/100 cm²	<0.001
CT/Comm	Antic (2011)	Proprietary QAC sanitiser wash with vacuum*	50°C, 6min	ACC	-1.00 (-1.61- -0.39) log_{10} CFU/cm²	<0.01
CT/Comm	Bosilevac (2005a)	Sodium hydroxide 1.5% spray wash/chlorine rinse with vacuum*	65°C, 700 lb/in²	ACC	-0.80 (-1.08- -0.52) log_{10} CFU/100 cm²	<0.001
CT/Comm	Bosilevac (2004)	Cetylpyridinium chloride 1% spray wash*	20°C, 3+1min, 500 lb/in131	EBC	-1.10 (-1.24- -0.96) log_{10} CFU/100 cm²	<0.001
CT/Comm	Antic (2011)	Proprietary QAC sanitiser wash with vacuum*	50°C, 6min	EBC	-1.30 (-1.85- -0.75) log_{10} CFU/cm²	<0.001
CT/Comm	Bosilevac (2005a)	Sodium hydroxide 1.5% spray wash/chlorine rinse with vacuum*	65°C, 700 lb/in²	EBC	-0.80 (-1.08- -0.52) log_{10} CFU/100 cm²	<0.001
CT/Comm	Antic (2011)	Shellac (23%) spray in ethanol hide coating*	20°C, 8min	E. coli	-1.30 (-1.87- -0.73) log_{10} CFU/cm²	<0.001
CT/Comm	Antic (2011)	Proprietary QAC sanitiser wash with vacuum*	50°C, 6min	E. coli	-1.20 (-1.81- -0.59) log_{10} CFU/cm²	<0.001
BA/Comm	Bosilevac (2005b)	Electrolyzed double water spray wash	70 ppm chlorine, 52°C, 10s, 700 lb/in² / water 60°C, 10s, 250 lb/in², model hide-wash	ACC	-3.50 (-3.69- -3.31) log_{10} CFU/100 cm²	<0.001
BA/Comm	Bosilevac (2005b)	Ozonated double water spray wash	2 ppm, 15°C, 10s, 700 lb/in²/ water 5s, 35 lb/in², model hide-wash	ACC	-2.10 (-2.27- -1.93) log_{10} CFU/100 cm²	<0.001

Study design/ conditions‡	Study	Intervention	Description	Micro-organism[a]	Log_{10} mean difference (95%-CI)	p-value
BA/Comm	Scanga (2011)	Acetic acid 5% spray wash (localised)	30°C, 3min, 2atm	ACC	-2.61 (-2.61- -2.61) log_{10} CFU/100 cm²	<0.001
BA/Comm	Scanga (2011)	Lactic acid 6% spray wash (localised)	30°C, 3min, 2atm	ACC	-2.29 (-2.29- -2.29) log_{10} CFU/100 cm²	<0.001
BA/Comm	Bosilevac (2005b)	Electrolyzed double water spray wash	70 ppm chlorine, 52°C, 10s, 700 lb/in² / water 60°C, 10s, 250 lb/in², model hide-wash	EBC	-4.30 (-4.49- -4.11) log_{10} CFU/100 cm²	<0.001
BA/Comm	Bosilevac (2005b)	Ozonated double water spray wash	2 ppm, 15°C, 10s, 700 lb/in²/ water 5s, 35 lb/in², model hide-wash	EBC	-3.40 (-3.62- -3.18) log_{10} CFU/100 cm²	<0.001
BA/Comm	Bosilevac (2005a)	Sodium hydroxide 1.5% spray wash/chlorine rinse with vacuum	65°C, 700 lb/in²	EBC	-3.40 (-3.68 - -3.12) log_{10} CFU/100 cm²	<0.001
BA/Comm	Scanga (2011)	Acetic acid 5% spray wash (localised)	30°C, 3min, 2atm	E. coli	-3.70 (-3.70- -3.70) log_{10} CFU/100 cm²	<0.001
BA/Comm	Scanga (2011)	Lactic acid 6% spray wash (localised)	30°C, 3min, 2atm	E. coli	-3.74 (-3.74- -3.74) log_{10} CFU/100 cm²	<0.001
BA/Comm	Scanga (2011)	Sodium hydroxide 2.7% spray wash	10°C, 3min, 2atm	E. coli	-3.47 (-3.47- -3.47) log_{10} CFU/100 cm²	<0.001
CT/Lab	Antic (2010)	Shellac (23%) in ethanol spray hide coating	20°C, 6min	ACC	-6.60 (-7.18- -6.02) log_{10} CFU/cm²	<0.001
CT/Lab	Antic (2010)	Shellac (23%) in ethanol spray hide coating	20°C, 6min	EBC	-4.79 (6.89- -2.69) log_{10} CFU/cm²	<0.001
CT/Lab	Antic (2010)	Shellac (23%) in ethanol spray hide coating	20°C, 6min	E. coli	-2.89 (-3.59 - -2.19) log_{10} CFU/cm²	<0.001

Study design/ conditions‡	Study	Intervention	Description	Micro-organism[a]	Log_{10} mean difference (95%-CI)	p-value
CT/Lab	Antic (2010)	Proprietary QAC sanitiser spray wash with vacuum	50°C, 1min	ACC	-4.90 (-5.24- -4.56) log_{10} CFU/cm²	<0.001
CT/Lab	Antic (2010)	Proprietary QAC sanitiser spray wash with vacuum	50°C, 1min	EBC	-3.40 (-4.62- -2.18) log_{10} CFU/cm²	<0.001
CT/Lab	Antic (2010)	Proprietary QAC sanitiser spray wash with vacuum	50°C, 1min	E. coli	-2.70 (-3.23- -2.17) log_{10} CFU/cm²	<0.001
ChT/RPP	Hasty (2018)	Hot water spray wash	82.2°C, 1min	E. coli	-4.50 (-5.74- -3.26) log_{10} CFU/100 cm²	<0.001
ChT/RPP	Hasty (2018)	Hot water wash/lactic acid 4.5% spray wash	82.2°C/20°C, 1+1min	E. coli	-6.10 (-6.77- -5.43) log_{10} CFU/100 cm²	<0.001
ChT/Lab	Antic (2010)	Shellac (23%) in ethanol spray hide coating	20°C, 6min	E. coli O157	-2.10 (-2.61- -1.59) log_{10} CFU/cm²	<0.001
ChT/Lab	Mies (2004)	Water spray wash	20°C, 7s	Salmonella	-0.70 (-1.58-0.18) log_{10} CFU/cm²	0.12

‡ CT-controlled trial; BA-before-and-after trial; ChT-challenge trial; Comm-commercial abattoir conditions; Lab-laboratory conditions; RPP-research/pilot plant conditions

* Reduction in hide-to-carcass transfer

[a] ACC-aerobic colony counts; EBC-*Enterobacteriaceae* counts

D3. Beef carcass interventions

D3.1. Standard processing procedures and GHP

Figure 87. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of improved hygiene during hide removal in reducing aerobic colony counts (log_{10} CFU) on resulting beef carcasses

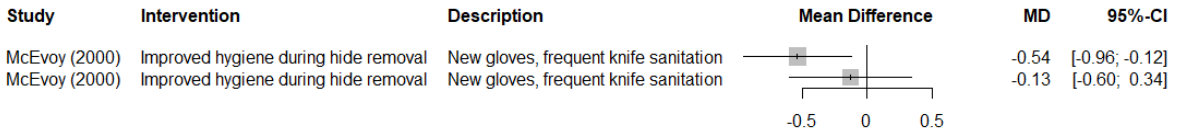
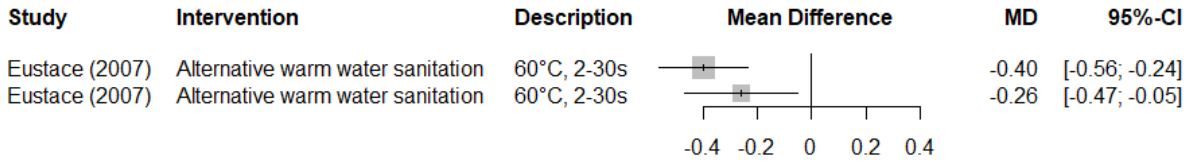


Figure 88. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of alternative knife sanitation with warm water comparing to hot water 82°C sanitation, in reducing aerobic colony counts (log_{10} CFU) on knives



D3.2. Pre-chill carcass treatments

D3.2.1 Water wash

Figure 89. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, no effect (MD 0.05, 95% CI: -0.37-0.47, I^2=80.0%)

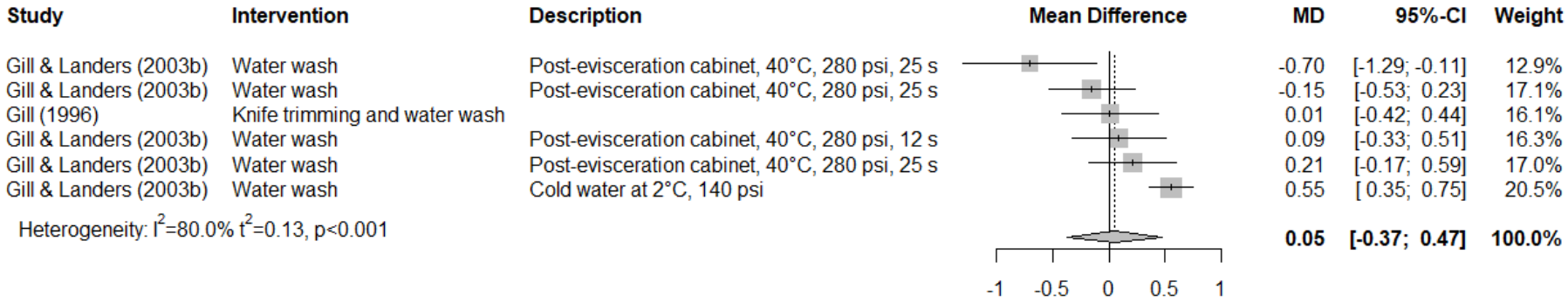


Figure 90 Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water wash in reducing generic *E. coli* counts (log_{10} CFU) on beef carcasses

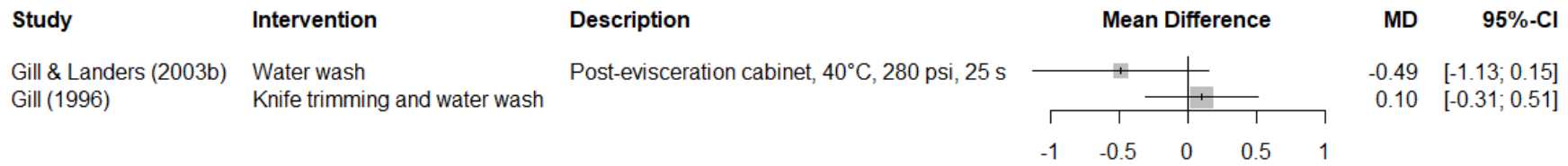


Figure 91. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water wash in reducing *E. coli* counts (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -2.33, 95% CI: -3.13- -1.53, I^2=72.1%)

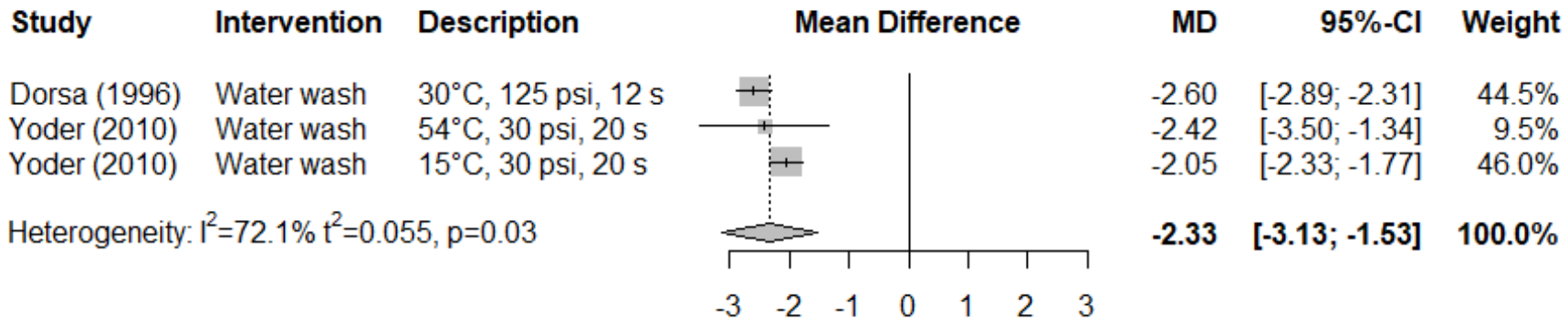


Figure 92. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water wash in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef

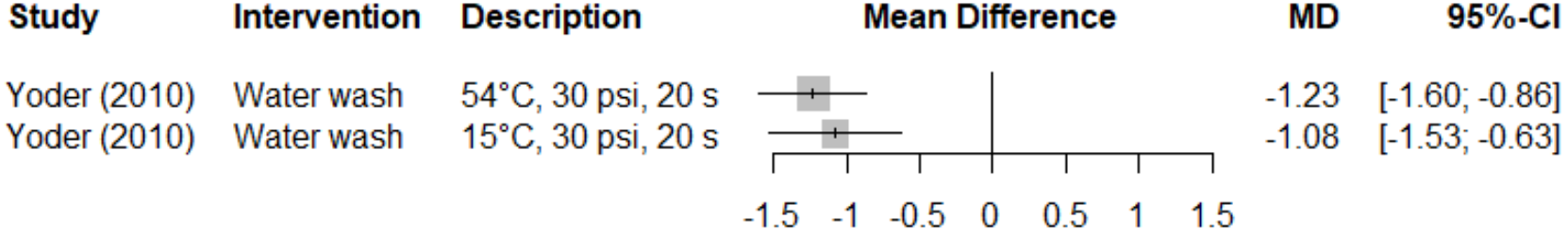
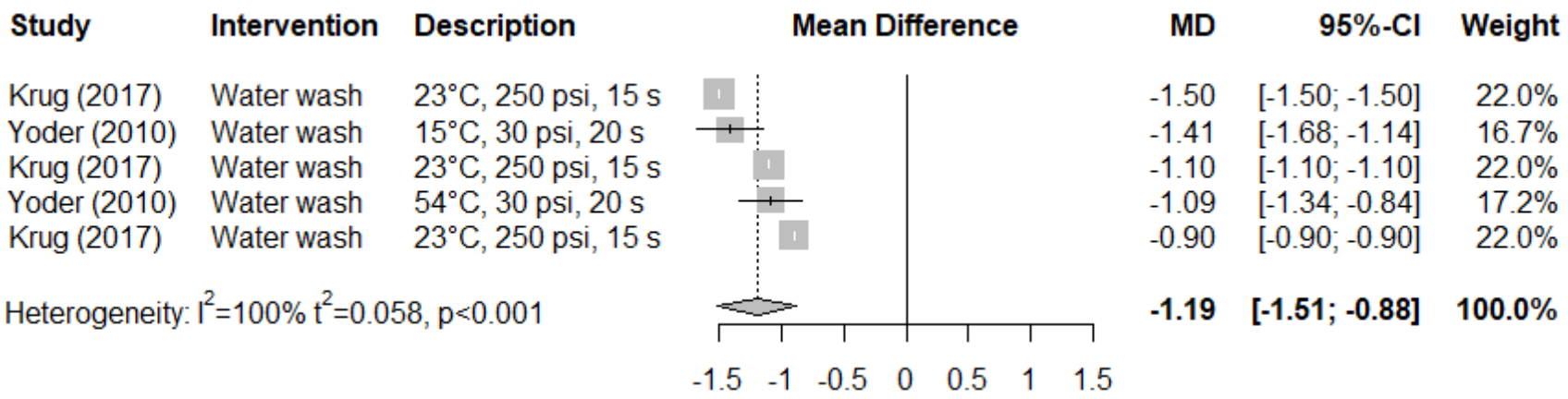
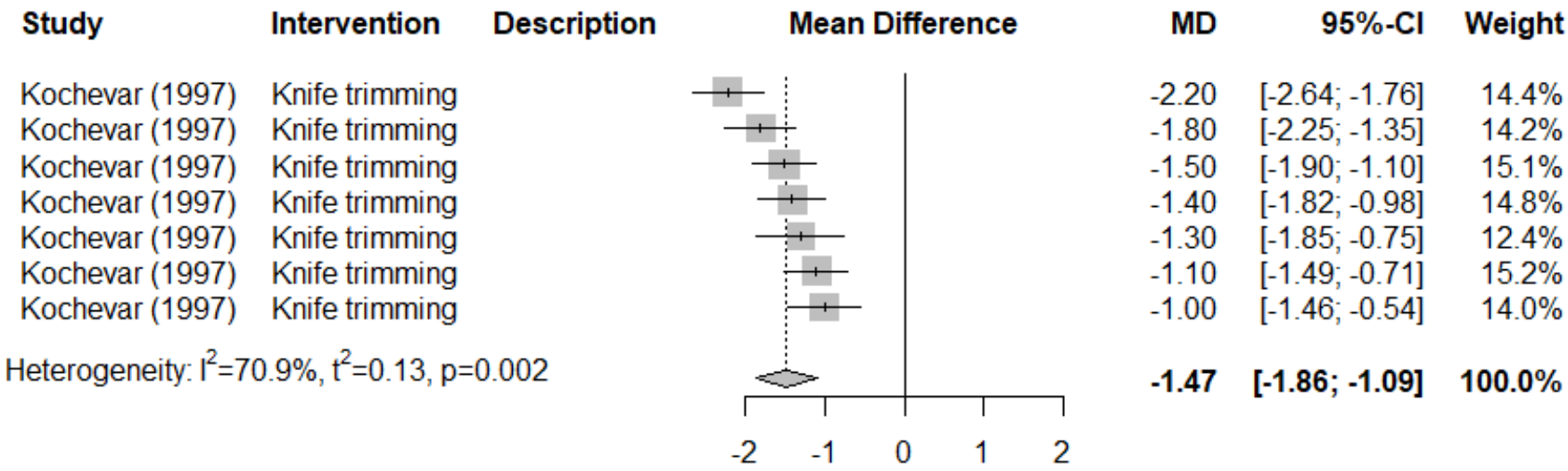


Figure 93. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water wash in reducing E. coli O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.19, 95% CI: -1.51- -0.88, I^2=100%)



D3.2.2 Knife trimming

Figure 94. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of knife trimming in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.47, 95% CI: -1.86- -1.09, I^2=70.9%)



D3.2.3 Hot water wash

Figure 95. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of hot water wash in reducing generic E. coli prevalence on beef carcasses. High heterogeneity, positive effect (RR 0.32, 95% CI: 0.17-0.58, I^2=69.0%)

Study	Intervention	Description	Risk Ratio	RR	95%-CI	Weight
Gill (1999)	Hot water wash	Post-evisceration cabinet, 85°C, 10 s		0.05	[0.01; 0.36]	5.5%
Gill (2000)	Hot water wash	Post-evisceration cabinet, 85°C, 10 s		0.15	[0.05; 0.44]	10.5%
Gill (2000)	Hot water wash	Post-evisceration cabinet, 85°C, 12 s		0.16	[0.05; 0.47]	10.5%
Gill (1999)	Hot water wash	Post-evisceration cabinet, 85°C, 10 s		0.28	[0.12; 0.63]	12.8%
Gill (1999)	Hot water wash	Post-evisceration cabinet, 85°C, 15 s		0.29	[0.13; 0.67]	12.7%
Gill (2000)	Hot water wash	Post-evisceration cabinet, 85°C, 11 s		0.42	[0.23; 0.78]	14.7%
Gill (2000)	Hot water wash	Post-evisceration cabinet, 85°C, 9 s		0.62	[0.41; 0.94]	16.3%
Gill (2000)	Hot water wash	Post-evisceration cabinet, 85°C, 8 s		0.67	[0.49; 0.90]	17.0%
Heterogeneity: I^2=69.0% t^2=0.43, p=0.002				**0.32**	**[0.17; 0.58]**	**100.0%**

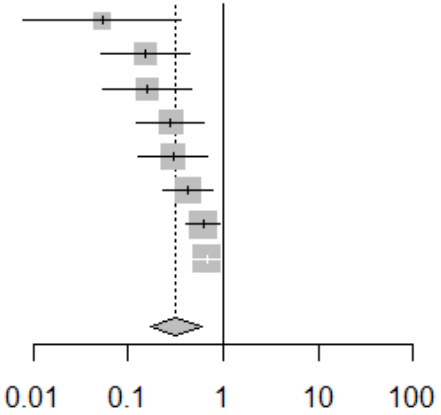


Figure 96. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of hot water wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.58, 95% CI: -1.95- -1.21, I^2=100%).

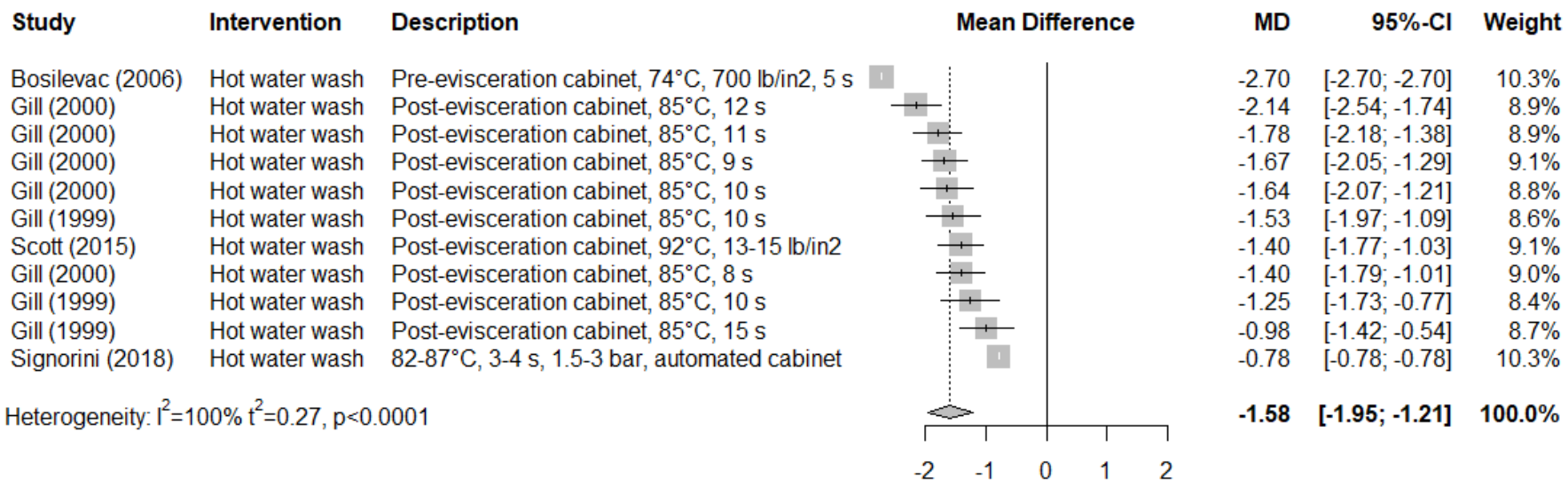


Figure 97. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of hot water wash in reducing *Enterobacteriaceae* counts (log_{10} CFU) on beef carcasses

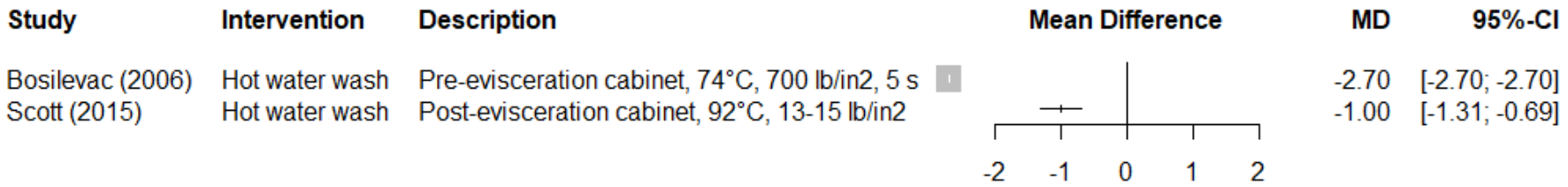


Figure 98. Forest plot of the results of challenge trials performed under commercial abattoir conditions to investigate the efficacy of hot water wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, no effect (MD -1.26, 95% CI: -6.08-3.55, I^2=99.6%)

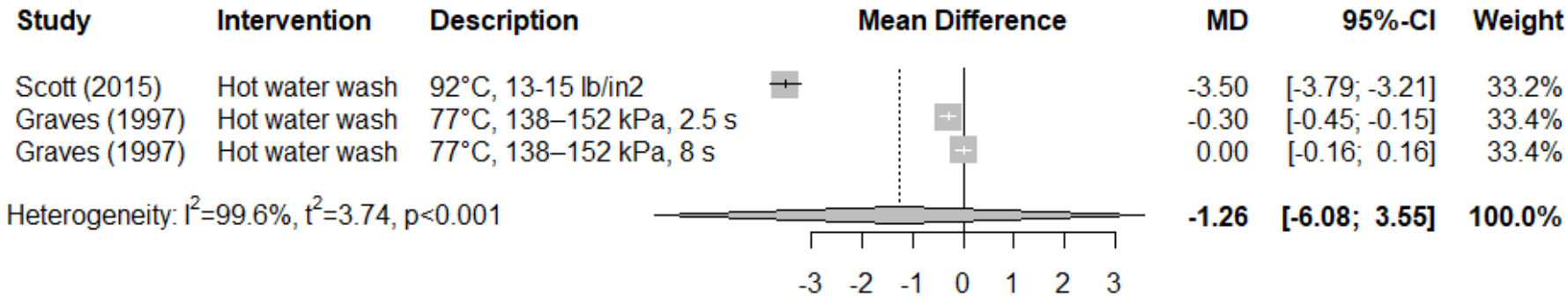


Figure 99. Forest plot of the results of challenge trials performed under commercial abattoir conditions to investigate the efficacy of knife trimming followed by hot water wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses

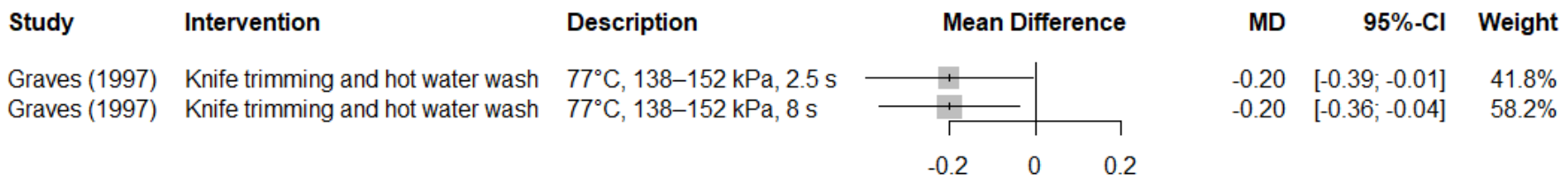


Figure 100. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of hot water wash in reducing *E. coli* counts (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -3.29, 95% CI: -3.93- -2.64, I^2=80.1%)

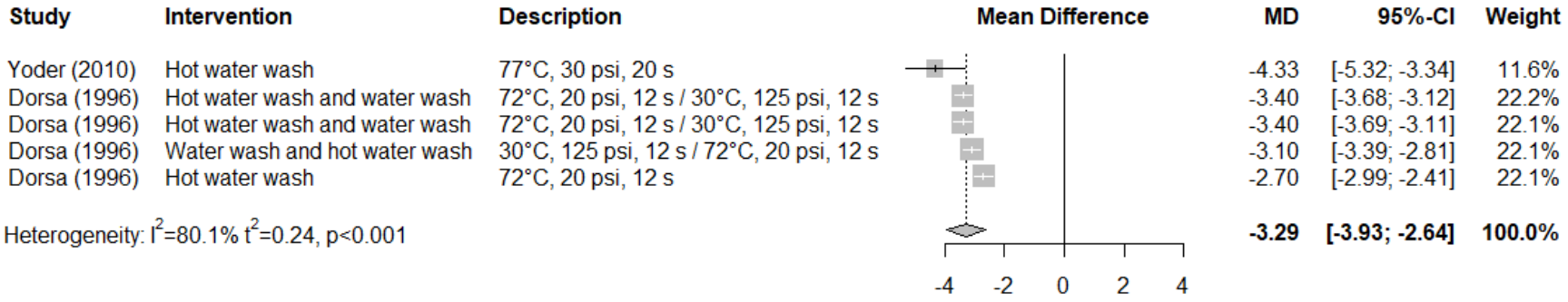
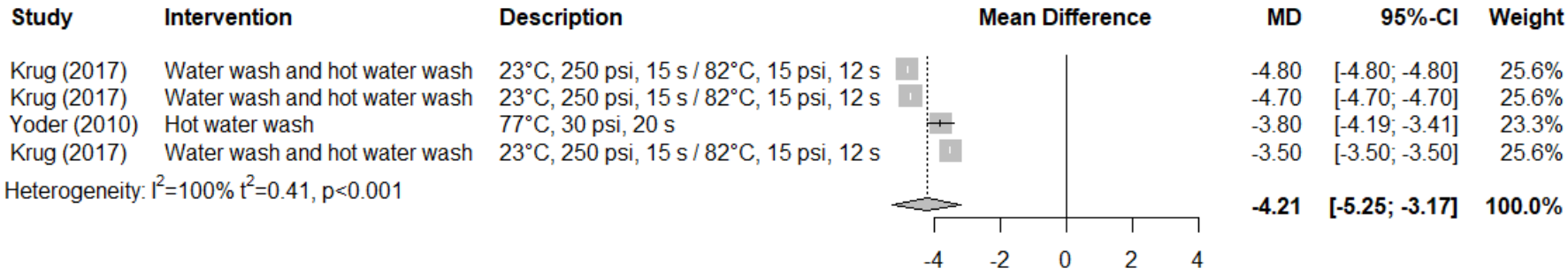


Figure 101. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of hot water wash in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -4.21, 95% CI: -5.25- -3.17, I^2=100%)



D3.2.4 Steam vacuuming

Figure 102. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of steam vacuuming carcass areas with no visible faecal contamination, in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. Medium heterogeneity, positive effect (MD -0.61, 95% CI: -0.89- -0.32, I^2=60.0%)

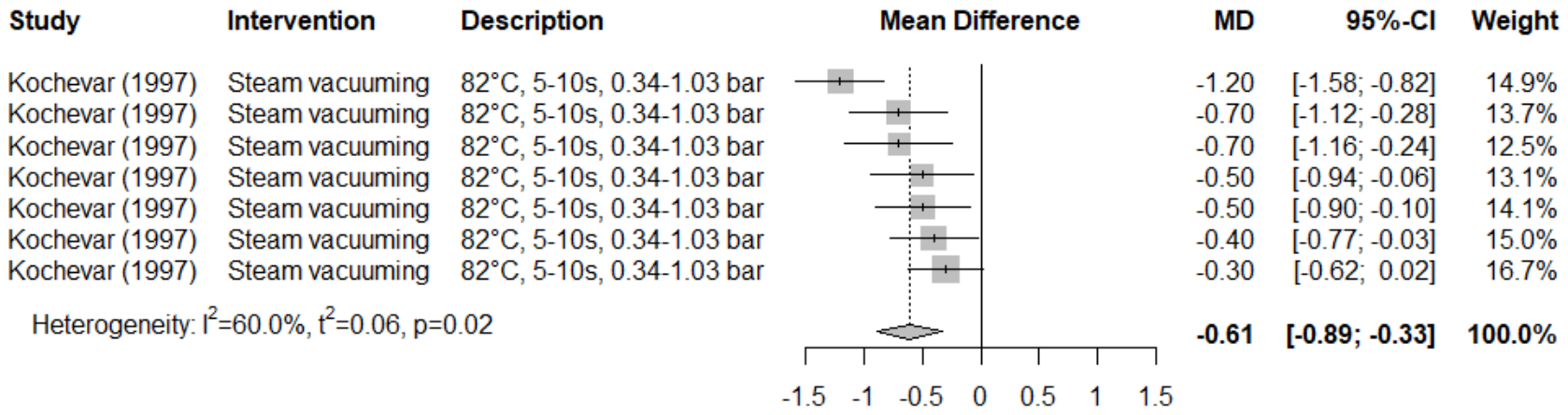


Figure 103. Forest plot of the results of controlled trials performed under commercial abattoir conditions to investigate the efficacy of steam vacuuming carcass areas with visible faecal contamination, in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.84, 95% CI: -2.17- -1.50, I^2=60.6%)

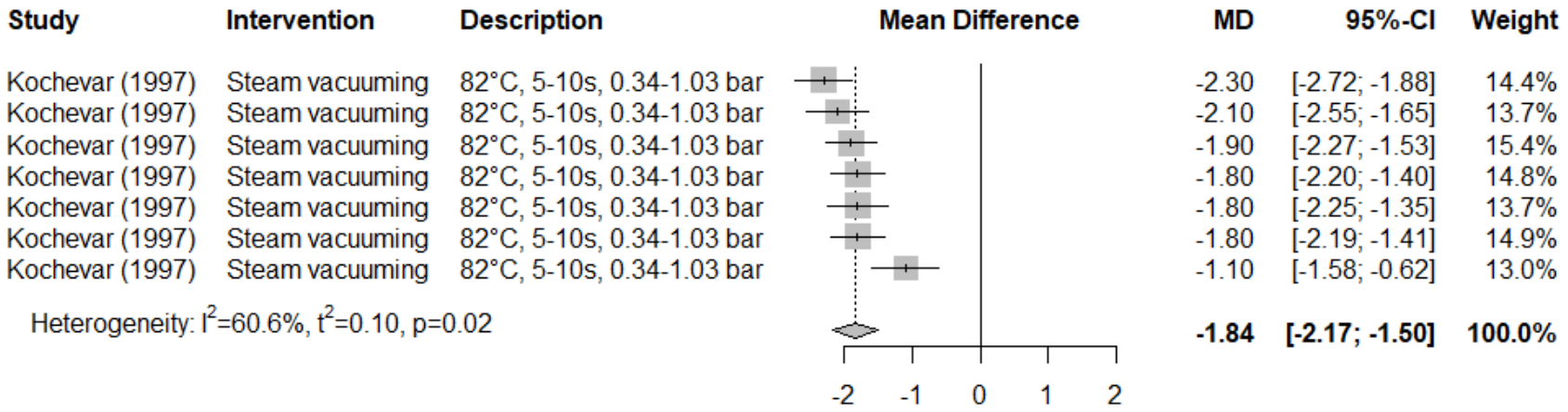


Figure 104. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam vacuuming in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. Medium heterogeneity, positive effect (MD -0.51, 95% CI: -0.70- -0.32, I^2=58.9%)

D3.2.5 Steam pasteurisation

Figure 105. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.14, 95% CI: -1.35- -0.93, I^2=100%)

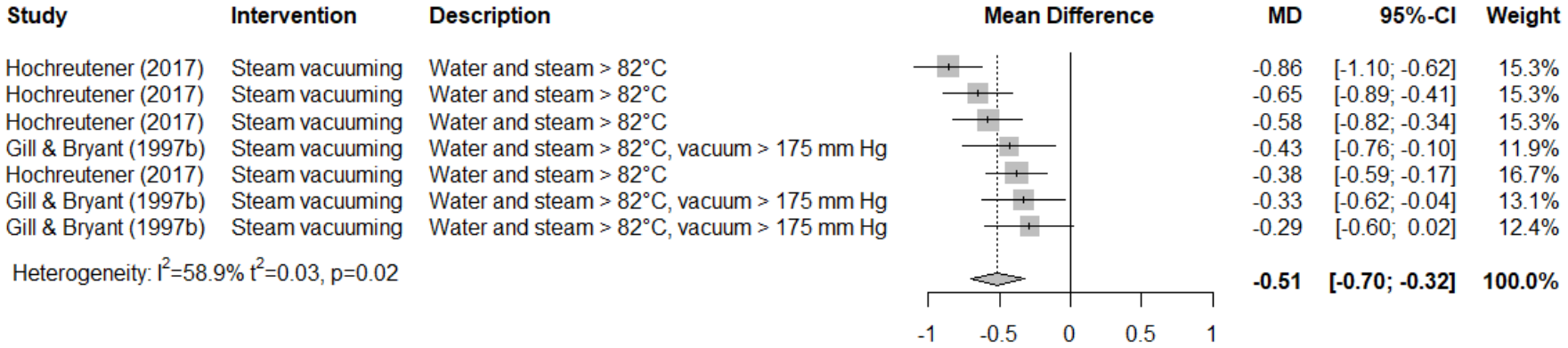


Figure 106. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing generic E. coli counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -0.54, 95% CI: -0.73- -0.34, I^2=91.7%)

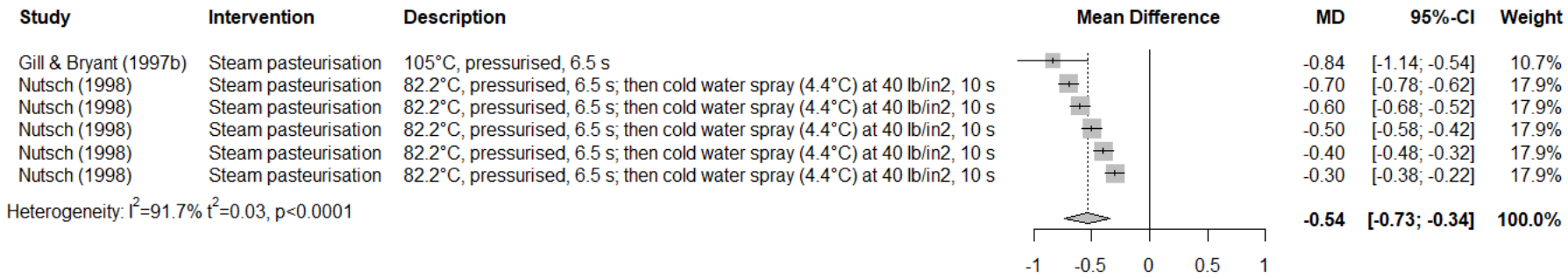
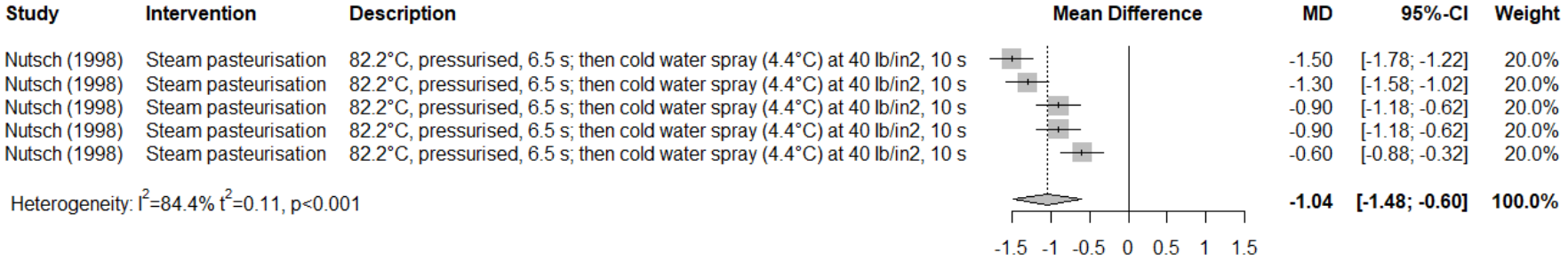


Figure 107. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of steam pasteurisation in reducing *Enterobacteriaceae* counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.04, 95% CI: -1.48- -0.60, I^2=84.4%)



D3.2.6 Lactic acid wash

Figure 108. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of lactic acid spray wash in reducing generic E. coli prevalence on beef carcasses. High heterogeneity, no effect (RR 0.93, 95% CI: 0.42-2.07, I^2=69.1%)

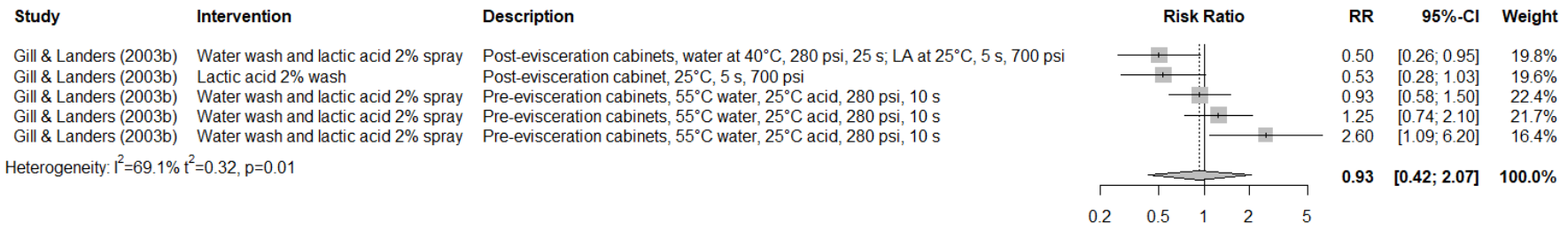


Figure 109. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of lactic acid spray wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -0.62, 95% CI: -1.08- -0.17, I^2=100%)

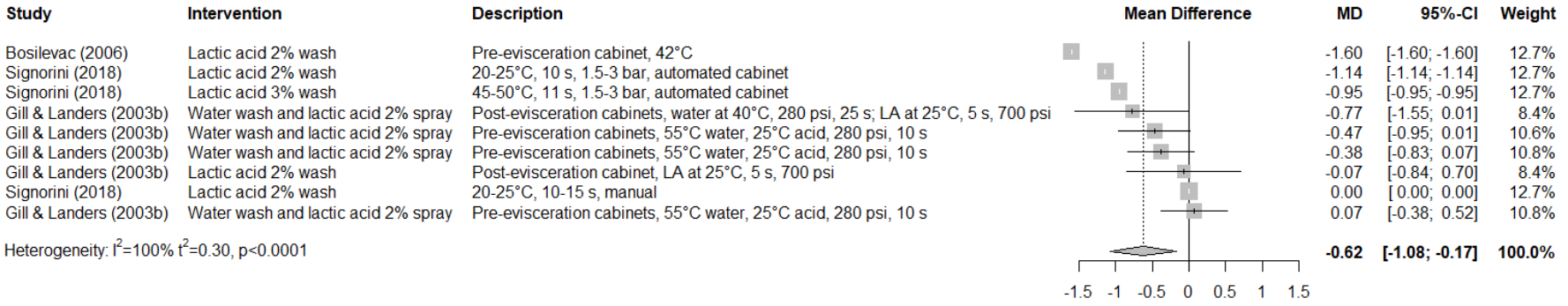


Figure 110. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of lactic acid spray wash in reducing generic *E. coli* counts (log_{10} CFU) on beef carcasses. High heterogeneity, no effect (MD -0.63, 95% CI: -1.89-0.62, I^2=97.1%)

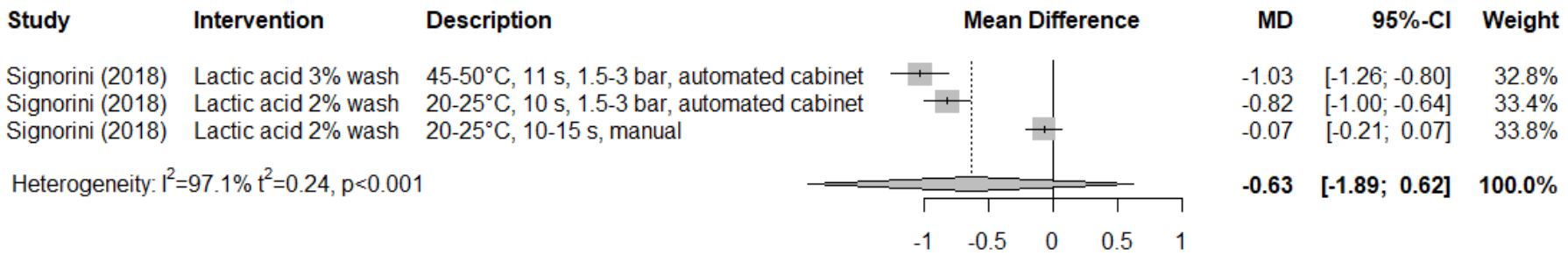


Figure 111. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid spray wash in reducing generic *E. coli* counts (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.03, 95% CI: -1.97- -0.09, I^2=76.4%)

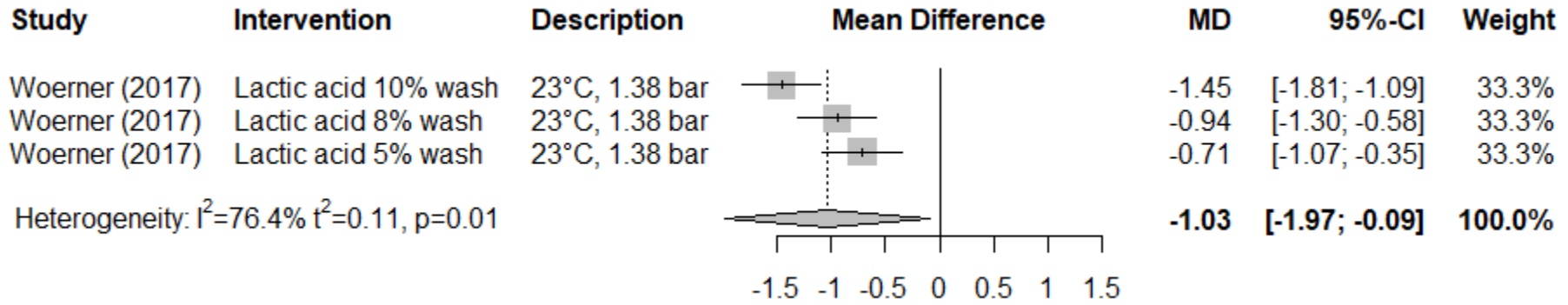


Figure 112. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid spray wash in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef

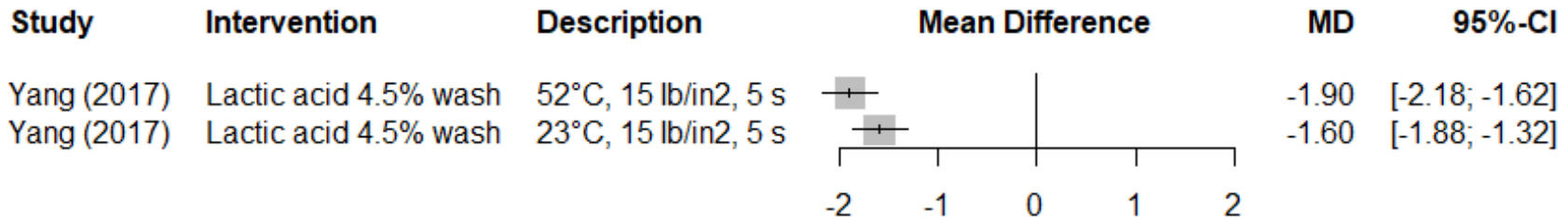
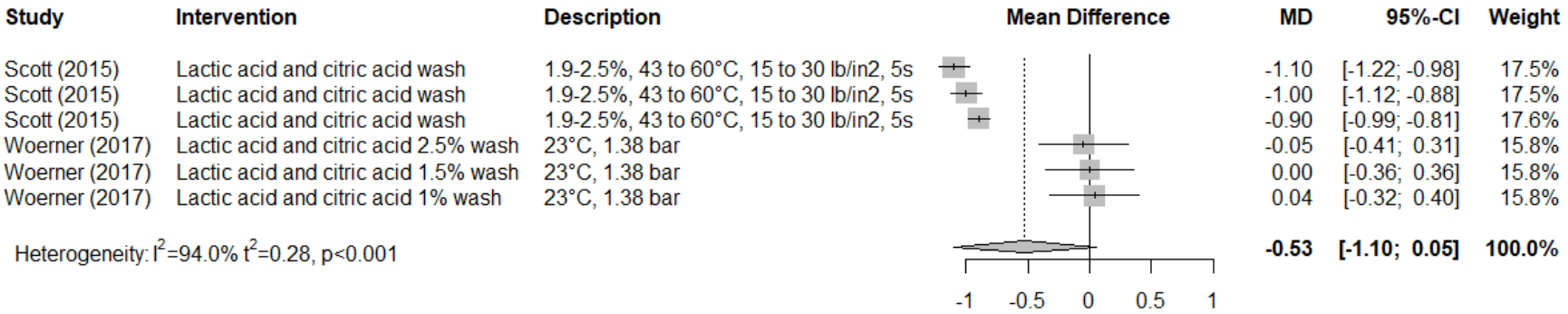


Figure 113. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid followed by citric acid spray wash in reducing *E. coli* O157:H7 and non-O157 numbers (log_{10} CFU) on beef. High heterogeneity, no effect (MD -0.53, 95% CI: -1.10-0.05, I^2=94.0%)



D3.2.7 Acid wash

Figure 114. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of various acids spray wash in reducing generic *E. coli* counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -0.36, 95% CI: -0.66- -0.06, I^2=78.8%)

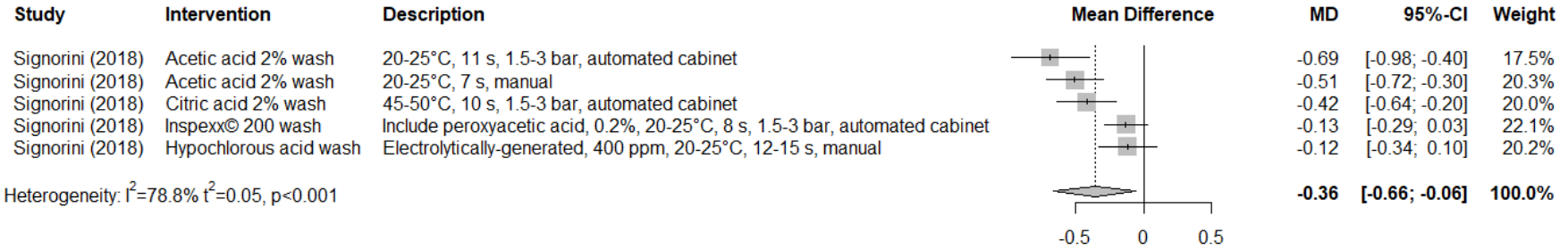


Figure 115. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of various acids spray wash in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -0.42, 95% CI: -0.81- -0.03, I^2=86.9%)

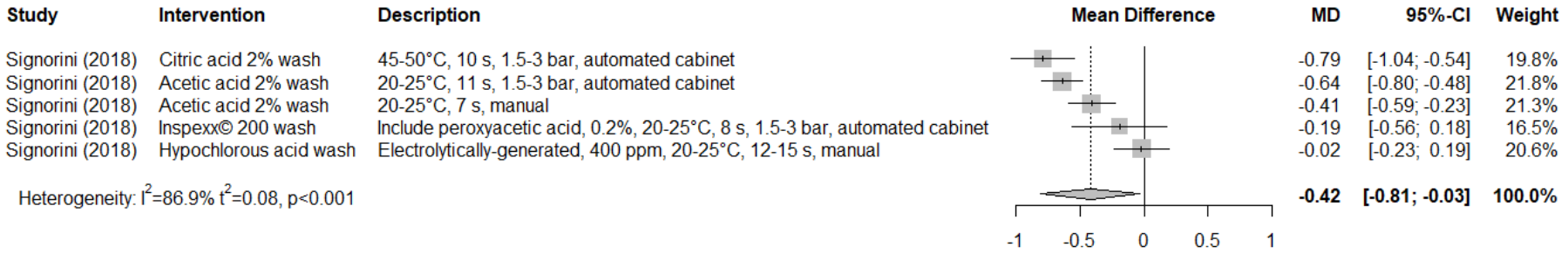


Figure 116. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of sulfuric acid and sodium sulfate spray wash in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef. High heterogeneity, no effect (MD -1.50, 95% CI: -3.05-0.05, I^2=94.9%)

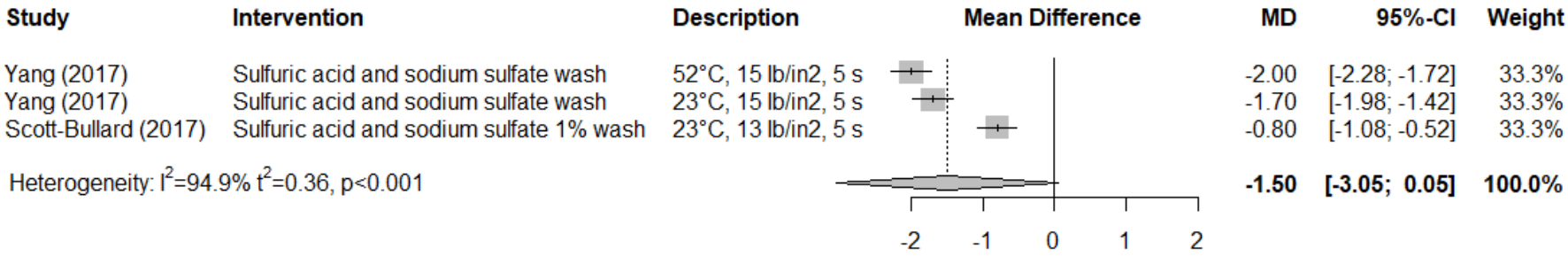
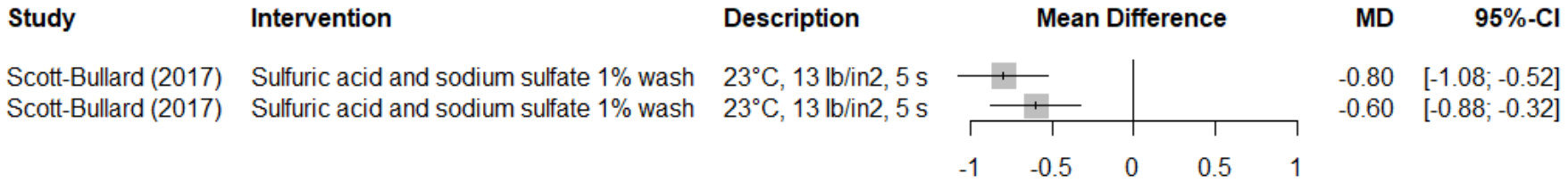


Figure 117. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of sulfuric acid and sodium sulfate spray wash in reducing *E. coli* O157:H7 and non-O157 numbers (log_{10} CFU) on beef



D3.2.8 Pasteurisation and acid wash

Figure 118. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of pasteurisation and acid spray washes in reducing aerobic colony counts (log_{10} CFU) on beef carcasses. High heterogeneity, positive effect (MD -1.41, 95% CI: -2.10- -0.72, I^2=97.2%)

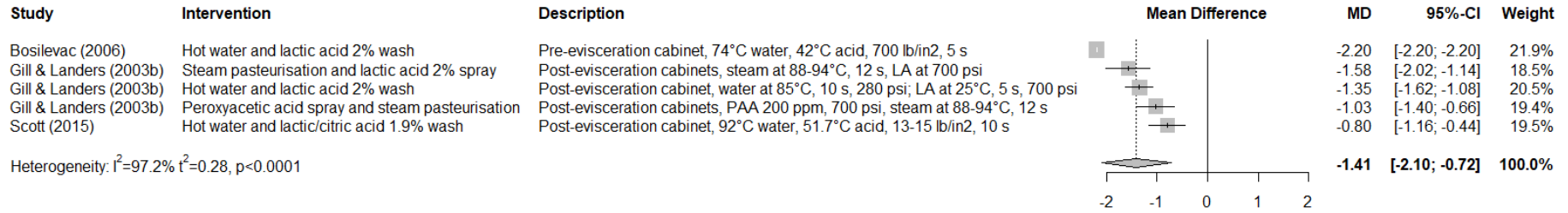
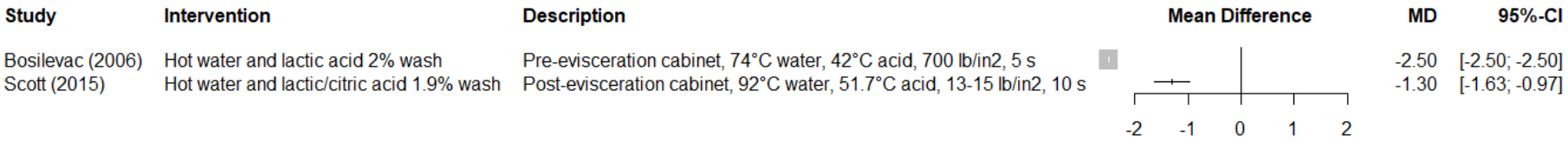


Figure 119. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of pasteurisation and acid spray washes in reducing *Enterobacteriaceae* counts (log_{10} CFU) on beef carcasses



D3.3. Chilling

D3.3.1 Dry chilling

Figure 120. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of dry chilling in reducing aerobic colony counts (log_{10} CFU) on beef carcass sides. High heterogeneity, positive effect (MD -1.11, 95% CI: -1.58- -0.63, I^2=93.5%)

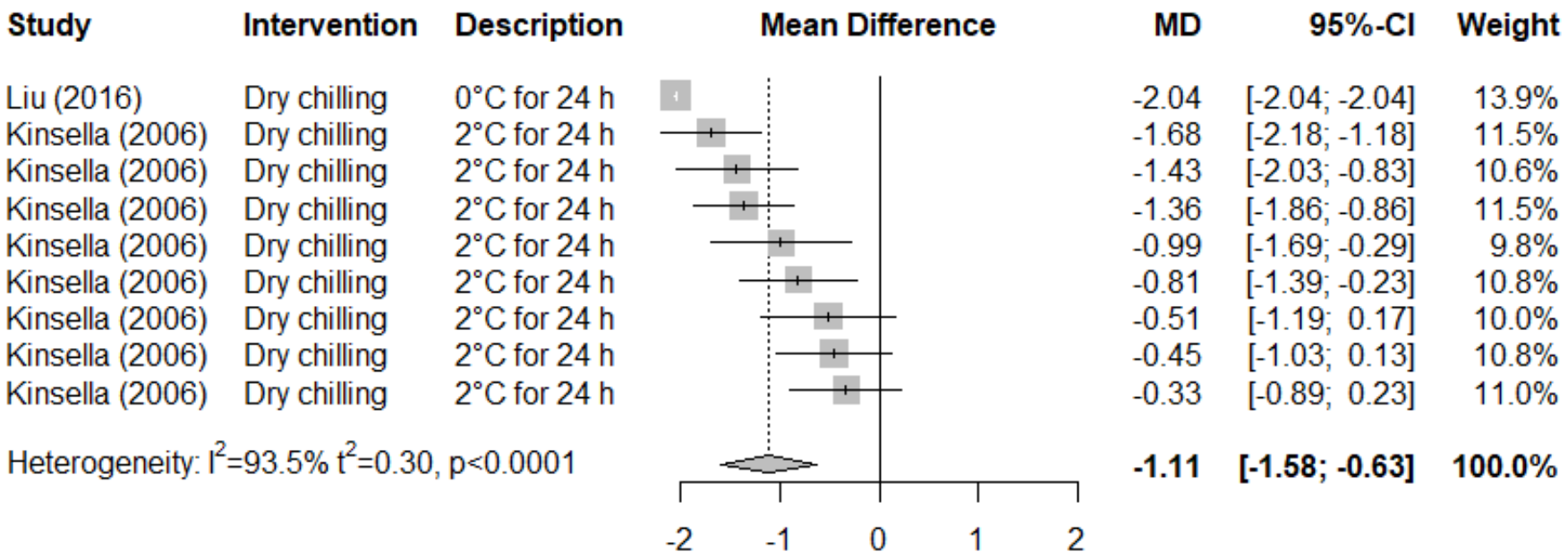

Study
Intervention
Description
Mean Difference
MD
95%-CI
Weight
Liu (2016) Dry chilling 0°C for 24 h -2.04 [-2.04; -2.04] 13.9%
Kinsella (2006) Dry chilling 2°C for 24 h -1.68 [-2.18; -1.18] 11.5%
Kinsella (2006) Dry chilling 2°C for 24 h -1.43 [-2.03; -0.83] 10.6%
Kinsella (2006) Dry chilling 2°C for 24 h -1.36 [-1.86; -0.86] 11.5%
Kinsella (2006) Dry chilling 2°C for 24 h -0.99 [-1.69; -0.29] 9.8%
Kinsella (2006) Dry chilling 2°C for 24 h -0.81 [-1.39; -0.23] 10.8%
Kinsella (2006) Dry chilling 2°C for 24 h -0.51 [-1.19; 0.17] 10.0%
Kinsella (2006) Dry chilling 2°C for 24 h -0.45 [-1.03; 0.13] 10.8%
Kinsella (2006) Dry chilling 2°C for 24 h -0.33 [-0.89; 0.23] 11.0%
Heterogeneity: I^2=93.5% t^2=0.30, p<0.0001
-1.11 [-1.58; -0.63] 100.0%
-2
-1
0
1
2

Figure 121. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of dry chilling following multiple slaughter line interventions in reducing aerobic colony counts (log_{10} CFU) on beef carcass sides. High heterogeneity, positive effect (MD -2.09, 95% CI: -2.78- -1.40, I^2=94.8%)

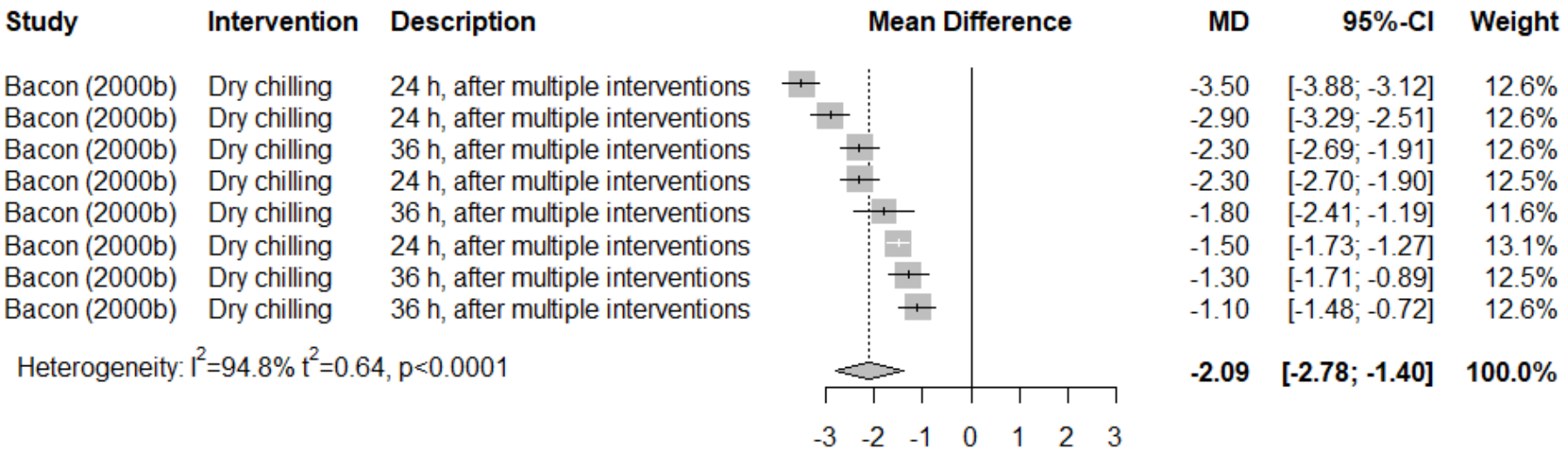


Figure 122. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of dry chilling following multiple slaughter line interventions in reducing generic *E. coli* counts (log_{10} CFU) on beef carcass sides. High heterogeneity, positive effect (MD -0.60, 95% CI: -1.13- -0.08, I^2=98.7%)

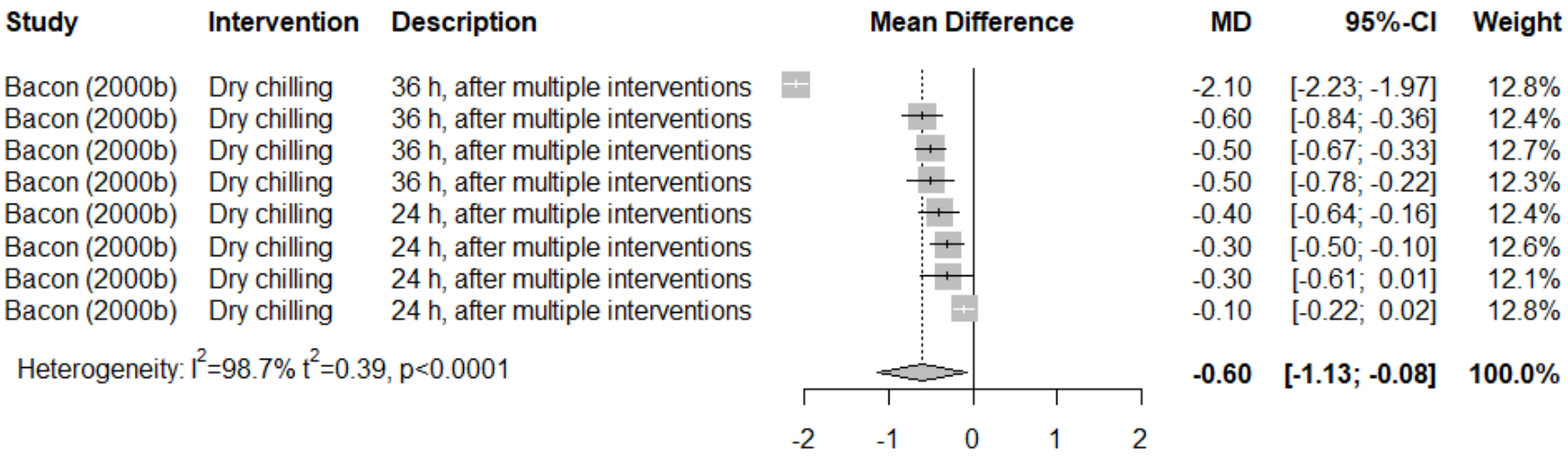


Figure 123. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 24 hours in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef. High heterogeneity, no effect (MD -0.24, 95% CI: -0.56-0.08, I^2=62.3%)

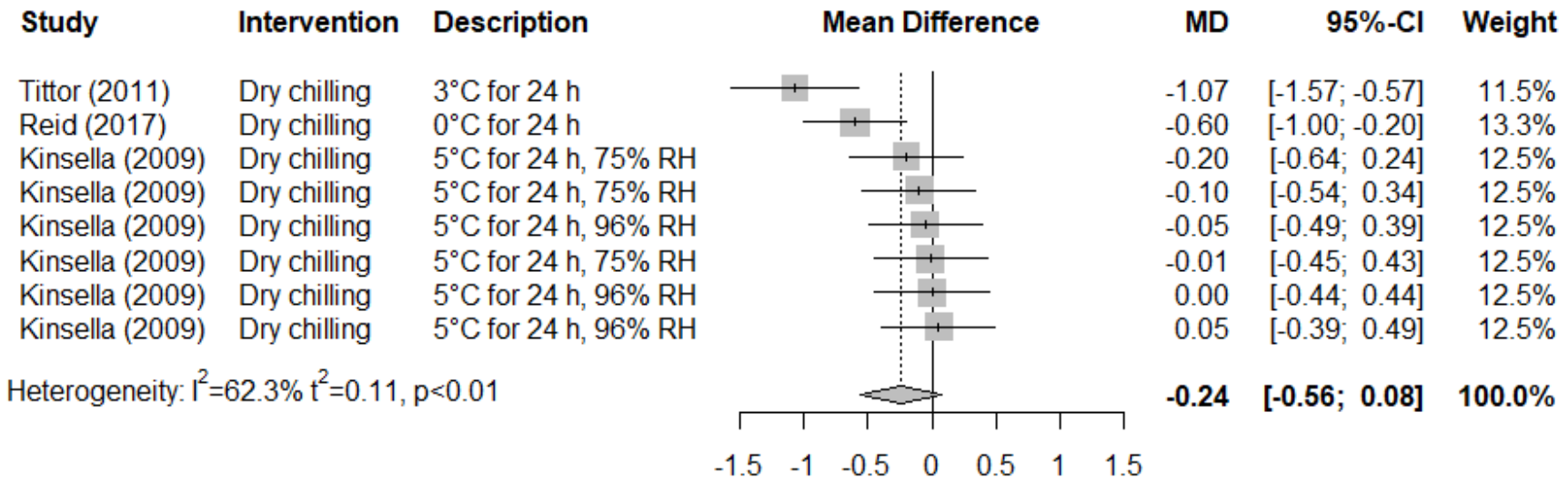


Figure 124. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 48 hours in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -0.63, 95% CI: -1.11- -0.15, I^2=81.4%)

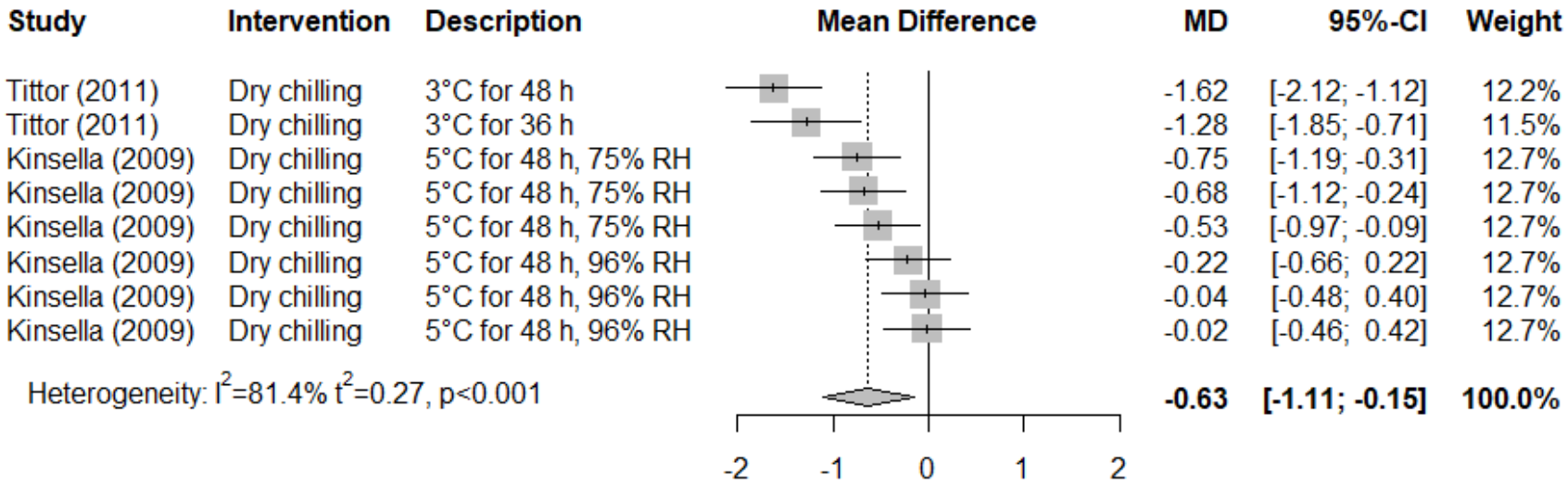


Figure 125. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling up to 72 hours in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -0.53, 95% CI: -1.16- -0.11, I^2=86.0%)

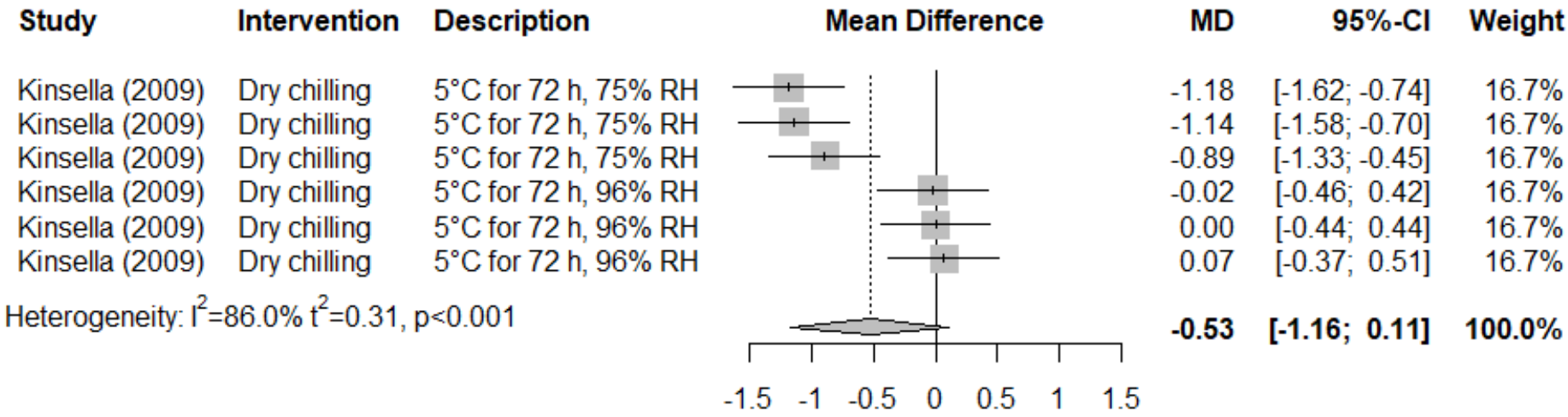


Figure 126. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling following chemical washes in reducing Salmonella spp. numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -3.48, 95% CI: -4.04- -2.92, I^2=70.8%)

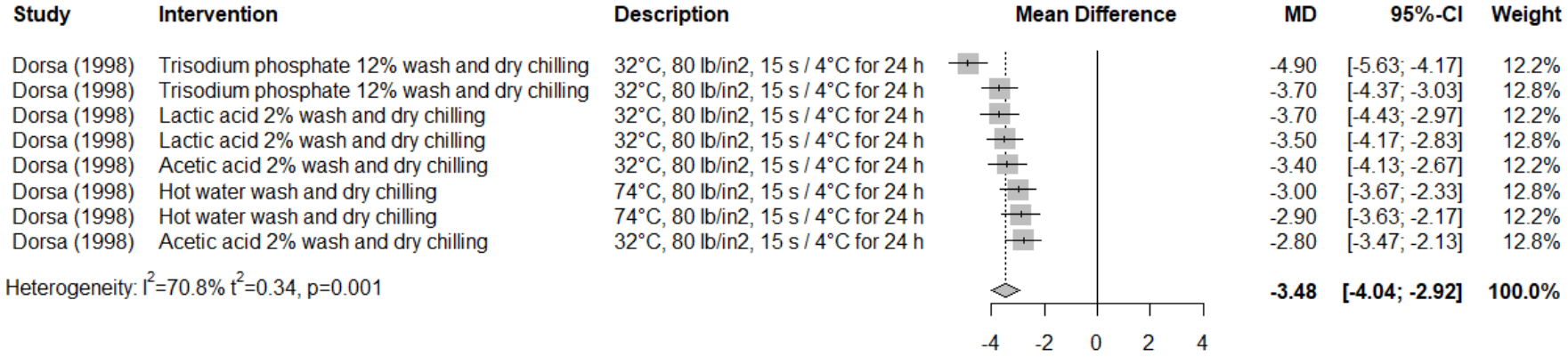


Figure 127. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling following chemical washes in reducing E. coli O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -2.86, 95% CI: -3.33- -2.39, I^2=82.4%)

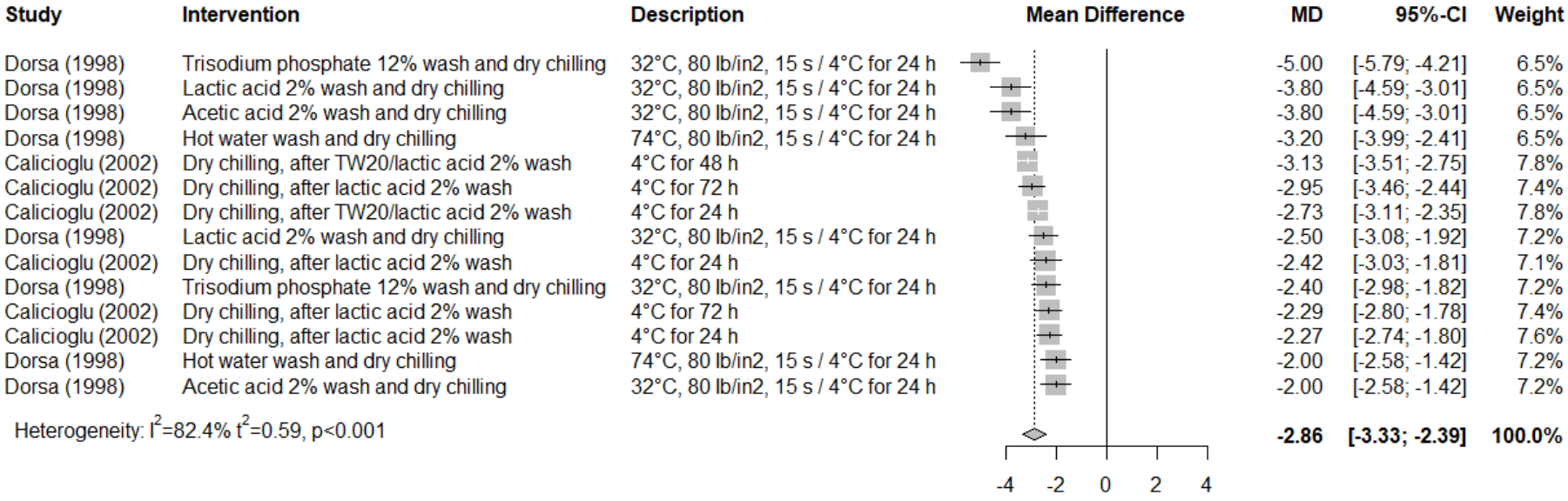
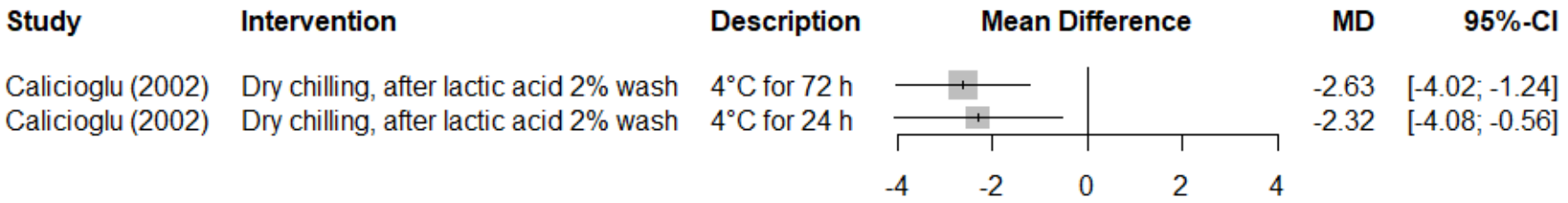


Figure 128. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry chilling following lactic acid wash in reducing generic *E. coli* counts (log_{10} CFU) on beef



D3.3.2 Dry aging

Figure 129. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry aging up to 14 days in reducing Salmonella spp. numbers (log_{10} CFU) on beef

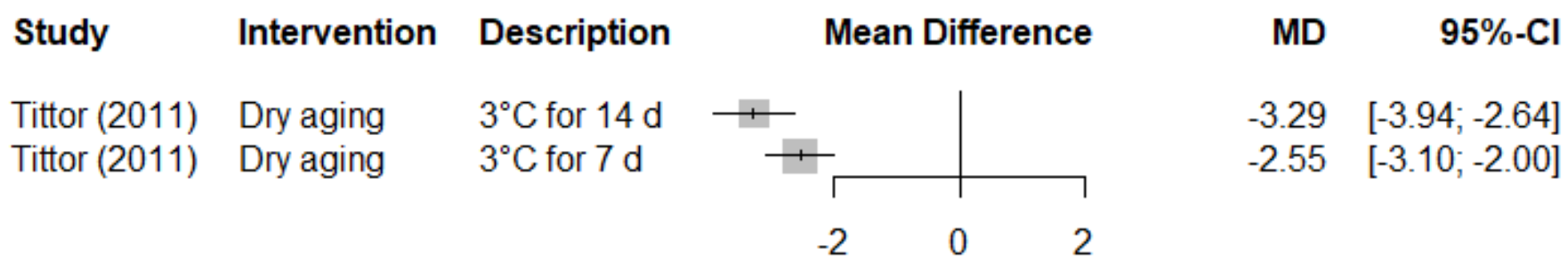
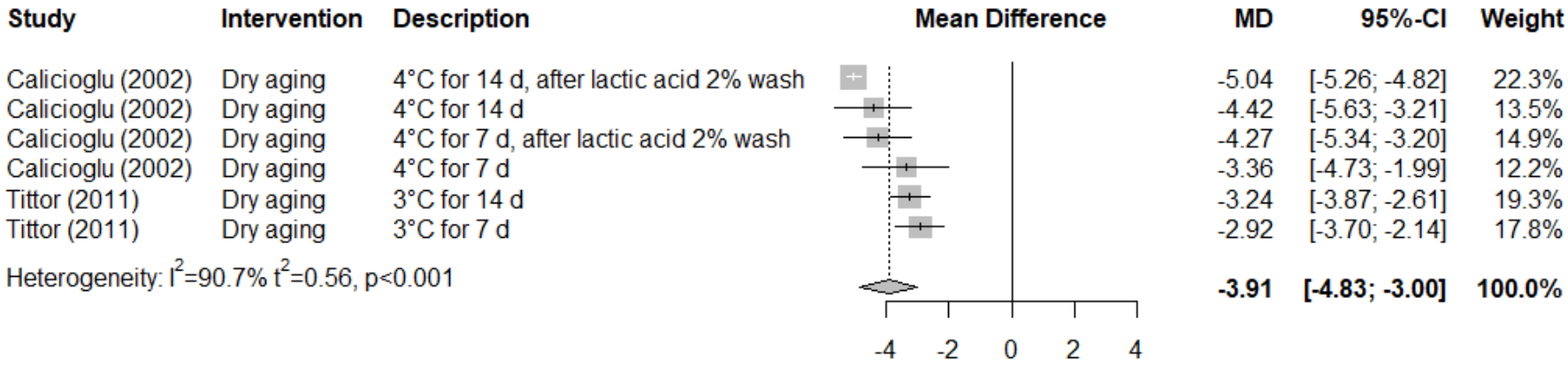


Figure 130. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of dry aging up to 14 days in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -3.91, 95% CI: -4.83- -3.00, I^2=90.7%)



D3.3.3 Water spray chilling

Figure 131. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water spray chilling in reducing generic E. coli prevalence on beef carcass sides. High heterogeneity, no effect (RR 0.67, 95% CI: 0.29-1.54, I^2=63.9%)

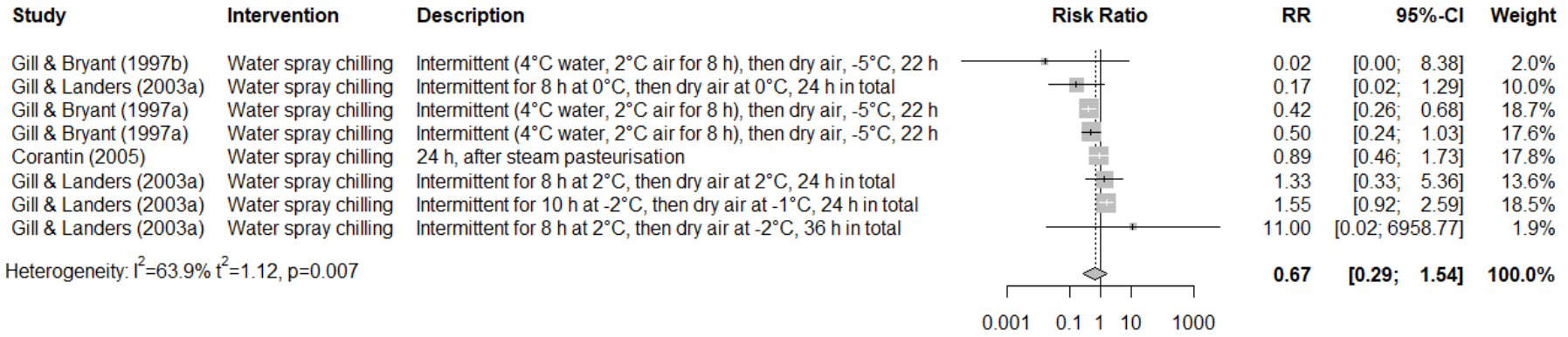


Figure 132. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water spray chilling in reducing aerobic colony counts (log_{10} CFU) on beef carcass sides. High heterogeneity, no effect (MD -0.44, 95% CI: -1.06-0.19, I^2=96.2%)

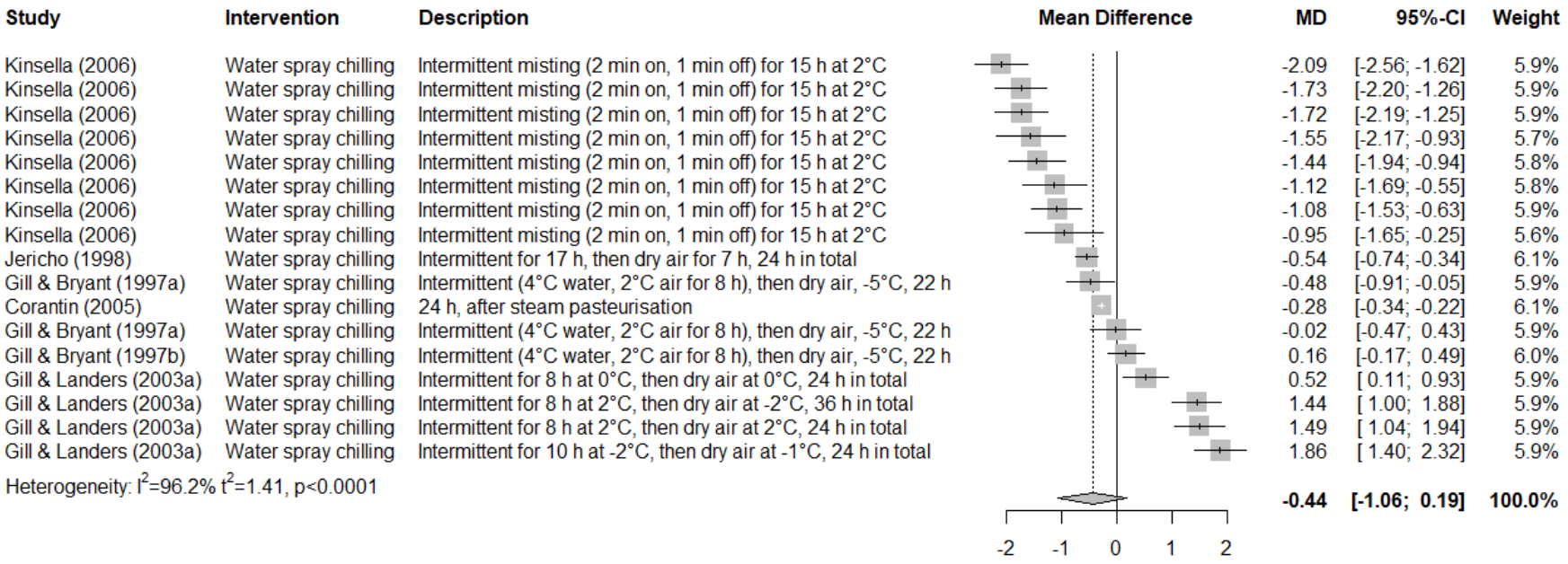


Figure 133. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water spray chilling in reducing generic *E. coli* counts (log_{10} CFU) on beef carcass sides

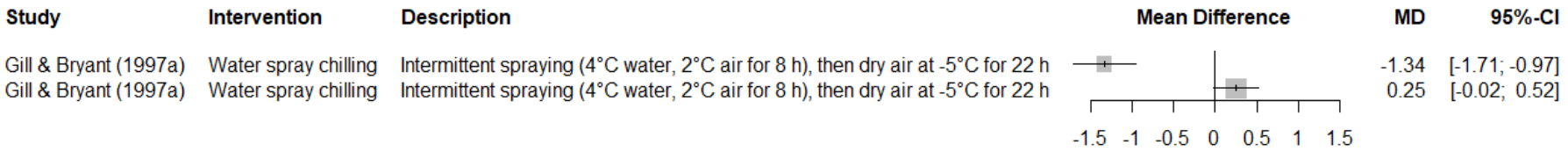


Figure 134. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of water spray chilling in reducing generic *E. coli* counts (log_{10} CFU) on beef carcass sides (converted values)

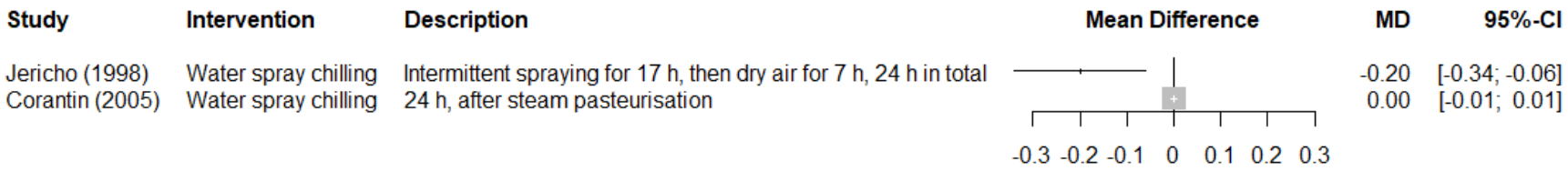


Figure 135. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.44, 95% CI: -1.86- -1.02, I^2=88.9%)

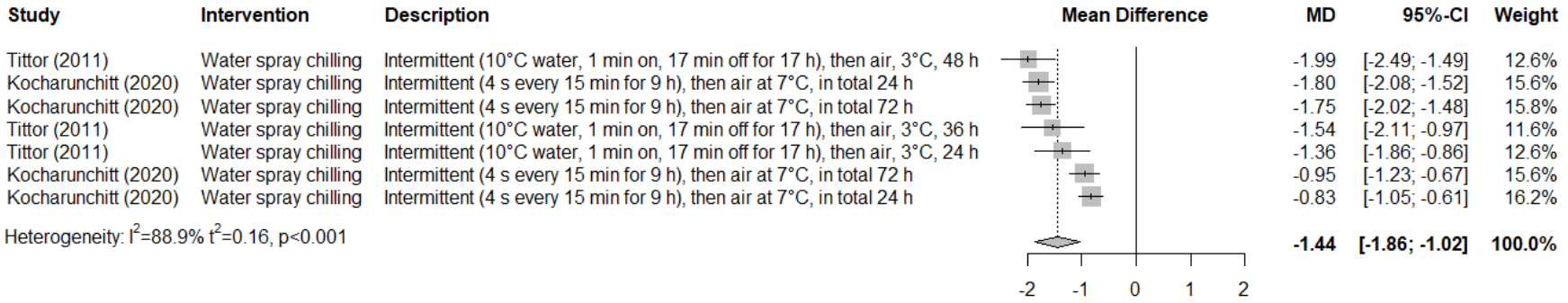


Figure 136. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.46, 95% CI: -2.06- -0.86, I^2=95.3%)

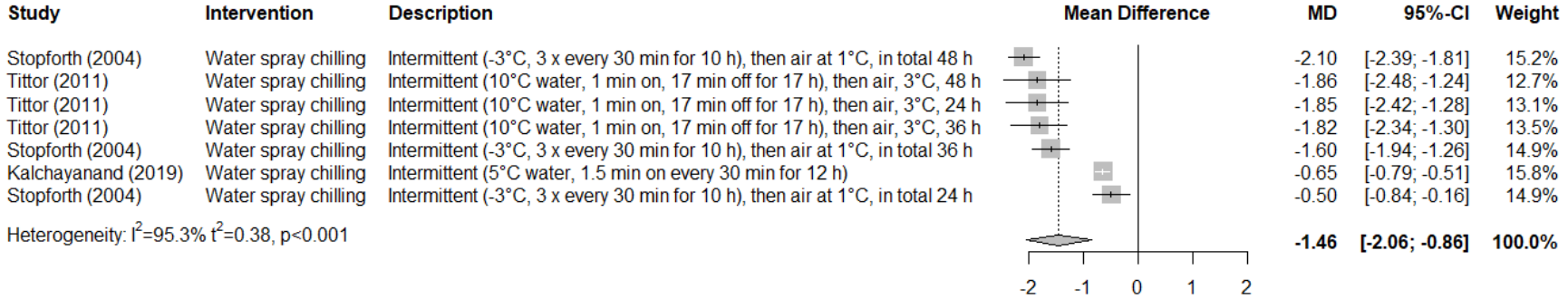


Figure 137. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling followed by aging up to 14 days, in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef

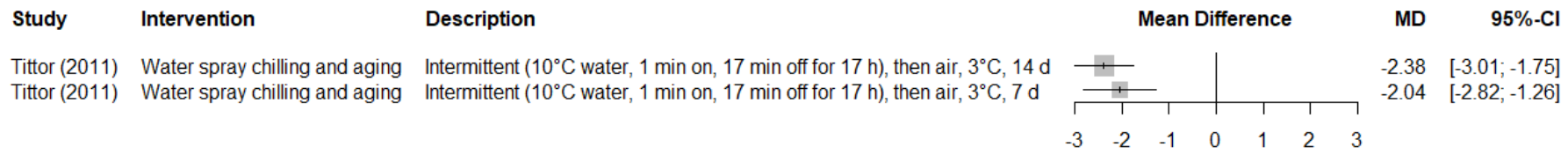


Figure 138. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling vs. dry chilling, followed by aging up to 14 days, in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef

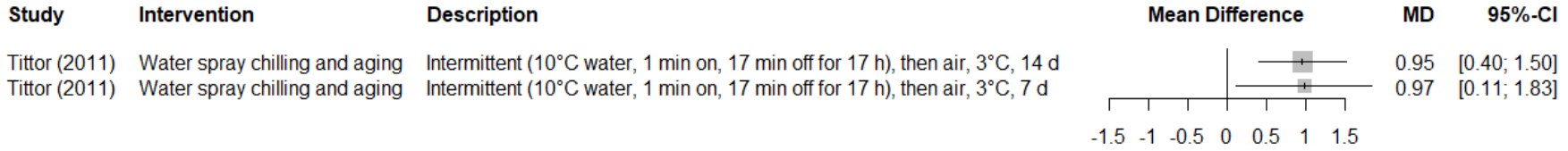


Figure 139. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling followed by aging up to 14 days, in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef

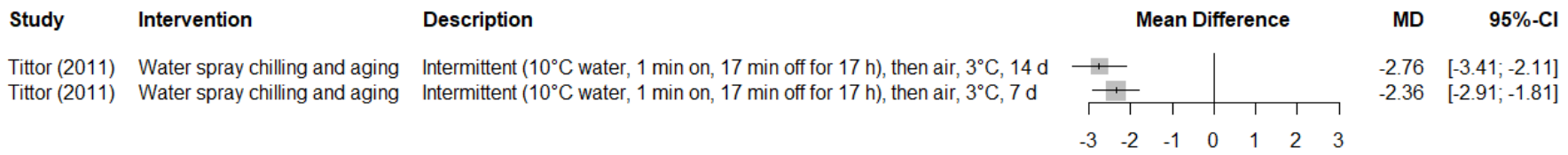
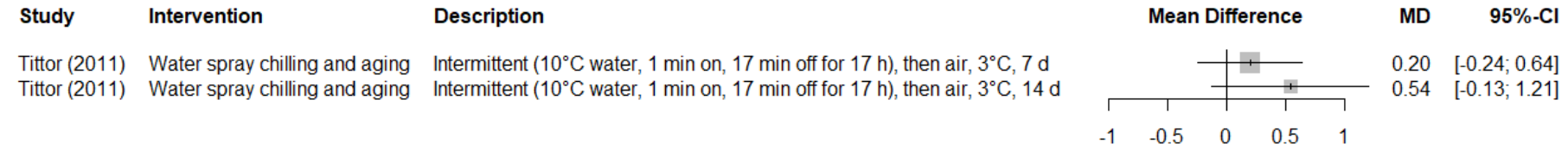


Figure 140. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of water spray chilling vs. dry chilling, followed by aging up to 14 days, in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef



D3.3.4 Spray chilling with chemicals

Figure 141. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals in reducing generic *E. coli* counts (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -2.40, 95% CI: -3.85- -0.94, I^2=99.8%)

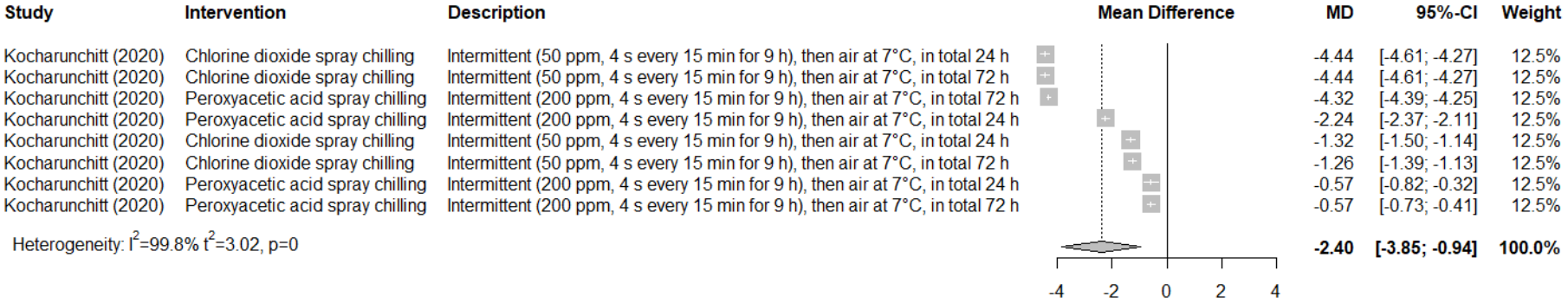


Figure 142. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals vs. water spray chilling in reducing generic *E. coli* counts (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.85, 95% CI: -3.12- -0.58, I^2=99.4%)

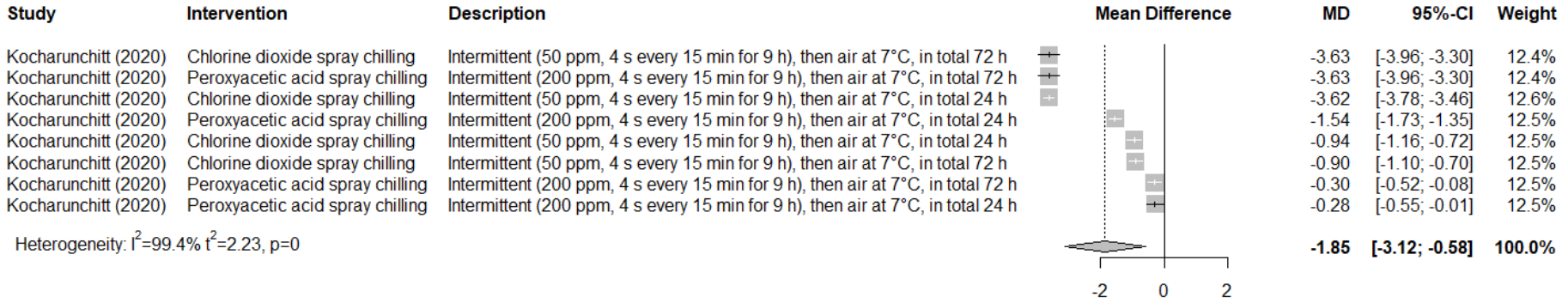


Figure 143. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals in reducing Salmonella spp. numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -2.28, 95% CI: -3.62- -0.94, I^2=99.8%)

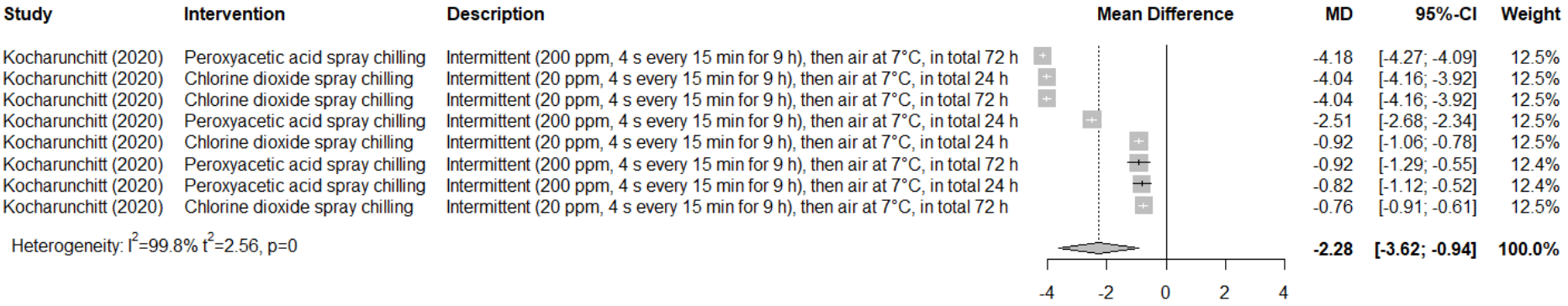


Figure 144. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals vs. water spray chilling in reducing Salmonella spp. numbers (log_{10} CFU) on beef. High heterogeneity, no effect (MD -0.96, 95% CI: -1.94-0.03, I^2=99.8%)

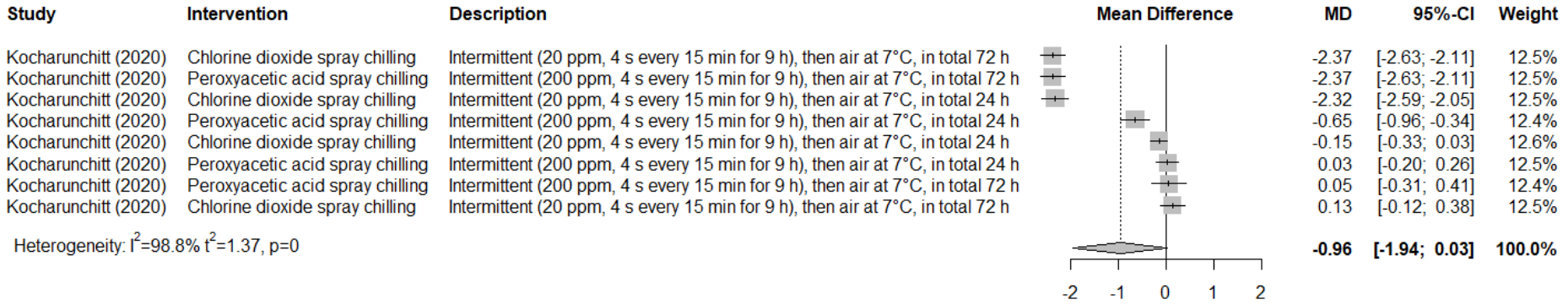


Figure 145. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals in reducing E. coli O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -2.85, 95% CI: -3.57- -2.13, I^2=98.4%)

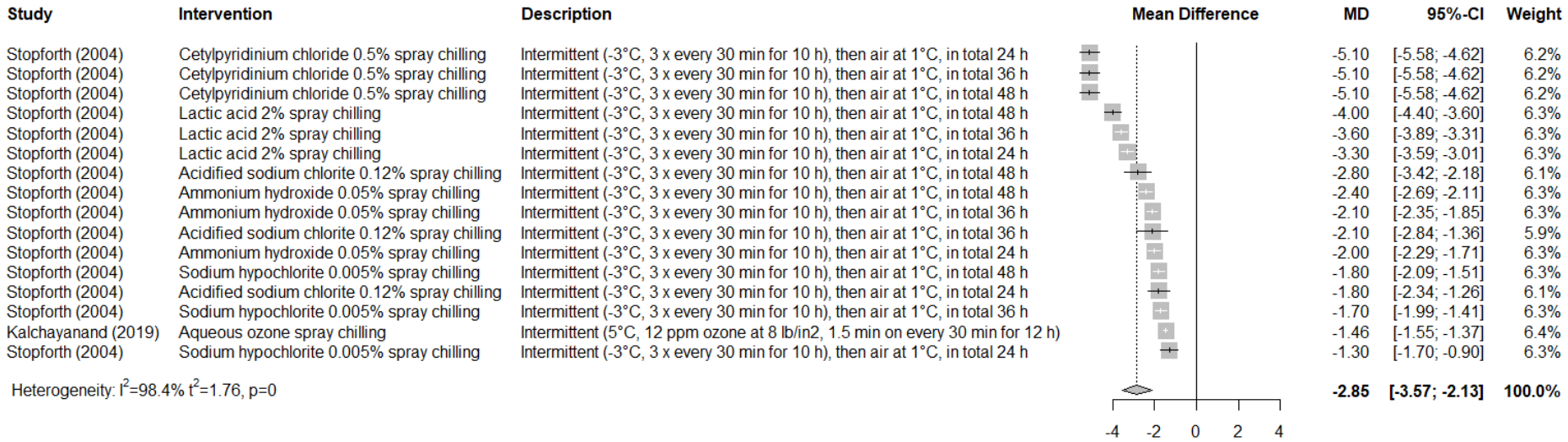
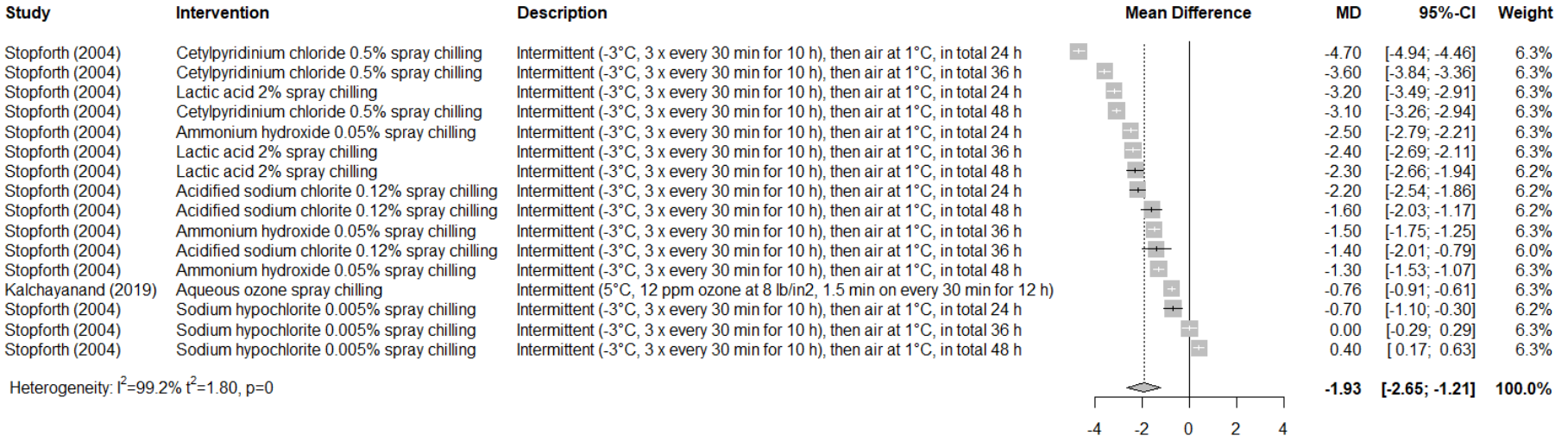


Figure 146. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of spray chilling with chemicals vs. water spray chilling in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef. High heterogeneity, positive effect (MD -1.93, 95% CI: -2.65- -1.21, I^2=99.2%)



D3.4. Multiple on-line interventions

Table 6. Description of multiple interventions

Multiple Intervention system	Description	Study
Pasteurisation and acid treatment system A	(i) steam vacuuming (104 to 110°C, 138 to 345 kPa steam, negative 7 to 12 mm of Hg vacuum), (ii) pre-evisceration carcass washing (29 to 38°C water at 193 to 331 kPa, 6 to 8 s), (iii) pre-evisceration acetic acid solution rinsing (1.6 to 2.6% acetic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s), (iv) thermal pasteurising (71 to 77°C water, 69 to 228 kPa, 10 to 14 s), (v) final carcass washing (16 to 32°C water, 483 to 897 kPa, 10 to 14 s), and (vi) post-evisceration acetic acid solution rinsing (1.6 to 2.6% acetic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s)	Bacon (2000b)
Pasteurisation treatment system B	(i) steam vacuuming (104 to 110°C, 138 to 345 kPa steam, negative 7 to 12 mm of Hg vacuum), (ii) pre-evisceration carcass washing (29 to 38°C water at 193 to 331 kPa, 6 to 8 s), (iii) thermal pasteurising (71 to 77°C water, 69 to 228 kPa, 10 to 14 s), and (iv) final carcass washing (16 to 32°C water, 483 to 897 kPa, 10 to 14 s)	Bacon (2000b)
Pasteurisation and acid treatment system C	(i) steam vacuuming (104 to 110°C, 138 to 345 kPa steam, negative 7 to 12 mm of Hg vacuum), (ii) thermal pasteurising (71 to 77°C water, 69 to 228 kPa, 10 to 14 s), (iii) final carcass washing (16 to 32°C water, 483 to 897 kPa, 10 to 14 s), and (iv) post-evisceration lactic acid solution rinsing (1.6 to 2.6% lactic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s)	Bacon (2000b)

Multiple Intervention system	Description	Study
Organic acid treatment system D	(i) steam vacuuming (104 to 110°C, 138 to 345 kPa steam, negative 7 to 12 mm of Hg vacuum), (ii) final carcass washing (16 to 32°C water, 483 to 897 kPa, 10 to 14 s), and (iii) post-evisceration acetic acid solution rinsing (1.6 to 2.6% acetic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s)	Bacon (2000a)
Organic acid treatment system E	(i) steam vacuuming (104 to 110°C, 138 to 345 kPa steam, negative 7 to 12 mm of Hg vacuum), (ii) pre-evisceration carcass washing (29 to 38°C water at 193 to 331 kPa, 6 to 8 s), (iii) pre-evisceration acetic acid solution rinsing (1.6 to 2.6% acetic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s), (iv) final carcass washing (16 to 32°C water, 483 to 897 kPa, 10 to 14 s), and (v) post-evisceration acetic acid solution rinsing (1.6 to 2.6% acetic acid solution, 43 to 60°C, 317 to 324 kPa, 2 to 4 s)	Bacon (2000a)
Pasteurisation and acid treatment system F	(i) pre-evisceration carcass washing (55°C water at 280 psi, 10 s), (ii) pre-evisceration spraying with 2% lactic acid (25°C); (iii) post-evisceration steam vacuuming of visible contamination from the rump, brisket and forelegs; (iv) post-splitting trimming visible contamination; (v) final carcass washing (40°C, 280 psi, 25 s); (vi) steam pasteurisation (steam at 88-94°C, 12 s); and (vii) final spraying with 2% lactic acid (700 psi)	Gill & Landers (2003b)
Pasteurisation and acid treatment system G	(i) pre-evisceration carcass washing (55°C water at 280 psi, 10 s), (ii) pre-evisceration spraying with 2% lactic acid (25°C); (iii) post-evisceration steam vacuuming of visible contamination from the rump, brisket and forelegs; (iv)	Gill & Landers (2003b)

Multiple Intervention system	Description	Study
	post-splitting trimming visible contamination; (v) final carcass washing (40°C, 280 psi, 12 s); (vi) peroxyacetic acid spray (200 ppm, 280 psi); and (vii) steam pasteurisation (steam at 88-94°C, 12 s)	
Pasteurisation and acid treatment system H	(i) pre-evisceration carcass washing (55°C water at 280 psi, 10 s), (ii) pre-evisceration spraying with 2% lactic acid (25°C); (iii) post-evisceration steam vacuuming of visible contamination from the rump, brisket and forelegs; (iv) post-splitting trimming visible contamination; (v) final carcass washing (40°C, 280 psi, 25 s); (vi) hot water wash (85°C, 10 s, 280 psi); (vii) final spraying with 2% lactic acid (700 psi); and (viii) cold water wash (2°C, 140 psi)	Gill & Landers (2003b)

D3.4.1 Multiple pasteurisation and acid interventions

Figure 147. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of multiple pasteurisation and acid interventions in reducing generic E. coli prevalence on beef carcass sides. High heterogeneity, positive effect (RR 0.30, 95% CI: 0.16-0.59, I^2=92.4%)

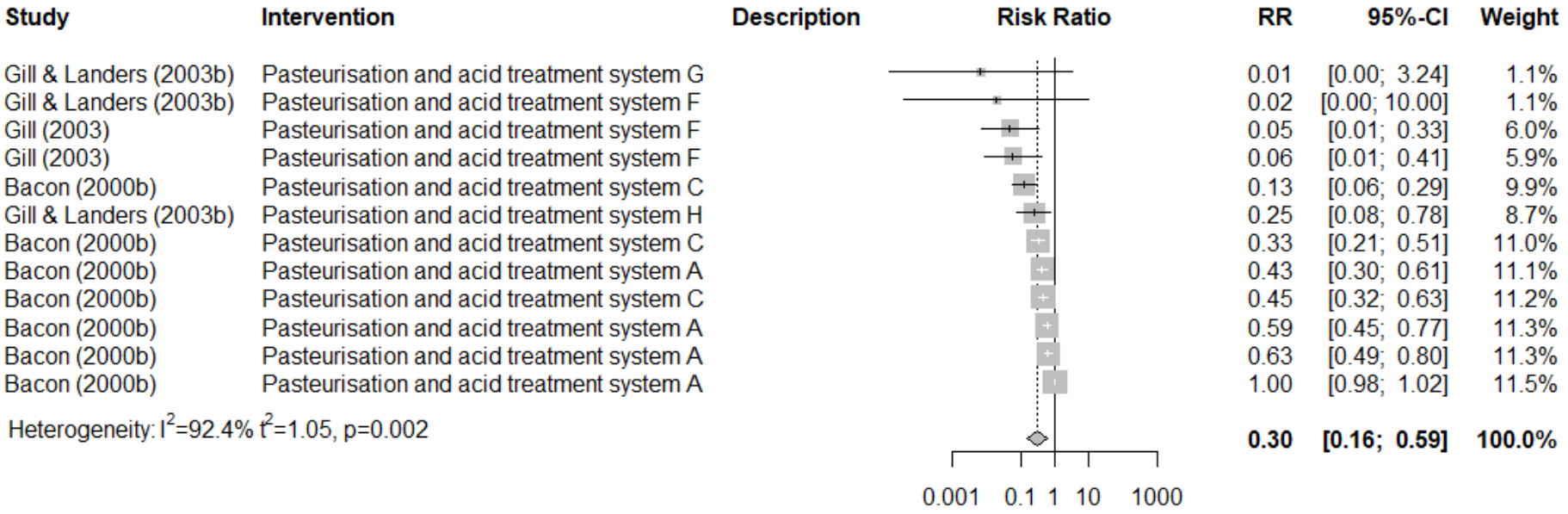


Figure 148. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of multiple pasteurisation and acid interventions in reducing aerobic colony counts (log_{10} CFU) on beef carcass sides. High heterogeneity, positive effect (MD -1.92, 95% CI: -2.33- -1.52, I^2=100%)

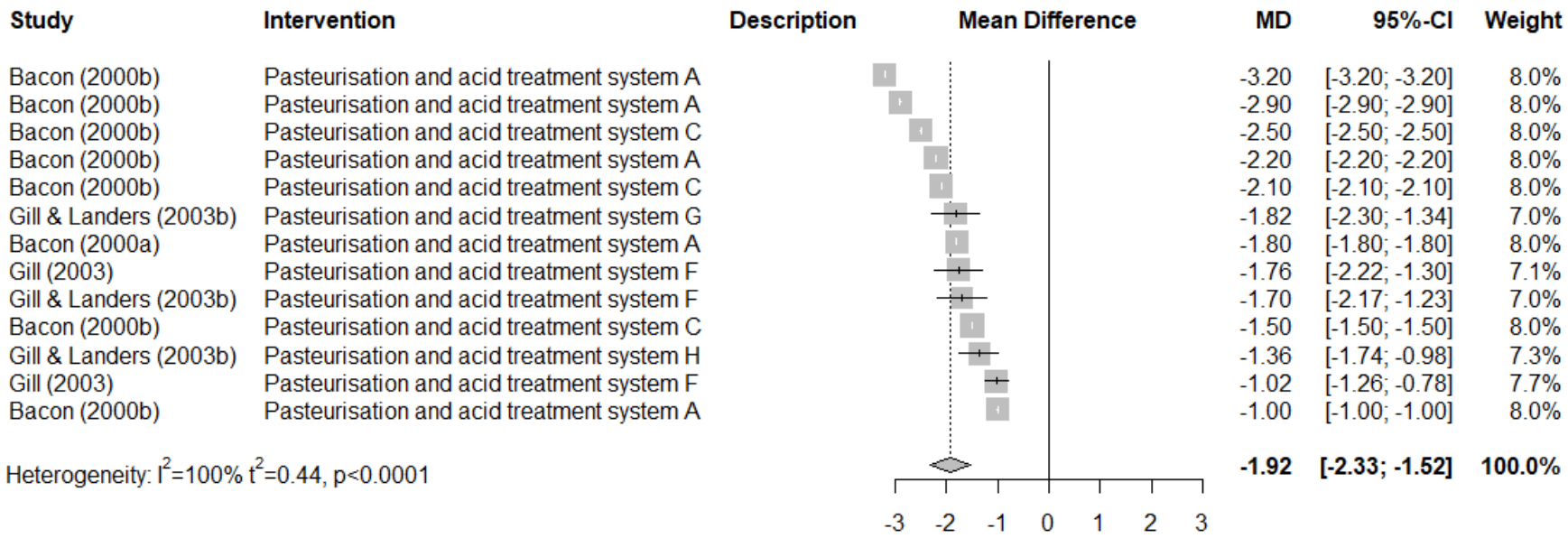
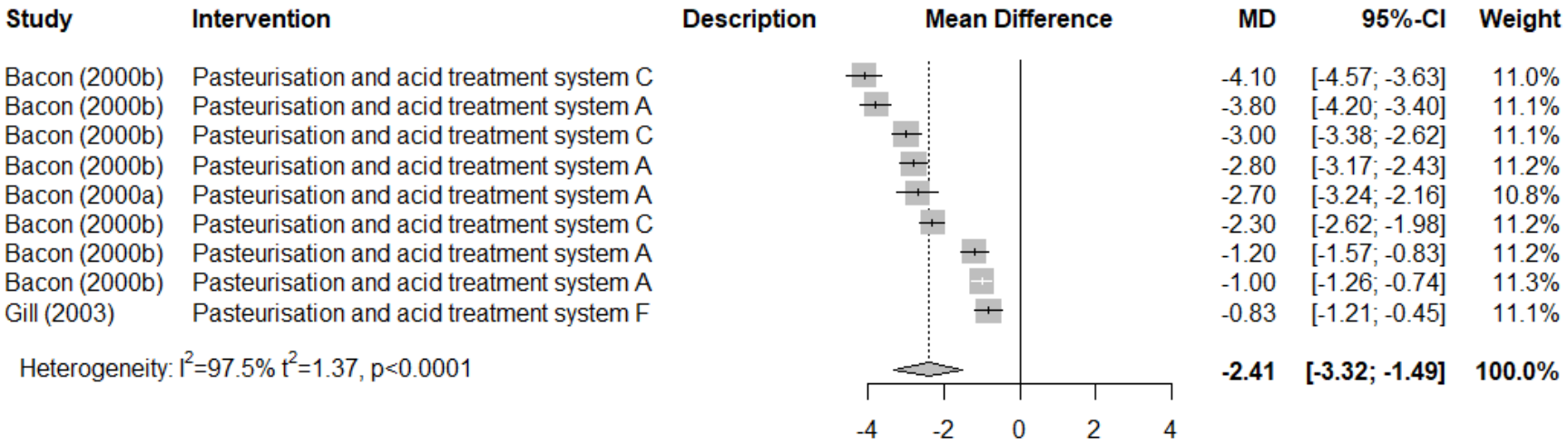


Figure 149. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of multiple pasteurisation and acid interventions in reducing generic *E. coli* counts (log_{10} CFU) on beef carcass sides. High heterogeneity, positive effect (MD -2.41, 95% CI: -3.32- -1.49 I^2=97.5%)



D3.4.2 Multiple acid interventions

Figure 150. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of multiple acid interventions in reducing aerobic colony counts (log_{10} CFU) on beef carcass sides

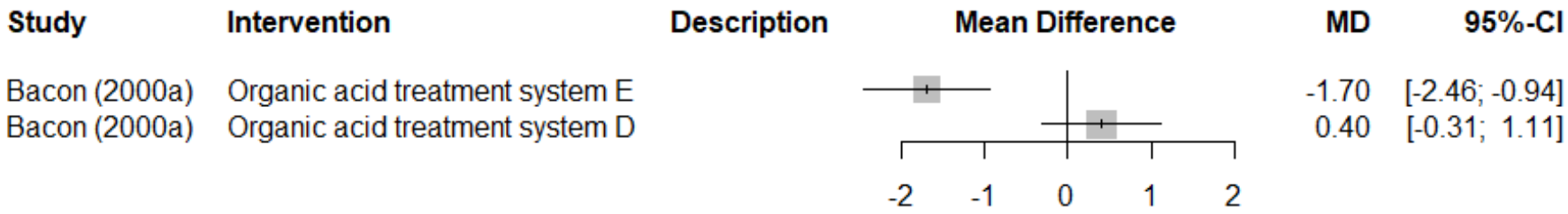


Figure 151. Forest plot of the results of before-and-after trials performed under commercial abattoir conditions to investigate the efficacy of multiple acid interventions in reducing generic *E. coli* counts (log_{10} CFU) on beef carcass sides

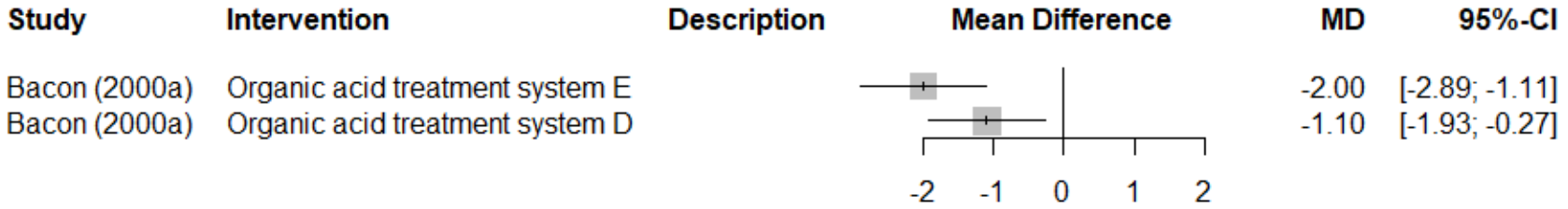


Table 7. Results on beef carcass intervention efficacies from studies with no direct comparisons investigating prevalence outcomes

Study design/ conditions‡	Study	Intervention	Description	Micro-organism[a]	RR (95%-CI)	p-value
CT/Comm	Stopforth (2006)	Bung bagging		STEC unspecified	0.60 (0.4-0.9)	0.01
CT/Comm	Stopforth (2006)	Bung bagging		E. coli O157:H7	0.33 (0.04-3.11)	0.34
CT/Comm	Stopforth (2006)	Bung bagging		Salmonella	0.02 (0.0-10.13)	0.22
BA/Comm	Algino (2007)	Dry aging	1.5°C for 4 days	EBC	0.34 (0.14-0.84)	0.02
BA/Comm	Algino (2007)	Dry aging	1.5°C for 4 days	E. coli	0.01 (0-5.22)	0.15
BA/Comm	Algino (2007)	Dry aging	2.5°C for 6 days	EBC	0.39 (0.3-0.52)	<0.001
BA/Comm	Algino (2007)	Dry aging	2.5°C for 6 days	E. coli	0.05 (0.02-0.15)	<0.001
BA/Comm	Algino (2007)	Dry aging	2.5°C for 7 days	EBC	0.62 (0.46-0.83)	<0.01
BA/Comm	Algino (2007)	Dry aging	2.5°C for 7 days	E. coli	0.52 (0.32-0.85)	<0.01
BA/Comm	Algino (2007)	Acetic acid 2.5% wash	Hand-held sprayer	EBC	0.52 (0.4-0.67)	<0.001
BA/Comm	Algino (2007)	Acetic acid 2.5% wash	Hand-held sprayer	E. coli	0.28 (0.17-0.46)	<0.001
BA/Comm	Algino (2007)	FreshBloomTM wash	Citric, ascorbic and erythorbic acid spray, hand-held sprayer	EBC	0.79 (0.62-0.99)	0.05
BA/Comm	Algino (2007)	FreshBloomTM wash	Citric, ascorbic and erythorbic acid spray, hand-held sprayer	E. coli	0.29 (0.15-0.57)	<0.001
BA/Comm	Algino (2007)	Hot water wash	Low pressure, 65-85°C, 70 s, hand-held sprayer	EBC	0.44 (0.29-0.69)	<0.001
BA/Comm	Algino (2007)	Hot water wash	Low pressure, 65-85°C, 70 s, hand-held sprayer	E. coli	0.13 (0.04-0.37)	<0.001
BA/Comm	Algino (2007)	Hot water wash	High pressure 1000 psi, 50°C, 75 s, hand-held sprayer	EBC	0.79 (0.62-1.00)	0.05

Study design/ conditions[‡]	Study	Intervention	Description	Micro-organism[a]	RR (95%-CI)	p-value
BA/Comm	Algino (2007)	Hot water wash	High pressure 1000 psi, 50°C, 75 s, hand-held sprayer	E. coli	0.17 (0.06-0.47)	<0.001
BA/Comm	Bacon (2000b)	Pasteurisation treatment system B		E. coli	0.30 (0.19-0.48)	<0.001
BA/Comm	Bosilevac (2006)	Hot water wash	Pre-evisceration cabinet, 74°C, 700 lb/in^2, 5 s	E. coli O157:H7	0.20 (0.12-0.35)	<0.001
BA/Comm	Bosilevac (2006)	Hot water and lactic a cid 2% wash	Pre-evisceration cabinet, 74°C water, 42°C acid, 700 lb/in^2, 5 s	E. coli O157:H7	0.19 (0.09-0.37)	<0.001
BA/Comm	Fegan (2005a)	Dry chilling	24 h	E. coli O157	0.02 (0-8.43)	0.20
BA/Comm	Fegan (2005b)	Dry chilling	24 h	Salmonella	1.50 (0.26-8.79)	0.65
BA/Comm	Fontcuberta (2016)	Blast and dry chilling	-4°C blast for 1 h, 4°C dry for 24-72 h	E. coli O157	0.24 (0.11-0.54)	<0.001
BA/Comm	Bosilevac (2006)	Lactic acid 2% wash	Pre-evisceration cabinet, 42°C	E. coli O157:H7	0.63 (0.46-0.86)	0.004

[‡] CT-controlled trial; BA-before-and-after trial; Comm-commercial abattoir conditions; Lab-laboratory conditions;

[a] EBC-*Enterobacteriaceae* counts

Table 8. Results on beef carcass intervention efficacies from studies with no direct comparisons investigating concentration outcomes

Study design/ conditions[‡]	**Study**	**Intervention**	**Description**	**Micro-organism**[a]	**Log_{10} mean difference (95%-CI)**	**p-value**
BA/Comm	Bacon (2000b)	Pasteurisation treatment system B		ACC	-2.50 (-/2.82 - –2.18) log_{10} CFU/100 cm^2	<0.001
BA/Comm	Bacon (2000b)	Pasteurisation treatment system B		E. coli	-2.70 (-3.03- -2.37) log_{10} CFU/100 cm^2	<0.001
BA/Comm	Bosilevac(2006)	Lactic acid 2% wash	Pre-evisceration cabinet, 42°C	EBC	-1.00 (-1.03- - 0.97) log_{10} CFU/100 cm^2	<0.001
BA/Comm	Liu (2016)	Dry chilling		E. coli	-1.42 (-1.73- -1.11) log_{10} CFU/4,000 cm^2	<0.001
BA/Comm	Signorini (2018)	Hot water wash	82-87°C, 3-4 s, 1.5-3 bar, automated cabinet	E. coli	-0.60 (-0.83- -0.37) log_{10} CFU/400 cm^2	<0.001
BA/Comm	Corantin (2005)	Steam pasteurisation	74.5°C, 95 to 100 psi, 5 s	E. coli	-0.05 (-0.06- -0.04) log_{10} CFU/cm^2	<0.001
ChT/Comm	Scott (2015)	Hot water and lactic/ citric acid 1.9% wash	Post-evisceration cabinet, 92°C water, 51.7°C acid, 13-15 lb/in^2, 10 s	ACC	-3.90 (-4.19 - - 3.61) log_{10} CFU/cm^2	<0.001
ChT/Comm	Graves (1997)	Knife trimming and water wash	26°C, 276 kPa/11 s & 1000 kPa/12 s	ACC	-0.40 (-0.58 - - 0.22) log_{10} CFU/cm^2	<0.001
ChT/Comm	Scott (2015)	Hot water and lactic/ citric acid 1.9% wash	Post-evisceration cabinet, 92°C water, 51.7°C acid, 13-15 lb/in^2, 10 s	EBC	-3.70 (-4.01- - 3.40) log_{10} CFU/cm^2	<0.001
ChT/Comm	Scott (2015)	Hot water wash	92°C, 13-15 lb/in^2	EBC	-3.20 (-3.52- -2.88) log_{10} CFU/cm^2	<0.001
CT/RPP	Van Ba (2018)	Lactic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	ACC	-2.46 (-2.85- -2.07) log_{10} CFU/10cm^2	<0.001
CT/RPP	Van Ba (2018)	Lactic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	E. coli	-1.65 (-1.85- -1.45) log_{10} CFU/10cm^2	<0.001

Study design/ conditions‡	Study	Intervention	Description	Micro-organism[a]	Log_{10} mean difference (95%-CI)	p-value
CT/RPP	Van Ba (2018)	Lactic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	Salmonella	-1.24 (-1.28- -1.20) log_{10} CFU/10cm²	<0.001
CT/RPP	Van Ba (2018)	Acetic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	ACC	-1.73 (-2.13- -1.33) log_{10} CFU/10cm²	<0.001
CT/RPP	Van Ba (2018)	Acetic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	E. coli	-1.47 (-1.67- -1.27) log_{10} CFU/10cm²	<0.001
CT/RPP	Van Ba (2018)	Acetic acid 3% wash	Hide and final carcass spray (manual), then 24 h chill, 2°C	Salmonella	-0.60 (-0.64- -0.56) log_{10} CFU/10cm²	<0.001
ChT/RPP	Dorsa (1996)	Steam vacuuming	88-94°C, 7-10 psi, 12s	E. coli	-4.00 (-4.28- -3.72) log_{10} CFU/cm²	<0.001
ChT/RPP	Dorsa (1996)	Multiple (steam vacuuming, hot water wash and water wash)	88-94°C, 7-10 psi, 12 s / 72°C, 20 psi, 12 s / 30°C, 125 psi, 12 s	E. coli	-4.30 (-4.58- -4.02) log_{10} CFU/cm²	<0.001
ChT/RPP	Calicioglu (2002)	Dry chilling	4°C for 24 h	E. coli	-1.99 (-3.80- -0.18) log_{10} CFU/cm²	0.03
ChT/RPP	Calicioglu (2002)	Dry chilling	4°C for 72 h	E. coli	-2.15 (-3.35- -0.95) log_{10} CFU/cm²	<0.001
ChT/Lab	Yoder (2010)	Hot water wash	77°C, 30 psi, 20 s	Salmonella	-4.08 (-4.62- -3.54) log_{10} CFU/cm²	<0.001
ChT/Lab	Scott (2015)	Lactic acid and citric acid wash	1.9-2.5%, 43 to 60°C, 15 to 30 lb/in², 5s	Salmonella	-1.50 (-1.59- -1.41) log_{10} CFU/cm²	<0.001
ChT/Lab	Scott-Bullard (2017)	Sulfuric acid and sodium sulfate 1% wash	23°C, 13 lb/in², 5 s	E. coli	-0.8 (-.1.00- -0.60) log_{10} CFU/cm²	<0.001

Study design/ conditions[‡]	**Study**	**Intervention**	**Description**	**Micro-organism**[a]	**Log_{10} mean difference (95%-CI)**	**p-value**
ChT/Lab	Woerner (2017)	Peroxyacetic acid wash	200 ppm, 23°C, 1.38 bar	E. coli O157+six non-O157	-0.12(-0.48- 0.24) log_{10} CFU/cm^2	0.51
ChT/Lab	Woerner (2017)	Peroxyacetic acid wash	200 ppm, 23°C, 1.38 bar	E. coli	-0.22 (-0.58-0.14) log_{10} CFU/cm^2	0.23

[‡] CT-controlled trial; BA-before-and-after trial; ChT-challenge trial; Comm-commercial abattoir conditions; Lab-laboratory conditions; RPP-research/pilot plant conditions

[a] ACC-aerobic colony counts; EBC-*Enterobacteriaceae* counts

D4. Post- carcass fabrication interventions

D4.1. Interventions for beef primals, subprimals and trim

D4.1.1. Chemical interventions for beef trim

Figure 152. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of lactic acid dipping vs. water dipping in reducing E. coli O157:H7 and non-O157 numbers (log_{10} CFU) on beef trim. High heterogeneity, positive effect (MD -0.88, 95% CI: -1.26- -0.51 I^2=68.2%)

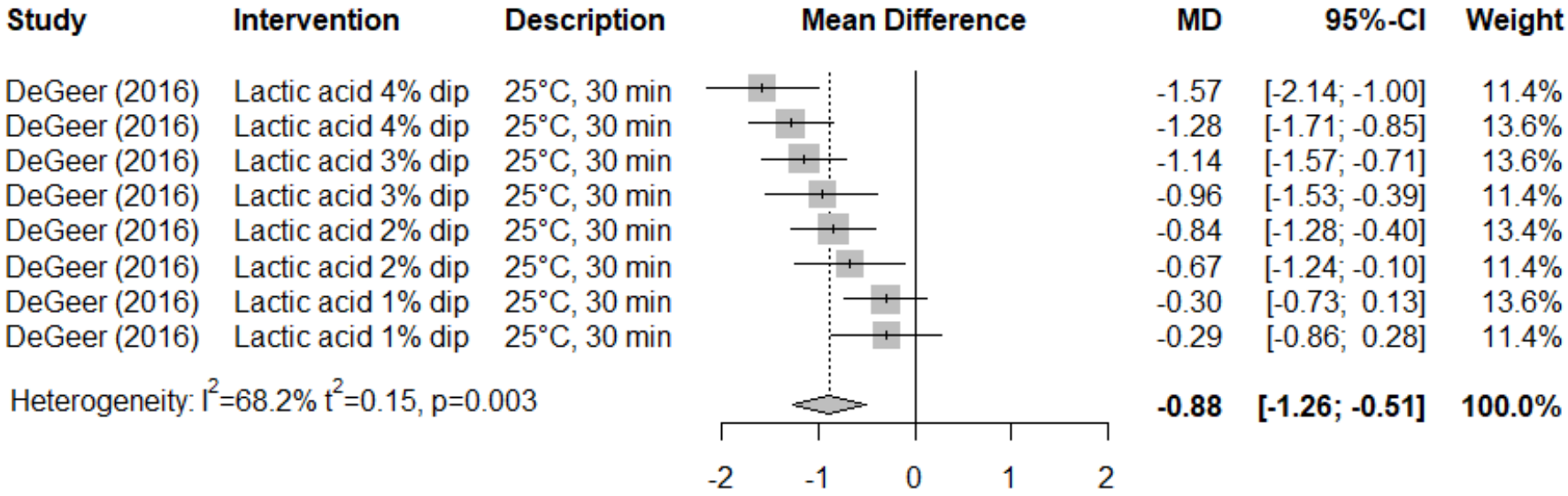


Figure 153. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of peroxyacetic acid dipping in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef trim. High heterogeneity, positive effect (MD -0.85, 95% CI: -1.12- -0.58 I^2=66.4%)

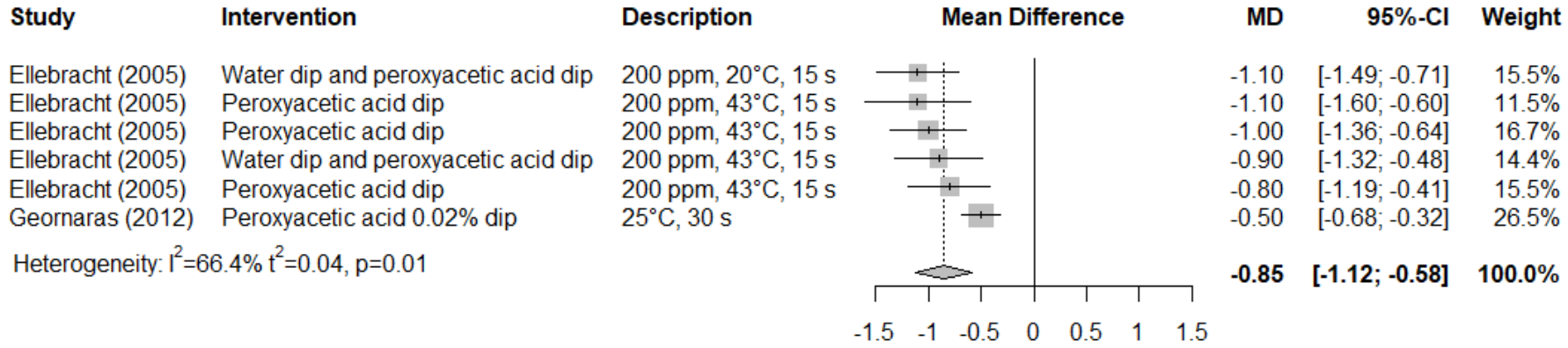


Figure 154. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of peroxyacetic acid dipping in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef trim. High heterogeneity, positive effect (MD -1.06, 95% CI: -1.49- -0.62 I^2=86.2%)

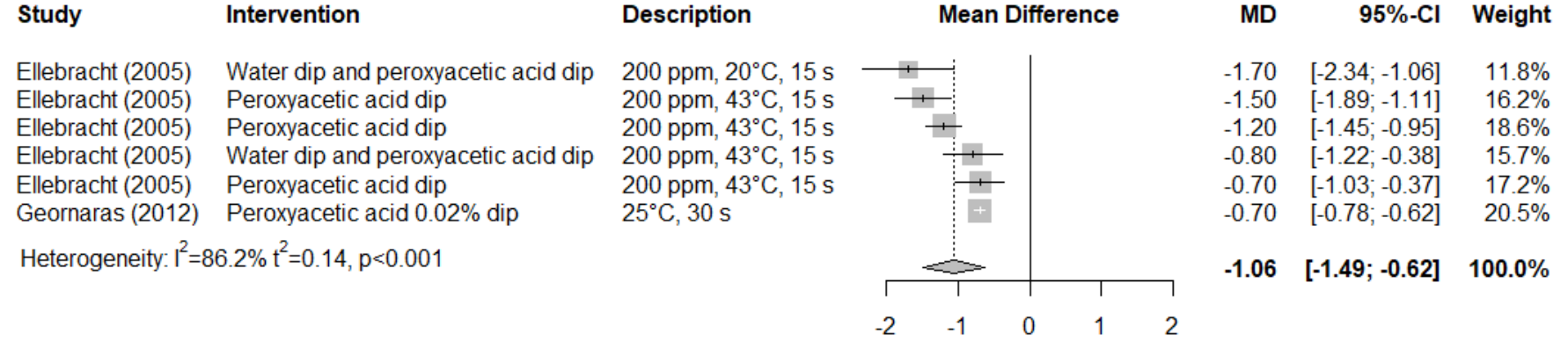


Figure 155. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of various chemicals dipping in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef trim. High heterogeneity, no effect (MD -1.42, 95% CI: -3.65- -0.81 I^2=96.2%)

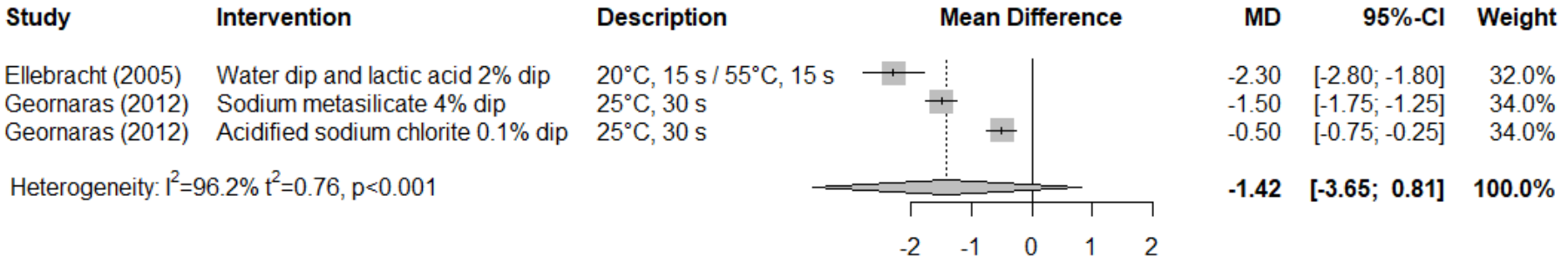


Figure 156. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of various chemicals dipping in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef trim. High heterogeneity, no effect (MD -1.12, 95% CI: -2.53- -0.30 I^2=98.0%)

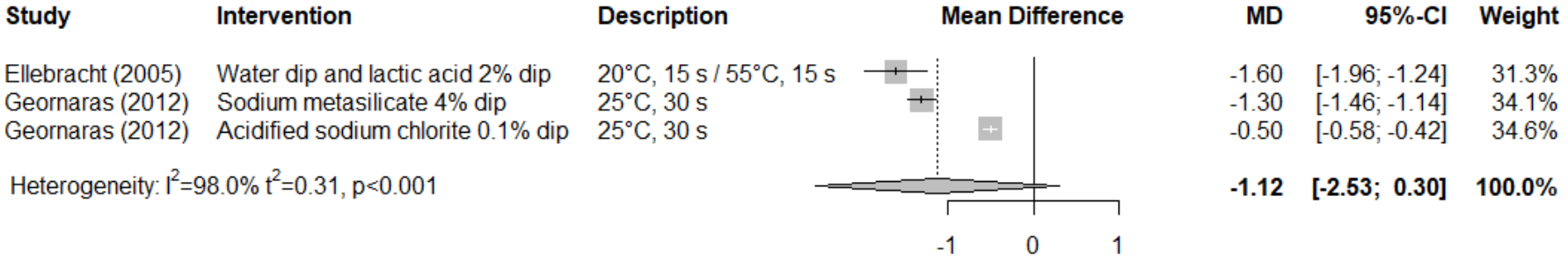


Figure 157. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of pasteurisation and/or lactic acid dipping in reducing *E. coli* O157:H7 numbers (log_{10} CFU) on beef trim

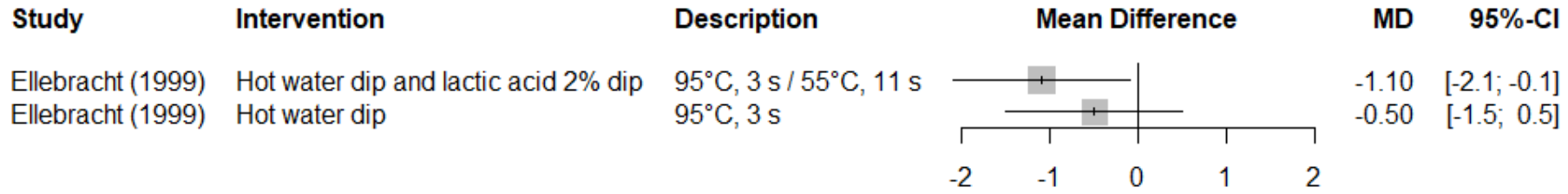


Figure 158. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of pasteurisation and/or lactic acid dipping in reducing *Salmonella* spp. numbers (log_{10} CFU) on beef trim

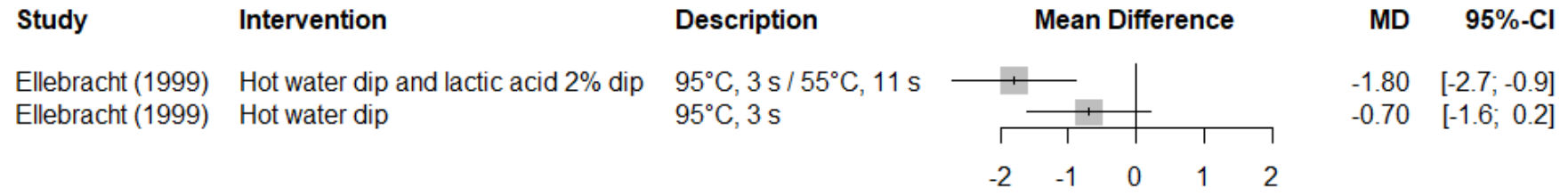


Figure 159. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of trisodium phosphate and cetylpyridinium chloride dipping in reducing generic *E. coli* counts (log_{10} CFU) on beef trim

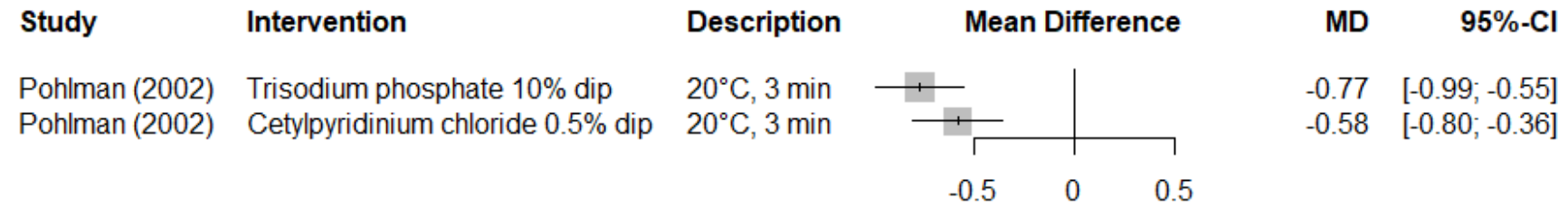
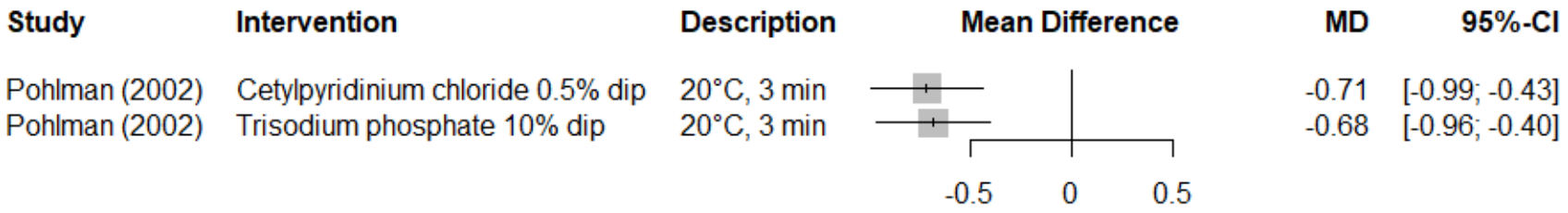


Figure 160. Forest plot of the results of challenge trials performed under laboratory conditions to investigate the efficacy of trisodium phosphate and cetylpyridinium chloride dipping in reducing Salmonella spp. numbers (log_{10} CFU) on beef trim



Appendix E: References for studies used in meta-analysis

The following 68 studies investigating beef interventions, judged to be at low risk-of-bias (*or some parts at low risk-of-bias), were used for further meta-analysis.

1. Algino, R. J., Ingham, S. C., & Zhu, J. (2007). Survey of antimicrobial effects of beef carcass intervention treatments in very small state-inspected slaughter plants. Journal of Food Science, 72(5), M173-M179.
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11. *Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., & Koohmaraie, M. (2005a). Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. Journal of food protection, 68(2), 265-272.
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Appendix G: Generic flow diagram of beef production processes for application of interventions

A generic flow diagram of the basic beef production processes is presented below. The steps are generic and the order may be varied in specific establishments. Intervention measures may be applied at one or multiple steps within the process flow.

The interventions are at the abattoir level (from receive and unload of animals to chilled carcasses) and post-abattoir level (further processing-storage-distribution of raw beef and packaging). Potential intervention measures for application at single or multiple points can be GHP- or hazard-based.

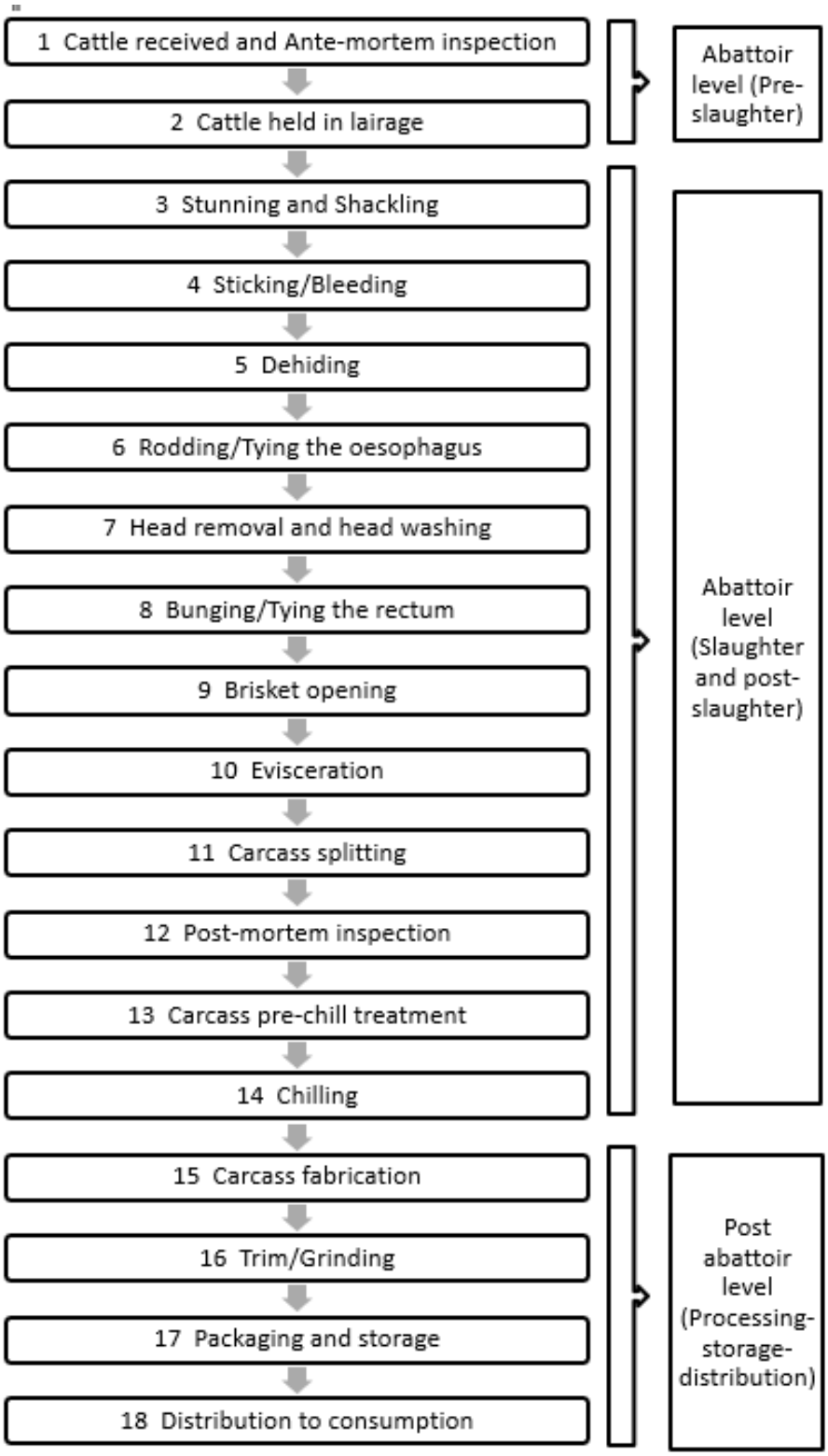
GHP-based measures are pre-requisites to hazard-based measures and are qualitative in nature and based on empirical knowledge and experience. Some examples of GHP-based control measures applied throughout slaughter and dressing process are: cleaning and disinfection of lairage-to-stunning areas, hide cleanliness assessment, bunging, rodding, hide removal methods, trimming, chilling, equipment and tools sanitation.

On the other hand, hazard-based intervention measures are developed from scientific research to specifically control certain hazards and are able to provide demonstrable and quantifiable reduction in bacterial load. Some examples of hazard-based intervention measures are:

i) at abattoir level for cattle hides pre- or post- exsanguination (ambient water washes, hide clipping, hide chemical decontamination and microbial immobilisation treatment of cattle hides with shellac) and carcass meat after dehiding but pre-chill (thermal washes such as hot water washes, steam vacuuming and steam pasteurisation; organic acid washes and other chemical solutions and oxidizers), during chilling (spray chilling with water or chemicals) and post-chill (carcass washes with chemicals); and

ii) at post-abattoir level for fabricated beef (large joints, small meat cuts, trimmings and minced meat): thermal (hot water) and chemical washes (organic acids and other chemicals), electron beam and gamma irradiation, ultraviolet (UV) light, use of bacteriophages, cold atmospheric plasma and high-pressure processing, modified packaging and preservation techniques (including active and bioactive packaging systems).

Generic flow diagram of beef production processes at abattoir and post abattoir level



Appendix H: List of interventions at abattoir and post abattoir level

Step 1: Cattle received and Ante-mortem inspection

The point where animals arrive at the abattoir. With the modern approach to meat inspection (to be risk based and orientated towards a whole meat chain), the animals should undergo categorisation into batches based on the risk they pose to public health. As a part of ante-mortem inspection, this is based on the analysis of Food Chain Information, hide cleanliness scoring and ante-mortem inspection per se. The batches assessed as posing a higher risk are expected to undergo additional interventions to reduce the risks and/or processed last.

GHP-based control measures

- Cleaning and disinfection of lairage-to-stunning areas;
- Hide cleanliness assessment and separation of excessively dirty animals.

Step 2: Cattle held in lairage

The point where the animals are held in lairage, shorter or longer, before slaughter. There is an increasing opportunity for cross-contamination between animals and animals and surfaces, particularly due to prolonged lairage time and/or increased stress. In this point, application of some pre-exsanguination, non-aggressive hide treatments of live cattle is possible.

GHP-based control measures

- Cleaning and disinfection of lairage-to-stunning areas;
- Lairage time kept to a minimum.

Hazard-based intervention measures

- Hide washing with ambient water;
- Hide clipping;
- Bacteriophage treatment applied to clean cattle.

Step 3: Stunning and Shackling

The point where animals are rendered unconscious. There is an increased possibility for hide cross-contamination due to cattle contact with contaminated floor in the stunning box and landing area.

GHP-based control measures

- Frequent cleaning of stunning box and area;
- Hygienic shackling to avoid contact between stick wounds (if sticking is performed in lying position) and contaminated areas.

Hazard-based intervention measures

- Some of the post- exsanguination hide treatments can/should be applied before sticking to avoid stick wound contamination.

Step 4: Sticking/Bleeding

The point where the animal is bled. There is a range of possible control measures for cattle hides at this point including post- exsanguination hide treatments. Some of these treatments have been investigated and trialled commercially but due to practical difficulties have not been used since.

GHP-based control measures

- Cleaning/scraping the hide surface area to remove dirt (if previous whole hide clipping is not performed) prior to sticking;
- Hygienic cut using two-knife system;
- Knife and tools cleaning and sanitation.

Hazard-based intervention measures

- Hide washing with ambient water;
- Hide clipping;
- Thermal interventions;
- Chemical dehairing;
- Organic acid washes;

- Oxidiser chemical washes;
- Other chemical washes;
- Microbial immobilisation treatment of cattle hides with ethanol or aqueous shellac.

Step 5: Dehiding

The point where the cattle hide is removed. Hide is the most significant source of microbial contamination for beef carcass and therefore there is a range of potential GHP- and hazard-based measures available for application at and after this step.

GHP-based control measures

- Using two-knife system with frequent changing knives;
- Knives, equipment and tools sanitation;
- Hide removal methods - mechanical hide pullers used in such way to pull hide away from the carcass (i.e. downward and backward motion).

Hazard-based intervention measures

A range of possible hazard-based pre-evisceration interventions for beef carcasses are available at this stage (particularly knife trimming, steam vacuuming, hot water and organic acid washes), but they may be also applied at other suitable stages (see step 13).

Step 6: Rodding/Tying the oesophagus

The oesophagus should be tied as soon as possible after stunning to prevent rumen spillage onto other carcass parts (including head).

GHP-based control measures

- The oesophagus should be tied to prevent rumen spillage;
- Equipment and tools sanitation.

Step 7: Head removal and head washing

Head is severed from the carcass in a hygienic manner.

GHP-based control measures

- Removing heads in a manner that avoids contamination with gut content;
- Adequate washing of heads but to limit splashing and contamination of cheek meat;
- Equipment and tools sanitation.

Step 8: Bunging/Tying the rectum

This is the process where a cut is made around the anus to free the rectum from the carcass and then it is tied off and/or bagged to prevent faecal spillage.

GHP-based control measures

- The rectum is tied and covered with plastic bag (bunging) to prevent faecal spillage;
- Equipment and tools sanitation.

Step 9: Brisket opening

GHP-based control measures

- Ensuring that the gastrointestinal tract is not ruptured;
- Equipment and tools sanitation.

Step 10: Evisceration

GHP-based control measures

- Knife trimming of potentially contaminated cut line before the cut is made;
- Ensuring that the gastrointestinal tract is not ruptured;
- Equipment and tools sanitation.

Step 11: Carcass splitting

GHP-based control measures

- Equipment and tools sanitation.

Step 12: Post-mortem inspection

Post-mortem inspection is the point where gross pathology is identified on carcasses, heads and offal, but at present is not an intervention measure to control microbiological contamination. There is, however, possibility for microbial cross-contamination of carcasses if inspection is not performed in a hygienic manner.

GHP-based control measures

- The procedure should be performed to avoid cross-contamination;
- Equipment and tools sanitation.

Step 13: Carcass pre-chill treatment

This step in the process is used to clean carcass before subjecting it to chilling. A range of possible hazard-based interventions are available at this stage, but they may be also applied at other suitable stages.

Hazard-based intervention measures

- Physical interventions aimed at removing microorganisms (knife trimming, spot steam vacuuming, ambient water washes);
- Thermal interventions (hot water washes, steam vacuuming, steam pasteurisation);
- Organic acid washes (acetic, citric, fumaric, lactic, levulinic, etc);
- Oxidiser chemical washes (electrolysed oxidised water, ozone, peroxyacetic acid, acidified sodium chlorate, hypobromous acid, chlorine dioxide, hydrogen peroxide);
- Other chemical washes (cetylpyridinium chloride, phosphoric acid, trisodium phosphate sodium metasilicate, etc);
- Other commercially available chemical formulations;
- Biological intervention measures (nisin, lactoferrin, bacteriophages).

Step 14: Chilling

After the completion of the carcass dressing on the slaughterline, carcasses enter the cold chain. The antibacterial activity of air chilling on beef carcasses is mainly based on the surface desiccation by high air velocity. Chilling also inhibits microbial growth.

GHP-based control measures

- Proper chilling conditions and parameters - carcass spacing, air flow, temperature and relative humidity.

Hazard-based intervention measures

- Spray chilling (with water or addition of lactic or acetic acid, CPC, ammonium hydroxide, ASC, TSP, peroxyacetic acid, sodium hydroxide or sodium hypochlorite)

Step 15: Carcass fabrication

This include cutting and deboning of the carcass meat which result in large primal joints and small meat cuts. A primal cut or cut of meat is a piece of meat initially separated from the carcass during fabrication. Examples of primals include the round, loin, rib, and chuck for beef. Each primal cut is then reduced into subprimal cuts. Individual portions derived from subprimal cuts are referred to as fabricated cuts.

GHP-based control measures

- Fat trimming;
- Temperature controls in boning and fabrication room;
- Timely flow of the products to avoid microbial growth;
- Equipment and tools sanitation (knives, saws, slicers and food contact surfaces) as frequently as necessary.

Hazard-based intervention measures

- Chemical washes (organic acids, peroxyacetic acid);
- Non-thermal interventions (electron beam (E-beam) irradiation).

Step 16: Trim/Grinding

During carcass fabrication, beef trim is generated and can be used for ground beef.

GHP-based control measures

- Temperature controls in boning and fabrication room;
- Sanitation of equipment, tools and food contact surfaces as frequently as necessary.

Hazard-based intervention measures

- Thermal interventions (hot water, steam, hot air)
- Non-thermal interventions (electron beam (E-beam) and ultraviolet (UV) light irradiation);
- Chemical washes (as in previous steps);
- Biological intervention measures (nisin, lactoferrin, bacteriophages).

Step 17: Packaging and storage

Packaging protects finished products from contamination post-processing. Packaging-based interventions include modifying the package environment (modified atmosphere, vacuum packaging), the addition of microbial inhibitors, such as chemicals, biological extracts and lactic acid bacteria, and the application of non-thermal technologies (irradiation is typically applied at the packaging step but it could also be applied earlier at post-fabrication).

GHP-based control measures

- Temperature controls in packaging room.

Hazard-based intervention measures

- Non-thermal interventions (electron beam (E-beam) and gamma irradiation, ultraviolet (UV) light irradiation, cold atmospheric plasma, high-pressure processing);
- Modified packaging (modified atmosphere packaging, vacuum packaging);
- Preservation and biopreservation (including active and bioactive packaging systems).

Step 18: Distribution to consumption

The main GHP-based control measure here is strict maintenance of the cold chain.