



Systematic Review

Effect of Critical Processing Stages and Interventions on *Campylobacter* in Poultry Meat at the Slaughterhouse Level: A Comprehensive Meta-Analysis

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Abstract

Campylobacter spp. are a major foodborne zoonosis, and poultry slaughterhouses are One Health interfaces where animal carriage, environmental reservoirs and worker exposure intersect. We performed a comprehensive systematic review and meta-regression to quantify how slaughter stages and in-plant interventions affect *Campylobacter* concentration and prevalence on poultry carcasses and meat. The Scopus literature search engine was accessed to collect articles that focused on observational studies and challenge tests, reporting results on *Campylobacter* concentrations or prevalence in both pre-stage/non-intervened and post-stage/intervened groups. From a total of 4080 studies initially retrieved, 71 were eligible for inclusion in the meta-analysis, yielding 1256 observations. Meta-regression models were adjusted according to the type of outcome measure: log₁₀ reduction for concentration outcomes and ln risk ratio (ln RR) for prevalence outcomes. Meta-analysis showed reductions in *Campylobacter* counts after scalding (0.898 log₁₀ reduction on neck skin $p < 0.001$), carcass rinsing with water (0.523 log₁₀, $p = 0.043$), and after sanitisation combined with chilling (0.692 log₁₀, $p < 0.001$). Although significant, chilling was found to slightly decrease *Campylobacter* prevalence by 8% (95% CI: 1–17%; ln RR = 0.082, $p = 0.032$). Intervention strategies reducing *Campylobacter* concentrations included processing in colder climates (autumn and winter) (1.927 log₁₀, $p = 0.005$), application of plant extracts (1.493 log₁₀, $p = 0.008$), use of organic acids (1.192 log₁₀, $p < 0.001$) and chemical carcass sanitisation (1.031 log₁₀, $p < 0.001$). Organic acids also reduced the prevalence of *Campylobacter* in poultry carcass slaughter groups (ln RR = 1.079, $p = 0.009$), whereas freezing did not reach statistical significance (0.740 log₁₀, $p = 0.288$). Environmental synthesis highlighted high pooled prevalence in transport crates (0.833, 95% CI 0.765–0.885) and among operators (0.732, 95% CI 0.404–0.917), supporting their role as reservoirs and vectors. Meta-regressions demonstrated that the measured effectiveness of slaughter stages and intervention strategies were driven by key moderators, namely, sample weight, type of sample, type of chemical/organic acid/extract and its concentration, mode of application and storage time. Despite substantial heterogeneity and small study effects for concentration, the evidence supports the implementation of a multi-barrier One Health strategy combining the control of incoming contamination, hygiene of equipment and personnel, and optimised rinsing/sanitisation and chilling to reduce consumer risks.

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1. Introduction

Campylobacteriosis is recognised as the leading cause of diarrheal diseases worldwide. Indeed, in 2010, of the 600 million cases of illness caused by 31 foodborne infectious agents, the majority—550 million cases—were due to diarrheal diseases. Among these, *Campylobacter* species were responsible for 96 million cases [1].

Fresh meat from chicken and other poultry species is considered the primary source of human campylobacteriosis worldwide [2–4]. This is supported by studies using multi-locus sequence typing and whole-genome sequencing, which have shown that poultry is the primary source of human *Campylobacter* infections across various geographical contexts. The findings of these studies provide a strong case for interventions targeting controls at the slaughter line, while recognizing that transmission can occur at various stages. [5–7]. This occurs due to cross-contamination, improper handling, and subsequent consumption of contaminated meat [2,8]. The EFSA (2011) suggested that reducing the number of *Campylobacter* in the intestines at slaughter by 3 log₁₀ units could reduce the risk to public health by at least 90% [9]. Therefore, numerous risk assessment studies indicate that, while challenging, understanding the dynamics of poultry processing stages in slaughterhouses is crucial for identifying key risk factors and preventing cross-contamination [10,11]. Additionally, understanding how each stage of the processing line affects *Campylobacter* levels can help reduce the incidence of campylobacteriosis in humans [11].

In practice, worldwide poultry slaughterhouses are designed with nine processing stages: stunning, bleeding, scalding, plucking or defeathering, evisceration, washing, chilling, portioning and packaging, as well as refrigeration [4,12,13]. During processing, the prevalence or concentration of *Campylobacter* changes across stages. For instance, scalding helps reduce *Campylobacter* contamination by applying high temperatures that inactivate bacteria on carcasses and wash away contaminants with water [12]. Meanwhile, plucking and evisceration are recognised as the most critical stages of the slaughter line, as they are associated with elevated levels of *Campylobacter* [12,14,15]. This is because, during the plucking or defeathering stage, cross-contamination of carcasses occurs due to the mechanical action of the machines and the tendency of microbial contaminants to disperse in all directions through aerosols [4]. At the same time, since evisceration involves removing internal organs from the carcass, it may occasionally occur that faecal contaminate the carcasses if the intestines of colonised birds rupture [14]. Furthermore, the microbial load and prevalence continue to decrease in subsequent processing stages [14,16]; moreover, during chilling, which is assumed to be the final stage of the slaughter process, contamination levels in carcasses decrease significantly, both in terms of microbial load and prevalence [12,14].

Due to the proximity of carcasses on the processing line, there is a likelihood of cross-contamination at all stages through aerosols, processing water, contaminated surfaces and equipment, as well as the hands of factory workers [4,17]. Therefore, to minimise the risk of contamination during processing in slaughterhouses, it is necessary to implement specific management strategies. These interventions should be based, firstly, on compliance with good hygiene practices, aimed at reducing faecal contamination during the slaughter process; and, secondly, on decontamination, aimed at reducing the number of *Campylobacter* through physical, chemical, and biological treatment [13,18–20]. Physical treatments include water-based treatments, air chilling, and freezing, while chemical treatments in-

volve adding organic acids, chlorine-based solutions, phosphate-based compounds, sulphate-based compounds, and others to the water [20]. Biological intervention consists of applying treatments such as bacteriophages, plant extracts, essential oils, and their derivative compounds to the carcass, neck skin, and other parts of the poultry [21–24].

Despite multiple review studies and meta-analyses have addressed the impact of *Campylobacter* in poultry slaughterhouses [25–28], few have conducted a quantitative synthesis that encompasses the effectiveness of processing steps, carcass interventions, and environmental risk factors that may contribute to the spread of this pathogen along the processing line. Therefore, this study aims to conduct a comprehensive systematic review and meta-analysis to (i) identify the critical processing steps involved in poultry slaughter and their effectiveness in reducing *Campylobacter* levels; (ii) determine the effectiveness of currently-implemented and/or proposed intervention strategies in lowering *Campylobacter* levels in poultry carcasses; and (iii) identify environmental risk factors that contribute to the spread of *Campylobacter* in poultry slaughterhouses.

2. Materials and Methods

2.1. Study Design and Research Question

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [29]. The research question was formulated using the Population, Intervention, Comparator, and Outcome (PICO) framework [30]. As in the meta-analysis conducted by Zefanias et al. [31], the population consisted of poultry (chickens, turkeys, and ducks at any stage of slaughter and processing. The interventions or treatments included processing stages and intervention strategies implemented in slaughterhouses. The comparator refers to the control group, understood as the pre-stage group when a processing stage is evaluated; or the non-intervened group when an intervention strategy is assessed. The measured outcomes are the concentration or prevalence of *Campylobacter* on poultry carcasses or poultry meat in the pre-stage/non-intervened group (control) and the post-stage/intervened (treated) group. Occurrence of *Campylobacter* in environmental samples of the slaughterhouse was also regarded as a measured outcome, although that specific meta-analysis (environmental elements) was of PO (Population, Outcome) type.

2.2. Literature Search Strategy

A targeted literature search was performed in July 2024 using the Scopus electronic database to identify original articles published from 1995 onwards. Searches were carried out in the title, abstract and keywords fields. Bibliographic searches combined terms related to *Campylobacter* infection or colonisation in poultry, on-farm interventions, control measures, processing stages, treatments or risk factors, connected by the logical operators “AND” and “OR”.

The following search string was employed:

((prevalence) OR (“risk factor”) OR (risk) OR (intervention*) OR (strateg*) OR (“observational study”) OR (“cohort”) OR (“longitudinal study”) OR (cross-sectional) OR (“cross sectional”) OR (farm*) OR (source*) OR (colonization) OR (colonisation) OR (“control measure”) OR (“control measures”) OR (husbandry) OR (management) OR (“free range”) OR (free-range) OR (slaughter*) OR (abattoir))

AND

((“avian campylobacteriosis”) OR (“campylobacter infection”) OR (campylobacter) OR (campylobacteriosis))

AND

((poultry OR (broiler) OR (hen*) OR (chicken*) OR (chick*) OR (duck) OR (turkey) OR (bird*) OR flock*)).

2.3. Eligibility and Exclusion Criteria

All papers retrieved from Scopus were imported into Rayyan software [32] for screening. Since only one database was used, no duplicate records were identified. Two researchers separately reviewed titles and abstracts of the studies using the pre-defined eligibility criteria. Studies were included if they met all of the following conditions: (i) written in English, Spanish or Portuguese; (ii) primary and original study; (iii) conducted in live (pre-slaughter) chickens, turkeys or ducks, their carcasses or meat produced at slaughterhouses anywhere in the world; (iv) reported on the effect of processing stages, interventions or treatments aimed at controlling *Campylobacter* at the slaughterhouse level (v) provided data on *Campylobacter* concentration and/or prevalence in both control and treated groups. In this study, the initial stage of the processing line was designated as the control group, while the subsequent phase was designated as the treatment group.

No restrictions were applied regarding type of intervention. Studies were excluded if: (i) no processing stage or intervention to control *Campylobacter* or no explicit environmental risk factor was evaluated; (ii) no sufficient data were provided to calculate the effect size for *Campylobacter* concentration or prevalence; or (iii) the article was a review, systematic review or meta-analysis.

In the present systematic review, only papers deemed “not potentially biased” were included. This means that articles that were suspicious of having any bias were excluded; namely, selection bias (i.e., not random samples such as infected chickens), methodological bias (i.e., methods not duly explained or at least referenced), detection bias (e.g., not stating the sample weight analyzed) and reporting quality (e.g., discrepancies in results between text and tables). Such bias types were defined according to Cochrane reviews’ recommendations for assessing risk of bias in a randomised trial [33]. As a consequence, there was no need to assess the effect of bias risk on the effect size or to conduct sensitivity analyses, since these papers were not included in the meta-analysis in the first place.

2.4. Data Extraction

After the preliminary screening, full texts of eligible articles were retrieved, and relevant data were extracted manually into a structured Microsoft Excel spreadsheet. Data extraction was performed by one reviewer and independently verified by other two reviewers. The following information was recorded for each study:

- (a) Study identification: reference code and full citation.
- (b) Country: country in which the study was conducted.
- (c) Bacterial species: *Campylobacter* species investigated.
- (d) Bird species carcass/meat: chickens, turkeys or ducks.
- (e) Processing stage: refers to bleeding, scalding, plucking, evisceration, washing, chilling, packaging, or any other stage reported in the study.
- (f) Intervention: refers to the intervention strategy or treatment adopted to control *Campylobacter* in poultry, such as use chemical sanitisers, organic acids, plant extracts, freezing, or any other intervention under investigation in the study. Colder climates are defined as processing in autumn and winter, and were included as a risk factor.
- (g) Type of treatment application: it refers to how the intervention/treatment was applied, for instance, whether immersion or spraying in the case of chemical sanitisation of carcasses.
- (h) Treatment duration: it refers to contact timing (in minutes) between chemical sanitisers and carcasses at the point of evisceration or rinsing.

- (i) Treatment concentration: it is the concentration of chemical sanitiser, organic acid or plant extract applied in the treatment (g/L or g/kg).
- (j) Temperature: refers to the temperature of water or sanitizing solutions used in stages such as scalding, plucking, evisceration or sanitizing.
- (k) Storage time: it refers to the time (days) the meat treated with plant extract or organic acids is cold stored before analysis.
- (l) Inoculum size: inoculum concentration in specific cases where studies reported results in which carcasses were artificially contaminated with *Campylobacter* strains.
- (m) Sample type: refers to the type of sample used for microbiological analysis; i.e., feathers, neck skin, breast, cloaca, carcass rinse water, scalding water, wings and drumsticks.
- (n) Environmental source: refers to one-group samples taken from the slaughterhouse environment, such as: live animals (cloaca), scalding tank, plucker, eviscerator, chill tank, operator hands/gloves, caecal content, and other miscellaneous.
- (o) Sample weight or volume: employed for *Campylobacter* analysis either for detection or enumeration.
- (p) Count results: consisting of sample size (total number of samples), measures of central tendency (mean and/or median concentration in CFU/g, CFU/mL or CFU/carcass) and standard deviation for both control and treated groups. When the standard error of the mean was reported, the sample standard deviation was derived accordingly.
- (q) Prevalence results: consisting of the sample size and the number of positive samples for both control and treated groups.

Whenever results were only presented graphically, numerical data were extracted using Plot Digitizer Software version 5.2 [34].

2.5. Meta Regression Models

The meta-analysis study considered three main effect sizes depending on the type of outcome variable: (i) an effect size measured as the $\log_{10}$ reduction attained by a processing stage or intervention using *Campylobacter* counts data; (ii) an effect size measured as the log risk ratio of a processing stage or intervention using *Campylobacter* prevalence data; and (iii) an effect size measured as the logit proportion [35] of the presence of *Campylobacter* in environmental elements from poultry slaughterhouses.

For concentration outcomes, each effect size was calculated as $\log_{10}$ reduction, that is, the logarithm base 10 of the ratio of the concentrations in the control and treated groups:

$$y_i = \log_{10} \frac{C_{control\ i}}{C_{treated\ i}}$$

where $C_{control\ i}$ and $C_{treated\ i}$ are the mean *Campylobacter* concentrations (CFU/g, CFU/mL, CFU/cm² or CFU/carcass) in the study i for the control and treated groups, respectively. Positive values of y_i indicate a reduction in concentration in the treated group, whereas negative values indicate increase in concentration in the treated group. The effect size variance, $V(y_i)$, for each study i was estimated as

$$V(y_i) = \frac{\sigma_{control\ i}^2}{n_{control\ i}} + \frac{\sigma_{treated\ i}^2}{n_{treated\ i}}$$

where $\sigma_{control\ i}$ and $\sigma_{treated\ i}$ are the standard deviations of the $\log_{10}$ concentrations of *Campylobacter* in the control and treated group, respectively; and $n_{control\ i}$ and $n_{treated\ i}$ are the number of samples analyzed in the control and treated groups, respectively. Most of the times, $n_{control\ i}$ and $n_{treated\ i}$ were the same.

Three types of random-effects meta-regression models were fitted to the $\log_{10}$ reduction effect size data: (i) a meta-regression model utilizing the complete dataset, placing processing stage/intervention as moderator, in order to summarise the pooled mean $\log_{10}$ reduction in *Campylobacter* load attained by the different stages and interventions; (ii) univariate meta-regressions separately fitted by processing stage/intervention, in which quantitative moderators were entered to the model one at a time to explore their association with the $\log_{10}$ reduction. The moderators included sample weight, duration of treatment, concentration applied in the treatment, temperature, storage time, and inoculum of *Campylobacter* strain in case of challenge tests; and (iii) multivariable meta-regressions separately fitted by processing stage/intervention, in which several moderators were now included simultaneously to determine the most important ones driving the reductions in *Campylobacter* load. It is worth mentioning that sample weight is not regarded as a moderator that could drive intervention effectiveness, but a covariate that could reflect methodological artifacts. For instance, low sample weights may not have sufficient detection sensitivity to point out significant effectiveness of an intervention.

For prevalence outcomes, the effect size y_i comparing post-stage/intervened (treated) group with pre-stage/non-intervened (control) group was expressed as the natural logarithm of the risk ratio (RR), defined as

$$y_i = \ln RR_i = \ln \frac{p_{control\ i}}{p_{treated\ i}}$$

where $p_{control\ i}$ and $p_{treated\ i}$ are the proportions of *Campylobacter*-positive samples in the control and treated groups, respectively, for study i . Values of y_i greater than zero indicate a reduction in prevalence in the treated group relative to the control. The effect size variance, $V(y_i)$, for each study i was estimated as

$$V(y_i) = \frac{n_{control\ i} - s_{control\ i}}{n_{control\ i}s_{control\ i}} + \frac{n_{treated\ i} - s_{treated\ i}}{n_{treated\ i}s_{treated\ i}}$$

where $n_{control\ i}$ and $n_{treated\ i}$ are the number of analyzed samples in the control and treated group, respectively; and $s_{control\ i}$ and $s_{treated\ i}$ are the number of *Campylobacter*-positive samples in the control and treated group, respectively.

The continuity correction used to prevent mathematical errors of dividing by zero or taking the logarithm of zero, was the Haldane–Anscombe correction, which consists of adding 0.5 to the each of the four cells in the 2×2 contingency table for any study that contains at least one zero.

Two types of random-effects meta-regression models were fitted to the log risk ratio effect size data: (i) an overall meta-regression model utilising the complete dataset, placing processing stage/intervention as moderator, in order to summarise the pooled log reduction in *Campylobacter* prevalence attained by the different treatments; and (ii) multivariable meta-regressions separately fitted by processing stage/intervention, in which several moderators were included simultaneously to determine the most important ones affecting the reductions in *Campylobacter* prevalence.

For single-group prevalence outcomes relative to live animals and environmental sources (i.e., entrance, scalding and plucking area, evisceration area, chilling room), the effect size y_i was defined as the logit transformation of the proportion of positive samples (p_i) for each source i ,

$$y_i = \ln \frac{p_i}{(1 - p_i)}$$

The effect size variance, $V(y_i)$, for each study i was estimated as

$$V(y_i) = \frac{1}{s_i} + \frac{1}{n_i - s_i}$$

where n_i is the total number of samples taken, and s_i is the number of *Campylobacter*-positive samples. For this type of data, one single meta-regression model was fitted to the logit proportions, placing source as moderator, in order to summarise the overall *Campylobacter* prevalence in live birds and environmental sources. In all meta-regressions, effect sizes were weighted by the inverse of their sampling variance [36,37].

For the three types of effect sizes described above, random-effects meta-regression models had the general form:

$$y_{ij} = \beta_0 + \sum_k (\beta_k \times x_{kij}) + u_i + \varepsilon_{ij}$$

where y_{ij} is the effect size for observation j belonging to study i , x_{kij} are qualitative or quantitative moderator variables, β_k are fixed-effect coefficients, u_i is the study-level random effect with variance τ^2 , and ε_{ij} is the within-study sampling error. In all meta-regressions—univariate and multilevel, the clustering variable was the primary study, and the random effects were placed only in the intercept (β_0). An unstructured covariance matrix was used [37]. All statistical analyses were performed using RStudio (version 2025.5.0; Posit Software, PBC) [38], using the metafor package for all meta-regressions and graphs [39] and sqldf for data manipulation [40].

2.6. Assessment of Heterogeneity, Accuracy and Consistency, and Publication Bias

As performed in Zefanias et al. [31], heterogeneity analysis was carried out in every prevalence and concentration meta-regression. To quantify the impact of this between-study variability, we used the I^2 statistic, which expresses the percentage of total variability in observed effect sizes that is attributable to heterogeneity rather than chance [28,32,33]. From a null random-effects model, the within-study variance (s^2) and the between-study variance (τ^2) were obtained, so that the I^2 statistic could be calculated as

$$I^2 = \frac{\tau^2}{(\tau^2 + s^2)} \times 100\%$$

A value of 0% can be interpreted as no observed heterogeneity, whereas values of ~25%, 50% and 75% are interpreted as low, moderate and high heterogeneity, respectively [41,42]. After fitting meta-regression models with moderators, the residual between-study variance (τ_{res}^2) could be obtained, which allowed for the calculation of R^2 , defined as the proportion of between-study variability explained by the set of moderators:

$$R^2 = \frac{(\tau^2 - \tau_{res}^2)}{\tau^2}$$

The accuracy and consistency of the study results were explored using Galbraith (radial) plots [43,44]. In these plots, the standardised effect size z_i for each observation i ($z_i = y_i/SE_i$) is plotted on the y-axis against precision ($1/SE_i$) on the x-axis (SE: standard error). Under a homogeneous random-effects model, points are expected to fall within approximate 95% reference bands around the regression line. Deviations from this pattern and points lying far outside the reference bands are used to visually assess heterogeneity; and to identify potential outliers.

Uncertainty around pooled effects and moderator coefficients was summarised through the standard errors reported in the tables, associated p -values, and the confidence intervals linked to these statistics. Galbraith plots provided a complementary graphical assessment of consistency and possible influential observations and outliers.

Publication bias [45] were evaluated using two approaches. First, by observing the level of asymmetry of funnel plots which points towards publication bias or other small study effects [45,46]. Secondly, by including the total sample size as a moderator in meta-regression models [47]. A statistically significant sample size ($p < 0.05$) is interpreted as

evidence of small study effects that could be compatible with publication bias and was considered alongside the funnel plot patterns.

3. Results

3.1. Description of the Meta-Analytical Data

The article selection process for this meta-analysis is compiled in the PRISMA flow diagram (Figure 1). A total of 4080 articles were retrieved from the Scopus database. After screening titles, abstracts and keywords according to the predefined inclusion and exclusion criteria, seventy-four articles were retained for full-text assessment, and 4006 records were excluded. Data was extracted from the 74 selected papers following complete reading. Of these, seventy-one articles met the criteria for inclusion in the meta-analysis, whereas the other 3 studies were excluded because effect sizes could not be reliably derived or other methodological concerns, including suspected bias. In total, 1256 effect size observations were extracted and were available for analysis. Although the review covered chickens, turkeys, and ducks, most intervention studies were conducted in chickens, which accounted for 94% of the data.

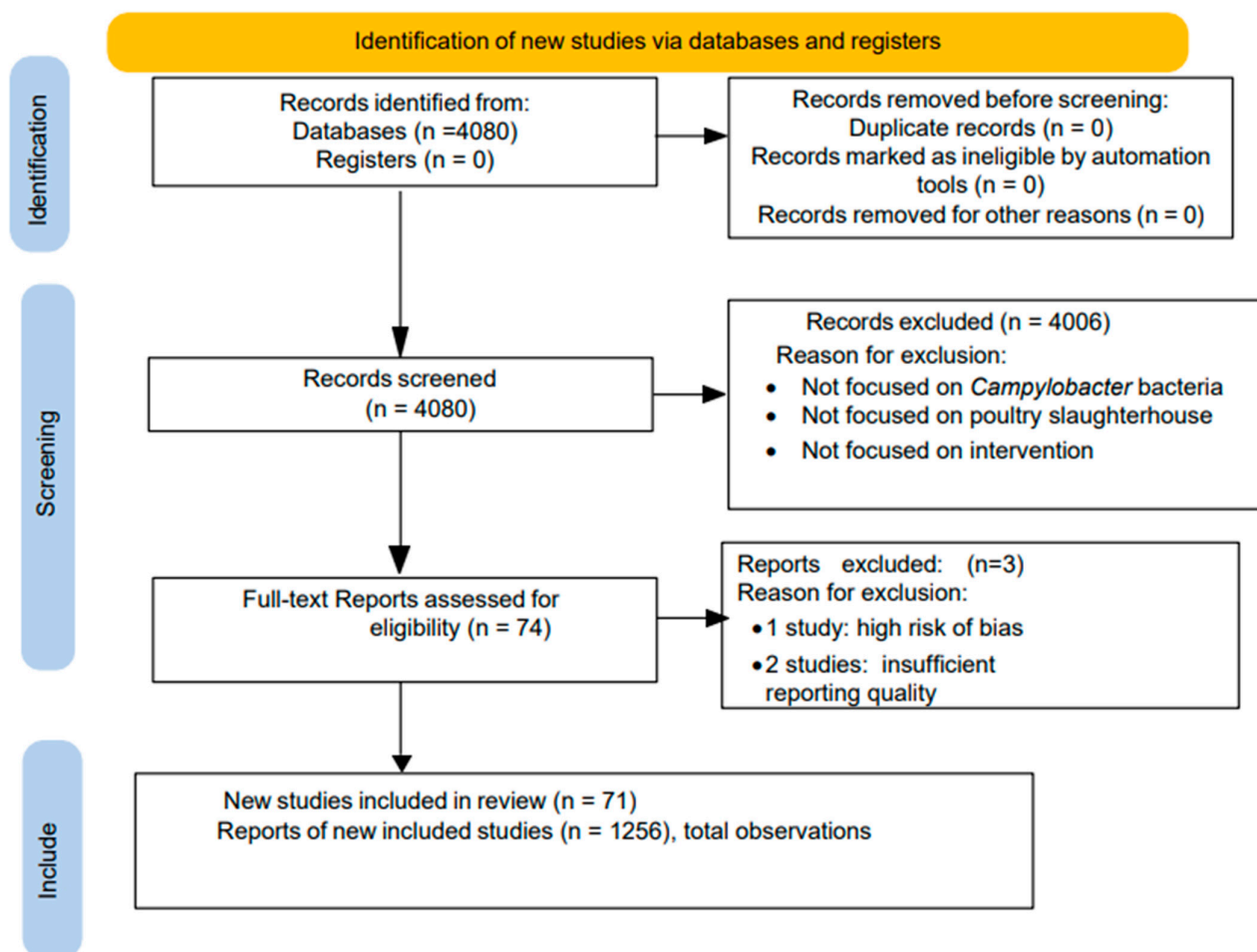


Figure 1. PRISMA chart describing the steps involved in selecting studies that assess the effect of slaughter-level processing stages or intervention strategies on the occurrence of *Campylobacter* in poultry carcasses and meat.

Campylobacter concentration data related to processing stages consisted of 435 observations taken from 41 studies [48–88]; whereas the concentration data on interventions comprised 431 observations extracted from 26 papers [49,55,66,68,71,72,79,88–105]. For prevalence, the *Campylobacter* data relative to processing stages consisted of 290 observations from 41 studies [48,54,57–60,62,64–66,68,71,73,74,76–83,86,87,89,91,106–120]; while those of interventions comprised 9 studies with 27 observations [68,71,79,86,94,100–102,110]. Finally, the meta-analysis of processing sources included 25 studies with 73 observations [49,59,64,68,71,78,82,86,87,108,112,114,116,118]. Some studies contributed data to more than one of these outcome categories.

3.2. Overall Effects of Processing Stages and Interventions on the Concentration of *Campylobacter* in Poultry

To estimate the overall effects of processing stages and interventions on *Campylobacter* concentration in poultry, meta-analyses were adjusted using the full dataset. Pooled effect sizes of $\log_{10}$ reduction are presented in Table 1.

For each processing stage and intervention, the meta-regression model also estimated the standard error and p -value, which are useful for comparing the effectiveness of the processing stages and interventions. Nonetheless, non-significance can also arise from lack of agreement (i.e., high heterogeneity) between studies.

Considering the results for the processing stages (Table 1), neck cutting and bleeding, as well as the evisceration stages yielded mean negative estimates (-0.315 ± 0.400 ; -0.110 ± 0.195 , respectively) although non-statistically significant. This indicates that in some studies, these stages contributed to an increase in *Campylobacter* concentrations. In contrast, the sanitisation/chilling and carcass rinsing stages showed a positive, highly significant effect ($p < 0.05$), indicating a mean decline in *Campylobacter* microbial loads of ~ 0.7 and $\sim 0.5 \log_{10}$, respectively.

Moreover, although the scalding, plucking, and routinary cleaning/disinfection were not statistically significant ($p > 0.1$), they still showed a positive effect. A notable effect was observed at the packaging stage, which showed the highest reduction in *Campylobacter* concentrations (0.966 ± 0.570 ; $p = 0.088$).

In general, the intervention strategies attained greater mean $\log_{10}$ reductions than the critical processing stages. Positive and highly significant effect ($p < 0.05$) were the mean *Campylobacter* reductions achieved by the application of plant extracts, organic acids, and chemical sanitisation, and colder climates. Interestingly, slaughtering poultry in autumn and winter (as opposed to spring and summer) yielded the highest reduction in *Campylobacter* concentrations, achieving a mean $\log_{10}$ of 1.927 ± 0.695 ($p = 0.005$). By contrary, chemical sanitisation showed the lowest mean $\log_{10}$ reduction of 1.031 ± 0.278 ($p < 0.001$). Although freezing showed a positive effect (0.740 ± 0.697 , $p = 0.288$), it did not reach statistical significance ($p > 0.05$) due to the few observations ($n = 4$) available for pooled effect estimation.

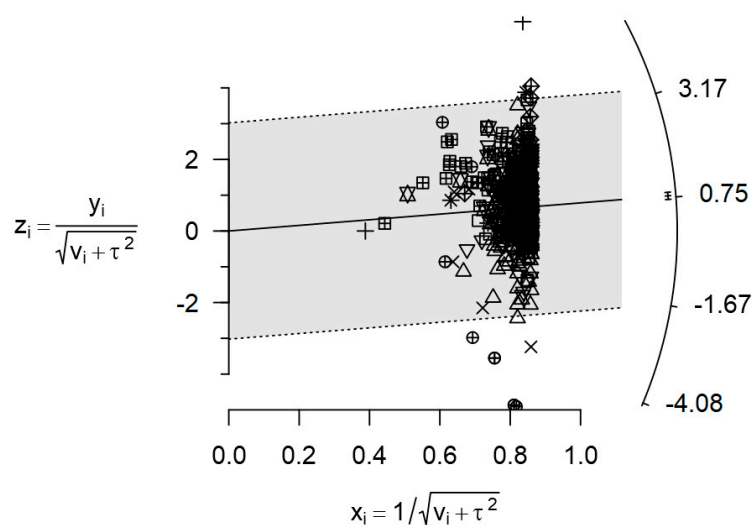
Regarding heterogeneity, the overall meta-analysis revealed an intraclass correlation (I^2) of 49%, which Higgins and Thompson would classify as moderate [41]. This value suggests that most of the variability between studies is due to heterogeneity and not mere chance [34]. Furthermore, a fraction of 27% of this heterogeneity (R^2) could be explained by the stages/interventions. Nevertheless, there are still some unexplained residual heterogeneity (Q-test for residual heterogeneity, $p < 0.001$).

The level of heterogeneity could also be inferred from the six-sigma radial plot (Figure 2, top), where, although most of the standardised effect sizes were concentrated in the range $x_i = <0.6–0.9>$, reflecting high precision and a greater contribution to the joint effect.

Table 1. Mean effects of the slaughter processing stages and interventions on the concentration of *Campylobacter* in poultry, showing heterogeneity analysis performed on the overall meta-regression model.

Stages and Interventions	Log ₁₀ Reduction (Control Group/Treated Group)			Heterogeneity Analysis
	Estimate [SE]	p-Value	n/N	
<i>Stages</i>				
Neck cutting and bleeding	−0.315 [0.400]	0.439	19/6	I ² = 48.7% p(Q) < 0.001 R ² = 26.2% τ _{Tres} ² = 0.9777
Scalding	0.403 [0.329]	0.221	25/9	
Plucking	0.330 [0.239]	0.163	55/18	
Evisceration	−0.110 [0.195]	0.583	138/25	
Carcass rinsing with water	0.523 [0.258]	0.043	35/12	
Sanitisation and chilling	0.692 [0.176]	<0.001	137/30	
Packaging and cold storage	0.966 [0.570]	0.088	22/3	
Routinary cleaning/disinfection	0.905 [0.585]	0.118	4/3	
<i>Interventions</i>				
Colder climate	1.927 [0.695]	0.005	14/2	Pub.bias p < 0.001
Freezing	0.740 [0.697]	0.288	4/2	
Plant extracts (carcasses/meat cuts)	1.493 [0.563]	0.008	196/3	
Organic acids (carcasses/meat cuts)	1.192 [0.272]	<0.001	144/13	
Chemical sanitisation of carcasses	1.031 [0.278]	<0.001	73/13	

Furthermore, the Galbraith plot suggests the presence of few outliers and overall consistency between the effects, given that the distribution of z_i values is mainly within the limits of $<2; +2>$. In relation to publication bias, the funnel plot (Figure 2, bottom) reveals certain asymmetry, whereas the p -value (<0.001 in Table 1) confirms the presence of strong study size effects on the pooled estimates. These results suggest that it is very likely that studies that failed to prove the efficiency of an intervention strategy or a critical stage remained unpublished, however, this conclusion should be interpreted with caution.



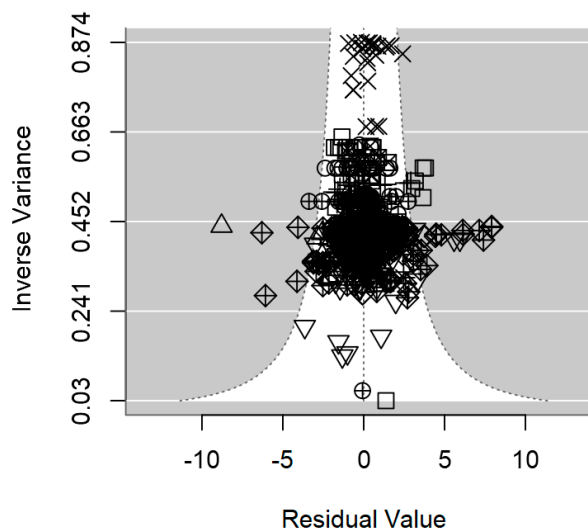


Figure 2. Six-sigma radial plot (**top**) and funnel plot (**bottom**) of the overall meta-regression on the effect of slaughter processing stages and interventions on the concentration of *Campylobacter* in poultry. Markers symbolise interventions: € = Chilling, ○ = Cleaning and disinfection, Δ = Evisceration, + = Freezing, × = Neck cutting and bleeding, ◇ = Packaging, ▼ = Plucking, ☒ = Scalding, * = Washing, ⬠ = Chemical sanitisation, ⊕ = Colder climates, ⋈ = Organic acids, ⊞ = Plant extracts.

3.3. Univariate Analysis of the Effects of Processing Stages and Interventions on the Concentration of *Campylobacter* in Poultry

The univariate analysis aimed at preliminarily identifying potential study characteristics moderating the effects of effects of processing stages and intervention strategies on *Campylobacter* concentrations in poultry. Table 2 compiles the p -values of the association between mean $\log_{10}$ reductions attained by each processing stage/intervention with available study characteristics.

Sample weight was consistently found to moderate the estimates of effectiveness of stages/interventions, having a direct relationship with the effects of neck cutting and bleeding ($p = 0.002$), plucking ($p = 0.010$), evisceration ($p < 0.001$), sanitizing and chilling ($p = 0.001$), and application of organic acids ($p < 0.001$) and plant extracts ($p < 0.001$) on *Campylobacter* population. In general, higher amounts or extent of the sample analysed increased the likelihood of detecting *Campylobacter*, leading to greater $\log_{10}$ reductions.

The duration of treatment also played a role in the impact of processing stages and interventions to suppress *Campylobacter* in poultry. In the processing stages of plucking ($p = 0.001$) and sanitizing and chilling ($p < 0.001$), the duration of the treatment with chemical sanitisers was positively related with the extent of *Campylobacter* $\log_{10}$ reduction in poultry carcasses. Similarly, for the intervention strategies of chemical sanitisation of carcasses/meat ($p = 0.083$) and application of organic acids on carcasses/meat ($p < 0.001$), the longer the treatment duration, the higher the observed mean $\log_{10}$ reductions in *Campylobacter* load (Table 2).

As encountered with the duration of the treatment with sanitisers, the sanitiser concentration was found to have a direct—and consistently strong—impact on the effectiveness of sanitising and chilling ($p = 0.033$), chemical sanitisation of carcasses/meat ($p < 0.001$), and application of organic acids ($p < 0.001$), and plant-based extracts ($p < 0.001$).

Table 2. Univariate analysis aiming to identify the potential study characteristics moderating the effect of slaughter processing stages and interventions on the concentration of *Campylobacter* in poultry. *p*-values are shown, and whenever significant ($\alpha = 0.10$), direct (+) or inverse (−) relationship with the quantitative moderator is indicated.

Quantitative Moderators Tested Individually	Log ₁₀ reduction (Control Group–Treated Group)					
	Sample Weight	Duration of Treatment	Treatment Concentration	Temperature	Storage Time	Inoculum in Challenge Test
<i>Stages</i>						
Neck cutting and bleeding	0.002 (+)	NA	NA	NA	NA	NA
Scalding	0.314	0.152	NA	0.140	NA	NA
Plucking	0.010 (+)	0.001 (+)	0.666	0.005 (−)	NA	NA
Evisceration	<0.001 (+)	0.025 (−)	0.227	0.723	NA	NA
Carcass rinsing with water	0.574	NA	NA	NA	NA	NA
Sanitizing and chilling	0.001 (+)	<0.001 (+)	0.033 (+)	<0.001 (+)	NA	NA
Packaging and cold storage	NA	NA	NA	NA	NA	NA
Routinary cleaning and disinfection	NA	NA	NA	NA	NA	NA
<i>Interventions</i>						
Chemical sanitisation	0.552	0.083 (+)	<0.001 (+)	<0.001 (−)	NA	0.576
Organic acids	<0.001 (+)	<0.001 (+)	<0.001 (+)	<0.001 (+)	<0.001 (+)	0.449
Plant extracts	<0.001 (+)	NA	<0.001 (+)	NA	<0.001 (+)	0.036 (−)
Freezing	NA	NA	NA	NA	NA	NA
Colder climates	NA	NA	NA	NA	NA	NA

NA: not applicable; no univariate meta-analysis could be adjusted because quantitative moderator was not available in the given stage/intervention dataset. *p*-values in bold indicate statistically significant association between variables.

The temperature at which the treatment took place was found to be negatively associated with the effectiveness of plucking (with sanitisers) ($p = 0.005$) and of sanitisation with chemicals ($p < 0.001$). This can be understood by the fact that many sanitisers are volatile compounds, and when temperature rise above 40–50°C the active ingredient may evaporate or decompose before it can eliminate the pathogens. For instance, sodium hypochlorite is very sensitive to heat. At higher temperatures, chlorine dissipates as a gas, therefore reducing the concentration of the sanitiser in the water [121,122].

In the case of intervention strategies, where studies were experimentally conducted through pathogen's inoculation, chill storage time of poultry carcass/meat treated with organic acids ($p < 0.001$) or plant extracts ($p < 0.001$) contributed to a greater mean log₁₀ reduction in *Campylobacter*. In other words, this finding points out that the concentration of *Campylobacter* in poultry meat treated with organic acids or plant extracts decreases during shelf life. This is likely to be a result of cumulative damage.

Finally, from the univariate analysis, it was found that in challenge studies entailing poultry carcasses or meat, the inoculum size can drive the measured effectiveness of an intervention. This is the case of the effectiveness of plant extracts, where it was found that the greater the concentration of *Campylobacter* inoculated on/in the meat, the lower the

mean $\log_{10}$ reductions quantified. This effect is well known in predictive microbiology and is called “active agent saturation”. The effectiveness of compounds like carvacrol or organic acids depends on the ratio between the number of agent molecules and the number of bacterial cells. At low inoculum, there is an excess of molecules for each bacterial cell, ensuring rapid death. However at high inoculum, the concentration of the agent can become the limiting factor. If each carvacrol molecule binds to a site on the membrane of a bacterium, a massive inoculum can simply sequester the available agent before all cells are affected [123,124].

3.4. Multilevel Meta-Analysis on the Effects of Processing Stages on the Concentration of *Campylobacter* in Poultry

Due to the observed heterogeneity in the overall meta-analysis, multilevel meta-regression models were adjusted within specific subgroups to more accurately examine the effects of various moderators. The results of these multilevel meta-analysis models on the effect of slaughter processing stages are presented in Table 3, and are described below.

Table 3. Best-fit multilevel meta-analysis models describing the effect of slaughter processing stages on the concentration of *Campylobacter* in poultry. Number of observations (n), heterogeneity analysis and *p*-value of the publication bias test are shown.

Measure ($\log_{10}$ Reduction)	Parameter	Estimate	Standard Error	<i>p</i> -Value	n	Heterogeneity Analysis ¹
Neck cutting and bleeding	Sample weight	0.003	0.001	0.014	17	$s^2 = 1.411$
	Sample type					$\tau^2 = 0.0541$
	Breast feathers	0.546	0.185	0.003		$I^2 = 3.7\%$
	Neck skin	−0.785	0.348	0.024		$p(QE) < 0.001$
	Carcass rinse	1.135	0.443	0.010		$R = 0.264$ Pub. bias $p = 0.941$
Scalding	Sample type				24	$s^2 = 0.8974$
	Neck skin	0.898	0.245	<0.001		$\tau^2 = 0.5689$
	Carcass rinse	1.618	0.253	<0.001		$I^2 = 38.8\%$ $p(QE) < 0.001$ $R = 0.761$ Pub. bias $p = 0.129$
Plucking	Sample weight	0.003	0.001	0.040	47	$s^2 = 1.6707$
	Treatment with sanitiser					$\tau^2 = 1.5963$
	None	−0.367	0.376	0.328		$I^2 = 51.1\%$
	Chlorine	0.053	0.424	0.900		$p(QE) < 0.001$
	Citric acid	0.061	1.088	0.955		$R = 0.338$
	Lactic acid	0.056	1.087	0.959		Pub. bias
	Sodium hypochlorite	2.172	1.189	0.067		$p = 0.092$
Evisceration	Trisodium phosphate	−0.182	1.089	0.867	99	
	Intercept	−0.623	0.194	0.001		$s^2 = 0.7473$
	Sample weight	0.003	0.001	<0.001		$\tau^2 = 0.4830$
	Treatment with sanitiser					$I^2 = 39.3\%$
	Cetylpyridinium chl.	0.912	0.571	0.110		$p(QE) < 0.001$
	Citric acid	0.923	0.800	0.249		$R = 0.506$
	Hot water sprayed	1.031	0.134	<0.001		Pub. bias
	Lactic acid	1.058	0.797	0.184		$p = 0.556$
	Peroxyacetic acid	1.025	0.568	0.071		

	Sodium hypochlorite	−0.931	0.816	0.254		
	Sodium phosphate	0.903	0.794	0.258		
	None	−	−			
Carcass rinsing with water (all by immersion)	Intercept	0.410	0.001	<0.001		$s^2 = 0.1121$
	Sample type					$\tau^2 = 0.0247$
	Breast skin	0.023	0.011	0.040		$I^2 = 18.0\%$
	Neck skin	0.028	0.005	<0.001	33	$p(QE) < 0.001$
	Carcass rinse	0.001	0.001	0.035		$R = 0.397$
						Pub. bias
						$p = 0.124$
Sanitizing and chilling	Sample type					$s^2 = 0.8533$
	Unknown	1.362	0.476	0.004		$\tau^2 = 0.4798$
	Breast skin	0.807	0.459	0.078		$I^2 = 36.0\%$
	Neck skin	0.506	0.157	0.001		$p(QE) < 0.001$
	Carcass rinse	1.017	0.166	<0.001		$R = 0.644$
	Swab	0.753	0.444	0.089	130	Pub. bias
	Thigh skin	0.292	0.645	0.650		$p = 0.222$
	Treatment with sanitiser					
	Acidified sodium chl.	0.578	0.125	<0.001		
	Chlorine	−0.860	0.345	0.013		
	Hot water sprayed	−0.121	0.138	0.377		
	Peroxyacetic acid (PA)	0.410	0.102	<0.001		
	PA + sodium hydroxide	−0.877	0.261	0.001		
	Sodium thiosulfate	−1.351	0.258	<0.001		
	None	−	−	−		
Packaging	Sample type					$s^2 = 1.1552$
	Neck skin	1.451	0.310	<0.001		$\tau^2 = 0.6851$
	Swab	−0.057	0.447	0.897		$I^2 = 37.2\%$
					22	$p(QE) < 0.001$
						$R = 0.624$
						Pub. bias
						$p = 0.403$
Routinary cleaning and disinfection	Environmental swabs	0.879	0.255	<0.001		$s^2 = 0.2967$
						$\tau^2 = 0.1534$
					4	$I^2 = 34.0\%$
						Pub. bias
						$p < 0.001$

¹ Heterogeneity analysis encompasses within-study variability (s^2), between-study variability (τ^2), and intra-class correlation (I^2) of the null model, and QE test of residual heterogeneity, and square root of the between-study variability explained by significant moderators (R^2) from the full model.

3.4.1. Neck Cutting and Bleeding Stage Meta-Regression Model

This model was adjusted based on 17 data points for the variables sample weight and sample type, both highly significant ($p < 0.05$; Table 3). The results exhibited a positive association between sample weight with estimate effect of 0.003 ± 0.001 and *Campylobacter* reduction during neck cutting/bleeding, suggesting that higher sample amounts enable a better measurement of the difference in *Campylobacter* on pre-bleeding and post-bleeding carcasses. This trend is also illustrated in Figure 3 (bottom left), which shows a slight positive correlation as the sample amount increases.

Additionally, our measurement of the reduction effect was heavily impacted by the type of sample obtained. Microbiological determinations made on carcass rinse (1.135 ± 0.443) led to higher estimates of *Campylobacter* reduction in comparison to breast feathers

(0.546 ± 0.185 ; Table 3). By contrary, neck skin (-0.785 ± 0.348) turned out to be the type of sample that would more likely show that bird killing increases *Campylobacter* concentrations in poultry. The impact of sample type can be explained by the greater area for *Campylobacter* recovery when sampling a full carcass, and the normally higher contamination found in neck skin [125,126]. Neck skin birds is considered the site of highest load of *Campylobacter* due to a combination of anatomical, physical, and processing factors [4].

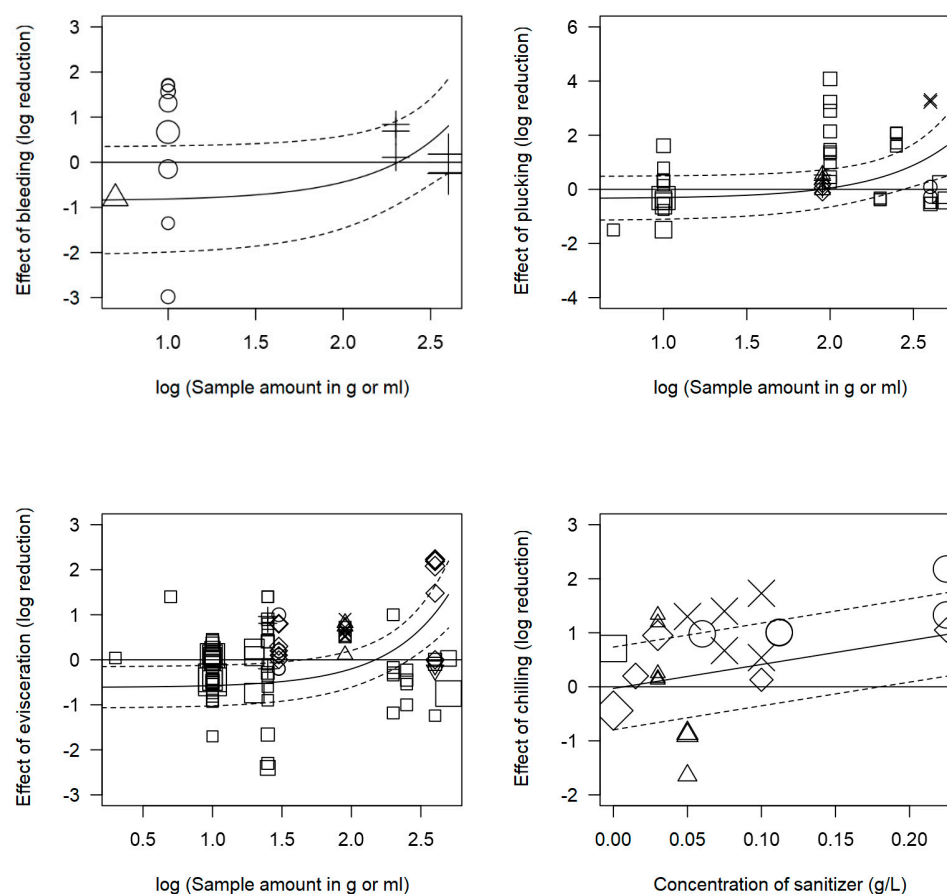


Figure 3. Meta-analytical bubble plots depicting the significant relationship ($p < 0.10$) between moderators and the effect of slaughter processing stages on the levels of *Campylobacter* in poultry. Dashed lines are 95% confidence bands. Top left: $\circ$ = Breast feathers, Δ = Neck skin, $+$ = Rinse; Top right: ∞ = No sanitiser, $\circ$ = Chlorine, Δ = Citric acid, $+$ = Lactic acid, $\times$ = Sodium hypochlorite, $\diamond$ = Trisodium phosphate; Bottom left: ∞ = No sanitiser, $\circ$ = Cetylpyridinium chloride, Δ = Citric acid, $+$ = Hot water sprayed, $\times$ = Lactic acid, $\diamond$ = Peroxyacetic acid, $\blacktriangledown$ = Sodium hypochlorite, $\boxtimes$ = Trisodium phosphate; Bottom right: ∞ = No sanitiser, $\circ$ = Acidified sodium chlorite, Δ = Chlorine, $\times$ = Peroxyacetic acid, $\diamond$ = Peroxyacetic acid and sodium hydroxide.

In terms of heterogeneity analysis, the neck cutting/bleeding stage meta-regression model revealed a low intraclass correlation ($I^2 = 3.7\%$), with the two moderators accounting for 26% of the variability between studies (R^2). However, some unexplained variability remains (Q_E test, $p < 0.001$), likely due to unmeasured variables, such as bird age at slaughter and bleeding method (manual or automatic). In addition, the observed p -value

($p = 0.941$) indicates that there is no evidence of publication bias, suggesting robustness of the parameter estimates [42].

3.4.2. Scalding Stage Meta-Regression Model

The meta-regression model examining the effect of scalding stage was fitted using 24 observations from different sample types, including neck skin and rinsed carcass taken before and after scalding. The results demonstrated that the scalding stage is effective in reducing *Campylobacter* regardless of the sample ($p < 0.1$). However, the overall reduction effect depends on the type of sample taken from the poultry carcass. The pooled reduction in *Campylobacter* due to scalding was found to be higher when measured by rinsing the entire carcass (1.618 ± 0.253) than when measured in neck skin samples (0.899 ± 0.245). As observed in the neck cutting/bleeding meta-regression, this arises from the greater odds of recovering *Campylobacter* from neck skin, even so after scalding, due to the numerous folds, crevices and deep follicles. The structure of the neck skin offers a larger surface area for bacterial adhesion, facilitating microcolony formation [127].

For the scalding stage meta-regression model, between-study heterogeneity was relatively low ($I^2 = 39\%$), and type of sample accounted for a high proportion of the variability across studies ($R^2 = 76\%$). Nevertheless, some variability remains unexplained (Q_E test, $p < 0.001$), because potential moderators such as scalding temperature and time were not reported in all studies. However, the model showed no evidence of publication bias ($p = 0.129$; Table 3), suggesting robustness of the findings [42].

3.4.3. Plucking Stage Meta-Regression Model

The meta-regression model, designed to identify the study characteristics affecting *Campylobacter* concentration during plucking, retained sample weight and treatment with sanitiser as significant moderators. Once again, it was found that as sample weight increased, the measurement uncertainty decreased and the likelihood of detecting *Campylobacter* increased; consequently, the reduction effect of plucking turned out to be higher (0.003 ± 0.001 ; $p = 0.040$; Table 3). Such a trend, although slight, can also be visualised in Figure 3 (top right).

According to the primary studies recovered, the chemicals applied during plucking were chlorine, citric acid, lactic acid, sodium hypochlorite, and trisodium phosphate. The sanitiser chosen to assist plucking was found to have a significant effect on the extent of *Campylobacter* reduction. Among all sanitisers, sodium hypochlorite produced the higher reduction (2.172 ± 1.189 ; $p = 0.067$) reaching significance. In contrast, chlorine, citric acid and lactic acid displayed positive reduction effects about the same order of magnitude (0.053 – 0.061) but did not reach statistical significant ($p > 0.1$). The effectiveness of sanitisers can be influenced by factors beyond temperature, including the chemical agent's properties, pH levels, and the presence of organic matter in the solution [128]. For instance, sodium hypochlorite is a strong oxidizing agent that acts as hypochlorous acid; therefore, its effectiveness against microorganisms increases when the pH is between 7.0 and 7.5, near neutral pH values as this facilitates penetration through cell membranes, whereas its efficacy decreases at high pH [129]. On the other hand, higher pH levels and the presence of organic matter can diminish the effectiveness of certain sanitisers on surfaces [121,130]. This reduction in effectiveness may explain the lower efficacy observed with sanitisers such as chlorine, citric acid, and lactic acid.

The heterogeneity measurement from the plucking stage meta-regression revealed moderate intraclass correlation ($I^2 = 51.1\%$). Sample weight and chemical treatment jointly accounted for ~34% of the between-study variability; and further analysis showed that there was significant remaining non-explained heterogeneity (Q_E test, $p < 0.001$). The study characteristic sanitiser's concentration could not be included as moderator due to

incomplete reporting. The test for small study effects did not indicate evidence of publication bias ($p = 0.092$; Table 3).

3.4.4. Evisceration Stage Meta-Regression Model

The meta-regression on the effectiveness of the evisceration stage can be regarded as robust as it was based on nearly 100 observations. As in plucking, the evisceration stage was able to reduce the *Campylobacter* populations in poultry only when a chemical treatment (or hot water sprayed) was incorporated. This can be deduced from the significant negative intercept (-0.623 ± 0.194 ; $p = 0.001$), and the positive ones for the different sanitisers (Table 3). Notice that in the variable treatment, the class “none” with a mean of zero represents the absence of a sanitiser; and therefore the coefficients reported for each sanitiser treatment indicate deviations from this baseline. Based on them, the treatment of carcasses with hot water spray (1.031 ± 0.134 ; $p < 0.001$), peroxyacetic acid (1.025 ± 0.568 ; $p = 0.071$) and cetylpyridinium chlorine (0.912 ± 0.571 ; $p = 0.110$) was proven to be effective in reducing *Campylobacter* counts on carcasses in about $1.0 \log_{10}$. This meta-regression failed to prove the efficacy of citric acid, lactic acid, sodium hypochlorite and sodium phosphate to control *Campylobacter* at least at the stage of evisceration. Although all three treatments showed similar reductions of $\sim 1 \log_{10}$, their mechanisms of action differ. For instance, hot water spray has both physical and thermal effects that cause protein denaturation and rapid partial removal of bacteria from the surface [58]. In contrast, peroxyacetic acid induces intracellular oxidative stress [129], while cetylpyridinium chloride disrupts the bacterial membrane via electrostatic interactions [131]. Nevertheless, their effectiveness during evisceration may be influenced by contact time, the presence of organic load, and the complex structure of poultry skin [129,132].

As already seen in bleeding and plucking, sample weight (0.003 ± 0.001 ; $p = 0.001$) was a significant variable affecting the measured effectiveness of evisceration, because large quantities sampled increase the likelihood of obtaining precise microbial concentration estimates. Reduced sample amounts are associated, on the contrary, with results heavily subjected to randomness, and therefore of greater uncertainty. Although there is dispersion in the relationship, the corresponding bubble plot shows the positive trend between sample amount and mean $\log_{10}$ reduction in *Campylobacter* (Figure 3, bottom left).

In this model, the between-study heterogeneity was relatively low ($I^2 = 39.3\%$), and the two moderators accounted for more than 50% of the variability between studies (R^2), leaving some residual variability unexplained by the model (Q_E test, $p < 0.001$). This may reflect that other key factors such as concentration, mode of application, and timing of contact with the sanitiser, could have explained more variability; yet, they were not available to the authors. Furthermore, the model's publication bias was not significant ($p = 0.556$), suggesting robustness in the meta-regression estimates [42].

3.4.5. Carcass Rinsing Meta-Regression Model

In the meta-regression on carcass washing data, only type of sample was found to drive the measured effectiveness of this stage for controlling *Campylobacter*. It is worth mentioning that this dataset comprised concentrations on pre-wash and post-wash carcasses only when water was employed. According to the overall model, immersing carcasses in water reduces *Campylobacter* concentrations in about $0.5 \log_{10}$ (Table 1). However, such an estimation of reduction is affected by sample type, with neck skin (0.028 ± 0.005) and breast skin (0.023 ± 0.011) leading to higher reduction estimates than the carcass rinse sampling method (0.001 ± 0.001 ; Table 3). This can be explained by the fact that neck and breast skin are known to accumulate microorganisms such as *Campylobacter* and *Salmonella*, because during processing poultry carcasses are hung by their feet, causing wash

water and other fluids to run down. Therefore, in pre-wash carcasses, *Campylobacter* recoveries are very likely to be higher in neck and breast skin samples than from carcass rinse samples.

For the stage of carcass rinsing, the publication bias test did not signal any file-drawer problem, as evidenced by the p -value of 0.222. Between-study heterogeneity value was low ($I^2 = 18\%$), but even so sample type accounted for 39% of the variability between studies (R^2), leaving some residual variability unexplained by the model (Q_E test, $p < 0.001$).

3.4.6. Sanitisation and Chilling Meta-Regression Model

The sanitisation/chilling dataset constitutes the stage with the highest number of observations available ($n = 130$), and comprises concentrations of *Campylobacter* in pre-sanitised poultry carcasses and post-chill carcasses (taken at ~18–24 h in chill storage). In this meta-regression, two moderators were found to affect the reduction effect of sanitisation/chilling: sample type and sanitiser (Table 3). Once again, the type of sample taken from the carcass heavily affected the estimation of the effectiveness of sanitisation/chilling for controlling *Campylobacter*. The greatest reduction was quantified in samples of carcass rinse (1.017 ± 0.166 ; $p < 0.001$), followed by carcass swab (0.753 ± 0.444 ; $p = 0.089$), breast skin (0.807 ± 0.459 ; $p = 0.078$), and neck skin (0.506 ± 0.157 ; $p = 0.001$). It is very likely that the sample type codified as “unknown” refers to the carcass rinse sampling method, yet it was not clearly specified in the studies.

The effectiveness of chilling as a key processing stage to reduce *Campylobacter* populations was further affected by the sanitiser used during carcass washing. Acidified sodium chlorine (0.578 ± 0.125 ; $p < 0.001$) and peroxyacetic acid (0.410 ± 0.102 ; $p < 0.001$) led to greater and comparable *Campylobacter* reductions, followed by chlorine (-0.860 ± 0.345 ; $p = 0.013$) and peroxyacetic acid + sodium hydroxide (-0.877 ± 0.261 ; $p = 0.001$). Among all available sanitisers, sodium thiosulfate appeared as the least effective (-1.351 ± 0.258 ; $p < 0.001$). In this case, the greater effect observed in acidified sodium chlorite and peroxyacetic acid can be attributed to their strong oxidative capacity and relative stability even in the presence of organic matter [132]. In addition, the cooling process itself may also indirectly increase the effect of these sanitisers by stressing *Campylobacter* cells. In contrast, the low effect observed with chlorine treatments is likely due to the low availability of free residual chlorine in chilled water and the high organic load. To note, peroxyacetic acid is stable in slightly acidic pH solutions [133]; therefore, its combination with sodium hydroxide may have reduced the solution's antimicrobial efficacy. Notably, sodium thiosulfate exhibited the lowest efficacy, consistent with its role as a chlorine-neutralising agent rather than an antimicrobial compound [134].

Even though, concentration of sanitisers was not retained in the meta-regression model, the bubble plot shown in Figure 3 (bottom right) evidences that higher concentrations tended to yield higher *Campylobacter* reductions, at least for acidified sodium chlorite, peroxyacetic acid and peroxyacetic acid + sodium hydroxide.

For the sanitisation/chilling meta-regression, the between-study heterogeneity was relatively low ($I^2 = 36\%$), and the two moderators retained by the model accounted for a large proportion of between-study variability ($R^2 = 64\%$). Nevertheless, according to the Q_E test of heterogeneity, the remaining residual variability was significant ($p < 0.001$). Furthermore, the non-significant p -value for publication bias ($p = 0.222$; Table 3) indicates that the meta-regression estimates can be regarded as robust.

3.4.7. Packaging Stage Meta-Regression Model

According to the meta-regression fitted on *Campylobacter* concentration data obtained from poultry meat before packaging in modified atmosphere and after cold storage (5–10 days), there was a significant pathogen's reduction of $1.451 \pm 0.310 \log_{10}$ ($p < 0.001$) when

microbiological determinations were carried out in neck skin. In the case of swabs, given the few observations available, the results were inconclusive.

Modified atmosphere packaging (MAP) could reduce the viability of *Campylobacter* if exposed to high oxygen concentrations (60–80%) since those levels cause oxidative stress. Studies have shown that mixtures with 80% O₂ and 20% CO₂ can reduce bacterial counts by approximately 0.7 to 1.2 log₁₀ after one week of storage. Furthermore, atmospheres with high O₂ can induce *Campylobacter* to enter a VBNC state. This means that the bacteria do not grow in traditional culture media [135], although remain alive and potentially capable of causing infection if ingested. Despite carbon dioxide inhibits spoilage bacteria, it has a limited effect on directly eliminating *Campylobacter*. However, it helps extend the shelf life of the product, which indirectly allows for more time for the natural reduction in the pathogen through desiccation or cold.

In this particular meta-regression model, the between-study heterogeneity was relatively low ($I^2 = 37.2\%$), and the only moderator retained—sample type—was able to explain a large proportion of the between-study variance ($R^2 = 62\%$). Nevertheless, some residual heterogeneity remained (Q_E test, $p = 0.001$). There was no evidence of publication bias according to the non-significant test for small study effects ($p = 0.403$; Table 3).

3.4.8. Routinary Cleaning and Disinfection Meta-Analysis Model

For the routinary cleaning and disinfection data, a simple random-effects meta-analysis was conducted due to the absence of moderators. The good hygiene strategy was able to significantly reduce *Campylobacter* populations by 0.879 ± 0.255 log₁₀ in the environmental elements of poultry slaughterhouses, as tested by the swabbing method (Table 3). Several studies indicate that plucking and evisceration can increase the risk of contamination. However, by strictly implementing good manufacturing and hygiene practices, particularly thorough cleaning and disinfection of equipment, the risk of cross-contamination between carcasses and meat from colonised batches during slaughter can be significantly reduced [9,136], as demonstrated in this meta-analysis. Despite this, there is still a risk that certain strains of *Campylobacter* may form biofilms in the slaughterhouse environment [137], likely due to inadequate hygiene. This persistence may explain their continued presence even after cleaning and disinfection, as noted in the literature [17].

The intra-class correlation ($I^2 = 34\%$) pointed out the presence of a relatively low between-study heterogeneity, which was encouraging, given that no moderators were available to explain such heterogeneity. Nonetheless, the pooled mean reduction estimate must be interpreted with caution, since according to the likely presence of publication bias ($p = 0.001$; Table 3), some studies encountering that cleaning and disinfection did not decrease *Campylobacter* in the environment may have remained unpublished.

3.5. Multilevel Meta-Analysis on the Effects of Interventions on the Concentration of *Campylobacter* in Poultry

Table 4 compiles the meta-regression estimates of the effect of intervention strategies on *Campylobacter* concentration in poultry carcasses and meat. Pre- and post-intervention data were available for chemical sanitisation, organic acids, plant extracts, freezing, and colder climate.

Table 4. Best-fit multilevel meta-analysis models describing the effect of slaughter-level interventions on the concentration of *Campylobacter* in poultry. Number of observations (n), heterogeneity analysis and *p*-value of the publication bias test are shown.

Measure (Log ₁₀ Reduction)	Parameter	Estimate	Standard Error	<i>p</i> -Value	n	Heterogeneity Analysis ¹
Chemical sanitisation of carcasses	Form of application					$s^2 = 0.9337$
	Immersion	1.839	0.254	<0.001		$\tau^2 = 0.7155$
	Spraying	1.303	0.268	<0.001		$I^2 = 43.4\%$
	Sample type					$p(QE) < 0.001$
	Cloaca	-2.267	0.758	0.003	68	$R = 0.559$
	Carcass (full rinse, neck, breast)	-	-			Pub. bias
						$p = 0.680$
	Sanitiser					
	Chlorine	-1.794	0.181	<0.001		
	Chlorine dioxide	-1.288	0.755	0.088		
	Sodium hypochl.	-0.777	0.259	0.003		
	None (water)	-1.042	0.058	<0.001		
	Sanitiser concentration					
	Cetylpyridinium chl.	0.047	0.001	<0.001		
Organic acids on carcass/meat cuts	Intercept	0.434	0.163	0.008		$s^2 = 0.6824$
	Form of application					$\tau^2 = 0.3714$
	Spraying	-0.067	0.028	0.019		$I^2 = 35.2\%$
	Immersion	-	-			$p(QE) < 0.001$
	Sample type					$R = 0.686$
	Carcass	0.213	0.178	0.232	142	Pub. bias
	Drumsticks	-0.129	0.244	0.598		$p = 0.004$
	Swabs	0.419	0.258	0.105		
	Wings	0.656	0.238	0.006		
	Breast	-	-			
	Sanitiser concentration					
	Acetic acid	-0.383	0.081	<0.001		
	Blends of OA	0.036	0.024	0.129		
	Citric acid	0.007	0.002	<0.001		
	Lactic acid	0.008	0.002	<0.001		
	Monocaprin emuls.	0.104	0.005	<0.001		
	Peroxyacetic acid	1.193	0.020	<0.001		
	Water	-	-			
Plant extracts on poultry meat	Intercept	-0.174	0.080	0.030		$s^2 = 0.6553$
	Sample weight	0.085	0.006	<0.001		$\tau^2 = 0.5131$
	Storage time	0.001	0.001	0.213		$I^2 = 44.0\%$
	Extract concentration					$p(QE) < 0.001$
	Carvacrol	0.074	0.006	<0.001	195	$R = 0.775$
	Carvacrol + Chitosan	0.030	0.002	<0.001		Pub. bias
	Carvacrol + Gum arabic	0.002	0.001	<0.001		$p = 0.082$
	Chitosan	0.012	0.003	<0.001		
	Cinnamaldehyde	0.051	0.004	<0.001		
	Linallol	0.031	0.008	<0.001		
Freezing (Carcasses)	Intercept	0.669	0.100	<0.001	4	$s^2 = 0.0760$
						$\tau^2 = 0.0000$

						I ² = 0.00%
						Pub. bias
						<i>p</i> = 0.462
Colder climates (Caecal samples)	Intercept	1.947	1.384	0.159	14	s ² = 12.43
						τ ² = 3.816
						I ² = 23.4%
						Pub. bias
						<i>p</i> < 0.001

¹ Heterogeneity analysis encompasses within-study variability (s²), between-study variability (τ²), and intra-class correlation (I²) of the null model, and QE test of residual heterogeneity, and square root of the between-study variability explained by significant moderators (R²) from the full model.

3.5.1. Chemical Sanitisation Meta-Regression Model

The chemical sanitisation to be presented in this subsection differs from that of sanitising and chilling (Section 3.4.6) in that whereas the latter combines the results of non-inoculated poultry samples taken at slaughterhouses, the former combines the experimental results of the efficacy of different sanitisers to decrease *Campylobacter* in inoculated poultry meat. According to the meta-regression for chemical sanitisation, four moderators significantly affect its effectiveness in the reduction in *Campylobacter* in poultry: form of application, type of sample obtained, sanitiser and sanitiser concentration, which explained approximately 56% of the total variability between studies (Table 4).

The results suggest that the application of sanitiser by immersion leads to a greater reduction effect of *Campylobacter* (1.839 ± 0.254 ; $p < 0.001$) in comparison to the spraying method (1.303 ± 0.268 ; $p < 0.001$). Prolonged contact time could be one of the main reasons for this difference. For instance, during immersion, the entire carcass surface is uniformly wetted, allowing for greater contact between the sanitiser and the microbial cells. In contrast, spraying may be hindered by rapid drying and droplet runoff, which can reduce the sanitiser's effectiveness [138]; although the reduction estimates are affected by the poultry zone where a sample is taken. Thus, quantifying *Campylobacter* in cloaca produces lower estimates of reduction due to sanitisation.

This is an expected outcome since *Campylobacter* in the poultry cloaca signals primary source infection through horizontal transmission (as opposed to post-contamination and cross-contamination). *Campylobacter* are specifically adapted to colonise the mucus layer of the lower gastrointestinal tract, particularly the ceca and cloaca; however, quantifying *Campylobacter* in the cloaca may not reflect the true bacterial load in the colonised intestine, as the cloaca may become positive due to transport or scalding processes [139].

When considering the efficacy of sanitisers, the meta-regression suggested that, on average at the usual concentrations applied, sodium hypochlorite leads to the greatest reduction effect (-0.777 ± 0.259 ; $p = 0.003$), while chlorine produces the smallest (-1.794 ± 0.181 ; $p < 0.001$). Nonetheless, an increase in sanitiser concentration of 1 g/kg causes a higher increase in the effectiveness of chlorine (17.46 ± 3.993 ; $p < 0.001$) than in sodium hypochlorite ($p = 0.488$). Despite the observable data dispersion (Figure 4, top left), the bubble plot between sanitiser concentration and effectiveness of sanitisation can be appreciated for cetylpyridinium chloride, chlorine, and sodium hypochlorite. Notice that most observations lay above the y-axis zero line, illustrating the effectiveness of sanitisers.

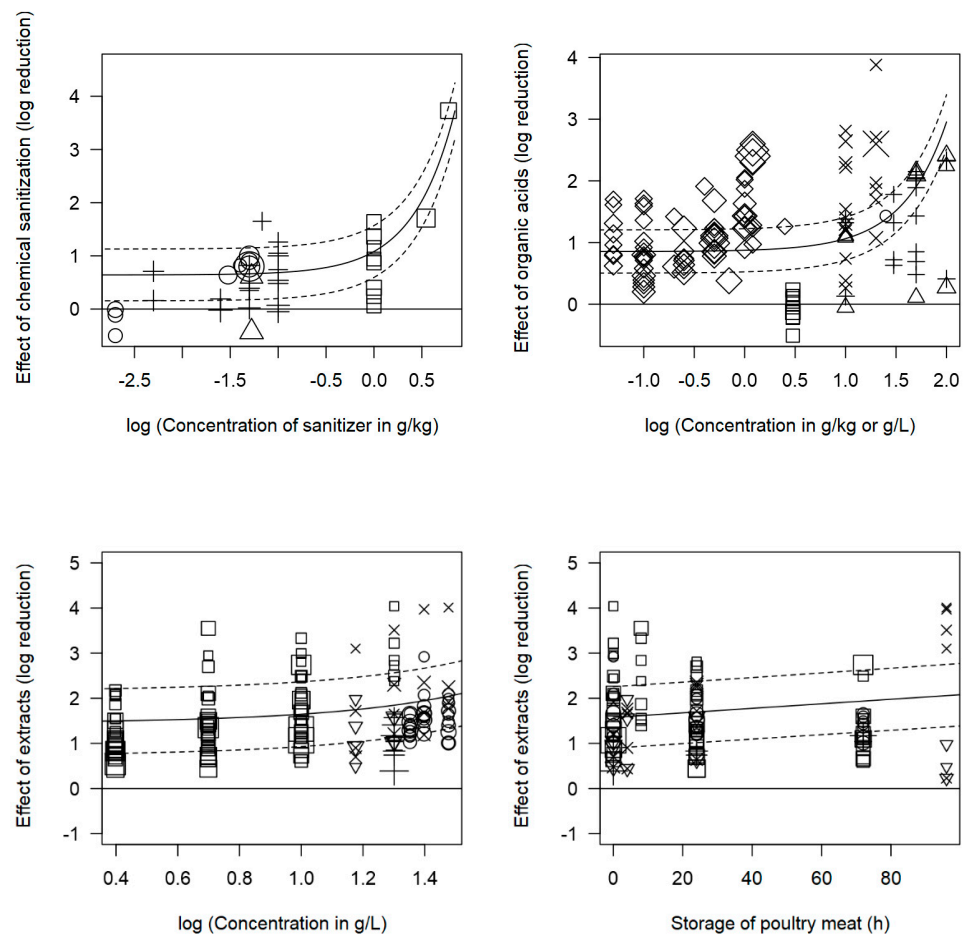


Figure 4. Meta-analytical bubble plots depicting the significant relationship ($p < 0.10$) between moderators and the effect of slaughter-level intervention strategies on the levels of *Campylobacter* in poultry. Top left: € = Cetylpyridinium chloride, ○ = Chlorine, Δ = Chlorine dioxide, + = Sodium hypochlorite; Top right: € = Acetic acid, ○ = Blend of OAs, Δ = Citric acid, + = Lactic acid, × = Monocaprin emulsion, ◇ = Peroxyacetic acid; Bottom left and right: € = Carvacrol, ○ = Carvacrol-chitosan, + = Chitosan, × = Cinnamaldehyde, ▼ = Linallol.

Although, in univariate analysis, treatment duration had a significant effect on the effectiveness of chemical sanitisers, this moderator was not significant in the multilevel meta-analysis. Despite both sodium hypochlorite and chlorine sanitisers have antimicrobial activity capable of reducing microbial populations, as described above, their action on bacteria is mainly determined by the available chlorine in solution. For example, due to its oxidising power, sodium hypochlorite is more active than chlorine. Furthermore, sodium hypochlorite is less affected by organic matter than chlorine, which may explain the difference in the magnitude of reduction between these two sanitisers [130]. However, increasing the concentration of the sanitiser has been shown to reduce the amount of organic matter [132], which explains the greater efficacy of chlorine with each 1 g/kg added to the solution.

The I^2 value of 43.4% in this meta-regression suggests moderate variability between studies; however, the four moderators jointly explained ~56% of such variance (R^2). Nevertheless, some unexplained residual variability remained (Q_E test, $p < 0.001$), which reflects that other factors such as pH or treatment duration could be important; yet these

were not reported in several studies. The test of the effect of small studies was non-significant ($p = 0.680$; Table 4), suggesting the absence of publication bias and therefore the reliability of estimates.

3.5.2. Organic Acid Meta-Regression Model

The meta-regression model adjusted for organic acids allowed for the evaluation of the effectiveness of acetic acid, citric acid, lactic acid, peroxyacetic acid, blends of organic acid and monocaprin emulsion in the control of *Campylobacter* populations in poultry carcasses. Such effectiveness is driven by the form of application, the sanitiser concentration and the type of sample. As in chemical sanitisation, the application of organic acids by spraying the solution on the carcass is less effective than by immersing the carcass (-0.067 ± 0.028 ; $p = 0.019$ versus immersion as baseline; Table 4).

In terms of sample type, when *Campylobacter* was enumerated on breast, the measured reductions tended to be the lowest (baseline), whereas samples of wings (0.656 ± 0.238 ; $p = 0.006$) and carcass swabs (0.419 ± 0.258 ; $p = 0.105$) were associated to greater estimates of *Campylobacter* reduction. Based on water as a reference, peroxyacetic acid appeared as the organic acid that achieved the greatest *Campylobacter* reduction (1.193 ± 0.020 ; $p < 0.001$), followed by monocaprin emulsion (0.104 ± 0.005 ; $p < 0.001$), and blends of organic acid (0.036 ± 0.024 ; $p = 0.129$). Lactic acid (0.008 ± 0.002 ; $p < 0.001$), citric acid (0.007 ± 0.002 ; $p < 0.001$) and acetic acid (-0.383 ± 0.081 ; $p < 0.001$) exhibited the lowest efficacy to reduce *Campylobacter* among all organic acids. The bubble plot shown in Figure 4 (top right) illustrates the effect of increasing the concentrations of citric acid, lactic acid, monocaprin emulsion and peroxyacetic acid on the reduction in *Campylobacter* on poultry carcasses, with the latter two being the most effective sanitisers. It is noteworthy mentioning that in univariate analysis, in addition to sanitiser's concentration, treatment duration and temperature exhibited a significant effect on the effectiveness of organic acids, although in multilevel meta-analysis these moderators did not reach significance.

Peroxyacetic acid is exceptionally effective against *Campylobacter* primarily because it acts as a high-level oxidizing agent that physically dismantles the bacteria's cellular structure. Unlike some traditional disinfectants, peroxyacetic acid remains potent even in the presence of the organic matter typically found in poultry processing environments [129]. Monocaprin emulsions are also highly effective against *Campylobacter*, during various stages of poultry production. Monocaprin (the 1-monoglyceride of capric acid) is a natural lipid that acts as a potent microbicidal agent, causing rapid death of the bacteria by disrupting their cell membranes [140]. *Campylobacter* reductions by 2.0–2.7 log₁₀ have been reported by immersing contaminated chicken parts in a 0.5% monocaprin emulsion for one minute [104].

In this data partition, the between-study heterogeneity was relatively low ($I^2 = 35.2\%$), and the inclusion of moderators explained 69% (R^2) of such variability. As often seen in meta-regressions, some residual variability remained unexplained (Q_E test, $p < 0.001$), suggesting that the solution pH, temperature and time of contact could have been important moderators. However, their reporting was limited in several studies. In addition, the model revealed evidence of publication bias ($p = 0.004$; Table 4), suggesting that some studies using organic acids may not have been published, in particular if they failed to prove a significant action against *Campylobacter*; however, this conclusion should be interpreted with caution.

3.5.3. Plant Extract Meta-Regression Model

The meta-regression model presented in this section identified the moderators having an impact on the effectiveness of plant extracts as inhibitors of *Campylobacter*. Sample weight and storage time were positively correlated with the effectiveness of plant extracts.

This indicates that the larger the amount of sample analysed, the higher the *Campylobacter* reduction estimate ($p < 0.001$), which is likely to stem from the higher certainty about the measurement. In the case of storage time, although there is no statistical significance ($p = 0.213$), the positive coefficient would suggest a trend of a progressively greater reduction in *Campylobacter* in poultry treated with extracts over time. This can be appreciated in the bubble plot of storage time versus *Campylobacter* reduction effect shown in Figure 4 (bottom, right). Greater reductions in time can be seen for carvacrol and cinnamaldehyde when treated poultry meat is stored for more than 60 h.

Even though, the compounds derived from the plant extracts were all highly significant ($p < 0.001$), their efficacy varied according to concentration applied, as shown in Table 4 and Figure 4 (bottom left). The treatment with increasing concentrations of carvacrol (0.074 ± 0.006) and cinnamaldehyde (0.051 ± 0.004) resulted in a greater *Campylobacter* reduction effect, followed by linalool (0.031 ± 0.008). Despite being used in combination, increasing concentrations of carvacrol and gum-arabic had the lowest reduction effect (0.002 ± 0.001) among all compounds. On the other hand, the mixture of carvacrol and chitosan had a greater inhibition effect (0.030 ± 0.002) than chitosan alone (0.012 ± 0.003), but a lower effect than carvacrol alone. The impact of sanitiser concentration on the effectiveness of the treatment is shown as a bubble plot in Figure 4 (bottom left), where the greater inhibitory effects can be appreciated for carvacrol and cinnamaldehyde.

Both carvacrol and cinnamaldehyde are lipophilic, which allows them to cross and destabilise the lipid membrane of *Campylobacter*. While cinnamaldehyde interacts directly with proteins and lipids of the cell wall, compromising the structural integrity of the bacterium; carvacrol binds to the membrane, increasing its permeability and causing cell depolarisation. This results in the leakage of vital components, such as ions and ATP, leading to bacterial death [141].

In this meta-regression, the intra-class correlation of 44% implies moderate between-study heterogeneity. The moderators retained accounted for 78% of the total variability between studies (R^2), although, as usual, some unexplained variability remained (Q_E test, $p < 0.001$). No publication bias was signalled in this meta-regression ($p = 0.082$), which supports the robustness of the model estimates.

3.5.4. Freezing and Colder Climate Meta-Analysis Models

For the freezing data partition, a simple fixed-effects model was adjusted for poultry carcasses to a very limited dataset ($n = 4$) that did not contain moderators. Nonetheless the *Campylobacter* mean $\log_{10}$ reduction due to freezing was significant and estimated at $0.669 \pm 0.100 \log_{10}$ ($p < 0.001$). There was no quantifiable between-study variability, nor likelihood of publication bias ($p = 0.462$; Table 4).

The freezing process and, especially, the thawing process generate free radicals (such as superoxides). *Campylobacter* is particularly sensitive to oxidative stress, which damages its DNA and cellular structures, hindering its recovery after freezing [142].

Although not an intervention strategy per se but a risk factor, the effect of processing poultry in autumn and winter (defined as colder climates) on the concentration of *Campylobacter* of caecal contents could be assessed. Fourteen observations were available for this analysis, and the absence of moderators led us to the choice of a random-effects meta-analysis model. Although not statistically significant ($p = 0.159$), there is a trend that slaughterhouses in colder climates could positively influence the reduction in *Campylobacter* infection (caecal contents) (1.947 ± 1.384 ; Table 4).

The test for small study effects indicates evidence of publication bias ($p < 0.001$), likely to arise from the fact that only two studies related to colder climates were available for the model.

Unlike other bacteria that only enter a hibernation state, *Campylobacter* is a fragile bacterium outside its host, and has a natural sensitivity against low temperatures. *Campylobacter* lacks certain cold shock proteins (CspA homologs), which explains its inability to grow below 30 °C [136]. On the other hand, *Campylobacter* survives better in the cold than at ambient temperature. In humid and chilled conditions, it can remain viable in chicken meat for weeks. The prolonged survival at 4 °C compared to 20–25 °C is due to a reduced metabolic rate and protection against oxidative stress [143–145].

3.6. Summarisation of the Effect of Processing Stages and Interventions on the Prevalence of *Campylobacter* in Poultry

Table 5 presents the overall effects of each processing stage and intervention in poultry slaughterhouses on *Campylobacter* prevalence across all included studies. The amount of extracted data available for prevalence of *Campylobacter* in pre-stage/non-intervened (control) and post-stage/intervened (treated) groups were much lower (Table 5) than those available for concentrations (Table 3). This is due to the fact that, unlike other pathogens such as *Salmonella* and *Listeria monocytogenes*, analysis of *Campylobacter* in poultry caecal contents, carcasses and meat is performed through quantification. Furthermore, all the experiments assessing intervention strategies are conducted on spiked samples, where their effectiveness is measured by quantitative analysis.

Table 5. Overall effects of slaughter processing stages and interventions on the prevalence of *Campylobacter* in poultry, showing heterogeneity analysis of the meta-regression.

Stages and Interventions	Log of Risk Ratio (Control Group/Treated Group)			Heterogeneity Analysis
	Estimate [SE]	<i>p</i> -Value	n/N	
<i>Stages</i>				
Neck cutting and bleeding	−0.144 [0.086]	0.094	34/5	I ² = 3.8% <i>p</i> (Q) < 0.001 R ² = 20.2% τres ² = 0.0309
Scalding	0.019 [0.101]	0.847	14/5	
Plucking	0.085 [0.060]	0.150	38/16	
Evisceration	−0.071 [0.050]	0.152	75/19	
Carcass rinsing with water	0.028 [0.063]	0.646	24/12	
Chilling	0.082 [0.038]	0.032	96/31	
Packaging and cold storage	−0.076 [0.093]	0.415	9/4	
<i>Interventions</i>				
Colder climate	0.045 [0.137]	0.741	8/2	Pub. bias <i>p</i> = 0.619
Freezing	0.358 [0.269]	0.462	7/3	
Organic acids (carcasses/meat cuts)	1.079 [0.422]	0.009	7/2	
Chemical sanitisation of carcasses	0.128 [0.264]	0.627	5/2	

In comparison to the concentration data, fewer processing stages and intervention strategies were represented by prevalence data, namely: neck cutting and bleeding, scalding, plucking, evisceration, carcass rinsing with water, chilling, packaging and storage, colder climate, freezing, organic acids and chemical sanitisation of carcasses. Taking together all data in a single meta-regression, the stage of neck cutting and bleeding (−0.144 ± 0.086; $p = 0.094$) was found to increase by 15% the prevalence of *Campylobacter* in slaughter groups of birds. Although the effect of chilling is significant ($p = 0.032$), it has a limited effectiveness in reducing *Campylobacter* prevalence (mean 8% and 95% CI: 1–17%; calculated from log RR of 0.082 ± 0.038). Plucking may also have a significant action in reducing the proportion of *Campylobacter*-contaminated birds in slaughter groups, although contra-

dictory results prevented to reach statistical significance ($p = 0.150$). Among the intervention strategies assessed, only the application of organic acids in carcasses and meat cuts was found to significantly reduce the prevalence of *Campylobacter* approximately by a factor of 3 (1.079 ± 0.422 ; $p = 0.009$), although this overall estimate was calculated with basis on only two primary studies. In contrast, the remaining interventions, such as colder climate, freezing, and chemical sanitisation, although they showed a positive effect, did not achieve statistical significance ($p > 0.05$).

In terms of between-study heterogeneity, this meta-analysis showed that, despite a very low ($I^2 = 3.8\%$) between-study heterogeneity was present, the moderators accounted for about 20% of the variability between studies (R^2). The low heterogeneity between effect sizes can be in the six-sigma radial plot (Figure 5, top), which shows that the studies occupy almost the full range ($x_i = 0.8$ to 4.2), reflecting high precision and the presence of only a few outliers. The overall consistency among the effects is maintained, as most of the z_i values fall within the limits of $<-2; +2>$. Additionally, the test for small study effects in this global meta-regression show no evidence of publication bias ($p = 0.619$; Table 5), which can be visually appreciated by the symmetry of the funnel plot (Figure 5, bottom).

3.7. Multilevel Meta-Analysis on the Effects of Processing Stages on the Prevalence of *Campylobacter* in Poultry

To estimate the effect of each processing stage and intervention on *Campylobacter* prevalence, multilevel random-effects meta-regression models were fitted individually by each moderator, and the results are illustrated in Table 6 and described in detail below.

3.7.1. Neck Cutting and Bleeding Stage Meta-Regression Model

According to this meta-regression, neck cutting and bleeding was found to increase the proportion of *Campylobacter* in slaughter birds, only when the pathogen was quantified in cloaca (-0.726 ± 0.155) ($p < 0.001$; Table 6). When samples were obtained from breast feathers and neck skin, the prevalence of *Campylobacter* in both groups were not significantly different ($p > 0.1$).

Regarding between-study heterogeneity, this meta-regression model indicated a very low intraclass correlation ($I^2 = 8.5\%$), with sample type accounting for 27% of the variability between studies (R^2) in the significant moderators from the full model. The small study test did not reveal existence of publication bias, as indicated by the p -value of 0.905 (Table 6).

3.7.2. Plucking Stage Meta-Regression Model

According to the meta-regression, the plucking stage yielded a reduction in the prevalence of *Campylobacter*, only when quantified by swabs (0.379 ± 0.098 ; $p < 0.001$) or by the carcass rinse method (0.635 ± 0.085 ; $p < 0.001$). However, the negative and significant coefficient for sample weight (-0.002 ± 0.001 ; $p < 0.001$) suggests that the apparent efficacy of plucking may be influenced by methodological factors since for higher sample weight, the measured estimates fade out. Probably for being sites of greater dirt accumulation, samples taken from breast skin and neck skin did not reveal any effect of plucking ($p > 0.10$).

Although the variability in effect size across studies was low ($I^2 = 7.4\%$), still the two included moderators explained 65% of the variance (R^2). Nonetheless, some caution must be taken in the interpretation of the estimates, as the current dataset likely suffers from publication bias ($p = 0.024$). It is likely that studies that failed to show a significant effect of plucking in the prevalence of slaughtered birds remained unpublished. It is noteworthy to mention that the current results for plucking contain data only for defeathering without the aid of chemical sanitisers. So it is probable that plucking does not have any positive effect on the prevalence of *Campylobacter* in groups of poultry.

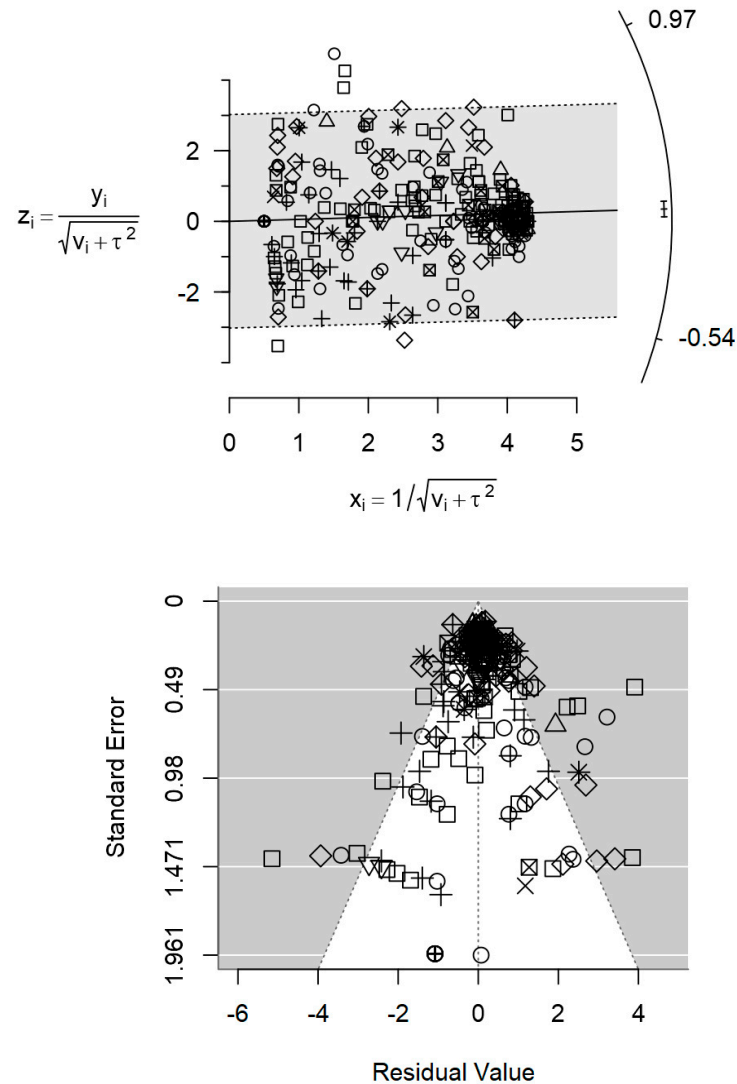


Figure 5. Six-sigma radial plot (top) and funnel plot (bottom) of the overall meta-regression on the effect of slaughter processing stages and interventions on the prevalence of *Campylobacter* in poultry. Markers symbolise interventions: € = Evisceration, ○ = Freezing, Δ = Neck cutting and bleeding, + = Packaging, × = Plucking, ◇ = Scalding, ▼ = Washing, ⊠ = Chemical sanitisation, * = Colder climates, ⊕ = Organic acids.

In fact, defeathering is considered one of the most critical points for increasing the prevalence and bacterial load of *Campylobacter* during poultry processing. Although prior scalding can reduce some of the surface load, mechanical defeathering often reverses this effect because the pressure exerted by the rubber “fingers” of the defeathering machines on the birds’ bodies can force the expulsion of intestinal and cloacal contents, which are often highly loaded with *Campylobacter*. This material spreads rapidly across the skin of multiple carcasses [146].

Table 6. Best-fit multilevel meta-analysis models describing the effect of slaughter processing stages and interventions on the prevalence of *Campylobacter* in poultry. Number of observations (n), heterogeneity analysis and *p*-value of the publication bias test are shown.

Measure (Log Risk Ratio)	Parameter	Estimate	Standard Error	<i>p</i> -Value	n	Heterogeneity Analysis ¹
Neck cutting and bleeding	Sample type					$s^2 = 0.8981$
	Breast feathers	0.005	0.067	0.936	32	$\tau^2 = 0.0837$
	Cloaca	−0.726	0.155	<0.001		$I^2 = 8.5\%$
	Neck skin	−0.010	0.029	0.724		$p(QE) = 0.063$
						$R = 0.274$ Pub. bias $p = 0.905$
Plucking (no added sanitiser)	Sample weight	−0.002	0.001	<0.001	32	$s^2 = 1.2052$
	Sample type					$\tau^2 = 0.0959$
	Breast skin	−0.016	0.036	0.654		$I^2 = 7.4\%$
	Neck skin	−0.017	0.058	0.766		$p(QE) < 0.001$
	Carcass rinse	0.635	0.085	<0.001		$R = 0.653$
	Swabs	0.379	0.098	<0.001		Pub. bias $p = 0.024$
Evisceration (no added sanitiser)	Intercept	0.001	0.013	0.938	71	$s^2 = 0.7301$
	Sample weight	−0.001	0.001	<0.001		$\tau^2 = 0.0440$
	Treatment					$I^2 = 5.6\%$
	Hot water sprayed	0.263	0.121	0.030		$p(QE) = 0.010$
	None	-	-			$R = 0.152$ Pub. bias $p = 0.344$
Sanitising and chilling	Treatment				78	$s^2 = 0.6341$
	Chlorine	0.017	0.045	0.711		$\tau^2 = 0.1160$
	Hot water sprayed	0.074	0.160	0.645		$I^2 = 15.5\%$
	Ice water	0.157	0.070	0.022		$p(QE) < 0.001$
	Salt	−0.142	0.074	0.053		$R = 0.458$
	Peroxyacetic acid	−0.085	0.062	0.175		Pub. bias
	None	0.000	0.026	0.999		$p < 0.001$
	Sample type					
	Neck skin	−0.001	0.030	0.977		
	Rinse	0.231	0.040	<0.001		
Chemical sanitisation of carcasses (all chlorine by immersion)	Swab	0.090	0.033	0.007	5	
	Breast skin	-	-			
	Intercept	0.932	0.890	0.295		$s^2 = 2.2738$
						$\tau^2 = 1.2121$
						$I^2 = 34.8\%$ Pub. bias $p = 0.006$
Organic acids in meat cuts (by spraying peroxyacetic acid)	Intercept	1.049	0.401	0.009	7	$s^2 = 0.2745$
						$\tau^2 = 0.0000$
						$I^2 = 0.00\%$
						Pub. bias $p = 0.138$
Freezing	Intercept	0.372	0.257	0.147	7	$s^2 = 0.5502$ $\tau^2 = 0.1418$ $I^2 = 20.5\%$ Pub. bias

						$p = 0.515$
Colder climates	Intercept	0.029	0.029	0.324	8	$s^2 = 0.3134$
						$\tau^2 = 0.000$
						$I^2 = 0.00\%$
						Pub. bias
						$p = 0.371$

¹ Heterogeneity analysis encompasses within-study variability (s^2), between-study variability (τ^2), and intra-class correlation (I^2) of the null model, and QE test of residual heterogeneity, and square-root of the between-study variability explained by significant moderators (R^2) from the full model.

3.7.3. Evisceration Stage Meta-Regression Model

The observed effect of evisceration on the prevalence of *Campylobacter* in poultry groups was found to be determined by sample weight and type of treatment. The spray of hot water significantly increase the odds of reducing the prevalence of *Campylobacter* in poultry carcasses (0.263 ± 0.121 ; $p = 0.030$). Nonetheless, as found in plucking, the extent of the prevalence reduction effect depends on sample weight, with higher amounts of sample analysed leading to lower estimates.

It is likely that the effectiveness of hot water spray against *Campylobacter* during evisceration is highly variable, not only because of the temperature and exposure time applied, but also because the pathogen has the ability to lodge in skin follicles and folds [58]. Even the decontamination tends to be more pronounced in poultry carcasses having high initial contamination levels [146].

Evisceration was one of the stages for which extensive prevalence data were available ($n = 71$). Despite the many publication sources, the between-study heterogeneity was nearly negligible ($I^2 = 5.6\%$), and still the two moderators jointly accounted for 15% of the variability between studies (R^2). Furthermore, the small study test did not show evidence of publication bias ($p = 0.344$; Table 6), which supports the reliability in the meta-regression estimates.

3.7.4. Sanitising and Chilling Meta-Regression Model

This particular meta-regression was based on the highest number of prevalence observations available ($n = 78$). Despite the multiple primary sources, the between-study variability was relatively low (15.5%), yet two study characteristics—chemical applied and sample type—were able to explain 45.8% of the between-study variability.

Among all the treatments available (chlorine, hot water sprayed, ice water, salt and peroxyacetic acid), only rinsing carcasses with ice water (0.157 ± 0.070 ; $p = 0.022$) was proven to reduce the proportion of contaminated poultry carcasses after 18–24 h chilling. The other treatments did not reach statistical significance. Greater reductions in the prevalence of *Campylobacter* due to sanitizing and chilling were estimated when the microbial enumeration was carried out in carcass rinses (0.231 ± 0.040 ; $p < 0.001$), followed by swabbed carcasses (0.090 ± 0.033 ; $p = 0.007$) and breast skin (baseline). As earlier found in the plucking meta-regression, when samples are taken from the neck skin (and even breast skin), there is lower odds of arriving to the conclusion that chilling effectively decreases the prevalence of *Campylobacter* in poultry carcasses.

This happens because bird skin has feather follicles and deep crevices that serve as safe havens for bacteria. During scalding and plucking, these follicles open, allowing for *Campylobacter* to enter. Furthermore, subsequent cooling can cause the follicles to close, therefore trapping the bacteria and protecting them from cleaning agents and environmental stress. Furthermore, *Campylobacter* is extremely sensitive to desiccation. The skin, especially on the neck, retains layers of water and organic matter that maintain the moist environment necessary for its survival, even when the outer surface appears cold [50,83].

Regarding between-study heterogeneity, the model revealed a low I^2 value (15.5%), with 46% of the variability across studies (R^2) being explained by the two moderators. Nevertheless, some variability remains unexplained by the model (Q_E test, $p < 0.001$). Furthermore, the small study test revealed publication bias, as assessed by the value ($p < 0.001$; Table 6), suggesting that some small studies on the effectiveness of chilling on the *Campylobacter* prevalence in poultry carcasses may have remained unpublished due to non-significant outcomes; however, some caution should be taken when interpreting this conclusion.

3.8. Multilevel Meta-Analysis on the Effects of Interventions on the Prevalence of *Campylobacter* in Poultry

Unlike the data available for intervention strategies based on *Campylobacter* concentration, the data available for prevalence was very limited and no study characteristic could be coded to allow for the adjustment of meta-regressions. Thus, for the *Campylobacter* prevalence data on interventions (e.g., chemical sanitisation of carcasses, use of organic acids, freezing and colder climates), only simple random-effect meta-analysis models (without moderators) could be fitted (Table 6).

According to these models, neither immersing poultry carcasses in chlorine solution ($p = 0.295$) nor slaughtering poultry in colder climates ($p = 0.324$) could be demonstrated to reduce the prevalence of *Campylobacter* in poultry meat. However, the strategy of spraying peroxyacetic acid in meat cuts can decrease the *Campylobacter* prevalence by a factor of ~ 3 (1.049 ± 0.401 ; $p = 0.009$). This finding is in line with the previous meta-regression output (Section 3.5.2) that purports peroxyacetic acid as the most effective sanitising solution to decrease *Campylobacter* population in poultry carcasses and meat.

In the case of poultry meat freezing, at a p -value of 0.147, there is a trend of freezing of decreasing the probability of recovery of *Campylobacter* in thawed carcasses. Freezing and thawing would decrease such probability by half (0.372 ± 0.257). The action of freezing and cold temperatures on the viability of *Campylobacter* has been discussed in Section 3.5.4.

Regarding between-study heterogeneity, the intra-class correlation values indicated from negligible to relatively low between-study variability ($I^2 = 0.00$ –34.8%). The small study test detected publication bias for the chemical sanitisation intervention by immersion in chlorine ($p = 0.006$; Table 6), likely to arise from the availability of only 2 studies furnishing data for this meta-analysis; therefore, the corresponding meta-analysed outcome must be interpreted with caution.

3.9. Multilevel Meta-Analysis on the Prevalence of *Campylobacter* in the Slaughterhouse Environment

Table 7 compiles the pooled prevalence of *Campylobacter* in slaughterhouse environmental elements by processing areas, including also in live animals cloaca and carcass caecal contents, for comparison. To pinpoint the main elements playing a role in the spread of *Campylobacter* in poultry slaughterhouses, a multilevel random-effects meta-analysis was conducted. Environmental sources included processing equipment and areas such as the entrance, scalding and plucking, evisceration, and chilling, as well as operator and other elements.

Overall, the pooled findings on *Campylobacter* prevalence indicated that all evaluated environmental sources represent the risk of contamination to be considered in slaughterhouses; however, incidence rates vary widely. Among all sources, the highest incidence, with a 95% confidence interval (CI) of 83% (76.6–88.5%), was found at the entrance to slaughterhouses, particularly in transport crates. Operators showed a prevalence of *Campylobacter* on hands/gloves of 73% (95% CI: 40.4–91.7), the second-highest contamination

rate. This was followed by evisceration equipment at 68% (95% CI: 43.4–85.2) and miscellaneous items at 67% (95% CI: 52.1–80.1). Miscellaneous refers to environmental samples collected from the floor, table surfaces, and vent cutters. On the contrary, the lowest prevalence was observed in the chill tank at 23% (95% CI: 6.7–54.2). Interestingly, the pooled *Campylobacter* prevalence in caecal contents from eviscerated carcasses was lower (26.5%; 95% CI: 4.3–74.2) than in most of the environmental elements.

Table 7. Pooled prevalence of *Campylobacter* sampled from live animals and environmental elements in poultry slaughterhouses, showing heterogeneity analysis.

Processing Area Source	Pooled Prevalence	95% CI	n/N	Heterogeneity Analysis
Entrance				
Live animals (cloaca)	0.384	[0.205–0.602]	8/5	I ² = 37% <i>p</i> (Q) < 0.001
Transport crates	0.833	[0.765–0.885]	4/2	
Scalding and plucking area				
Scalding tank	0.320	[0.160–0.537]	5/3	R ² = 13.6% τ _{res} ² = 3.852
Plucker	0.422	[0.136–0.773]	2/1	
Evisceration area				
Caecal	0.265	[0.043–0.742]	19/5	
Eviscerator	0.678	[0.434–0.852]	6/2	
Chilling room				
Chill tank	0.226	[0.067–0.542]	15/3	Publication bias
Unknown				
Miscellaneous	0.677	[0.521–0.801]	6/2	<i>p</i> = 0.619
Operators	0.732	[0.404–0.917]	8/2	

Several studies on poultry slaughterhouses have reported higher levels of *Campylobacter* due to cross-contamination of carcasses by equipment. Indeed, transport crates are recognised as a major source of pathogen introduction into processing plants, even after cleaning and disinfection [17,147,148]. Additionally, a risk analysis study highlighted environmental surfaces, evisceration machines, and workers' hands as key points for the transmission of *Campylobacter* along the broiler slaughter [149]. Although chilling may reduce bacterial loads, water tanks can also facilitate bacterial transfer between carcasses due to inadequate hygiene [150]. Regarding between-study heterogeneity, the pooled prevalence data displayed a relatively low intraclass correlation ($I^2 = 37\%$), with sources accounting for 14% of the variability between studies (R^2). The relatively low heterogeneity was corroborated by the radial plot (Figure 6, top) which shows symmetry in the distribution of points and absence of extreme points. Likewise, the symmetrical distribution of the effect sizes in the funnel plot (Figure 6, bottom) is in agreement with the non-significant p -value of 0.619 (Table 7), both indicating the absence of publication bias.

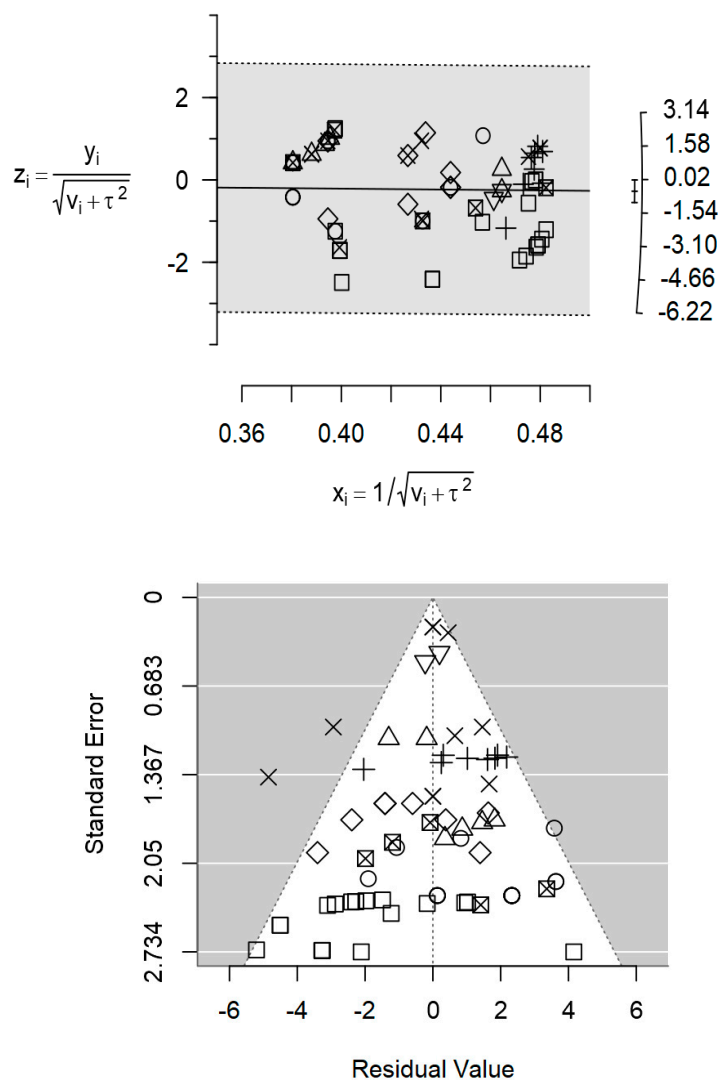


Figure 6. Six-sigma radial plot (**top**) and funnel plot (**bottom**) of the meta-analysis of prevalence of *Campylobacter* sampled from live animals and environmental elements in poultry slaughterhouses. Markers symbolise sample sources: € = Caecal contents, o = Chill tank, Δ = Eviscerator, + = Live animals (cloaca), × = Miscellaneous elements, ◇ = Operators, ▼ = Pluckers, ☒ = Scalding tank.

4. Discussion

4.1. Effectiveness of Processing Stages in the Reduction in *Campylobacter* on Poultry

The general results of this meta-analysis indicate that the effectiveness of processing stages varies in terms of *Campylobacter* concentration and prevalence. Based on the overall meta-analysis, *Campylobacter* contamination in poultry carcasses increases significantly during cutting/bleeding, as reflected in the average values of bacterial load and prevalence. On the contrary, when evaluated with moderators, the effect of cutting/bleeding is determined by sample weight. Larger quantities allow for more accurate measurement and a higher likelihood of detecting *Campylobacter*, potentially leading to greater estimated reductions. Furthermore, the results showed that the sample type also determined this reduction. Greater reductions were observed in the carcasses rinse, whereas the neck skin and the cloaca showed a tendency toward increased counts and prevalence during cutting/bleeding. These findings are consistent with previous observational studies,

which suggested cross-contamination during this process [151], likely due to the use of automated equipment; consequently, carcass contamination may arise from the accumulation of organic debris on equipment or from inadequate cleaning of environmental elements workers' hands in slaughterhouses [12,152].

After bleeding, the carcasses are scalded in hot water, typically between 50 and 65°C, depending on the type of slaughterhouse. This process loosens the feathers, making the subsequent plucking stage easier [12,153]. At the same time, hot water helps eliminate microorganisms from the carcass surface, thereby reducing the bacterial population [16,154].

The overall decrease in *Campylobacter* counts observed in this meta-analysis underscores the effectiveness of the scalding process in reducing bacterial load. The reason for this reduction is that *Campylobacter* is heat-sensitive, meaning roughly 1 log₁₀ may drop at temperatures above 52 °C [155,156]. This suggests that the success of this process relies heavily on maintaining the temperature of the scalding water, as outlined in previous studies [17,157,158].

The most significant reduction in bacterial load of *Campylobacter* observed in the carcass rinse compared to neck skin and scalding water suggests that the scalding water's high temperature effectively removed a large portion of the bacterial population from the carcasses, preventing its accumulation in the water [153]. This also explains the low recovery of bacteria from hot water. In contrast, the limited reduction observed on the neck skin is probably due to its characteristic structure, which consists of folds and crevices that tend to accumulate residual organic matter such as fat, feathers, mucus, and faeces during the scalding process [127], which may physically protect *Campylobacter* from heat and water flow [127,153].

Similar findings were found with other investigators [159]. In that work, the authors observed *C. jejuni* counts of 1.39 ± 0.70 log₁₀ CFU in the scalding water and 2.93 ± 0.31 log₁₀ CFU in the carcasses after scalding at a pH of 8.0. They concluded that, in addition to maintaining high water temperatures, it is essential to supply clean water to dirty scalding tanks to minimise *C. jejuni* contamination in the scalding water. This step is critical to prevent cross-contamination of carcasses, a common issue during scalding [153,158,159].

As mentioned above, scalding is a critical step in preparing poultry for plucking; Plucking is the removal of feathers from poultry carcasses [12]. As this process involves mechanised equipment, the risk of cross-contamination between carcasses is also to be expected [12]. This may explain the 42% prevalence (95% CI: 13.6–77.3) detected on the equipment (plucker) in this study. One major contributing factor is worn or cracked rubber fingers, which may allow for bacteria to penetrate surfaces where practices such as cleaning and disinfection are inadequate [12]. Furthermore, bacteria can be transferred to birds and enter the feather follicles during removal, before the openings are sealed [12,160].

Another factor contributing to cross-contamination is the escape of small amounts of intestinal contents from the cloaca, as noted in an experimental study conducted in laboratory processing facilities [161]. That study reported a significant increase in contamination prevalence, from 0.8% to 79%, with bacterial counts ranging from 2.9 to 3 log₁₀ CFU on the breast skin of naturally contaminated chickens before and after plucking [161]. Similar to previous studies [27], the present meta-analysis found a substantial increase in the prevalence of *Campylobacter*, particularly on breast and neck skin when sanitiser was not applied; however, the difference was not statistically significant. In contrast, studies examining *Campylobacter* concentrations during the plucking process observed a decrease in the microbial population, particularly when sodium hypochlorite was used to sanitise the equipment. Therefore, to minimise cross-contamination during plucking, it is essential to ensure the complete removal of faecal material from carcasses and proper sanitisation of

equipment [20,26]. While decontamination after plucking and before evisceration may not be entirely practical, it can help reduce contamination levels.

Evisceration is the process of removing the internal organs from a poultry carcass [12]. This stage is considered the most critical in terms of contamination risk, as highlighted in various studies [17,27,162]. This is often observed when the viscera of colonised birds rupture during the removal of intestinal contents, thus allowing for faeces to and contaminate the carcasses [157]. However, the risk of contamination during evisceration may be less significant if the gastrointestinal contents of the slaughtered birds are less contaminated [17]. Despite all this, the results of this meta-analysis show that significant reductions in *Campylobacter* concentrations and prevalence can be achieved when chemical sanitisers or hot water spray are used as interventions during the carcass evisceration process. The higher prevalence of *Campylobacter* found in the eviscerator compared to the caecal content in the evisceration area indicates that special attention should be paid during this process to ensure the safety of the final product.

After evisceration, carcasses are typically washed to eliminate debris, loose tissue, residual blood, and some microorganisms [12]. Washing with water is one of the physical interventions used to decontaminate carcasses during the poultry slaughtering process [20]. However, the effectiveness of this process depends on several factors, including water temperature, pressure, nozzle type and arrangement, line speed, and the use of chemical additives, with chlorine being the most widely used in the processing industry [12,118,163]. The studies included in this meta-analysis generally demonstrated that washing poultry carcasses with water by immersion helps lower levels of both the prevalence and counts of *Campylobacter*. This finding confirms previous reports from other meta-analyses, which showed reductions in *Campylobacter* levels after carcass washing [25,27,157].

The final stage of processing in poultry slaughterhouses is rapid chilling until 4 °C, a critical step that reduces microbial growth, improves carcass appearance, and increases overall shelf life before packaging or distribution to consumers [12]. Overall, the studies included in this meta-analysis showed reductions in both counts and prevalence of *Campylobacter*, suggesting that sanitisation/chilling is a valuable approach to suppress microbial growth. Interestingly, for reductions in concentrations, a greater overall effect was observed when acidified sodium chlorine and peroxyacetic acid were used as treatment. In contrast, for reductions in prevalence levels, a greater effect was observed in treatment with ice water. Leone et al. in their systematic review reported similar results [28]. In that study, comparing post-chill interventions, they found significant reductions in populations after treatment with chlorine immersion, peroxyacetic acid immersion, and peroxyacetic spraying. Nonetheless, the effectiveness of peroxyacetic acid could not be proven at prevalence level, as data could be obtained from only two studies.

Additionally, in this meta-analysis the chilling process led to higher *Campylobacter* reductions when samples were obtained by rinsing carcasses than other samples, such as breast and neck skin. This suggests a high microbial load on the neck skin, which is encountered by many authors who report that, after chilling, the neck skin was more contaminated than other areas of the skin and the entire rinsed carcass [125,164,165]. In addition, given that poultry neck skin consists of multiple folds and follicles where bacteria lodge, physical or temperature-based interventions, such as cooling, would appear to be less effective than on smooth/or rinsed surfaces. This is because chilling with cold air dries the skin, leading to stronger binding of bacteria to the skin surface, whereas rinsing only removes the loosely adherent fraction of bacteria from the skin [125].

Although relatively moderate, the prevalence observed in the chilling tank in this meta-analysis was expected. This is because during chilling, cross-contamination occurs through the transfer of bacteria population from carcasses into the tank, with subsequent

acquisition by other carcasses [150]. Therefore, chemical additives such as chlorine should be added to the cooler water, particularly in countries where legislation permits the addition of chemical additives during the processing of poultry carcasses, as they can reduce cross-contamination by killing bacteria suspended in the water [163,166].

Additionally, the positive effects on packaging and cold storage as well as regular cleaning and disinfection identified in this meta-analysis suggest that improving hygiene management through standardised procedures and cleaning slaughter equipment can substantially reduce pathogen levels in the facilities, thereby helping to minimise the occurrence of foodborne illnesses.

4.2. Effectiveness of Slaughter-Level Interventions in the Reduction in *Campylobacter* on Poultry

Although processing stages such as scalding, washing, and chilling tend to reduce bacterial populations in carcasses, their effectiveness is partial; therefore, depending specifically on governmental regulation, additional interventions such as chemical substances, are applied during and after slaughter to reduce the number and/or prevalence of pathogenic microorganisms [20,167].

In general terms, the antimicrobial activity of chemical substances used in poultry processing is based on the destruction of cell membranes, other cell constituents and physiological processes [20,132]. These mechanisms are particularly relevant for *Campylobacter* spp., which have a structurally thin and fragile Gram-negative cell wall, an outer membrane that is very sensitive to oxidation, and low stress tolerance, all of which contribute to their high sensitivity to these substances [168,169].

In this meta-analysis, interventions targeting *Campylobacter* on poultry at the slaughter level, were grouped into chemical sanitisation, organic acid, plant extracts, cold versus warm climate, as well as freezing. Overall, all interventions were effective in reducing *Campylobacter* concentrations. For prevalence outcomes, only organic acid had a significant effect against *Campylobacter*. However, plant extracts, chemical sanitisation, colder climate, and freezing did not reach statistical significance effect. Nevertheless, the results revealed that treatment efficacy varies across interventions and may also be influenced by other factors, including sanitiser type, concentration applied, method of application, and the quantified sample amount.

As can be seen, the chemical sanitisers included in this meta-analysis are largely chlorine-based. Chlorine-based sanitisers are commonly used to reduce the microbial load on the surface of meat and poultry and to sanitise food contact surfaces [132,170]. To note, the effectiveness of chlorine-based sanitisers depends on various factors such as, temperature, pH, concentration, and the amount of organic material present [132,171].

As chlorine-based compounds act by oxidation, their effectiveness on poultry carcasses is generally limited because the organic material on the skin quickly neutralises the active chlorine. As a result, their antimicrobial effect against *Campylobacter* is usually lower and less consistent under commercial processing conditions [9,132,167].

In this meta-analysis, among the chemical sanitisation moderators, sodium hypochlorite proved more efficient against *Campylobacter*, while chlorine was the least. This was to be expected, since, compared to sodium hypochlorite and chlorine dioxide, chlorine is more affected by pH and organic material, thereby reducing its effectiveness [130]. The results presented here disagree with those reported previously, demonstrating that spray washing by sodium hypochlorite was less effective than electrolyzed oxidizing water in reducing total aerobic bacteria, *E. coli*, *Campylobacter*, and *Salmonella* levels [172]. In addition, a more recent study found that sodium hypochlorite was less effective at reducing the enumeration of total viable microorganisms and *Campylobacter*, as well as lowering the proportion of *Salmonella*, compared to peroxyacetic acid and acidified sodium chlorine in carcasses washed inside and outside [173].

Moreover, among the methods of application in this meta-analysis, immersion was more effective than spraying. These findings are consistent with those reported by other researchers [138]. In that study, evaluating immersion and spraying applications for reducing *C. jejuni* on chicken wings, they found reductions of 0.5 to 1.2 log₁₀ with spraying. In contrast, immersion reduced initial pathogen levels by 1.7 to 2.2 log₁₀.

Additionally, the results from this meta-analysis showed that increasing the concentrations of chemical sanitiser significantly improved the treatment's effectiveness. However, the greater effectiveness observed for chlorine rather than cetylpyridinium chloride, sodium hypochlorite, and trisodium phosphate for each 1 g/kg increase is unclear, since the last three sanitisers have greater antimicrobial activity than chlorine [132,174]. Furthermore, other studies indicate that cetylpyridinium chloride and trisodium phosphate are more effective, with reductions that are relevant from a public health perspective. For instance, concentrations of 0.5% cetylpyridinium chloride achieved reductions of *C. jejuni* of 1.5 and 4.2 log₁₀ on inoculated chicken skin [175,176]. On the other hand, 10% trisodium phosphate reduced *Campylobacter* counts on chicken skin by 1.63 and 2.3 log₁₀ [175,177]. Likewise, among the chemical treatments evaluated in the meta-analysis by Dogan and colleagues, cetylpyridinium chloride, acidified sodium chlorine, and trisodium phosphate produced higher estimates, with a combined average reduction of 1.9 log₁₀ [27].

The results related to chemical sanitiser also indicate that the treatment was more effective when *Campylobacter* was quantified on the carcass rather than in the cloaca. This result aligns with findings from our previous meta-analysis [31], which assessed the effectiveness of chemical treatments against *Campylobacter* in litter, feed, and caecal content. Our analysis indicated that chemical agents tend to be more effective on surfaces where *Campylobacter* is more exposed, rather than in the cecum, where the intestinal mucosa offers protection. This may also explain the higher effectiveness of chemical sanitisers on carcasses than in the cloaca.

In this meta-analysis, the interventions related to organic acids include acetic acid, citric acid, lactic acid, monocalcium emulsion, and peroxyacetic acid. Among them, peroxyacetic acid was the most effective in reducing the enumeration and prevalence of *Campylobacter*. In contrast, acetic acid was less effective in suppressing microbial growth. These findings are consistent with previous meta-analyses, which reported greater reductions in both counts and prevalence of *Campylobacter* after using peroxyacetic acid treatment [162,178,179].

To note, the inactivation mechanism of organic acids against *Campylobacter* involves penetration into bacterial cells in their undissociated form and reduction in intracellular pH [180,181]. This acidification disrupts enzyme activity, interferes with nutrient transport, and causes metabolic failure [182]. Since *Campylobacter* is sensitive to acid stress, its effectiveness varies depending on the type of acid, contact conditions, microorganism species, contamination level, concentration, pH, part/location of the carcass or meat to which it is applied, and mode of application [128]. For example, in this meta-analysis, it was found that across sample types, treatment efficacy was higher when *Campylobacter* was quantified in wings and lower when quantified in drumsticks. However, this observation should be interpreted with caution, as it may be due to several factors, including methodological variability among the included studies and/or the morphology of the carcass parts, treatment contact conditions, or contamination levels.

While most chemicals used to treat meat typically do not leave residue, there are concerns about potential oxidation products in the final product. Therefore, in response to concerns about conventional chemicals, several investigators [23,24,183–188] have extensively investigated plant extracts and essential oils, and their derived compounds, for controlling foodborne pathogens, including *Campylobacter*. These alternatives have shown promising potential to reduce *Campylobacter* contamination and enhance poultry safety.

It is important to note that all data used in this meta-analysis and related to the efficacy of plant extracts, essential oils, or their compounds against *Campylobacter* were obtained under laboratory conditions following artificial contamination of the microorganism on the neck skin or other parts of the carcass and/or meat.

The results from this meta-analysis indicate that although all plant extracts tested have a reducing effect, carvacrol compound had greater effectiveness in reducing *Campylobacter* concentrations. In contrast, carvacrol and gum-arabic combined showed little effect. This result can be interpreted in two ways. For example, because carvacrol is lipophilic when applied directly, it will be more readily available to interact with the cell and act directly on the cell membrane, disrupting it [189,190], which may explain the greater effect observed. On the other hand, the reduced effect observed when carvacrol was combined with gum-arabic may be due to decreased availability of the active compound, resulting from encapsulation or delayed release [191]. However, this conclusion should be interpreted with caution, as the 96 observations in this meta-analysis come from only three primary studies; therefore, the results should not be generalised. These results can be compared with those from an experimental study that compared the efficacy of peroxyacetic acid with carvacrol [192]. Peroxyacetic acid with 3.3 log₁₀ on chicken skin and 0.5 to 0.9 log₁₀ on chicken breast resulted in greater efficacy after 48 h and 24 h of refrigerated storage, respectively. At the same time, carvacrol showed reductions, but these could not be maintained after 24 to 48 h. When peroxyacetic acid was combined with carvacrol, efficacy was drastically reduced to 0.4 log₁₀ on chicken breast. The authors attributed this phenomenon to differences in properties and mechanisms of action between the two substances [192].

Meta-analyses carried out on the application of antimicrobial substances showed, either in univariate or multilevel meta-regressions, that concentration, duration treatment or contact time, treatment temperature, and storage time post-treatment have a direct and significant impact on the effectiveness of the antimicrobial substances. These findings should inform plant Standard Operating Procedures (SOPs) and validation studies.

Freezing was evaluated in this meta-analysis as a method to reduce bacterial contamination after poultry carcasses are slaughtered. The results indicated that the freezing process decreased both the concentration and prevalence of *Campylobacter* in poultry carcasses. Similar reductions due to freezing were also found in the meta-analysis by Dogan and colleagues [27]. In that analysis, the authors reported mean reductions of 1.29 log₁₀ (95% CI: 1.10–1.48) and a mean prevalence reduction of 0.12 (95% CI: 0.03–0.54). However, unlike the present meta-analysis, they found high heterogeneity between studies.

Numerous experimental and review studies have also shown that freezing carcasses, neck skin, or poultry parts at temperatures between −25 and −18 °C, followed by long-term storage for 21 to 35 days, can lead to significant reductions in *Campylobacter* levels, typically 0.5 to 3.4 log₁₀ [17,193–196]. The most substantial reductions, approximately 1 log₁₀, are generally observed within the first 24 h of freezing [194,196]. Extending the storage period beyond 3 to 5 weeks yields minimal additional reductions in *Campylobacter* levels, as negligible changes occur afterward [197]. It is important to note that freezing does not completely eliminate the pathogen; therefore, surviving *Campylobacter* cells can persist even after prolonged storage and remain detectable qualitatively and quantitatively [196,197]. However, according to EFSA [9], proper freezing practices for 2–3 weeks can significantly reduce the risk of campylobacteriosis in humans by more than 90%.

The last variable evaluated in this meta-analysis was the effect of climate, which essentially involved comparing reductions in *Campylobacter* levels in poultry carcasses between colder and warmer climates. The results showed that colder climates were associated with reductions in both concentration and prevalence of *Campylobacter*, although the prevalence reduction was not significant. However, this result should be interpreted with

caution, given the small number of studies ($n = 2$), which yielded 14 concentration outcomes and 8 prevalence outcomes. These results support previous reports describing correlations between seasonality, climatic conditions, and the survival and spread of *Campylobacter* [198,199]. Several studies [200–202] have reported a higher incidence of *Campylobacter* on poultry during the spring and summer months, which correspond to temperatures around 42 °C—optimal for growth under laboratory conditions [156] and similar to the body temperature of birds [203]. In contrast, the incidence of *Campylobacter* generally decreases in the autumn and winter months. Rosenquist et al. [204] reported a high incidence of *Campylobacter* (78%) in broiler chickens slaughtered in warm weather, and a lower incidence in colder weather. Similarly, another study evaluating the influence of temperature, humidity, and precipitation on poultry slaughter found a higher prevalence of *Campylobacter* in warmer climates than in colder ones [205]. In that study, the overall prevalence found in positive batches was 63.8% (95% CI 59.6–67.9%, $n = 542$). Each 1 °C increase in average monthly temperature up to 26 °C resulted in a 16.4% increase. In addition, for each 10 mm increase in total monthly precipitation up to 300 mm, positive batches increased significantly by 0.8%. Already, for every 1% increase in relative humidity up to 80%, positive batches increased by 4.2%.

From a “One Health” perspective, the findings of this study are significant for antimicrobial resistance (AMR). Although this study did not directly evaluate resistance profiles, the observed reduction in *Campylobacter* contamination at poultry slaughter could indirectly decrease human exposure to antimicrobial-resistant strains, while responsible management of antibiotics in poultry primary production upstream remains fundamental [205].

5. Conclusions

In conclusion, this meta-analysis emphasised the significant effects of processing stages and interventions on *Campylobacter* contamination in poultry slaughterhouses. The fact that this meta-analysis identified cutting/bleeding and evisceration as the two critical stages for increased concentration and prevalence of *Campylobacter* suggests that special attention should be paid to these stages during poultry carcass processing to control cross-contamination. Nevertheless, this meta-analysis highlighted that *Campylobacter* reductions during these two processing stages can be achieved through chemical interventions, with sodium hypochlorite, acidified sodium chlorine, and peroxyacetic acid being the most promising. On the other hand, scalding, carcass washing, and chilling were highlighted as the leading stages that reduce *Campylobacter* levels, both in concentration and prevalence. In general, for all stages of processing, the greatest reductions in both concentration and prevalence were estimated when samples were obtained by carcass rinses rather than sampling breast and neck skin. Moreover, the interventions/treatments examined in this meta-analysis were generally effective at reducing *Campylobacter* levels in poultry. However, their effectiveness varied according to several factors, including the type of intervention, mode of application, concentration, and the sample measured. For instance, among all sanitisers, sodium hypochlorite was the most effective, while chlorine was the least effective. When comparing application methods, immersion proved to be more effective than spraying. Additionally, treatments were deemed as more effective when *Campylobacter* was quantified from the carcass rather than from the cloaca. Among organic acids, peroxyacetic acid was the most effective at reducing *Campylobacter* enumeration and prevalence, while acetic acid was less effective in suppressing microbial growth. The efficacy of treatments also varied according to the sampled part of the poultry; it was higher when quantifying *Campylobacter* in wings and lower when quantifying in drumsticks. Additionally, among plant extract interventions, carvacrol was the most effective at reducing *Campylobacter* levels, whereas the combination of carvacrol and gum-arabic

had lower effect. Furthermore, freezing and poultry processing in colder climates led to lower concentrations and prevalence of *Campylobacter* in poultry carcasses, although the prevalence reduction did not reach significance in cold climates. In environmental meta-analyses, the highest risk factor for *Campylobacter* was observed at the entrance to slaughterhouses, notably in transport crates, while the lowest prevalence was observed in the chill tank.

Overall, the findings of this meta-analysis can inform decision-making tools and optimisation frameworks, facilitating the data-driven selection of effective intervention strategies in poultry processing. At the same time, given that no single method could fully eliminate *Campylobacter* from poultry carcasses, the results of this meta-analysis also highlight the need for a multi-barrier approach that combines controlled processing steps, effective intervention/treatment measures between processing steps, optimised packaging, and routine cleaning/disinfection to ensure the safety of the final product. High-prevalence environmental nodes (transport crates, operator hands/gloves) underscores the need for targeted hygiene interventions and training.

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