Meta-Analysis on the Effect of Interventions Used in Cattle

Processing Plants to Reduce *Escherichia coli* Contamination

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ABSTRACT

Cattle coming from feedlots to slaughter often harbor pathogenic *E. coli* that can contaminate final meat products. As a result, reducing pathogenic contamination during processing is a main priority. Unfortunately, food safety specialists face challenges when trying to determine optimal intervention strategies from published literature. Plant intervention literature results and methods vary significantly, making it difficult to implement interventions with any degree of certainty in their effectiveness. To create a more robust understanding of plant intervention effectiveness, a formal systematic literature review and meta-analysis was conducted on popular intervention methods. Effect size or intervention effectiveness was measured as raw log reduction, and modeled using study characteristics, such as intervention type, temperature of application, initial microbial concentration, etc. Least-squares means were calculated for intervention effectiveness separately on hide and on carcass surfaces. Heterogeneity between studies (I^2) was assessed and factors influencing intervention effectiveness were identified. Least-squares mean reductions ($\log \text{CFU}/\text{cm}^2$) on carcass surfaces ($n=249$) were 1.44 [95% CI: 0.73 – 2.15] for acetic acid, 2.07 [1.48 – 2.65] for lactic acid, 3.09 [2.46 – 3.73] for steam vacuum, and 1.90 [1.33 – 2.47] for water wash. On hide surfaces ($n=47$), least-squares mean reductions were 2.21 [1.36 – 3.05] for acetic acid, 3.02 [2.16 – 3.88] for lactic acid, 3.66 [2.60 – 4.72] for sodium hydroxide, and 0.08 [-0.94 – 1.11] for water wash. Meta-regressions showed that initial microbial concentrations and timing of extra water washes were the most important predictors of intervention effectiveness. Unexplained variation remained high in carcass, hide, and lactic acid meta-regressions, suggesting that other significant moderators are yet to be identified. The results will allow plant managers and risk assessors to evaluate plant interventions, variation, and factors more effectively.

Keywords: Beef, Cattle, *E. Coli*, Intervention, Meta-analysis, O157

1. Introduction

Shiga-toxin producing *Escherichia coli* (STEC) has been recognized as a serious source of illness since it was first identified in 1982 (CDC, 2015). Young children, the elderly, and immunocompromised individuals are especially susceptible to illness and death from STEC infections (CDC, 2015). An estimated 176,000 U.S. foodborne STEC infections occur annually, with approximately 63,000 due to *E. coli* O157:H7 and 113,000 from non-O157 STEC (Batz, Hoffmann, & Morris, 2012; Scallan, Hoekstra, Angulo, Tauxe, Widdowson, Roy, Jones, & Griffin, 2011). STEC is estimated to cause 1% of food borne illnesses in England and 3% in Scotland. O157 is the predominant STEC organism in both the U.S. and the U.K. Continental Europe generally has a lower outbreak rate than the U.S or U.K., but they are caused by a broader range of STEC organisms (Vanaja, Jandhyala, Mallick, Leong, & Balasubramanian, 2013). In the U.S., thirty-nine percent of O157 infections and 30% of non-O157 STEC infections are linked to beef sources (Painter, Hoekstra, Ayers, Tauxe, Braden, Angulo, & Griffin, 2013).

Consequently, reducing STEC concentration and prevalence in beef is a high priority (Sofos, 2008). Through the implementation of plant hazard analysis critical control point (HACCP) principles, sanitary conditions at cattle processing plants have improved (Ropkins & Beck, 2000; Sofos, 2008). The risk and impact of product contamination has significantly decreased through plant interventions (Antic, Blagojevic, Ducic, Nastasijevic, Mitrovic, & Buncic, 2010; Arthur, Bosilevac, Nou, Shackelford, Wheeler, Kent, Jaroni, Pauling, Allen, & Koohmaraie, 2004; Sheridan, 1998). However, current plant intervention literature provides conflicting results. Some authors, for instance, report very high reductions, such as 5.05 log CFU/cm² for a water wash spray, while others recorded increases in bacterial counts from water

washes on cattle surfaces (Scanga, Buschow, Kauk, Burk, Koohmaraie, De La Zerda, Motlagh, Samadpour, & Koohmaraie, 2011; Yoder, Henning, Mills, Doores, Ostiguy, & Cutter, 2010). These discrepancies among reported intervention effectiveness are found throughout the literature and make it difficult to determine optimal decontamination strategies. It is likely that variations in experimental design (i.e., temperature, surface type, indicator organism, etc.) contribute to these discrepancies.

A systematic literature review coupled with meta-analysis is one method used to address differences between experimental methods and results within a body of literature (O'Connor, Sargeant, & Wang, 2014; Sargeant, Rajic, Read, & Ohlsson, 2006). Reported results, as intervention effectiveness, can be aggregated to provide weighted averages, or summary effects, among similar trials. Summary effects draw from a larger pool of information and therefore, create a more robust estimate of an intervention's effectiveness. When heterogeneity between trials is high, other tools, such as meta-regressions, can be used to explain the differences in intervention effectiveness (O'Connor et al., 2014; Prado-Silva, Cadavez, Gonzales-Barron, Rezende, & Sant'Ana, 2015). Systematic reviews and meta-analysis are powerful tools that are currently being used in food safety to measure intervention effectiveness with reduced bias and increased transparency (Bucher, Fazil, Rajić, Farrar, Willis, & McEwen, 2012; Greig, Waddell, Wilhelm, Wilkins, Bucher, Parker, & Rajić, 2012; Sargeant et al., 2006). A recent report on abattoir-level plant intervention studies supported current industry practices as effective methods for the reduction of STEC (Greig et al., 2012). However, the report was only limited to abattoir-level studies and did not appear to account for substitution practices in the recorded data. Substitution practices refer to the replacement of a non-detection or zero count (i.e., either a true zero or a value below the limit of detection) by some fraction of the detection limit to calculate

descriptive statistics. These substitution methods are an issue because they often lead to biased and inaccurate summary statistics.

This meta-analysis research had two objectives: (i) to determine the effectiveness of various plant interventions to mitigate Shiga-toxin producing *E. coli* using all published intervention data since 1990; and (ii) to apply meta-regressions to determine significant moderators, or covariates, (e.g., temperature of rinse, pressure of application, etc.) that could explain the variability observed across studies. It is expected that this research will help plant operators determine which combination of interventions and intervention parameters are optimal for the reduction of STEC.

2. Methods

2.1. Intervention Selection and Search Design

The 2011 Food Safety Inspection Service report was used to compile the list of potential plant interventions (Alvares, Lim, & Green, 2008). Only primary interventions that were (a) continuously applied throughout the year and (b) applied at 5% or more of plants surveyed were included as potential candidates for this meta-analysis. This method was chosen because a meta-analysis on each intervention should include several studies, but the number of studies for uncommon interventions was expected to be low (Borenstein, Hedges, Higgins, & Rothstein, 2009). Nine interventions that met the above criteria were: rinsing with water, lactic acid, acetic acid, sodium hydroxide, peroxyacetic acid, steam vacuum, citric acid, hypochlorite, and acidified sodium chlorite.

In June 2015, a published systematic literature review process (Knobloch, Yoon, & Vogt, 2011; Moher, Liberati, Tetzlaff, & Altman, 2009) was followed in order to effectively search for preliminary interventions and identify potential explanatory variables that could influence the

effectiveness of interventions. A full search of databases including Google Scholar, PubMed, Agricola, CAB, and Food Science and Technology Abstracts was completed in August of 2015. Journal articles within the previous 25 years were used. The general format of the searches was: *intervention type AND (beef OR carcass OR subprimal OR hide) AND ("Escherichia coli" OR O157 OR "non-O157" OR coliform OR "E. coli")*.

When these terms were too broad, restrictive terms against other products (e.g., poultry, produce, etc.) were added. A full list of search terms are available in Table 1, and a diagram of the systematic review procedure (Knobloch et al., 2011; Moher et al., 2009) is available in Figure 1. All search results were screened for relevance, except for Google Scholar where only the first 40 results were screened. All the papers that passed the first round of screening were collected for further evaluation.

2.2. Screening and Eligibility Criteria

The screening criteria followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) method (Knobloch et al., 2011; Moher et al., 2009). Primary screening was purposefully broad; titles and abstracts from the initial searches were checked for any possible relevance to plant interventions. Papers were more rigorously screened in the second round by two independent reviewers. Differences between reviewers' findings, that could not be reconciled initially, were brought to a third reviewer for a final decision. Articles in the second round were required to have information to obtain a mean difference in log CFU/cm² of bacteria and a variance of the difference. If not explicitly provided, mean differences could be extracted from papers reporting concentrations on a before and after group. Visual information, such as graphs, representing bacterial reduction were not considered due to reduced precision.

Studies that applied interventions by dipping samples in solutions were also excluded. Only primary studies reporting the author's original work were included. Furthermore, the treated specimens must have originated from cattle. The tracked organism must have been one of the STEC-7 (O157, O26, O45, O103, O111, O121, and O145), generic *E. coli*, or coliforms. The STEC-7 were chosen because they are the pathogenic organism of interest, while generic *E. coli* and coliforms are often used as surrogates (Ingham, Algino, Ingham, & Schell, 2010). Trials that reported log reduction statistics from substituted values were not included if 20% or more of the data was substituted. This occurred when experimenters recorded initial or final concentrations below a detection limit. Values below the detection limit were often substituted for some fraction of the detection limit and incorporated into the analysis of the original papers. The procedure for identification and screening is summarized in Table 1.

Table 1: Procedure for Identification and Screening During Systematic Review (Knobloch et al., 2011; Moher et al., 2009)

Step	Procedure
Identification	<p>Plant intervention studies were searched in Google Scholar, PubMed, Agricola, CAB, and Food Safety and Technology Abstracts databases using the following terms: (<i>"sodium hydroxide" OR "lactic acid" OR "citric acid" OR "acidified sodium chlorite" OR ASC OR hypochlorite OR "sodium hypochlorite" OR bleach OR chlorine OR "steam vacuum" OR "peroxyacetic acid" OR "water wash" OR "hot water" OR "water washes" OR "water rinse" OR "water spray" OR "spray washing" OR "spray wash" OR "water rinsing" OR "water washing"</i>) AND (<i>"Escherichia coli" OR O157 OR "non-O157" OR coliform OR "E. coli"</i>) AND (<i>beef OR carcass OR hide OR subprimal</i>).</p> <p>Restrictive terms applying only to the abstracts were: (<i>poultry OR chicken OR cilantro OR lettuce OR dairy OR milk OR biofilm OR brine OR broiler OR pigs OR pork</i>).</p>

**Primary
Screening**

Initial screening included scanning abstracts and figures to check if studies were relevant. In other words, the studies must have included at least one of the nine interventions (water wash, lactic acid, acetic acid, sodium hydroxide, peroxyacetic acid, steam vacuum, citric acid, hypochlorite, or acidified sodium chlorite), on cattle surfaces, and one of the organisms of interest (STEC-7 [O157, O26, O45, O103, O111, O121, and O145], generic *E. coli*, or coliforms).

**Secondary
Screening**

Articles must have contained the following information to pass secondary screening: Reported intervention effectiveness in log CFU/area and standard error (SE) of the intervention effectiveness or information to calculate effectiveness and SE; published after 1990; sampled within 2 hours of intervention application; and measured reductions must have been from previously uncleaned surfaces (i.e., before any other treatment).

Experiments were excluded if the above criteria were not met, if the data was only presented visually, if the interventions were applied by dipping/soaking samples instead of spraying, or if more than 20% of values were being substituted.

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Figure 1: Results from the Systematic Review: Identification and Screening

^a Only the first 40 results of Google Scholar were checked.

^b Table 3 defines procedures for identification and screening.

^c Some articles were already gathered from previous research on plant interventions.

Based on initial screening, insufficient data were available for peroxyacetic acid, citric acid, hypochlorite, and acidified sodium chlorite. Data were considered insufficient when there were less than three eligible studies on a particular intervention. These interventions were not considered further.

2.3. Assessing Critical Variables and Data Extraction

After the preliminary search, several factors were selected as possible explanatory variables. In an effort to explain the differences in intervention effectiveness between trials, the following study characteristics were extracted from the papers: temperature of application, intervention type, duration of application, sampling method (viz. excision or sponge), pressure of application, microbial concentrations on samples before intervention, concentration of antimicrobial, microorganism being tracked (viz. coliforms, *E. coli* O157, non-O157 STEC, or generic *E. coli*), surface type (viz. hide or carcass), type of contamination (viz. natural or inoculated), and rinsing with water (viz. no rinse, rinse before/after main treatment). For further details on study characteristics, see Table 2. These study characteristics were used as moderating variables in the meta-regressions. The final list of journal articles used in this meta-analysis is available in Supplemental Information, Appendix A. For clarification, in this analysis, a “study”, “article”, or “paper” refers to a single, unique, peer-reviewed publication. “Trials” are separate experiments within a publication. “Carcass” trials are those experiments that were conducted on the dehided carcass surface while “hide” trials were conducted on the hair and epidermis sections of the cattle.

Table 2: Covariates Used in the Meta-Regressions

Variable	Definition	Values Taken ^a	Baseline used in meta-regressions ^b
Organism Type	Microbial organisms used to track reductions	Coliforms, <i>E. coli</i> O157, non-O157 STEC, or generic <i>E. coli</i>	Coliforms
Sample Method	Sample collection techniques used for enumeration	Excision or sponge	Excision
Duration	Intervention application time	2.5 to 150 seconds	0 seconds
Inoculation Type	Origin of the microbe being tracked	Naturally contaminated or artificially inoculated	Natural contamination
Surface Type	Sample surface	Hide or carcass	Carcass
Extra Water Wash	Water wash(es) in addition to main treatment ^c	No wash, wash after, wash before, or both before and after	No extra wash
Temperature	Maximum temperature of intervention applied on samples	15 to 95°C	0°C
Pressure	Maximum pressure of the intervention applied	23 to 8274 kPa	0 kPa
Initial Microbial Concentration (IMC)	Levels of contamination before sanitization	-0.32 to 8 log CFU/cm ²	0 log CFU/cm ²
Intervention Type	Method or antimicrobial used to remove contamination	Water wash, acetic acid, lactic acid, sodium hydroxide, steam vacuum	Water wash
Antimicrobial Concentration (AMC)	Concentration of antimicrobial	1.6 to 10% antimicrobial	0% antimicrobial

^a Values taken represents the range of categorical and continuous variables observed across all trials.

^b The baseline refers to the one option within a variable that is set as the default in the meta-regression. For example, meta-regression results including inoculation type as a variable will only explicitly show the effect of inoculated samples, as the effect of naturally contaminated samples, is accounted for in the intercept term. Regressions use a baseline of 0 for continuous variables as a

default to calculate intercepts. However, for predictive purposes, it is not recommended to use values outside those observed for continuous moderators.

^c The water wash applied at a higher temperature was considered the main intervention for sequential water wash treatments.

2.4. Data Description

Table 3 is provided to give some understanding of the data structure used in the meta-regressions. A key characteristic of the data is the skewed information among covariates and intervention types. For example, the most information collected on interventions was for lactic acid, with 135 trials, and water wash, with 99 trials. On the other hand, the total trials for acetic acid, steam vacuum, and sodium hydroxide were 21, 18, and 11, respectively. The same issue exists within covariate data; there are a total of 241 trials on carcass surfaces while only 43 trials on hides. A total of 251 inoculated trials were collected to 33 naturally contaminated ones. The organism type, temperature, and initial microbial concentration variables were generally more uniformly distributed; while the extra wash, inoculation type, surface type, sample method, pressure, antimicrobial concentration, and duration variables had frequent issues with skewed representation. Additionally, there were issues with missing data; specifically, the pressure, initial microbial concentration, and duration of application were not always reported for every trial used in the analysis. An alternative version of the data structure based on surface type is provided in Supplemental Information Table SI-1.

The non-uniformity and sparseness of the data in certain covariates and interventions impede the analysis of all the trials under one meta-regression. As a result, several meta-regressions were split by surface type, intervention type, and available covariate data. Details of each model and methods are given in the *Meta-Regression* section.

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Intervention (# of trials)	Organisms type	Count	Extra water wash	Count	Inoculation type	Count	Surface type	Count	Sample method	Count		Continuous Variables ^a				
												Temp	Pres	IMC	AMC	Duration
Acetic acid (21)	Coliforms	7	After	6	Inoculated Natural contamination	15	Hide	12	Excision	6	Min	23	203	3.4	2	5.6
	<i>E. coli</i>	8	Before	5							Max	55	207	6.6	10	15
	O157	6	B & A	4							Mean	47.6	206.5	4.93	6.2	7.2
	Non-O157	0	No wash	6							Count	21	21	11	21	21
											% Reported	100	100	52	100	100
Lactic acid (135)	Coliforms	9	After	6	Inoculated Natural contamination	127	Hide	12	Excision	123	Min	15	69	1.23	2	7
	<i>E. coli</i>	45	Before	17							Max	55	850	8	10	60
	O157	49	B & A	0							Mean	37.2	273.5	5.76	3.8	14.4
	Non-O157	32	No wash	112							Count	135	127	125	135	33
											% Reported	100	94	93	100	24
Sodium hydroxide (11)	Coliforms	4	After	7	Inoculated Natural contamination	4	Hide	11	Excision	3	Min	10	203	3.7	1.6	7
	<i>E. coli</i>	3	Before	1							Max	60	8274	5.2	3	30
	O157	4	B & A	0							Mean	24	1214	4.63	2.8	11.7
	Non-O157	0	No wash	3							Count	11	8	4	11	11
											% Reported	100	73	36	100	100
Steam vacuum (18)	Coliforms	11	After	6	Inoculated Natural contamination	12	Hide	0	Excision	15	Min	82	23	-0.32	-	6
	<i>E. coli</i>	5	Before	0							Max	95	103	5.3	-	6
	O157	2	B & A	0							Mean	90.9	40.5	3.93	-	6
	Non-O157	0	No wash	12							Count	18	15	18	-	6
											% Reported	100	83	100	-	33
Water wash (99)	Coliforms	39	After	8	Inoculated Natural contamination	93	Hide	8	Excision	82	Min	15	138	1.9	-	2.5
	<i>E. coli</i>	37	Before	9							Max	95	2760	8	-	150
	O157	23	B & A	2							Mean	47.4	717	5.02	-	30.5
	Non-O157	0	No wash	80							Count	99	79	93	-	87
											% Reported	100	80	94	-	88

^a Temp = temperature in °C; Pres = pressure in kPa; IMC = initial microbial concentration in log CFU/cm²; AMC = antimicrobial concentration in percent; Duration in seconds

2.5. Summary Effects

In anticipation of high heterogeneity between trials, a random effects model was used to produce summary effects for the interventions that were listed earlier (Borenstein et al., 2009). High or statistically significant heterogeneity occurs when intervention results differ more than expected by random error alone (Higgins & Green, 2006). Statistically significant heterogeneity is often the product of differences in experimental design or application that influences trial results, causing results to vary significantly (Higgins & Green, 2006). Summary effects are the weighted averages of trial results. In the random-effects model, the weights for each trial is the inverse of the sum of trial variance (w_i) and between-trial variance (τ^2) (Borenstein et al., 2009):

$$Weight_i = \frac{1}{w_i + \tau^2}$$

The “meta” package v4.3-0 in R v3.2.2 (R Development Core Team, 2015) was used to obtain single summary effects, forest plots, funnel plots, and heterogeneity (I^2) measurements for each intervention. I^2 , which is calculated on the Q statistic and by degrees of freedom, allows for heterogeneity to be compared on a relative scale by measuring the amount of true heterogeneity over the total observed variation (Borenstein et al., 2009; Gelman, 2015; R Development Core Team, 2015). The Q statistic is based on a chi-squared test; it has low power as a test for heterogeneity when the number of trials are low and too much power when trial size is high (Higgins & Green, 2006). As a result, the I^2 is usually regarded as a better gauge of heterogeneity as it measures the percentage of variability that is due to heterogeneity rather than sampling error (Borenstein et al., 2009; Higgins & Green, 2006). Generally, I^2 between 0-25% is considered low, 25-75% is moderate, and 75-100% is high (Higgins & Green, 2006). Meta-regressions were run when moderate to high heterogeneity was found in the summary effects.

Microbial concentrations in log CFU/cm² along with their respective standard deviations were used as inputs into the meta-analysis. The mean difference was used when an author failed to report before and after values. Trials within a single paper were entered as independent entries into the meta-analysis. This procedure likely underestimated the variance of the effects and overestimated the precision of the model (Borenstein et al., 2009). This issue was more thoroughly addressed in the meta-regressions by nesting the effect of trials within a paper as additional random effects to account for the correlation among trials (Borenstein et al., 2009; Pinheiro & Bates, 2000).

2.6. Meta-Regressions

Meta-regressions are similar to simple linear regressions except they use the variance of each trial to allocate more weight to trials with smaller variances. This procedure is similar in effect to the random-effects model summary effect, except that meta-regressions can incorporate continuous and categorical variables as moderators. Results from a meta-regression can be used to assess the impact of a unit increase in explanatory variable on the effect size, which is intervention effectiveness (Higgins & Green, 2006). Following recommendations, meta-regressions were only carried out on interventions that had more than one study and over ten total trials (Borenstein et al., 2009; Higgins & Green, 2006). Meta-regressions, performed with the “metafor” package v1.9-7 and “nlme” package v3.1-124, could further reduce heterogeneity by incorporating the continuous and categorical variables into the meta-analysis (Pinheiro, Bates, DebRoy, & Sarkar, 2016; Viechtbauer, 2010). However, papers did not always report all of the variables of interest. As mentioned previously, surface type, temperature, inoculation type, antimicrobial concentrations, extra wash type, and organism type were always reported, while

duration, pressure, and initial microbial concentrations were not always stated. Data were split into 7 categories before regression analysis. The first two subsets were separated by surface type, with trials conducted on hide surfaces analyzed separately from those on carcass surfaces. The other five subsets were arranged by intervention type, with trials on water wash, lactic acid, acetic acid, steam vacuum, and sodium hydroxide analyzed separately.

For each of the seven subsets, two meta-regressions were conducted in order to utilize and obtain as much information as possible. The first meta-regression approach only included the variables of interest that were always reported in the literature as moderators; it utilized all the trials within a data set, thus, it is referred to as the “full-trial” meta-regression. An analysis of 20 trials, for instance, with each reporting a temperature, but with incomplete reporting of initial concentrations, would be run with temperature as a covariate, but not initial concentration. Given adequate information, a second meta-regression was performed that also contained the less frequently reported variables (e.g., initial microbial concentration, duration of application, or pressure) to observe their impact on log reductions. This second model is referred to as the “full-variable” meta-regression because it encompassed more variables, but often had fewer trials. Covariates were added to the full-variable meta-regression when they were reported in less than 100% of trials, but more than 75%. Seventy-five percent was chosen as a limit below which too many trials were being lost. As a minimum, two trials per study were required to calculate the random effects terms. Therefore, studies with only one trial were excluded from mixed model meta-regressions.

2.7. Meta-regression Models

Non-significant predictors were removed from the models so that their correlation with other covariates did not adversely affect the standard error estimates of other predictors (O'Brien, 2007). A simple backward selection process was used to determine which variables to eliminate from each model and the results were confirmed by a forward selection process (Chatterjee & Hadi, 2006). All significance tests to determine covariate significance used an alpha of 0.10. Table 4 shows all of the covariates that remained significant after backward selection for each data subset. The full-trial meta-regression is explicitly reproduced below as an example:

$$LR_{ijmnqr} = \beta_0 + \beta_{1i} + \beta_{2j} + \beta_{4im} + \beta_{5n} + \varepsilon_{ijmnqr}$$

Where LR_{ijmnqr} is the expected log reduction in log CFU/cm²; β_0 is the mixed effects intercept term equal to $\bar{B} + v_q + \eta_{qr}$ for paper q and trial r ; and $\bar{B}$ is the fixed effect intercept. The random effects terms, v_q and η_{qr} , were added to separate the between-trial variation from the between-paper variation. This allowed the correlated between-trial results to be entered as separate entries and provided more information on the relationship between covariates and log reduction (Pinheiro & Bates, 2000).

β_{1i} is the effect of intervention i (i = lactic acid, sodium hydroxide, water wash, or acetic acid), β_{2j} is the effect of inoculation type j (j = lab inoculation or natural contamination), β_{4im} is the nested effect of an extra wash m (m = no extra water wash, water wash before intervention, water wash after intervention) within intervention i , and β_{5n} is the effect of sample method n (n = excision or sponge). The nested effect for water wash allows the effect to be calculated within each intervention, e.g., the effect of water wash within the lactic acid data is calculated separately from the effect of water wash within the sodium hydroxide data. The random effect terms for paper (v_q), trial (η_{qr}), and residual error (ε) were expected to follow normal

distributions with mean of zero and variance of s_p^2 , s_t^2 , and s^2 , respectively. These definitions for v_q , η_{qr} , and ε are the same for all meta-regressions.

Table 4: Final Meta-Regression Model Structure From Backward Variable Selection^a

Equation Number	Data Group ^b	Model Type ^c	Meta-regressions after backward selection procedure for each data sub-group
1	<i>Hide</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{1i} + \beta_{2j} + \beta_{4im} + \beta_{5n}$
NA	<i>Hide^d</i>	<i>FV</i>	
2	<i>Carcass</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{1i} + \beta_{2j} + \beta_{3k} + \beta_{4m} + \beta_7 * \text{IMC} + \beta_8 * T$
NA	<i>Carcass^d</i>	<i>FV</i>	
4	<i>WW</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{2j} + \beta_{4m} + \beta_{5n} + \beta_{6p} + \beta_8 * T$
5	<i>WW</i>	<i>FV</i>	Log Reduction = $\beta_0 + \beta_{5n} + \beta_7 * \text{IMC} + \beta_8 * T + \beta_9 * t$
6	<i>LA</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{3k} + \beta_{4m}$
7	<i>LA</i>	<i>FV</i>	Log Reduction = $\beta_0 + \beta_{4m} + \beta_{6p} + \beta_7 * \text{IMC}$
8	<i>AA</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{3k}$
NA	<i>AA^d</i>	<i>FV</i>	
9	<i>SH</i>	<i>FT</i>	Log Reduction = $\bar{B}^e + \beta_{2j} + \beta_{4m} + \beta_{11} * \text{AMC}$
NA	<i>SH^d</i>	<i>FV</i>	
10	<i>SV</i>	<i>FT</i>	Log Reduction = $\beta_0 + \beta_{4m} + \beta_7 * \text{IMC}$
NA	<i>SV^f</i>	<i>FV</i>	

^a β_0 is the mixed effects intercept term equal to $\bar{B} + v_q + \eta_{qr}$ for paper q and trial r , β_{1i} is the effect of intervention i (i = lactic acid, sodium hydroxide, water wash, steam vacuum, or acetic acid), β_{2j} is the effect of inoculation type j (j = lab inoculation or natural contamination), β_{3k} is the effect of organism k (k = generic *E. coli*, *E. coli* O157, non-O157 STEC, or coliforms), β_{4m} is the effect of an extra wash m (m = no extra water wash, water wash before, water wash after, or both before and after), β_{4im} is the nested effect of an extra wash m (m = no extra water wash, water wash before, water wash after) within intervention i , β_{5n} is the effect of sampling method n (n = excision or sponge), β_{6p} is the estimated effect of surface p (p = carcass or hide), β_7 is the increase in log reduction observed with each log increase in IMC, β_8 is the unit increase in effectiveness given a Celsius increase in T for temperature, β_9 is the estimated effect of a unit increase in t for time (s), β_{10} is the effect of an incremental increase in P for pressure (kPa), and β_{11} is the unit increase in effectiveness given a percent concentration increase in antimicrobial.

^b Data subset by trial design, Carcass = Regression on intervention data for carcass surface only, Hide = Regression on intervention data for hide surface only, WW = Water Wash, AA = Acetic Acid, SH = Sodium Hydroxide, SV = Steam Vacuum.

^c FT = Full-Trial, FV = Full-Variable.

^d Meta-regression was conducted but converged to the same model as in the Full-Trial

^e The sodium hydroxide model did not have trial or paper as random effects.

^f There was not enough data to test the Full-Variable regression. Quality information on time and temperatures were missing.

Sodium hydroxide had the smallest pool of data, thus, the mixed effects model could not be used while meeting the minimum trial criteria. Therefore, the random effects terms, v_q and η_{qr} ,

were dropped to incorporate the two studies with only one trial each so the total number of trials could pass the 10 trial minimum.

3. Results

3.1. Summary Effects

Sodium hydroxide had the highest estimated log reduction, with a summary effect at 3.17 log CFU/cm², while steam vacuum had an estimated log reduction of 3.08 log CFU/cm². The estimated impact of acetic acid, lactic acid, and water wash were similar at approximately 2 log CFU/cm². Moderate to high I² was observed in all data sets (Table 5). Additionally, all summary effects were for overall intervention effectiveness and those presented were not sub-grouped by factor (e.g., effectiveness of lactic acid on hide vs. on carcass surfaces). Examples of intervention forest plots of extract data from the different intervention studies can be found in Supplemental Information, Appendix C, Figures SI-1 through SI-5.

Table 5: Meta-Analysis Summary Effects Results

Intervention	Summary Effects ^a	Between-Trial Variation (τ^2)	Heterogeneity (I ²)	Trials	Papers
Sodium Hydroxide	3.17	0.906	89.7	11	4
Steam Vacuum	3.08	3.233	99.3	19	6
Acetic Acid	2.10	0.717	95.9	23	5
Lactic Acid	2.01	0.171	99.4	139	14
Water Wash	1.81	0.254	98.5	105	20

^a Measured in log CFU/cm² reduction

3.2. Meta-Regressions

327 The results for the full-trial and the full-variable meta regressions are given in Tables 6
328 and 7 respectively. The full-trial meta-regression maximized the amount of trials being used, but
329 limited the covariates considered in the backward selection process.
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332 **Table 6: Full-Trial Meta-Regression Results (mean and standard error)^a**

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Covariates	Carcass (Trials = 249, papers = 22)	Hide (Trials = 47, papers = 4)	Acetic acid (Trials = 21, papers = 3)	Lactic acid (Trials = 135, papers = 10)	Water wash (Trials = 99, papers = 14)	Sodium hydroxide (Trials = 11, papers = 4)
Fixed Effects						
Intercept	-0.79 (0.43) ^c	-1.63 (0.49) ^b	2.28 (0.60) ^b	2.21 (0.55) ^b	3.17 (0.82) ^b	1.06 (0.54) ^b
Acetic acid	-0.46 (0.30)	3.22 (0.43) ^b	NT	NT	NT	NT
Lactic acid	0.16 (0.18)	3.94 (0.45) ^b	NT	NT	NT	NT
Sodium hydroxide	NT	3.91 (0.45) ^b	NT	NT	NT	NT
Steam vacuum	1.19 (0.23) ^b	NT	NT	NT	NT	NT
Inoculation	0.92 (0.45) ^c	0.82 (0.31) ^b	NS	NS	-1.66 (0.74) ^b	0.55 (0.24) ^b
Generic <i>E. coli</i>	0.19 (0.14)	NT	0.72 (0.35) ^c	0.06 (0.34)	NS	NS
<i>E. coli</i> O157	-0.55 (0.15) ^b	NT	-0.86 (0.46) ^c	-0.70 (0.34) ^b	NS	NS
Non-O157 STEC	-0.58 (0.15) ^b	NT	NT	-0.72 (0.34) ^b	NS	NS
Wash after	1.02 (0.203) ^b	NT	NS	-0.5 (0.72)	0.74 (0.35) ^b	-1.06 (0.27) ^b
Wash before	0.80 (0.206) ^b	NT	NS	1.47 (0.74) ^c	0.43 (0.28)	0.69 (0.67)
Wash before & after	1.01 (0.301) ^b	NT	NT	NT	-1.22 (0.67) ^c	NT
IMC	0.31 (0.043) ^b	NT	NT	NT	NT	NT
AMC	NS	NS	NS	NS	NT	0.93 (0.18) ^b
Temperature	0.003 (0.001) ^c	NS	NS	NS	0.014 (0.003) ^b	NS
Sponge	NS	1.65 (0.43) ^b	NS	NS	-1.21 (0.43) ^b	NS
Hide	NT	NT	NS	NS	-2.53 (0.63) ^b	NT
Wash after AA	NT	-0.92 (0.43) ^b	NT	NT	NT	NT
Wash after LA	NT	-1.41 (0.45) ^b	NT	NT	NT	NT
Wash after SH	NT	-1.48 (0.46) ^b	NT	NT	NT	NT
Wash after WW	NT	1.11 (0.37) ^b	NT	NT	NT	NT
Wash before AA	NT	-0.94 (0.72)	NT	NT	NT	NT

Wash before LA	NT	-0.16 (0.73)	NT	NT	NT	NT
Wash before SH	NT	1.92 (0.96) ^c	NT	NT	NT	NT
Wash before WW	NT	0.33 (0.92)	NT	NT	NT	NT
Random Effects^d						
S_p^2	0.285	0.024	0.86	1.603	0.23	
S_t^2	0.026	0.113	0.455	0.021	0.355	
S^2	11.049	1.669	<0.001	4.04	<0.001	<0.001

^a NT = not tested because of insufficient data, NS = not statistically significant at $\alpha=0.10$.

^b Significant at $\alpha=0.05$

^c Significant at $\alpha=0.10$

^d Estimated variance for paper (s_p^2), trial (s_t^2) and residual error (s^2)

Table 7: Full-Variable Meta-Regression Results (mean and standard error)^a

Covariates	Lactic acid (Trials = 125, papers = 9)	Water wash (Trials = 81, papers = 12)	Steam vacuum (Trials = 18, papers = 5)
Fixed effects			
Intercept	-0.27 (0.29)	-0.50 (0.45)	0.23 (0.36)
Wash after	NT	NS	1.43 (0.34) ^b
Wash before	2.00 (0.18) ^b	NS	NT
IMC	0.36 (0.05) ^b	0.27 (0.07) ^b	0.57 (0.09) ^b
Sponge	NS	-1.44 (0.50) ^b	NS
Temperature	NS	0.02 (0.003) ^b	NT
Hide	2.24 (0.59) ^b	NT	NT
Duration	NT	0.013 (0.003) ^b	NT
Random Effects			
S _p ²	<0.001	0.49	0.021
S _t ²	0.154	0.27	0.322
S ²	2.822	<0.001	0.569

^a NT = not tested because of insufficient data, NS = not statistically significant at $\alpha=0.10$.

^b Significant at $\alpha=0.05$

For the full-trial regressions on carcass, steam vacuum had the highest estimated impact at 1.19 log CFU/cm² higher reduction than water wash. Increases in temperature resulted in higher reductions by 0.003 log CFU/cm² per °C, which translates to approximately 0.3 log CFU/cm² at 95°C. Washing with water before, after, or both before and after the main treatment added to microbial reduction by 0.80, 1.02, and 1.01 log CFU/cm², respectively. The data limitations of the extra wash data within the carcass trials did not allow for a nested analysis of extra wash within intervention type. Higher initial concentrations were predicted to increase reported log reductions at a slope of 0.31 log CFU/cm² per log CFU/cm² increased starting concentration. Samples that were inoculated were predicted to have higher levels of reported reduction, by 0.92 log CFU/cm², than those that were naturally contaminated.

The full-trial meta-regression on hide sample results estimated that acetic acid, lactic acid, and sodium hydroxide were over 3 log CFU/cm² more effective than water wash alone

(Table 6). Inoculated samples were associated with higher reductions than their naturally contaminated counterparts by 0.82 log CFU/cm². Adding an extra water wash after the application of an acid or base was predicted to substantially decrease effectiveness. The use of water after the application of acetic acid, lactic acid, or sodium hydroxide reduced the effectiveness by 0.92, 1.41, and 1.48 log CFU/cm², respectively. So for example, a lactic acid application followed by a water wash had a combined effectiveness of only 2.53 log CFU/cm² (the lactic acid effectiveness of 3.94 log CFU/cm² together with the negative water wash after effectiveness of -1.41 log CFU/cm²).

For the full-trial meta-regression on acetic acid, organism type was the only variable that remained significant after testing, with generic *E. coli* associated with increased reductions by 0.72 log CFU/cm² when compared to trials using coliforms (Table 6). *E. coli* O157, on the other hand, was estimated to decrease reduction by 0.86 log CFU/cm² when compared to coliforms. For example, the estimated 2.28 log reduction for coliforms with acetic acid would yield a 3.00 log reduction for generic *E. coli*, but only a 1.42 log reduction for O157.

Lactic acid data was available to produce both the full-trial meta-regression and full-variable meta-regression (Table 6 and Table 7). Having an extra wash before was estimated to increase effectiveness by 1.47 and 2.00 log CFU/cm² in the full-trial and full-variable meta-regressions, respectively. Organism type was significant in the full-trial meta-regression, with the pathogenic strains of *E. coli* associated with increased resistance to intervention, but not in the full-variable. Hide samples increased reductions by 2.24 log CFU/cm² when compared to carcass samples. Initial microbial concentration, in the full-variable meta-regression, was highly significant with a slope of 0.36 log CFU/cm² per increased log CFU/cm² starting concentration.

Water wash also produced both full-trial and full-variable meta-regressions. The full-trial water meta-regression showed inoculation type, extra wash, sample method, surface type, and temperature as significant predictors (Table 6). Temperature increased effectiveness by 0.014 log CFU/cm² per °C. Initial microbial concentration and duration were both estimated to have substantial impacts on final log reductions with slopes of 0.27 log CFU/cm² per increased log CFU/cm² and 0.013 log CFU/cm² per second, respectively. Sampling with a sponge was predicted to decrease reported reductions by approximately 1.21 and 1.44 log CFU/cm² in the full-trial and full-variable water wash regressions, respectively.

For the full-trial sodium hydroxide meta-regression, the number of trials available for analysis fell below the 10 trial minimum so only the residual error term remained to account for variance in the full-trial regression for sodium hydroxide (Table 6). The results showed that antimicrobial concentration, extra water wash, and inoculation were all statistically significant predictors. The resulting residual term was less than 0.001 (log CFU/cm²)² as the three covariates described all of the between-trial variability. Concentration was estimated to have the most significant impact at a slope of 0.93 log CFU/cm² per percent NaOH. Following previous trends, the water rinse after the sodium hydroxide was linked to decreased effectiveness by about 1 log CFU/cm². Inoculated samples were associated with higher reported reduction.

The full-variable steam vacuum meta-regression found that extra water wash and initial microbial concentration variables were the only covariates found to be statistically significant. Each unit increase in initial concentration was estimated to increase reductions by 0.57 log CFU/cm², while adding an extra wash after would further increase reductions by 1.43 log CFU/cm² (Table 7). Variability between trials and residual variation remained high.

3.3. Least-Means Squares

The results of the surface models for intervention effectiveness are summarized as least-squares means (Figure 2 and Figure 3). Based on the hide model (Table 6), sodium hydroxide was estimated to be the strongest sanitizer followed by lactic acid, acetic acid, and water in the hide trials at reductions of 3.66, 3.02, 2.21, and 0.08 log CFU/cm², respectively (Figure 2). In the full-trial carcass meta-regression, steam vacuum had the greatest reductions followed by lactic acid, water wash, and acetic acid at reductions of 3.09, 2.07, 1.90, and 1.44 log CFU/cm², respectively (Figure 3). The full-trial carcass regression, however, estimates that the application of water, lactic acid, and acetic acid on carcasses are all statistically the same.

Figure 2: Least-Squares Means for Full-Trial Hide Model

Figure 3: Least-Squares Means for Full-Trial Carcass Model

4. Discussion

4.1. Summary Effects

Caution should be taken when interpreting the summary effect results presented in Table 5 because of the high unexplained heterogeneity. The range of reported reductions vary substantially between trials, more than would be expected by sampling error alone (Borenstein et al., 2009; Higgins & Green, 2006). Therefore, the use of meta-regressions is appropriate to explain the variability between trials.

4.2. Meta-regressions: Explanatory Variables

Although heterogeneity remained high in some of the meta-regression models, the results clearly support that moderators are statistically significant predictors of intervention effectiveness. Initial microbial concentration and extra water wash were the most consistent predictors of log reduction, both in terms of being frequently statistically significant and estimated impact in effectiveness. Antimicrobial concentration, sample method, inoculation type, organism type, duration of application, temperature, and surface type were also observed as important explanatory variables, but their impact was not as distinct and/or significant. Also, it is important to reiterate that all meta-regressions used baselines of 0 for any continuous variables as a default to calculate intercepts. However, for predictive purposes, it is not recommended to use values outside those observed for continuous covariates (e.g., setting temperature equal to 0°C in a regression is not recommended for predictions).

Initial microbial concentration was arguably the most critical predictor of intervention effectiveness. Initial microbial concentrations were highly statistically significant ($p < 0.001$) predictors of intervention effectiveness in every meta-regression that tested initial concentration as a variable (Tables 6 and 7). These results show that interventions become less effective as concentrations decrease, even when excluding papers with major detection limit issues. Direct comparisons of similar trials with different initial concentrations in the literature show the same effect (Delmore, Sofos, Schmidt, & Smith, 1998; Youssef, Yang, Badoni, & Gill, 2012). As the top layers of contamination are washed away or disinfected, microorganisms may continue to survive in the microenvironments within the hide or subcutaneous tissue. This attribute of reduced effectiveness at low concentrations has critical implications for pathogenic contamination in plants. Specifically, food safety specialists are at risk of overestimating the effectiveness of interventions if they are using studies with moderate to high initial

concentrations to estimate intervention effectiveness at plants. This can have negative effects on HACCP plans and plant sanitary conditions. For example, food safety managers deciding between implementing two or more sanitizers that were tested at different initial concentrations may choose the less effective intervention if the impact of initial microbial concentration is not considered.

The significance of initial concentration as a critical predictor also has implications on the use of intervention data more broadly. Some epidemiologists argue that analysis of intervention effectiveness should be based on naturally contaminated studies in the field rather than the inoculated lab studies (Greig et al., 2012). The general sentiment is that the different environments produce systematically different results and therefore, it is often misleading to extrapolate findings from inoculated labs samples to the real world (Greig et al., 2012). The physiological state of cells also varies between inoculated samples versus naturally occurring samples so that results of inoculation studies cannot be easily extrapolated to naturally contaminated samples. However, if these differences can be adequately explained by the covariates of the experimental design, such as initial concentration, then it allows for a more effective use of a larger body of information in the realms of risk assessment and risk management.

While water washes before the main intervention often increased reductions, water rinses after antimicrobial treatments were linked to decreased reductions in the hide, sodium hydroxide, and acetic acid meta-regressions (Tables 6 and 7). This effect is likely the result of the water rinse removing or diluting the antimicrobial, leading to decreased application times, concentrations, and ultimately reductions (Carlson, Geornaras, Yoon, Scanga, Sofos, Smith, & Belk, 2008; Sapers, Miller, Jantschke, & Mattrazzo, 2000). For this reason, the same negative

effect is not seen on the water wash and steam vacuum trials, where washing after improves reductions.

Following trends seen in previous research, temperature was statistically significant in the carcass and water wash regressions (Tables 6 and 7) (Anderson & Marshall, 1989; Fouladkhah, Geornaras, Yang, Belk, Nightingale, Woerner, Smith, & Sofos, 2012; Gorman, Sofos, Morgan, Schmidt, & Smith, 1995; Yoder et al., 2010). Temperature, however, was not significant in the other meta-regressions although this may be due to the limitations of the data available.

The antimicrobial concentration was largely seen as a non-significant factor in the meta-regressions. The sodium hydroxide data, composed of 11 trials, was the only set of information that showed a statistically significant effect from increased antimicrobial concentration (Table 6). Both the lactic acid and acetic acid data sets had sufficient ranges of applied concentrations, 2 to 10%, but failed to show them as statistically significant predictors (Tables 6 and 7). Although increases in antimicrobial concentrations are expected to increase reductions, previous research has shown that the effectiveness is not always significantly different (Anderson & Marshall, 1989; Heller, Scanga, Sofos, & Belk, 2007). It is also possible that the presence of an extra wash has an interaction with the antimicrobial concentration, making it difficult to assess the impact of both individually. As mentioned, residual water left on before or added after may change the actual antimicrobial concentration on the sample surface by diluting the sanitizer, leading to concentrations on the surface that are substantially different than those expected by the concentration reported in the original mixture (Carlson et al., 2008; Sapers et al., 2000).

The duration of application was the least reported covariate, and therefore, its impact is difficult to compare to the other covariates. The water wash trials, however, did have sufficient information on application times and estimated the reduction to be 0.013 log CFU/cm² per

second (Table 7). Food safety specialists may be able to increase reductions by exploring increased application times.

Washing with water was shown to be less effective on hide than carcass samples, while washing with lactic acid was more effective on hide samples than carcass (Tables 6 and 7). This may be due to the means by which each intervention reduces bacteria. It is possible that the hair on hide samples makes removal by physical means more difficult, but antimicrobial effects of acids may actually benefit in the environments of hair samples. While no research directly comparing the same intervention on hide and carcass samples were available, comparison of clipped versus unclipped hides shows significant differences (Baird, Lucia, Acuff, Harris, & Savell, 2006). Reinforcing the idea that the surface type plays a major role, these results suggest plant operators may gain increased reductions if cattle are sprayed with acidic interventions before dehiding rather than solely after dehiding.

The inoculation variable was frequently significant, showing that there are statistical differences between reductions reported on artificially inoculated samples and naturally contaminated ones. Inoculated organisms were more easily removed from hide and carcass surfaces compared to naturally contaminated organisms. Specifically, researchers should be wary of overestimating the effectiveness of a particular intervention on naturally contaminated specimens if inoculated samples are used as a reference.

Information from the meta-regressions on organism type indicated that pathogenic strains of *E. coli* were less vulnerable to intervention than indicator organisms (Tables 6). Specifically, the carcass, acetic acid, and full-trial lactic acid meta-regressions suggest *E. coli* O157 and non-O157 STEC may be more resistant to intervention than generic *E. coli* or coliforms. As previous research has suggested, both non-O157 and O157 STEC were predicted to behave similarly

(Fouladkhah et al., 2012). While some studies do show O157 being less responsive to certain interventions, the opposite effect, or negligible differences, have also been recorded (Castillo, Lucia, Goodson, Savell, & Acuff, 1998a, 1998b; Ingham et al., 2010; Yoder, Henning, Mills, Doores, Ostiguy, & Cutter, 2012). Nevertheless, the ability of O157 to survive in low pH environments has been established in the literature (Byrne, O'Kiely, Bolton, Sheridan, McDowell, & Blair, 2002; Feng, 1995). Therefore, the current meta-regression results coupled with the findings in the literature should encourage food safety specialists to be wary of overestimating pathogenic reductions when using indicator organisms to track effectiveness for acidic interventions. In addition, other than for water wash, there were too few studies to evaluate the overall effect of different interventions applied sequentially, e.g., an acetic acid wash followed by a steam vacuum. Further research is needed in this area.

4.3. Random Effects

The moderators used in the meta-regressions explained some variation among trial results, reducing unexplained heterogeneity particularly for water wash and sodium hydroxide meta regressions. However, the variation attributed to residual error and between-papers remained moderate to high for some of the meta-regressions (Tables 6 and 7).

While variation between trials, studies, and due to residual error is expected, the estimates are likely artificially high for two reasons. First, without a sufficient number of trials, the models cannot accommodate all of the covariates that could explain heterogeneity and variation. The results have shown that many of the covariates tested are highly statistically significant. The data, however, are limited with many covariates highly correlated. For instance, sponge sampling was often done on naturally contaminated samples, which only measured

coliform or generic *E. coli* levels, and never STEC concentrations. Testing all possible covariates becomes impossible and the result is unexplained heterogeneity.

Additionally, although incorporating trials with high levels of substitution was avoided to a large extent, many experiments did not report whether they used substitution methods. It is possible that fabricated results were incorporated into the meta-analysis if authors failed to mention the use of substitution. If true, the variation within-trials would decrease and the between-trial variation would increase, resulting in higher levels of heterogeneity.

4.4. Real World Applications

Several recommendations can be made following the meta-analysis results. First, on hide surfaces, water washes should be avoided, as they are largely ineffective and may contribute to diluting any antimicrobial applied. Instead, sodium hydroxide, or possibly lactic acid, should be used for hide decontamination (Figure 2). Although they were not statistically different in the least-squares means, lactic acid was predicted to be less effective on STEC in the full-trial lactic acid regression. Sodium hydroxide may be a more appropriate solution to STEC contamination, however the sodium hydroxide data were scarce and therefore its estimated effect should be used with caution.

For decontamination after dehiding, steam vacuum presented the largest reductions (Figure 3). Lactic acid is also an effective sanitizer on carcass surfaces and is recommended if steam vacuum is not available. While water washes are viable interventions on carcass surfaces, they should be implemented before any antimicrobial. Finally, further microbial reductions can be gained by increasing the application temperature of any water washes.

5. Conclusion

The meta-regressions revealed that initial microbial concentration, extra water washes, intervention type, surface type, inoculation type, temperature, duration, antimicrobial concentration, organism type, and sample method all had impacts on the intervention effectiveness. The most compelling evidence was for initial microbial concentration and extra water washes as the constant and most impactful predictive variables across interventions. The steam vacuum was the most effective intervention on the carcass and sodium hydroxide was the most effective on the hide. High heterogeneity remained, but this may be due to data limitations and substitution issues. Overall, the models and covariates helped explained differences across study results, and these findings can be used by the industry and risk assessors to improve safety and sanitary conditions.

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