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SCIENTIFIC OPINION

Scientific Opinion on the evaluation of the safety and efficacy of lactic acid for the removal of microbial surface contamination of beef carcasses, cuts and trimmings[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 4]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) [3, 4]

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ABSTRACT

Studies evaluating the safety and efficacy of lactic acid treatment for decontamination of beef carcasses, cuts and trimmings were assessed. Treatments considered consisted of using 2 % to 5 % lactic acid solutions at temperatures of up to 55 °C applied either by spraying or misting. It is concluded that these treatments will be of no safety concern provided the substance used complies with the European Union specifications for food additives. A total of 25 papers of the 52 submitted were selected as meeting certain criteria and were included in the assessment of the antimicrobial efficacy of lactic acid. No studies applying water rinsing of lactic acid after treatment of beef were submitted, and therefore, this issue was not addressed. As the studies described in the selected papers used a wide range of experimental designs, the assessment did not attempt to differentiate efficacy based on factors such as lactic acid concentration and temperature, that might influence efficacy. It was concluded that, although variable, microbial reductions achieved by lactic acid treatment of beef are generally significant compared to untreated or water treated controls. Development of enzymatic resistance to therapeutic antimicrobials as a result of exposure to lactic acid and the possibility of mutational changes resulting in the development of resistance to therapeutic antimicrobials are unlikely. An environmental risk assessment was not carried out as the lactic acid concentration before entering the wastewater treatment system is considered as negligible. It is recommended that, according to HACCP principles, during use, business operators verify lactic acid concentration, temperature of application and other factors affecting its efficacy as a decontaminating agent and validate the antimicrobial efficacy under their specific processing conditions.

1 On request from the European Commission, Question No EFSA-Q-2011-00032, adopted on 7 July 2011 by the BIOHAZ Panel and No EFSA-Q-2011-00081, adopted on 18 May 2011 by the CEF Panel.

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KEY WORDS

Decontamination, beef, lactic acid, efficacy, toxicological safety assessment, antimicrobial resistance, environmental impact

SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) were asked by the European Food Safety Authority (EFSA) to deliver a Scientific Opinion on an application dossier submitted by the U. S. Department of Agriculture (USDA) for the approval of lactic acid for uses to reduce microbial contamination of beef hides, carcasses, cuts and trimmings. More specifically, the approval was sought for treatments using lactic acid solution concentrations from 2 % to 5 % (wt/wt) at temperatures of up to 55 °C applied either by spraying or misting.

The Commission asked EFSA to issue a Scientific Opinion on the assessment of the safety and efficacy of lactic acid when used to reduce microbial surface contamination on beef hides, carcasses, cuts and trimmings. Specifically, the task was to consider the toxicological safety of the substance, its antimicrobial efficacy, the potential emergence of reduced microbial susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance, and any risk related to the release of the slaughterhouse and/or processing plant effluents containing the substance into the environment. The assessment was based on the document "Guidelines on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption" published by EFSA[5].

Concerning the human toxicological safety of the substance, it was concluded that the treatments, as described, would be of no safety concern provided that the substance used complies with the European Union specifications for food additives[6]. This was based on the expected low level of exposure deriving from the use of lactic acid on carcasses, cuts and trimmings, and the fact that it is an endogenous substance.

A total of 25, of the 52 papers submitted by the applicant, were selected based on identified criteria and were used in the assessment of the efficacy of lactic acid as a decontaminating agent for beef hides, carcasses, cuts and trimmings. Since no studies were submitted for the evaluation of the lactic acid efficacy when its application was followed by water rinsing, this sequence of treatments was not assessed. Evaluation of the efficacy of lactic acid for decontamination of hides was also not performed since all relevant studies submitted evaluated 10 % lactic acid (not the requested maximum of 5 %) or the application method used in the studies was not requested for approval.

The studies described in the selected papers used a wide range of experimental designs and thus differed in relation to products, settings, method of application, lactic acid concentration, use of controls, microorganisms studied, time and temperature of storage, etc. All of these factors impacted on the efficacy both within and between studies. Given this wide range of application conditions, the evaluation did not attempt to differentiate effects due to different factors, such as lactic acid concentration and temperature of application, within the limits considered, which might influence its efficacy.

Studies on industrial scale and pilot scale which are representative of industrial scale with naturally contaminated products were considered as providing high strength of evidence. Pilot studies with naturally contaminated products and with inoculated pathogenic microorganisms and laboratory

[5] EFSA Journal 2010;8(4):1544

[6] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union, 31.12.2008, L 354/16.

studies with naturally contaminated products were considered as providing medium strength of evidence. Laboratory studies with inoculated pathogenic microorganisms were considered as providing low strength of evidence. Based on studies classified by the Panel as of high strength of evidence, lactic acid reduced counts of naturally occurring *Enterobacteriaceae* on beef carcasses, cuts and trimmings to a variable degree. However, these reductions were usually significantly higher compared to untreated or water treated controls. According to studies classified as of high or medium strength of evidence, lactic acid reduced the prevalence of *Salmonella* and/or Shigatoxin-producing/Verotoxin-producing *Escherichia coli* (STEC/VTEC) on carcasses, beef cuts and trimmings to varying degrees depending on study design and contamination level. Based on studies classified as of medium strength of evidence, lactic acid was shown to reduce counts of inoculated pathogens (*Salmonella* and/or STEC/VTEC) on beef carcasses, cuts and trimmings to a variable degree. Usually reductions were higher on carcasses compared to meat cuts and trimmings.

Data to address the issue of the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance were not provided. It was however concluded that the development of enzymatic resistance to therapeutic antimicrobials as a result of exposure to lactic acid is unlikely. Considering the extensive natural presence of lactic acid in fermented food, the possibility of mutational change resulting in the development of resistance to therapeutic antimicrobials is also unlikely to be a significant issue. There is some evidence that repeated exposure to lactic acid can select for reduced susceptibility to the substance. Under good hygienic practices (GHP), this possibility is not considered a significant issue.

This Scientific Opinion further points out that the concentration of lactic acid just before entering the wastewater treatment system is considered as negligible. For this reason, an environmental risk assessment was considered as not necessary.

It is recommended that, according to HACCP principles, during use, food business operators verify lactic acid concentration, temperature of application and other factors affecting its efficacy as a decontaminating agent. Because of the variability between various studies, it is also recommended that food business operators validate the antimicrobial efficacy under their specific processing conditions.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The EU food hygiene legislation is aimed at protecting consumers against potential risks to health and maintaining a high level of consumer protection at all stages of the food chain. That objective must be achieved by applying the appropriate measures, including good hygiene practices and hazard control measures at each step of the food chain.

According to EU scientific advice[7], decontamination practices can constitute a useful tool in further reducing the number of pathogenic microorganisms but the use of substances intended to remove microbial surface contamination should only be permitted if a fully integrated control programme is applied throughout the entire food chain. Those substances shall be assessed thoroughly before their use is authorised.

Article 3 (2) of Regulation (EC) No 853/2004 provides a legal basis to approve, and therefore authorise, the use of substances other than potable water to remove surface contamination from products of animal origin.

In addition to the safety of the substance, are also a matter of concern the potential emergence of reduced susceptibility to biocides and/or the resistance to therapeutic antimicrobials and the impact of the substance or its by-products on the environment.

Therefore, before taking any risk management decision on their approval, a risk analysis should be carried out taking into account the results of a risk assessment based on the available scientific evidence and undertaken in an independent and transparent manner.

EFSA GUIDANCE AS PROVIDED BY THE EUROPEAN COMMISSION

On 14 April 2010, the European Food Safety Authority (EFSA) issued a revision of a guidance document[8] on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption.

APPLICATION FOR APPROVAL AS PROVIDED BY THE EUROPEAN COMMISSION

On 14 December 2010, the Commission received an application dossier from the U. S. Department of Agriculture (USDA) for the approval of lactic acid for uses to reduce microbial contamination of beef carcasses, cuts and trimmings.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is requested to evaluate the safety and efficacy of lactic acid to remove microbial surface contamination of beef carcasses, cuts and trimmings, considering:

- the toxicological safety of the substance;
- the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic microorganisms;
- the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance;
- the risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment.

[7] SCVPH (Scientific Committee On Veterinary Measures Relating To Public Health), 1998. Report on the benefits and limitations of antimicrobial treatments for poultry carcasses, 30 October 1998. SCVPH (2003) Opinion on the evaluation of antimicrobial treaments for poultry carcasses (http://ec.europa.eu/food/fs/sc/scv/out14_en.pdf).

[8] EFSA Journal 2010;8(4):1544

Clarification of the terms of reference:

Following discussion with the Commission services, the following issues were clarified:

- to also consider the safety and efficacy of decontamination of beef hides in this Scientific Opinion since this is relevant for carcass contamination; and
- to consider in the Scientific Opinion both non-rinsing and water rinsing of lactic acid after treatment.

APPROACH TAKEN TO ANSWER THE TERMS OF REFERENCE

After having received this request from the European Commission, EFSA assigned the mandate to the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel). Chapter 1 "Introduction", Chapter 3 "The efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic microorganisms", Chapter 4 "The potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance", and Chapter 5 "The risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment", and the respective conclusions were adopted by the BIOHAZ Panel on 7 July 2011. Chapter 2, "The toxicological safety of the substance to humans" and the respective conclusions were adopted by CEF Panel on 18 May 2011.

ASSESSMENT

1. INTRODUCTION

The terminology and procedure used in this assessment conform with the "Guidelines on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption" prepared by the European Food Safety Authority (EFSA, 2010a).

Approval was sought for treatments using up to 5 % (wt/wt) lactic acid solution and at temperatures of up to 55 °C for the treatment of beef hides, carcasses, cuts and/or trimmings in a variety of applications as follows:

- spray washing hides prior to hide removal;
- spray washing or misting skinned animals pre-evisceration;
- spray washing or misting post-evisceration carcasses, either whole or split pre-chill;
- misting carcasses, either whole or split, during chilling;
- spray washing or misting carcass sections or primal cuts post-chill; and
- spray washing or misting meat cuts or trimmings prior to packaging, grinding, or tenderizing.

The aim of the present Opinion is to assess the safety and efficacy of lactic acid to reduce microbial surface contamination on beef carcasses, cuts and trimmings, and beef hides considering (1) the toxicological safety of the substance, (2) the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic microorganisms, (3) the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance, and (4) the risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment. Each of these assessments is described subsequently.

2. THE TOXICOLOGICAL SAFETY OF THE SUBSTANCE TO HUMANS

2.1. Evaluation

2.1.1. Technical data

The applicant has provided information about a lactic acid solution in a concentration of up to 5 % (wt/wt). However, the impurities that might be present in the solution are not clearly specified by the applicant.

The manufacturing processes of lactic acid for which approval is requested, especially regarding production controls and quality assurance, are not described in detail.

According to the applicant, this solution may be used for the treatment of beef hides, carcasses, cuts and/or trimmings in a variety of applications as described in the literature.

Regarding data on reactions and fate of the decontaminating agent of the formulated product on the treated foods, lactic acid is a naturally occurring component of (beef) meat so that it is unlikely to form degradation or reaction products that do not occur naturally in meat. However, lactic acid should comply with the European Union specifications for food additives[9]. Two methods for analysis of lactic acid in the solutions used were specified in the application dossier.

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union, 31.12.2008, L 354/16.

2.1.2. Consumer exposure assessment

Information on estimated residue levels of lactic acid in carcasses and trimmings was provided in the application dossier. Lactic acid is present in a variety of foods, like yogurt containing 9 g/kg (Boylston, 2006), traditional cheese with 8 g/kg (Dolci et al., 2008), dry fermented sausages with 9-15 g/kg (Talon et al., 2004), and beef meat with a content of 1.4 – 5.0 g/kg (Greaser, 1986; Nassos et al., 1988). The amount of lactic acid that can be absorbed in the beef meat from lactic acid treatment may be estimated to be within the range 50-190 mg/kg. So, the overall concentration of lactic acid in beef will not be majorly affected by those residual levels. For high consumers of meat like those in Spain eating 3.3 g livestock meat/kg body weight (bw)/day (EFSA, 2011a), the consumption of the treated beef meat would correspond to an additional daily intake up to 650 micrograms of residual lactic acid/kg bw/day.

2.1.3. Toxicological data

No toxicological data were provided by the applicant in view that lactic acid is a permitted food additive (E 270) that may be used in a variety of foods other than meats (i.e. nectars, jam, jellies, marmalades, mozzarella and whey cheese, fats of animal or vegetable origin for cooking and/or frying, canned and bottled fruits and vegetables, fresh pasta, beer, etc.) according to Regulation (EC) No 1333/2008[10] on food additives. Specifications for purity are laid down in Directive 2008/84/EC[11].

Lactate is an endogenous substance (in carbohydrate and amino acid metabolism) and a natural component of very many foods, in particular fruits and fermented milk products. Under conditions of heavy energy demand (and thus high oxygen need) skeletal muscles convert glucose anaerobically into lactic acid, which is excreted from the muscle cells into the blood. In the liver this lactic acid is reduced to glucose. Ultimately any absorbed lactic acid will be oxidised to give carbon dioxide and water. In 1973 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) derived an Acceptable Daily Intake (ADI) "not limited" for lactate and several salts (JECFA, 1974). In 1991, this view (ADI "not specified") was also supported by the Scientific Committee of Food (SCF) (SCF, 1991); and in 2006 iterated in the evaluation of lactate and sodium lactate for poultry carcass treatment (EFSA, 2008). The amount of lactic acid that can be absorbed from lactic acid treatment may be estimated to be about within the range 50-190 mg/kg beef meat that would correspond to a daily intake up to 650 microgram of residual lactic acid/kg bw/day in a high consumer of meat. The amount of endogenous lactic acid in human blood is about 90 mg/L in a resting condition. Based on such estimates, the potential increase in lactic acid in the body after consumption of treated meat is negligible. Moreover, considering the fact that it is an endogenous substance, the use of lactic acid on beef carcasses, cuts and trimmings is not expected to be of safety concern.

2.2. Conclusions

The described treatments (both with and without rinsing off) are expected to leave small amounts of residual lactic acid on the surface of the beef hides, carcasses, trimmings or cuts. Considering the expected low level of exposure deriving from the use of lactic acid in such treatments and the fact that it is an endogenous substance, it was concluded that the treatments as described will be of no safety concern provided the substance used complies with the European Union specifications for food additives.

[10] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union, L 354, p 16-33.

[11] Commission Directive No 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. Official Journal of the European Union, 20.9.2008, L 253/1.

3. THE EFFICACY, I.E. DOES THE USE OF THE SUBSTANCE SIGNIFICANTLY REDUCE THE LEVEL OF CONTAMINATION OF PATHOGENIC MICROORGANISMS

3.1. Introduction

In order to assist in assessing the efficacy of a decontaminating agent, EFSA issued in 2010 a revised guidance document (EFSA, 2010a) which points out the major components and data that an application dossier should contain in order to demonstrate that the substance intended to be used for the reduction of microbial surface contamination of foods of animal origin is efficacious. These guidelines have been used in this assessment of lactic acid for use in the decontamination of beef hides, carcasses, cuts, and/or trimmings.

According to the EFSA Guidance document, the use of substance(s) as decontaminating treatments will be regarded efficacious when any reduction of the prevalence and/or numbers of pathogenic target microorganisms is statistically significant as compared to the control (e.g. water) and, at the same time, this reduction has a positive impact on reduction of human illness cases (EFSA, 2010a). Risk assessment studies on other microbial species (EFSA, 2011b, 2011c) have shown that even 0.5 $\log_{10}$ unit microbial reductions may reduce consumer risks to a significant extent. In addition, there is a linear correlation between reductions in prevalence and reductions of consumer risks. Efficacy depends on a range of factors such as concentration of the decontaminating agent, contact time, temperature, mode of application, the microbial load of the surface, and other conditions of application.

3.2. Comments on the application

The primary objective of the lactic acid application is to reduce the incidence of foodborne illness by lowering the prevalence and/or numbers of human pathogens on beef. Secondly, when used, lactic acid may also reduce spoilage organisms and increase the storage time of beef cuts and products.

The application dossier summarizes the data from 52 peer-reviewed papers documenting the efficacy of lactic acid treatment at various steps in beef processing, ranging from the cleaning of carcasses before skinning to treatment of trimmings before grinding. The submission sought approval for treatments using up to 5 % (wt/wt) lactic acid solution at temperatures of up to 55 °C applied to product categories referred to above.

The studies range from experiments on small pieces of meat in laboratory settings to simulated plant conditions and measurements in commercial plants. Included studies evaluate inoculated or naturally present pathogens such as *Salmonella* and Shigatoxin-producing/Verotoxin-producing (STEC/VTEC) *Escherichia coli*, as well as natural bacterial contamination including total viable counts, *Enterobacteriaceae*, coliforms and *Escherichia coli*. Studies were categorized in three groups: (1) those where the treatment was compared to a water control or water washing, (2) studies where the data are from before/after treatment or treated/non-treated samples, and (3) studies where the effect of the treatment on microbial flora was followed over time during storage.

3.3. Evaluation

Of the 52 papers submitted, twenty-seven were excluded for the evaluation either because the studies were outside the scope for which the applicant is seeking approval or because they evaluated only aerobic plate count (APC) and not specific pathogens or indicator organisms. More specifically, the first set of eight papers were excluded because lactic acid concentrations used were below 2 % or exceeding 5 %. The lower limit of lactic acid concentration considered in the assessment was set at 2 % as the applicant clarified that lactic acid solutions in the range of 1 % are not particularly effective and that the industry typically uses at least 2 % lactic acid.

Then, 16 papers were excluded because lactic acid was applied in ways other than spraying or misting (e.g. immersion, dipping, tumbling, sponging or centrifugation). Thus, all studies evaluating treatment of hides prior to hide removal were excluded from the evaluation because the lactic acid concentration

used was 10 % or it was applied with a sponge, neither of which was applied for. One study was excluded because offals were treated which also was not part of the application for approval.

The assessment included two pathogenic bacterial groups (*Salmonella* and STEC/VTEC, including *E. coli* O157:H7, *E. coli* O111:H8 and *E. coli* O26:H11), and indicator organisms other than APC, collectively grouped as *Enterobacteriaceae,* including coliforms and *E. coli*. *Enterobacteriaceae* are regarded as indicator bacteria; i.e. if a decontaminating agent is efficient in reducing *Enterobacteriaceae* this evidence is supportive of the efficacy to reduce enteric pathogens. The limitation to these three bacterial groups resulted in the exclusion of two more studies that evaluated only APCs. Therefore, the assessment of the efficacy of using lactic acid to decontaminate beef was based on 25 of the 52 papers included in the application dossier. All the studies in these papers were completed before the EU guidance document for such studies (EFSA, 2010a) was published and no single study addresses all the requirements in the guidance document (EFSA, 2010a). However, considered together, the studies in the 25 papers address all the requirements of the guidance.

Data from water control treatments were included in the assessment when water temperatures used were below 72 °C since hot water of 72 °C or above is a decontaminating procedure in itself (EFSA, 2010b). Both, water rinsing and non-rinsing of lactic acid after treatment of beef were to be considered in the assessment, but no studies that included rinsing after lactic acid treatment were submitted. Data using both the L (+) enantiomer and the D, L racemic mixture were included in the assessment and it was assumed that both forms have the same efficacy.

Papers included in the evaluation described studies with both inoculated and naturally contaminated beef. The studies in the papers evaluated a wide range of experimental designs and thus differed in relation to products, settings, method of application, lactic acid concentration applied, types of controls used, microorganisms studied, storage time after application, etc. All of these parameters impacted on the lactic acid decontaminating efficacy both within and between studies. Given this wide range of application conditions, the assessment did not attempt to identify contribution differences among factors, such as lactic acid concentration and application temperature.

The assessment of the body of evidence of the studies took into account whether the studies were done in the laboratory, a pilot plant or a slaughterhouse, and whether they used inoculated or naturally contaminated beef. Table 1 presents how combinations of industrial-, pilot- or laboratory-scale study settings and evaluation of natural or inoculated contamination were used to classify the strength of evidence of the data in each study. The criteria were originally presented in the FAO/WHO report on Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing (FAO/WHO, 2008), and were adapted from a previous EFSA Opinion (EFSA, 2011c).

Table 1: Relative strength of the contribution of study data to the general body of evidence, based on study type (based on EFSA (2011c))

Study type	Natural contamination[a]	Inoculated studies[b]
Industrial	High	Not applicable
Pilot-scale[c]	High[d]/medium	Medium[e]
Laboratory	Medium[e]	Low[f]

[a] Includes studies with faecal material used for inoculating the meat surface.
[b] Includes studies where the meat surface was inoculated with pathogens in faecal material or pure culture suspensions.
[c] Experiments using industrial equipment in non-industrial settings.
[d] If the pilot process is representative of the industrial process; otherwise, evidence makes a "medium" contribution to the body of evidence.
[e] Data would not be sufficient to inform a quantitative microbial risk assessment or to allow definitive conclusions on risk reduction.
[f] Data are indicative of a disinfectant effect that may be reproducible in practice, but individually do not allow definitive conclusions on risk reduction.

A table with all 52 papers submitted by the applicant and the reasons for exclusion of the 27 papers can be found in Appendix A. An overview of the 25 papers included in the assessment is given in Table 2. For detailed data on relevant characteristics (e.g. microorganisms studied, type of product treated, lactic acid concentration used, temperature of application, time and temperature of storage, $\log_{10}$ cfu reductions achieved, etc.), and strength of evidence, please refer to Appendices B to E.

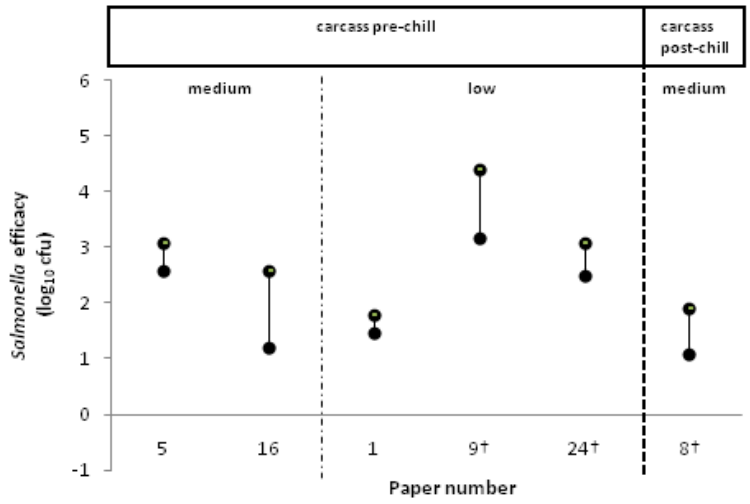
Table 2: Overview of the 25 papers included in the assessment of the efficacy of lactic acid

Paper number	Reference	Product group	Strength of evidence	Microorganisms[a]
1	Arthur et al. (2008)	Carcass pre-chill	Low	*Salm*, STEC/VTEC
2	Bacon et al. (2002)	Carcass post-chill	High	*Eb*
		Meat cuts	High	*Eb*
3	Bosilevac et al. (2006)	Carcass pre-chill	High	STEC/VTEC, *Eb*
4	Calicioglu et al. (2002)	Meat cuts	Low	STEC/VTEC, *Eb*
5	Castillo et al. (1998)	Carcass pre-chill	High	*Eb*
			Medium	*Salm*, STEC/VTEC
6	Castillo et al. (1999)	Carcass pre-chill	High	*Eb*
7	Castillo et al. (2001a)	Carcass post-chill	High	*Eb*
8	Castillo et al. (2001b)	Carcass post-chill	High	*Eb*
			Medium	*Salm*, STEC/VTEC
9	Cutter and Rivera-Betancourt (2000)	Carcass pre-chill	Low	*Salm*, STEC/VTEC
10	Cutter and Siragusa (1994)	Carcass pre-chill	Medium	STEC/VTEC
11	Dormedy et al. (2000)	Carcass pre-chill	High	*Eb*
12	Dorsa et al. (1997)	Carcass pre-chill	Low	STEC/VTEC
13	Echeverry et al. (2009)	Meat cuts	Medium	*Salm*, STEC/VTEC
14	Gill and Badoni (2004)	Meat cuts	Medium	*Eb*
15	Gill and Landers (2003)	Carcass post-chill	High	*Eb*
16	Hardin et al. (1995)	Carcass pre-chill	Medium	*Salm*, STEC/VTEC
17	Harris et al. (2006)	Trimmings	Medium	*Salm*, STEC/VTEC
18	Heller et al. (2007)	Meat cuts	Medium	STEC/VTEC
19	Kalchayanand et al. (2008)	Carcass pre-chill	Medium	STEC/VTEC
20	Kang et al. (2001)	Trimmings	High	*Eb*
21	Marshall et al. (2005)	Carcass pre-chill	Medium	STEC/VTEC, *Eb*
22	Rodriguez et al. (2004)	Carcass pre-chill	High	*Eb*
23	Ruby et al. (2007)	Carcass pre-chill	High	*Salm*, *Eb*
		Carcass post-chill	High	*Salm*, *Eb*
24	Sawyer et al. (2008)	Carcass pre-chill	Low	*Salm*, STEC/VTEC
25	Smulders and Woolthuis (1985)	Meat cuts	High	*Eb*

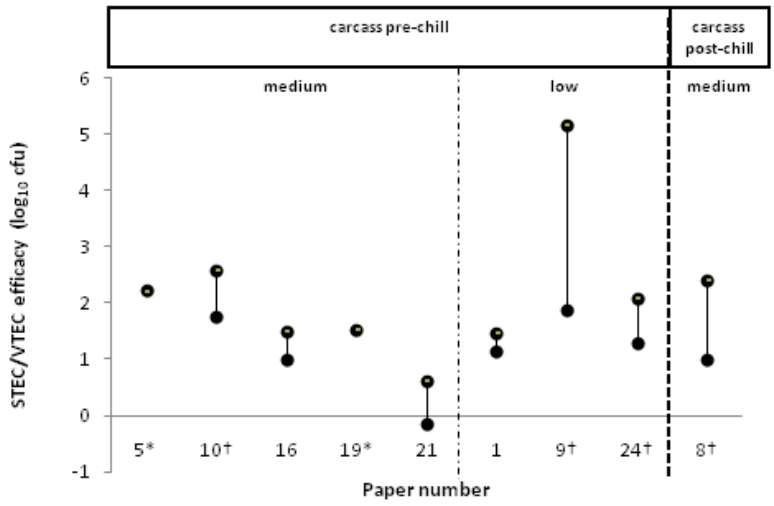
[a] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin producing *Escherichia coli*; *Eb: Enterobacteriaceae*

The ranges of lactic acid efficacies (expressed as $\log_{10}$ cfu reductions) for different conditions used in each study, are shown in Figures 1 and 2. The ranges of efficacies over a control treatment are depicted in Figure 3. The relative microbial prevalence reductions by lactic acid are shown in Table 3.

1a



1b



1c

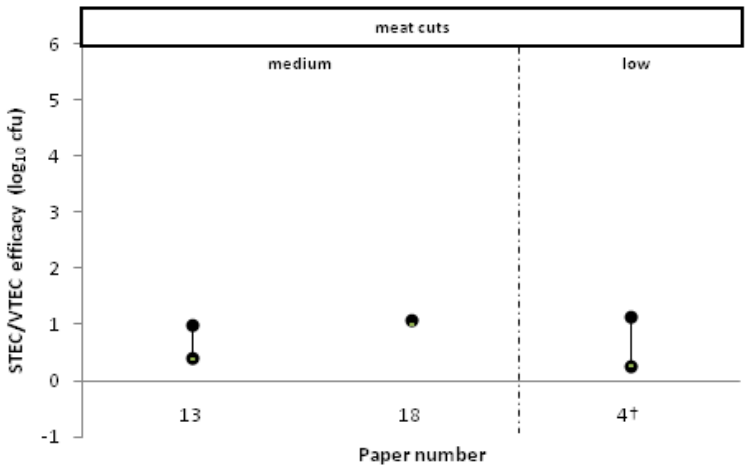
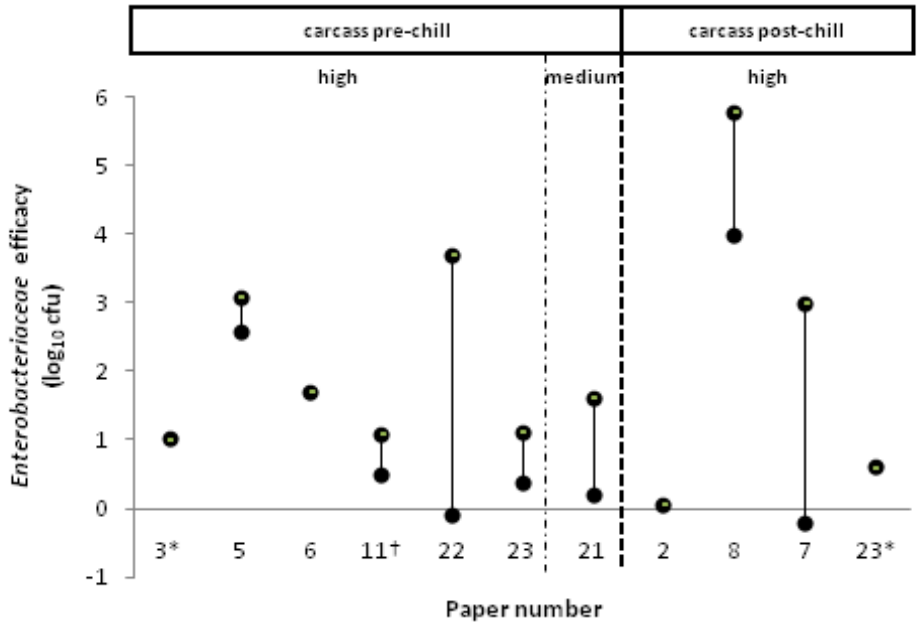


Figure 1: Efficacy of lactic acid treatment (in ranges of $\log_{10}$ cfu reductions) for *Salmonella* on beef carcasses pre-chill or post-chill (1a), STEC/VTEC on beef carcasses pre-chill or post-chill (1b), and STEC/VTEC in meat cuts (1c). The segments represent the range of efficacies for different conditions used in each study (the paper number is given in the x-axis). Dashed lines separate carcass pre-chill from post-chill studies. The dotted lines categorise the studies according to their strength of evidence as high, medium and low. Paper numbers followed by an * indicate point estimates for efficacies; an † indicates studies where storage data were also considered (ranging from 24 hours to 35 days).

2a



2b

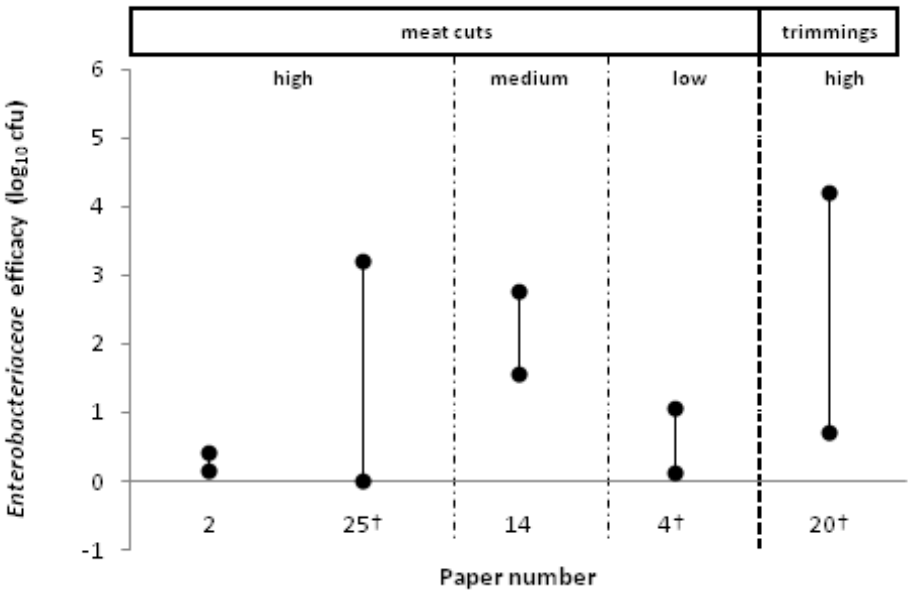
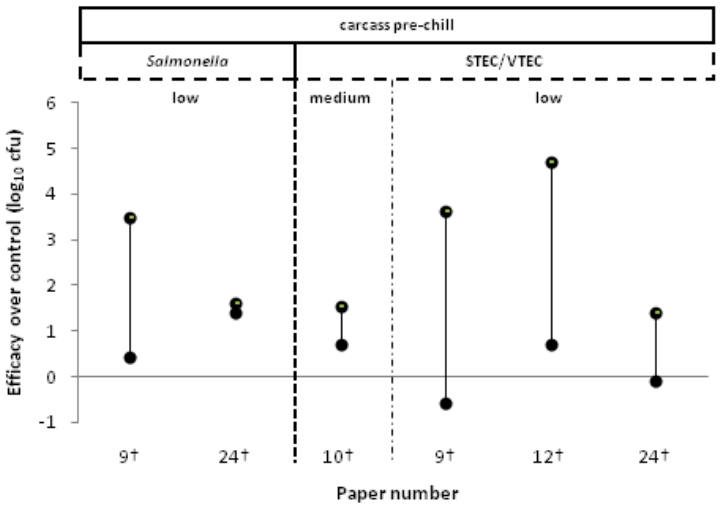
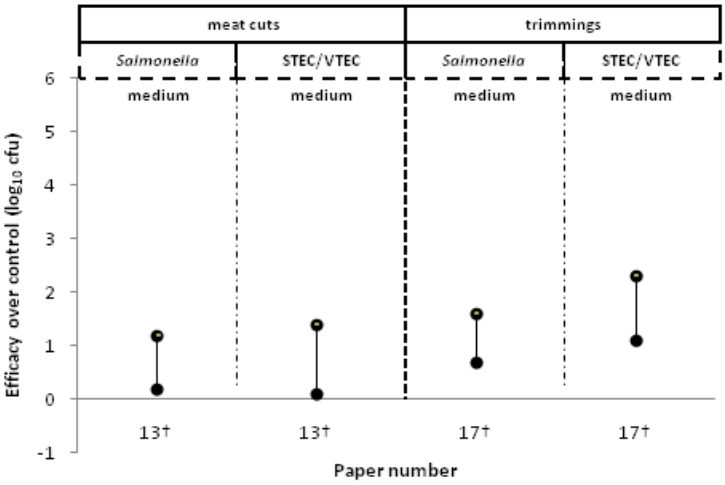


Figure 2. Efficacy of lactic acid treatment (in ranges of $\log_{10}$ cfu reductions) for *Enterobacteriaceae* on beef carcasses pre-chill or post-chill (2a), and *Enterobacteriaceae* in meat cuts and trimmings (2b). The segments represent the range of efficacies for different conditions used in each study (the paper number is given in the x-axis). Dashed lines separate carcass pre-chill from post-chill studies as well as meat cuts from trimmings. The dotted lines categorise the studies according to their strength of evidence as high, medium and low. Paper numbers followed by an * indicate point estimates for efficacies; an † indicates studies where storage data were also considered (ranging from 24 hours to 35 days).

3a



3b



3c

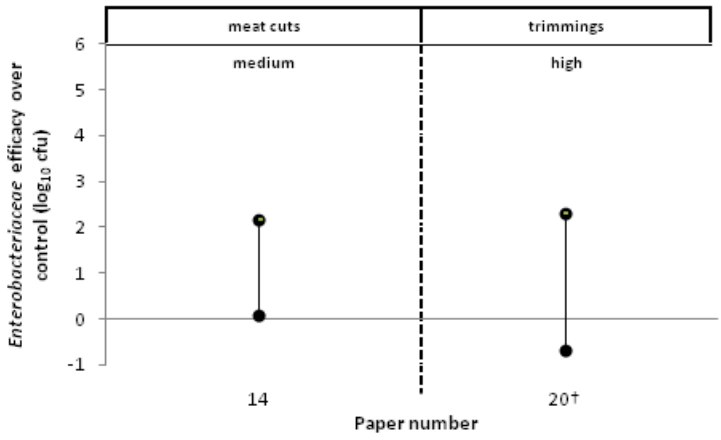


Figure 3. Efficacy of lactic acid treatment *over* control (in ranges of $\log_{10}$ cfu reductions) for *Salmonella* and STEC/VTEC on beef carcasses pre-chill (3a), *Salmonella* and STEC/VTEC in meat cuts and trimmings (3b), and *Enterobacteriaceae* in meat cuts and trimmings (3c). The segments represent the range of efficacies for different conditions used in each study (the paper number is given in the x-axis). Dashed lines separate meat cuts from trimmings studies. The dotted lines categorise the studies according to their strength of evidence as high, medium and low. Paper numbers followed by an † indicate studies where storage data were also considered (ranging from 24 hours to 35 days).

Table 3: Relative prevalence reductions[a] calculated for different microorganism groups on carcasses pre-chill and post-chill, and meat cuts and trimmings from all selected relevant studies.

Product group	Strength of evidence	Microorganisms[b]	Relative prevalence reduction (%)[c]	Paper number
Carcass pre-chill	High	Salm	37.7 to 91.8	23
		STEC/VTEC	35.5	3
		Eb	88.9	6
	Medium	Salm	12.1 to 100	16
		STEC/VTEC	17.0 to 67.0	16
Carcass post-chill	High	Salm	54.8	23
		Eb	25.0 to 38.5	2
			100.0	7
			35.3 to 46.7	15
Meat cuts	High	Eb	-4.2 to 100	25
	Medium	Eb	31.8 to 92.3	14
Trimmings	Medium	Salm	0 to 100	17
	Medium	STEC/VTEC	0	17

[a] Relative prevalence reduction = $(P_{before} - P_{after})/P_{before}$ with P = prevalence
[b] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin-producing *Escherichia coli*; *Eb: Enterobacteriaceae*
[c] Range of all selected studies

Based on the results of the selected studies, the decontamination efficacy of lactic acid as an antimicrobial intervention for **beef carcasses**, is summarized as follows:

- Microbial reductions, through application of lactic acid solutions on beef carcass surfaces pre-chill, were as follows:
    - *Salmonella* reductions, based on medium and low strength of evidence studies, ranged from 1.2 to 3.1 (Castillo et al., 1998; Hardin et al., 1995) and 1.5 to 4.4 $\log_{10}$ units (Arthur et al., 2008; Cutter and Rivera-Betancourt, 2000; Sawyer et al., 2008), respectively (Figure 1a);
    - reductions of STEC/VTEC in medium and low strength of evidence studies ranged from -0.2 to 2.6 (Castillo et al., 1998; Cutter and Siragusa, 1994; Hardin et al., 1995; Kalchayanand et al., 2008; Marshall et al., 2005) and from 1.2 to 5.2 (Arthur et al., 2008; Cutter and Rivera-Betancourt, 2000; Sawyer et al., 2008) $\log_{10}$ units, respectively (Figure 1b); and
    - based on results of studies considered as being of high and medium strength of evidence, reductions of *Enterobacteriaceae* ranged from -0.1 to 3.7 (Bosilevac et al., 2006; Castillo et al., 1998, 1999; Dormedy et al., 2000; Rodriguez et al., 2004; Ruby et al., 2007) and from 0.2 to 1.6 $\log_{10}$ units (Marshall et al., 2005), respectively (Figure 2a).
- Based on medium strength of evidence studies, reductions achieved when lactic acid solutions were applied on beef carcass surfaces post-chill ranged from 1.6 to 1.9 and from 1.0 to 2.4 $\log_{10}$ units for *Salmonella* (Castillo et al., 2001b) (Figure 1a) and STEC/VTEC (Castillo et al., 2001b) (Figure 1 b), respectively. Based on high strength of evidence studies, reductions achieved ranged from -0.2 to 5.8 $\log_{10}$ units for *Enterobacteriaceae* (Bacon et al., 2002; Castillo et al., 2001a; Castillo et al., 2001b; Ruby et al., 2007) (Figure 2a).
- Efficacy of lactic acid decontamination on beef carcass surfaces over that of control decontamination treatments was as follows:
    - reductions of *Salmonella*, based on low strength of evidence studies were 0.4 to 3.5 $\log_{10}$ units (Cutter and Rivera-Betancourt, 2000; Sawyer et al., 2008) (Figure 3a); and

- STEC/VTEC reductions ranged from 0.7 to 1.5 $\log_{10}$ units (Cutter and Siragusa, 1994), based on medium strength of evidence studies, and -0.6 to 4.7 $\log_{10}$ units (Cutter and Rivera-Betancourt, 2000; Dorsa et al., 1997; Sawyer et al., 2008) based on low strength of evidence studies (Figure 3a).

- Based on studies that evaluated naturally occurring pathogen prevalence on carcasses without and with pre-chill application of lactic acid interventions, relative prevalence reductions by lactic acid were (see Table 3):

  - for pre-chill applications, in high strength of evidence studies, *Salmonella* and STEC/VTEC were reduced by 38 % to 92 % (Ruby et al., 2007) and 36 % (Bosilevac et al., 2006), respectively, while corresponding reductions in medium strength of evidence studies ranged from 12 % to 100 % and 17 % to 67 %, respectively (Hardin et al., 1995). *Enterobacteriaceae* in a high strength of evidence study were reduced by 89 % (Castillo et al., 1999); and

  - for post-chill applications, in high strength of evidence studies, *Salmonella* were reduced by 55 % (Ruby et al., 2007) and *Enterobacteriaceae* by 25 % to 39 % (Bacon et al., 2002), 100 % (Castillo et al., 2001a) and 35 % to 47 % (Gill and Landers, 2003).

Based on examination of the studies used to derive the above ranges of reductions of microbial contamination, decreases of less than 0.5 to 1.0 $\log_{10}$ unit are generally associated with low (<2 $\log_{10}$ units) initial microbial counts or prevalences (below 50 %) on carcass surfaces used in the studies. This included situations in which lactic acid treatment was preceded by a control decontamination intervention.

Overall, reductions in microbial counts presented above exceeded 1 $\log_{10}$ unit and in many cases were much higher, reaching levels of 5.2, 5.8 and 4.7 $\log_{10}$ units respectively, when lactic acid was applied on carcasses pre-chill, post-chill and pre-chill or post-chill over a control decontamination treatment.

Based on the results of the selected studies, the decontamination efficacy of lactic acid as an antimicrobial intervention for **meat cuts and trimmings**, is summarized as follows:

- Microbial reductions, through application of lactic acid solutions on meat cuts and trimmings, were as follows:

  - reductions of STEC/VTEC in medium and low strength of evidence studies on meat cuts ranged from 0.4 to 1.1 (Echeverry et al., 2009; Heller et al., 2007) and 0.3 to 1.1 (Calicioglu et al., 2002) $\log_{10}$ units (Figure 1c); and

  - based on results of studies on meat cuts considered as being of high, medium and low strength of evidence, reductions of *Enterobacteriaceae* were 0 to 3.2 (Bacon et al., 2002; Kang et al., 2001; Smulders and Woolthuis, 1985), 1.6 to 2.8 (Gill and Badoni, 2004) and 0.1 to 1.1 (Calicioglu et al., 2002) $\log_{10}$ units, respectively. Based on studies on trimmings with a high strength of evidence, reductions of *Enterobacteriaceae* were 0.7 to 4.2 (Kang et al., 2001) $\log_{10}$ units (Figure 2b).

- Efficacy of lactic acid decontamination on meat cuts and trimmings over that of control treatments was as follows:

  - based on medium strength of evidence studies, reductions of *Salmonella* on meat cuts were 0.2 to 1.1 (Echeverry et al., 2009) and on trimmings 0.7 to 1.6 (Harris et al., 2006) $\log_{10}$ units, respectively (Figure 3b);

  - based on medium strength of evidence studies STEC/VTEC reductions on meat cuts ranged from 0.1 to 1.4 (Echeverry et al., 2009) and on trimmings from 1.1 to 2.3 (Harris et al., 2006) $\log_{10}$ units (Figure 3b); and

- based on results of studies on meat cuts considered as being of medium strength of evidence, reductions of *Enterobacteriaceae* ranged from 0.1 to 2.2 (Gill and Badoni, 2004) $\log_{10}$ units. Based on studies on trimmings with a high strength of evidence, reductions of *Enterobacteriaceae* ranged from -0.7 to 2.3 (Kang et al., 2001) $\log_{10}$ units (Figure 3c).

- Based on studies that evaluated naturally occurring pathogen prevalence on meat cuts and trimmings, relative prevalence reductions by lactic acid were (see Table 3):

  - for meat cuts, in high and medium strength of evidence studies, *Enterobacteriaceae* were reduced by -4 % to 100 % (Smulders and Woolthuis, 1985) and 32 % to 92 % (Gill and Badoni, 2004), respectively; and

  - for trimmings, in medium strength of evidence studies, *Salmonella* were reduced by 0 % to 100 % (Harris et al., 2006) and STEC/VTEC by 0 % (Harris et al., 2006).

In one study (Kang et al., 2001), the reduction by lactic acid was lower than that of a water control in one experiment. However, the water control was sprayed at a pressure of 4.48 bar while the lactic acid was sprayed at a pressure of 2.07 bar.

Overall, reductions in microbial counts presented above typically ranged between less than 1 up to just over 2 $\log_{10}$ unit over the effects of a control treatment.

3.4. Conclusions

- A total of 25 of the 52 submitted papers were included in the assessment of the efficacy of lactic acid as a decontaminating agent for beef hides, carcasses, cuts and trimmings.

- The studies described in the 25 papers used a wide range of experimental designs and thus differed in relation to products, settings, method of application, lactic acid concentration, use of controls, microorganisms studied, storage time after application, etc. All these parameters impacted the efficacy both within and between studies.

- The total volume of data from the 25 papers is regarded sufficient to draw overall conclusions on the efficacy of lactic acid to reduce pathogenic and indicator bacteria on beef carcasses, cuts and trimmings.

- In the 11 studies classified as of high strength of evidence, lactic acid was shown to reduce counts of naturally occurring *Enterobacteriaceae* on beef carcasses, cuts and trimmings to a variable degree, but, usually, these reductions were significantly higher compared to untreated or water treated controls.

- In studies classified as of high or medium strength of evidence, lactic acid was shown to reduce the prevalence of *Salmonella* and/or STEC/VTEC on carcasses, beef cuts and trimmings to varying degrees depending on study design and pretreatment prevalence.

- In studies classified as of medium strength of evidence, lactic acid was also shown to reduce counts of inoculated pathogens (*Salmonella* and/or STEC/VTEC) on beef carcasses, cuts and trimmings to a variable degree. Usually these reductions were higher on carcasses compared to meat cuts and trimmings.

- Since no studies were submitted to evaluate the efficacy of lactic acid followed by water rinsing after its application, only the efficacy of lactic acid treatment without subsequent rinsing was assessed.

- Evaluation of the efficacy of lactic acid for decontamination of hides was not performed since all studies submitted evaluated 10 % lactic acid concentrations or an application method that was not requested for approval.

3.5. Recommendations

- It is recommended that, according to HACCP principles, during use, food business operators verify lactic acid concentration, temperature of application and other factors affecting its efficacy as a decontaminating agent.

- Because of the variability between various studies, it is also recommended that food business operators validate the antimicrobial efficacy under their specific processing conditions.

4. THE POTENTIAL EMERGENCE OF REDUCED SUSCEPTIBILITY TO BIOCIDES AND/OR RESISTANCE TO THERAPEUTIC ANTIMICROBIALS LINKED TO THE USE OF THE SUBSTANCE

4.1. Evaluation including comments to application

The main issue relates to the paragraph in the application dossier mentioning *"it is extremely improbable that use of lactic acid as a meat treatment would lead to any new enzymatic-based resistance of microbes to therapeutic antibiotics"*. Although this statement is probably correct in that the use of lactic acid is unlikely to induce 'new enzymatic-based resistance of microbes to therapeutic antibiotics', the possibility of mutational changes in global regulatory genes as a consequence of exposure to lactic acid either at high concentrations or for long periods has not been fully considered. Horizontal transfer of such resistances from non-pathogens to pathogens may occur not only by conjugation, which is for the most part confined to plasmids, transposons and integrons, but is also theoretically possible, albeit at very low level, by natural genetic transformation of the mutated global regulatory genes (Courvalin, 2008; EFSA, 2010a). This in turn may lead to the dissemination of such resistances in the environment. Such considerations (i.e., changes in global regulatory genes) may also apply to the development of low-level resistance to biocides (Karatzas et al., 2008). The possibility and public health significance of mutational changes from prolonged exposure to lactic acid through various uses should be considered.

A further possibility that has not been addressed by the applicant is the selection pressure imposed by the use of lactic acid on the transfer via the food chain from animals to humans of lactobacilli that are resistant to antimicrobial agents and that are naturally present on carcasses. Studies have indicated that such organisms frequently exhibit multiple resistance to clinically-relevant antimicrobials encoded by genes with high sequence similarities to genes in pathogenic bacteria (Aquilanti et al., 2007; Mathur and Singh, 2005; Teuber et al., 1999). Such genes are plasmid-encoded and are thus capable of transfer to pathogenic organisms. The public health significance of antimicrobial-resistant *Lactobacillus* in the diet should be considered.

There is some evidence that repeated exposure to repetitive cycles of mild bactericidal treatments, including exposure to lactic acid, can induce reduced susceptibility of pathogens such as *Listeria monocytogenes*, *E. coli* O157:H7 and *Campylobacter jejuni* to such compounds (Rajkovic et al., 2009). Under such circumstances reduced susceptibility to lactic acid could be a problem if cleaning in the plant was insufficient. Therefore under good hygienic practices (GHP), this possibility is not considered a significant issue. Nevertheless it should be stressed that lactic acid treatment of beef should not be a substitute for GHP.

4.2. Conclusions

- Data to address the issue of the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance were not provided.

- The development of enzymatic resistance to therapeutic antimicrobials as a result of exposure to lactic acid is unlikely.

- Considering the extensive natural presence of lactic acid in fermented food, the possibility of mutational change resulting in the development of resistance to therapeutic antimicrobials is also unlikely to be a significant issue.

- There is some evidence that repeated exposure to lactic acid can select for reduced susceptibility to the substance. Under GHP, this possibility is not considered a significant issue.

5. THE RISK RELATED TO THE RELEASE OF THE SLAUGHTERHOUSE AND/OR PROCESSING PLANT EFFLUENTS, LINKED TO THE USE OF THE SUBSTANCE, INTO THE ENVIRONMENT

According to the application dossier, it is estimated that there is about 10 mg of lactic acid per litre of wastewaters just before entering the wastewater treatment system. This concentration is based on data for water use and lactic acid use in a US meat plant. The contribution of such lactic acid concentration (10 mg/L) to pH decrease in the wastewater can be considered as negligible. As lactic acid is fully biodegradable, this concentration would be further reduced during wastewater treatment. The Biological Oxygen Demand (BOD) of slaughterhouse wastewater is in the order of several grams per litre (Doble and Kumar, 2005), hence 100 to 1000-fold higher than the lactic acid concentration. For these reasons an environmental risk assessment is not considered necessary.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Conclusions in relation to the toxicological safety of the substance

- Considering the expected low level of exposure deriving from the use of lactic acid in carcasses, cuts and trimmings and the fact that it is an endogenous substance, it was concluded that the treatments, as described, will be of no safety concern, provided the substance used complies with the European Union specifications for food additives.

Conclusions in relation to the efficacy, i.e. does the use of the substance significantly reduce the level of contamination of pathogenic microorganisms

- Of the 52 papers submitted by the applicant, twenty-seven were excluded for the evaluation either because the studies were outside the scope for which the applicant is seeking approval or because they evaluated only aerobic plate count and not specific pathogens or indicator organisms.

- The studies described in the remaining 25 papers used a wide range of experimental designs and thus differed in relation to products, settings, method of application, lactic acid concentration, use of controls, microorganisms studied, time of analysis after application, etc. All these parameters impacted the efficacy both within and between studies.

- Studies on industrial scale and pilot scale which are representative of industrial scale with naturally contaminated products were considered as providing high strength of evidence. Pilot studies with naturally contaminated products and with inoculated pathogenic microorganisms and laboratory studies with naturally contaminated products were considered as providing medium strength of evidence. Laboratory studies with inoculated pathogenic microorganisms were considered as providing low strength of evidence.

- In the studies classified as of high strength of evidence, lactic acid was shown to reduce counts of naturally occurring *Enterobacteriaceae* on beef carcasses, cuts and trimmings to a variable degree, but usually these reductions were significantly higher compared to untreated or water treated controls.

- In studies classified as of high or medium strength of evidence, lactic acid was shown to reduce the prevalence of *Salmonella* and/or STEC/VTEC on carcasses, beef cuts and trimmings to varying degrees depending on study design and contamination level, but reductions were generally significantly higher compared to controls.

- In studies classified as of medium strength of evidence, lactic acid was shown to reduce counts of inoculated pathogens (*Salmonella* and/or STEC/VTEC) on beef carcasses, cuts and trimmings to a variable degree. Usually these reductions were higher on carcasses compared to meat cuts and trimmings.

- Evaluation of the efficacy of lactic acid for decontamination of hides was not performed since all studies evaluating hides used 10 % lactic acid or application was through methods not requested for approval.

Conclusions in relation to the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance

- Data to address the issue of the potential emergence of reduced susceptibility to biocides and/or resistance to therapeutic antimicrobials linked to the use of the substance were not provided.

- The development of enzymatic resistance to therapeutic antimicrobials as a result of exposure to lactic acid is unlikely.

- Considering the extensive natural presence of lactic acid in fermented food, the possibility of mutational change resulting in the development of resistance to therapeutic antimicrobials is also unlikely to be a significant issue.

- There is some evidence that repeated exposure to lactic acid can select for reduced susceptibility to the substance. Under GHP, this possibility is not considered a significant issue.

Conclusions in relation to the risk related to the release of the slaughterhouse and/or processing plant effluents, linked to the use of the substance, into the environment.

- The concentration of lactic acid just before entering the wastewater treatment system can be considered as negligible and an environmental risk assessment was therefore considered not necessary.

RECOMMENDATIONS

- It is recommended that, according to HACCP principles, during use, food business operators verify lactic acid concentration, temperature of application and other factors affecting its efficacy as a decontaminating agent.

- Because of the variability between various studies, it is also recommended that food business operators validate the antimicrobial efficacy under their specific processing conditions.

DOCUMENTATION PROVIDED TO EFSA

1. Letter Ref. Ares(2010)991913 received on 19 January 2011 including the request form the Commission and the application dossier from U.S. Department of Agriculture (USDA) "Submission of data for the authorization of lactic acid for uses to reduce microbial contamination of beef carcasses and tissues".

2. Reply to questions posed on 18 February 2011 by the EFSA Secretariat to the Contact Person at USDA. Received from the USDA on 16 March 2011.

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APPENDICES

A. TABLE WITH ALL 52 PAPERS SUBMITTED BY THE APPLICANT AND THE REASONS FOR EXCLUSION OF 27 PAPERS FROM THE ASSESSMENT

Reference	Include in assessment (reason for exclusion)
Acuff et al. (1987)	No (lactic acid concentration: 1 %)
Anderson and Marshall (1990)	No (way of application: dipping)
Anderson et al. (1992)	No (way of application: dipping)
Arthur et al. (2008)	Yes
Bacon et al. (2002)	Yes
Baird et al. (2006)	No (way of application: sponge used)
Bosilevac et al. (2006)	Yes
Bracket et al. (1994)	No (lactic acid concentration: 1 %)
Calicioglu et al. (2002)	Yes
Carlson et al. (2008a)	No (lactic acid concentration: 10 %)
Carlson et al. (2008b)	No (lactic acid concentration: 10 %)
Castillo et al. (1998)	Yes
Castillo et al. (1999)	Yes
Castillo et al. (2001a)	Yes
Castillo et al. (2001b)	Yes
Cubon et al. (2006)	No (bacteria: only APC)
Cutter and Rivera-Betancourt (2000)	Yes
Cutter and Siragusa (1994)	Yes
Delmore et al. (2000)	No (offals treated)
Dickson & Kunduru (1995)	No (way of application: dipping)
Dormedy et al. (2000)	Yes
Dorsa et al. (1997)	Yes
Dorsa et al. (1998)	No (inoculated after washing with lactic acid solution)
Echeverry et al. (2009)	Yes
Ellebracht et al. (1999)	No (way of application: submersion)
Gill and Badoni (2004)	Yes
Gill et al.(1996)	No (no lactic acid used)
Gill and Landers (2003)	Yes
Hamby et al. (1987)	No (lactic acid concentration: 1 %)
Hardin et al. (1995)	Yes
Harris et al. (2006)	Yes
Heller et al. (2006)	No (bacteria: only APC)
Heller et al. (2007)	Yes
Ikeda et al (2003)	No (way of application: dipping)
Kalchayanand et al. (2008)	Yes
Kang et al. (2001)	Yes
Kotula et al. (1994)	No (way of application: dipping)
Marshall et al. (2005)	Yes
Ozdemir et al. (2006)	No (way of application: dipping)
Podolak et al. (1996)	No (way of application: dipping)
Prasai et al. (1991)	No (lactic acid concentration: 1 % and bacteria: only APC)
Ransom et al. (2003)	No (way of application: dipping)
Rodriguez et al. (2004)	Yes
Rose et al. (2004)	No (way of application: immersion)
Ruby et al. (2007)	Yes
Sawyer et al. (2008)	Yes
Signorini et al. (2006)	No (way of application: dipping)
Smulders and Woolthuis (1985)	Yes
Stivarius et al. (2002)	No (way of application: tumbling)
Stopforth et al. (2007)	No (way of application: dipping)
Visser et al. (1988)	No (way of application: centrifugation)
Woolthuis and Smulders (1985)	No (lactic acid concentration: < 2 % and bacteria: only APC)

B. TABLE WITH DETAILLED DATA OF LACTIC ACID TREATMENT OF BEEF CARCASSES PRE-CHILL USING THE 25 PAPERS INCLUDED IN THE ASSESSMENT

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Temperature[g]		
HIGH STRENGTH OF EVIDENCE																					
3	Bosilevac et al. (2006)	*Eb*	1.0			***	Carcass pre-evisceration	*Eb*	Natural	L	2 %	42 °C	NS	Spray	NS	Ind	UC	NS	NS	Sponge	255
5	Castillo et al. (1998)	*Eb*	2.6			***	Outside round, brisket, clod	*Eb*	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
5	Castillo et al. (1998)	*Eb*	2.7			***	Outside round, brisket, clod	Total coliforms	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
5	Castillo et al. (1998)	*Eb*	2.6-3.1			***	Outside round, brisket, clod	Thermotolerant coliforms	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
5	Castillo et al. (1998)	*Eb*	2.6-3.1			***	Outside round, brisket, clod	Generic *E. coli*	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
6	Castillo et al. (1999)	*Eb*	1.7			***	Outside round, brisket, clod	*Eb*	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
6	Castillo et al. (1999)	*Eb*	1.7			***	Outside round, brisket, clod	Total coliforms	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
6	Castillo et al. (1999)	*Eb*	1.7			***	Outside round, brisket, clod	Thermotolerant coliforms	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
6	Castillo et al. (1999)	*Eb*	1.6			***	Outside round, brisket, clod	*E. coli*	FM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
11	Dormedy et al. (2000)	*Eb*	0.9			***	Carcass	Coliforms	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	30
11	Dormedy et al. (2000)	*Eb*	1.1			***	Carcass	Generic *E. coli*	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	30
11	Dormedy et al. (2000)	*Eb*	1.0			***	Carcass	Coliforms	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	24 h	Chill	Sponge	30
11	Dormedy et al. (2000)	*Eb*	1.1			***	Carcass	Generic *E. coli*	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	24 h	Chill	Sponge	30
11	Dormedy et al. (2000)	*Eb*	0.5			***	Carcass	Coliforms	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	30
11	Dormedy et al. (2000)	*Eb*	0.8			***	Carcass	Generic *E. coli*	Natural	NS	2 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	30
22	Rodriguez et al. (2004)	*Eb*	0.5			NS	Rump	Coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	-0.1			NS	Clod	Coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	1.0			NS	Brisket	Coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	2.5			***	Rump	Total coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	2.0-3.0			***	Clod	Total coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	2.7-3.7			***	Brisket	Total coliforms	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	2.0-3.0			***	Rump	*E. coli*	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	1.1-2.1			***	Clod	*E. coli*	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
22	Rodriguez et al. (2004)	*Eb*	2.2-3.2			***	Brisket	*E. coli*	Natural	NS	2 %	55 °C	20 s	Spray	2.76 bar	Pilot	UC	NS	NS	Sponge	
23	Ruby et al. (2007)	*Eb*	0.39			***	Carcass	*Eb*	Natural	NS	4-5 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	3184
23	Ruby et al. (2007)	*Eb*	1.12			***	Carcass	*Eb*	Natural	NS	4-5 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	3184
MEDIUM STRENGTH OF EVIDENCE																					
5	Castillo et al. (1998)	*Salm*	2.6-3.1			***	Outside round, brisket, clod	*S.* Typhimurium	IFM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
16	Hardin et al. (1995)	*Salm*	1.2			***	Inside rounds, outside rounds, briskets, clods	*S.* Typhimurium	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	*Salm*	1.8			***	Inside rounds, outside rounds, briskets, clods	*S.* Typhimurium	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	*Salm*	2.5			***	Inside rounds, outside rounds, briskets, clods	*S.* Typhimurium	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	*Salm*	2.6			***	Inside rounds, outside rounds, briskets, clods	*S.* Typhimurium	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
5	Castillo et al. (1998)	STEC/VTEC	2.2			***	Outside round, brisket, clod	*E. coli* O157:H7	IFM	L	2 %	55 °C	11 s	Spray	2.76 bar	Pilot	UC	NS	NS	Excision	9
10	Cutter and Siragusa (1994)	STEC/VTEC	1.76	1.06	0.70	NP	Beef carcass tissue from outer surface of carcass	*E. coli* O157:H7	PC	DL	3 %	24 °C	NS	Spray	5.51 bar	Pilot	Water (24 °C)	24 h	4 °C	Excision	3
10	Cutter and Siragusa (1994)	STEC/VTEC	2.60	1.06	1.54	NP	Beef carcass tissue from outer surface of carcass	*E. coli* O157:H7	PC	DL	5 %	24 °C	NS	Spray	5.51 bar	Pilot	Water (24 °C)	24 h	4 °C	Excision	3
16	Hardin et al. (1995)	STEC/VTEC	1			***	Inside rounds, outside rounds, briskets, clods	*E. coli* O157:H7	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	STEC/VTEC	1.5			***	Inside rounds, outside rounds, briskets, clods	*E. coli* O157:H7	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	STEC/VTEC	1.2			***	Inside rounds, outside rounds, briskets, clods	*E. coli* O157:H7	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
16	Hardin et al. (1995)	STEC/VTEC	1.2			***	Inside rounds, outside rounds, briskets, clods	*E. coli* O157:H7	IFM	L	2 %	55 °C	11 s	Mist	2.76 bar	Pilot	UC	NS	NS	Excision	18
19	Kalchayanand et al. (2008)	STEC/VTEC	1.52			***	Cheek area of bovine heads	*E. coli* O157:H7	PC	DL	2 %	25 °C	26 s	Spray	1.72 bar	Pilot	UC	10 min	20-25 °C	Excision	40
21	Marshall et al. (2005)	STEC/VTEC	0.62			NP	Cutaneous trunci, adepose carcass trim	*E. coli* O157:H7	IFM	NS	2 %	20 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	STEC/VTEC	0.31			NP	Cutaneous trunci, adepose carcass trim	*E. coli* O157:H7	IFM	NS	2 %	55 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	STEC/VTEC	-0.16			NP	Cutaneous trunci, adepose carcass trim	*E. coli* O157:H7	IFM	NS	2 %	20 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.81			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P1	IFM	NS	2 %	20 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.38			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P3	IFM	NS	2 %	20 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Temperature[g]		
21	Marshall et al. (2005)	*Eb*	0.64			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P8	IFM	NS	2 %	20 °C	3 s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.92			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P14	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.98			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P68	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.89			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P1	IFM	NS	2 %	55°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.34			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P3	IFM	NS	2 %	55°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.20			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P8	IFM	NS	2 %	55°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.69			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P14	IFM	NS	2 %	55°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.70			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P68	IFM	NS	2 %	55°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	1.61			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P1	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.38			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P3	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.74			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P8	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.85			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P14	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
21	Marshall et al. (2005)	*Eb*	0.73			NP	Cutaneous trunci, adepose carcass trim	*E. coli* P68	IFM	NS	2 %	20°C	3s	Spray	1.38 bar	Pilot	UC	NS	NS	Excision	
LOW STRENGTH OF EVIDENCE																					
1	Arthur et al. (2008)	*Salm*	1.80			NP	Carcass	*S.* Newport MDR[h]	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
1	Arthur et al. (2008)	*Salm*	1.61			NP	Carcass	*S.* Newport susceptible	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
1	Arthur et al. (2008)	*Salm*	1.46			NP	Carcass	*S.* Typhimurium MDR[h]	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
1	Arthur et al. (2008)	*Salm*	1.57			NP	Carcass	*S.* Typhimurium susceptible	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.16	2.71	0.45	NP	Shortplates or cutaneous trunci	*S.* Typhimurium	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.22	2.80	0.42	NP	Shortplates or cutaneous trunci	*S.* Typhimurium DT 104	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.96	1.41	2.55	NP	Shortplates or cutaneous trunci	*S.* Typhimurium	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.78	1.59	2.19	NP	Shortplates or cutaneous trunci	*S.* Typhimurium DT 104	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.57	1.12	2.45	NP	Shortplates or cutaneous trunci	*S.* Typhimurium	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.60	1.29	2.31	NP	Shortplates or cutaneous trunci	*S.* Typhimurium DT 104	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.95	0.84	3.11	NP	Shortplates or cutaneous trunci	*S.* Typhimurium	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	3.82	1.81	2.01	NP	Shortplates or cutaneous trunci	*S.* Typhimurium DT 104	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	4.30	0.82	3.48	NP	Shortplates or cutaneous trunci	*S.* Typhimurium	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	*Salm*	4.41	2.20	2.21	NP	Shortplates or cutaneous trunci	*S.* Typhimurium DT 104	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6
24	Sawyer et al. (2008)	*Salm*	3.1	1.5	1.6	***	Outside round of carcass	*S.* Typhimurium	IFM	L	2.5 %	55°C	11s	Mist	0.69 bar	Lab	Water spray (35°C, up to 27.58 bar)	NS	NS	Excision	2[i]
24	Sawyer et al. (2008)	*Salm*	2.5	1.1	1.4	NS	Outside round of carcass	*S.* Typhimurium	IFM	L	2.5 %	55°C	11s	Mist	0.69 bar	Lab	Water spray (35°C, up to 27.58 bar)	24 h	4 °C	Excision	2[i]
1	Arthur et al. (2008)	STEC/VTEC	1.47			NP	Carcass	*E. coli* O157:H7 HDA[j]	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
1	Arthur et al. (2008)	STEC/VTEC	1.15			NP	Carcass	*E. coli* O157:H7 non HDA[j]	PC	DL	2 %	25°C	20s	Spray	1.72 bar	Lab	UC	30 s	Ambient	Excision	12
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.00	0.87	1.13	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.00	1.54	1.46	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Temperature[g]		
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.73	2.12	0.61	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	5.18	1.54	3.64	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.36	2.24	0.12	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.28	1.45	1.83	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	1.87	2.12	-0.25	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	4.39	2.65	1.74	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.94	3.53	-0.59	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.86	3.44	0.42	NP	Shortplates or cutaneous trunci	*E. coli* O157:H7	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6
12	Dorsa et al. (1997)	STEC/VTEC			0.7		Lean surface of beef carcass	*E. coli* O157:H7	IFM	NS	3 % (or 1.5 %)	32°C	15 s	Spray	5.52 bar	Lab	Water (32 °C, 15 s, 5.52 bar)	0 d	5 °C	Excision	10
12	Dorsa et al. (1997)	STEC/VTEC			2.3-3.6		Lean surface of beef carcass	*E. coli* O157:H7	IFM	NS	3 % (or 1.5 %)	32°C	15 s	Spray	5.52 bar	Lab	Water (32 °C, 15 s, 5.52 bar)	2 d	5 °C	Excision	10
12	Dorsa et al. (1997)	STEC/VTEC			3.4-4.7		Lean surface of beef carcass	*E. coli* O157:H7	IFM	NS	3 % (or 1.5 %)	32°C	15 s	Spray	5.52 bar	Lab	Water (32 °C, 15 s, 5.52 bar)	7 d	5 °C	Excision	10
12	Dorsa et al. (1997)	STEC/VTEC			3.2-4.5		Lean surface of beef carcass	*E. coli* O157:H7	IFM	NS	3 % (or 1.5 %)	32°C	15 s	Spray	5.52 bar	Lab	Water (32 °C, 15 s, 5.52 bar)	14 d	5 °C	Excision	10
12	Dorsa et al. (1997)	STEC/VTEC			3.4-4.7		Lean surface of beef carcass	*E. coli* O157:H7	IFM	NS	3 % (or 1.5 %)	32°C	15 s	Spray	5.52 bar	Lab	Water (32 °C, 15 s, 5.52 bar)	21 d	5 °C	Excision	10
24	Sawyer et al. (2008)	STEC/VTEC	1.3	1.4	-0.1	NS	Outside round of carcass	*E. coli* O157:H7	IFM	L	2.5 %	55°C	11s	Mist	0.69 bar	Lab	Water spray (35°C, up to 27.58 bar)	NS	NS	Excision	2[i]
24	Sawyer et al. (2008)	STEC/VTEC	2.1	0.7	1.4	NS	Outside round of carcass	*E. coli* O157:H7	IFM	L	2.5 %	55°C	11s	Mist	0.69 bar	Lab	Water spray (35°C, up to 27.58 bar)	24 h	4 °C	Excision	2[i]
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.36	1.85	0.51	NP	Shortplates or cutaneous trunci	*E. coli* O111:H8	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.26	1.59	1.67	NP	Shortplates or cutaneous trunci	*E. coli* O26:H11	IFM	NS	2 %	35°C	15s	Spray	8.62 bar	Lab	Water (35 °C)	0 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	2.89	2.11	0.78	NP	Shortplates or cutaneous trunci	*E. coli* O111:H8	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.74	1.78	1.96	NP	Shortplates or cutaneous trunci	*E. coli* O26:H11	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	2 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.01	2.00	1.01	NP	Shortplates or cutaneous trunci	*E. coli* O111:H8	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.02	1.75	1.27	NP	Shortplates or cutaneous trunci	*E. coli* O26:H11	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	7 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.15	1.44	1.71	NP	Shortplates or cutaneous trunci	*E. coli* O111:H8	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	5.05	2.47	2.58	NP	Shortplates or cutaneous trunci	*E. coli* O26:H11	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	21 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.62	2.33	1.29	NP	Shortplates or cutaneous trunci	*E. coli* O111:H8	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6
9	Cutter and Rivera-Betancourt (2000)	STEC/VTEC	3.78	2.62	1.16	NP	Shortplates or cutaneous trunci	*E. coli* O26:H11	IFM	NS	2 %	35°C	15 s	Spray	8.62 bar	Lab	Water (35 °C)	35 d	4 °C	Excision	6

[a] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin-producing *Escherichia coli*; *Eb: Enterobacteriaceae*
[b] ***: significant; NS: not significant; NP: not provided
[c] FM: faecal material; IFM: pathogen inoculated in faecal material; PC: pure culture suspension
[d] NS: not specified
[e] Ind: industrial scale; Lab: lab-scale
[f] UC: untreated control
[g] NS: no storage
[h] MDR: multidrug resistant
[i] Number of replicated experiments
[j] HDA: human disease associated

C. TABLE WITH DETAILLED DATA OF LACTIC ACID TREATMENT OF BEEF CARCASSES POST-CHILL USING THE 25 PAPERS INCLUDED IN THE ASSESSMENT

Paper no	Reference	Microorganisms[a]	Microbial reduction by treated group	Signi-ficant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
								Enantiomer[d]	Concentration	Tempe-rature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Tempe-rature[g]		
HIGH STRENGTH OF EVIDENCE																			
2	Bacon et al. (2002)	*Eb*	0.06	***	Carcass	Total coliforms	Natural	NS	1.5 to 2.5 %	29.5 °C	3 s	Mist	1.79 bar	Ind	UC	NS	NS	Sponge	105
2	Bacon et al. (2002)	*Eb*	0.03	***	Carcass	*E. coli*	Natural	NS	1.5 to 2.5 %	29.5 °C	3 s	Mist	1.79 bar	Ind	UC	NS	NS	Sponge	105
8	Castillo et al. (2001b)	*Eb*	4.2	NS	Carcass rounds	*E. coli*	FM	L	2 %	55 °C	15 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.7-5.7	***	Carcass rounds	*E. coli*	FM	L	2 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.0	***	Carcass rounds	*E. coli*	FM	L	2 %	65 °C	15 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.4-5.4	***	Carcass rounds	*E. coli*	FM	L	2 %	65 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.2	***	Carcass rounds	*E. coli*	FM	L	4 %	55 °C	15 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.8-5.8	***	Carcass rounds	*E. coli*	FM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.5-5.5	***	Carcass rounds	*E. coli*	FM	L	4 %	65 °C	15 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
8	Castillo et al. (2001b)	*Eb*	4.6-5.6	***	Carcass rounds	*E. coli*	FM	L	4 %	65 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	16
7	Castillo et al. (2001a)	*Eb*	0-1.4	***	Brisket	Coliforms	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
7	Castillo et al. (2001a)	*Eb*	1.6-3.0	***	Clod	Coliforms	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
7	Castillo et al. (2001a)	*Eb*	0.3-1.4	***	Neck	Coliforms	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
7	Castillo et al. (2001a)	*Eb*	0-1.4	***	Brisket	*E. coli*	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
7	Castillo et al. (2001a)	*Eb*	-0.2-1.4	***	Clod	*E. coli*	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
7	Castillo et al. (2001a)	*Eb*	0-1.4	***	Neck	*E. coli*	Natural	L	4 %	55 °C	35 s	Mist	NS	Pilot	UC	NS	NS	Sponge	40
23	Ruby et al. (2007)	*Eb*	0.59	***	Carcass	*Eb*	Natural	NS	4-5 %	NS	NS	Spray	NS	Ind	UC	NS	NS	Sponge	3139
MEDIUM STRENGTH OF EVIDENCE																			
8	Castillo et al. (2001b)	*Salm*	1.9	***	Carcass rounds	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	6
8	Castillo et al. (2001b)	*Salm*	1.6	***	Carcass rounds	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	6
8	Castillo et al. (2001b)	*Salm*	1.3	***	Carcass rounds ground	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	4 °C	Excision	6
8	Castillo et al. (2001b)	*Salm*	1.5	***	Carcass rounds ground	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	7 d	4 °C	Excision	6
8	Castillo et al. (2001b)	*Salm*	1.1	***	Carcass rounds ground	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	14 d	4 °C	Excision	6
8	Castillo et al. (2001b)	*Salm*	1.7	***	Carcass rounds ground	*S.* Typhimurium	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	21 d	4 °C	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	2.4	***	Carcass rounds	*E. coli* O157:H7	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	2.0	***	Carcass rounds	*E. coli* O157:H7	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	NS	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	1.0	***	Carcass rounds ground	*E. coli* O157:H7	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	7 d	4 °C	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	1.0	***	Carcass rounds ground	*E. coli* O157:H7	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	14 d	4 °C	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	1.2	***	Carcass rounds ground	*E. coli* O157:H7	IFM	L	4%	55°C	30 s	Spray	0.69 bar	Pilot	UC	21 d	4 °C	Excision	6
8	Castillo et al. (2001b)	STEC/VTEC	1.8	***	Carcass rounds ground	*E. coli* O157:H7	IFM	L	4 %	55 °C	30 s	Spray	0.69 bar	Pilot	UC	NS	4 °C	Excision	6

[a] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin-producing *Escherichia coli*; *Eb: Enterobacteriaceae*
[b] ***: significant
[c] FM: faecal material; IFM: pathogen inoculated in faecal material
[d] NS: not specified
[e] Ind: industrial scale
[f] UC: untreated control
[g] NS: no storage

D. TABLE WITH DETAILLED DATA OF LACTIC ACID TREATMENT OF BEEF CUTS USING THE 25 PAPERS INCLUDED IN THE ASSESSMENT

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Temperature[g]		
HIGH STRENGTH OF EVIDENCE																					
2	Bacon et al. (2002)	*Eb*	0.41			***	Subprimal cuts	Total coliforms	Natural	NS	1.5-2.5 %	29.5 °C	NS	Mist	NS	Ind	UC	NS	NS	Sponge	35
2	Bacon et al. (2002)	*Eb*	0.32			***	Subprimal cuts	*E. coli*	Natural	NS	1.5-2.5 %	29.5 °C	NS	Mist	NS	Ind	UC	NS	NS	Sponge	35
2	Bacon et al. (2002)	*Eb*	0.15			NS	Subprimal cuts after lactic acid treatment	Total coliforms	Natural	NS	1.5-2.5 %	29.5 °C	NS	Mist	NS	Ind	UC	NS	NS	Sponge	35
2	Bacon et al. (2002)	*Eb*	0.32			***	Subprimal cuts after lactic acid treatment	*E. coli*	Natural	NS	1.5-2.5 %	29.5 °C	NS	Mist	NS	Ind	UC	NS	NS	Sponge	35
25	Smulders and Woolthuis (1985)	*Eb*	0.3			***	Carcass + meat cut	*Eb*	Natural	L	1.25 % + 2 %	NS	30 s	Spray	1.01 bar	Pilot	UC	45 min	3 °C	Excision	
25	Smulders and Woolthuis (1985)	*Eb*	0-1.3			***	Carcass + meat cut	*Eb*	Natural	L	1.25 % + 2 %	NS	30 s	Spray	1.01 bar	Pilot	UC	2.5 h	3 °C	Excision	
25	Smulders and Woolthuis (1985)	*Eb*	0.8-2.1			***	Carcass + meat cut	*Eb*	Natural	L	1.25 % + 2 %	NS	30 s	Spray	1.01 bar	Pilot	UC	7 d	3 °C	Excision	
25	Smulders and Woolthuis (1985)	*Eb*	1.5-2.8			***	Carcass + meat cut	*Eb*	Natural	L	1.25 % + 2 %	NS	30 s	Spray	1.01 bar	Pilot	UC	9 d	3 °C	Excision	
25	Smulders and Woolthuis (1985)	*Eb*	1.9-3.2			***	Carcass + meat cut	*Eb*	Natural	L	1.25 % + 2 %	NS	30 s	Spray	1.01 bar	Pilot	UC	14 d	3 °C	Excision	
MEDIUM STRENGTH OF EVIDENCE																					
13	Echeverry et al. (2009)	*Salm*			0.2	NS	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	Chill	Excision	
13	Echeverry et al. (2009)	*Salm*			0.9	***	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	14 d	Chill	Excision	
13	Echeverry et al. (2009)	*Salm*			1.2	***	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	21 d	Chill	Excision	
13	Echeverry et al. (2009)	*Salm*			0.2	NS	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	Chill	Excision	
13	Echeverry et al. (2009)	*Salm*			1.1	***	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	14 d	Chill	Excision	
13	Echeverry et al. (2009)	*Salm*			0.9	***	Boneless beef strip loin	*S.* Typhimurium	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	21 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC	1.0	0.5	0.5	NS	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	NS	Excision	
13	Echeverry et al. (2009)	STEC/VTEC	0.4	0.3	0.1	NS	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	NS	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			0.4	NS	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			0.7	***	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	14 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			1.4	***	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	21 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			0.4	NS	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	0 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			0.1	NS	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	14 d	Chill	Excision	
13	Echeverry et al. (2009)	STEC/VTEC			0.8	***	Boneless beef strip loin	*E. coli* O157:H7	PC	NS	3 %	Ambient	NS	Spray	1.38 bar	Pilot	Water (ambient)	21 d	Chill	Excision	
18	Heller et al. (2007)	STEC/VTEC	1.0			***	Subprimal cuts	*E. coli* O157:H7	PC	NS	2.5 %	55 °C	20 s	Spray	3.1 bar	Pilot	UC	NS	NS	Excision	6 plants
18	Heller et al. (2007)	STEC/VTEC	1.1			***	Subprimal cuts	*E. coli* O157:H7	PC	NS	5 %	55 °C	20 s	Spray	3.1 bar	Pilot	UC	NS	NS	Excision	6 plants
14	Gill and Badoni (2004)	*Eb*	2.68	0.78	1.90	NS	Brisket	*E. coli*	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.70	1.21	1.49	NS	Brisket	Coliforms	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.68	1.71	0.97	NS	Brisket	*E. coli*	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.70	1.89	0.81	NS	Brisket	coliforms	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.68	0.55	2.13	***	Brisket	*E. coli*	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.70	0.80	1.90	***	Brisket	Coliforms	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.55	0.76	1.79	***	Brisket	*E. coli*	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.78	1.09	1.69	***	Brisket	Coliforms	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	1.58	0.76	0.82	NS	Brisket	*E. coli*	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.38	1.30	1.08	NS	Brisket	Coliforms	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	1.58	1.12	0.46	NS	Brisket	*E. coli*	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.38	2.28	0.10	NS	Brisket	Coliforms	Natural	L	2 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	1.58	-0.60	2.18	***	Brisket	*E. coli*	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.38	0.58	1.80	***	Brisket	Coliforms	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	5 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	1.90	0.48	1.42	***	Brisket	*E. coli*	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
14	Gill and Badoni (2004)	*Eb*	2.37	0.85	1.52	***	Brisket	Coliforms	Natural	L	4 %	7 °C	NS	Mist	NS	Lab	Water (7 °C)	60 min	7 °C	Swab	25
LOW STRENGTH OF EVIDENCE																					
4	Calicioglu et al. (2002)	STEC/VTEC	0.53			NS	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	1 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	0.54			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	3 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	0.79			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	7 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	0.50			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	14 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	0.27			NS	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	1 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	0.68			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	3 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	1.14			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	7 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	STEC/VTEC	1.01			***	Subprimal cuts	*E. coli* O157:H7	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	14 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	0.37			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	1 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	0.52			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	3 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	0.88			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	7 d	4 °C	Sponge	

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment[f]	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature[d]	Contact time[d]	Mode	Pressure[d]	Scale[e]		Time[g]	Temperature[g]		
4	Calicioglu et al. (2002)	*Eb*	0.46			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	14 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	0.13			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	1 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	1.00			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	3 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	1.07			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	7 d	4 °C	Sponge	
4	Calicioglu et al. (2002)	*Eb*	1.05			***	Subprimal cuts	*E. coli* biotype I	IFM	NS	2 %	38-46 °C	NS	Spray	NS	Lab	UC	14 d	4 °C	Sponge	

[a] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin-producing *Escherichia coli*; *Eb: Enterobacteriaceae*

[b] ***: significant; NS: not significant

[c] IFM: pathogen inoculated in faecal material; PC: pure culture suspension

[d] NS: not specified

[e] Ind: industrial scale; Lab: lab-scale

[f] UC: untreated control

[g] NS: no storage

E. TABLE WITH DETAILLED DATA OF LACTIC ACID TREATMENT OF BEEF TRIMMINGS USING THE 25 PAPERS INCLUDED IN THE ASSESSMENT

Paper no	Reference	Microorganisms[a]	Microbial reduction		Efficacy *over* control group	Significant reduction[b]	Product treated	Target strain[a]	Inoculum type[c]	Lactic acid		Application					Control treatment	Storage criteria before analysis		Sampling method	No samples tested
			Treated group	Control group						Enantiomer[d]	Concentration	Temperature	Contact time[d]	Mode	Pressure[d]	Scale		Time[e]	Temperature[e]		
HIGH STRENGTH OF EVIDENCE																					
20	Kang et al. (2001)	*Eb*	0.73	1.10	-0.37	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	1s-3s/1 pass	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	NS	NS	Excision	3
20	Kang et al. (2001)	*Eb*	0.88	1.30	-0.42	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	1s-3s/1 pass	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	NS	NS	Excision	3
20	Kang et al. (2001)	*Eb*	1.00	1.60	-0.60	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	1s-3s/1 pass	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	NS	NS	Excision	3
20	Kang et al. (2001)	*Eb*	1.11	1.80	-0.69	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	1s-3s/1 pass	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	NS	NS	Excision	3
20	Kang et al. (2001)	*Eb*	1.3	1.1	0.2	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	0 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	1.3	1.0	0.3	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	1 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	1.2	0.9	0.3	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	7 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	1.3	1.1	0.2	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	0 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	1.5	1.1	0.4	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	1 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	1.4	0.7	0.7	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	7 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	2.5	1.8	0.7	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	0 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	3.5	2.0	1.5	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	1 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	4.0	1.7	2.3	***	Beef loins trims[f]	*E. coli*	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	7 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	2.7	1.9	0.8	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	0 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	3.6	2.0	1.6	***	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	1 d	4 °C	Excision	4
20	Kang et al. (2001)	*Eb*	4.2	1.9	2.3	NS	Beef loins trims[f]	Coliforms	FM	NS	2 %	15 °C	3s/3 passes	Spray	2.07 bar	Pilot	Water (15-17 °C, 4.48 bar)	7 d	4 °C	Excision	4
MEDIUM STRENGTH OF EVIDENCE																					
17	Harris et al. (2006)	*Salm*			1.2	NS	Beef trim[g]	*S.* Typhimurium	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	NS	4 °C	Excision	3[h]
17	Harris et al. (2006)	*Salm*			1.6	NS	Beef trim[g]	*S.* Typhimurium	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	NS	4 °C	Excision	3[h]
17	Harris et al. (2006)	*Salm*			1.3	***	Beef trim[g]	*S.* Typhimurium	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	0 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			1.0	***	Beef trim[g]	*S.* Typhimurium	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	0 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			1.2	***	Beef trim[g]	*S.* Typhimurium	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	1 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			0.7	***	Beef trim[g]	*S.* Typhimurium	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	1 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			0.8	***	Beef trim[g]	*S.* Typhimurium	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	5 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			0.8	***	Beef trim[g]	*S.* Typhimurium	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	5 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			1.1	***	Beef trim[g]	*S.* Typhimurium	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	30 d	Frozen	Grounded[i]	3[h]
17	Harris et al. (2006)	*Salm*			1.5	***	Beef trim[g]	*S.* Typhimurium	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	30 d	Frozen	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.2	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	NS	4 °C	Excision	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.1	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	NS	4 °C	Excision	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.2	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	0 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			1.9	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	0 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			1.7	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	1 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.0	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	1 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.0	NP	Beef trim[g]	*E. coli* O157:H7	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	5 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			1.1	NP	Beef trim[g]	*E. coli* O157:H7	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	5 d	4 °C	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			2.3	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	2 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	30 d	Frozen	Grounded[i]	3[h]
17	Harris et al. (2006)	STEC/VTEC			1.9	***	Beef trim[g]	*E. coli* O157:H7	PC	NS	4 %	Ambient	NS	Spray	NS	Pilot	Water spray (ambient)	30 d	Frozen	Grounded[i]	3[h]

[a] *Salm: Salmonella*; STEC/VTEC: Shigatoxin-producing/Verotoxin-producing *Escherichia coli*; *Eb: Enterobacteriaceae*
[b] ***: significant; NS: not significant; NP: not provided
[c] FM: faecal material; PC: pure culture suspension
[d] NS: not specified
[e] NS: no storage
[f] Beef loins trims post-chill
[g] Beef trim with a 75% lean and 25% fat blend
[h] Number of replicated experiments
[i] Grounded beef sampled

GLOSSARY AND ABBREVIATIONS

ADI	Acceptable Daily Intake
APC	Aerobic Plate Count
BOD	Biological Oxygen Demand
bw	Body weight
cfu	Colony Forming Unit
GHP	Good Hygienic Practices
HACCP	Hazard Analysis Critical Control Point
Eb	*Enterobacteriaceae*
Salm	*Salmonella*
STEC	Shigatoxin-producing *Escherichia coli*
VTEC	Verotoxin-producing *Escherichia coli*