UNIVERSITY OF LIVERPOOL

Report

A critical literature review to assess the significance of intervention methods to reduce the microbiological load on beef through primary production

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Abbreviations and Glossary

ACC	Aerobic Colony Counts
ASC	Acidified sodium chlorite
B/A	Before-and-after trial
CFU	Colony Forming Units
ChT	Challenge trial (with artificially inoculated microorganisms)
CPC	Cetylpyridinium Chloride
CrS	Cross-sectional study
CT	Controlled trial
EBC	*Enterobacteriaceae* Counts
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FBO	Food Business Operator
FDA	(United States) Food and Drug Administration
FSA	Food Standards Agency
FSMS	Food safety management system(s)
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
HPP	Hydrostatic pressure processing
LTTC	Less than thoroughly cooked burgers
MAP	Modified atmosphere packaging
No treatment	Untreated control (CT) or Before treatment (B/A)
PAA	Peroxyacetic acid (peracetic acid and hydrogen peroxide)
PCR	Polymerase chain reaction
QMRA	Quantitative microbial risk assessment
SCF	(EU) Scientific committee for Food
SOP	Standard Operating Procedure
TSP	Trisodium phosphate
UK	United Kingdom
USA	United States of America
VTEC	Verocytotoxin-producing *Escherichia coli,* Verocitotoxigenic *Escherichia coli*

Executive Summary

Background and introduction

The sale and consumption of burgers served less than thoroughly cooked (LTTC) and pink in the middle is a steadily increasing trend and a number of catering chains and outlets now offer this option to customers. This prompted concerns that there may be an increased risk of exposure to *E. coli* O157 for consumers who prefer this type of food. The Food Standards Agency's Board concluded that burgers served LTTC should be delivered to the same level of protection as thorough cooking provides the consumer (a 6 log reduction in microbial load).

However, reduced cooking procedures at the catering establishment outlets are unlikely to achieve 6 log reduction in burgers LTTC. Therefore, the safe production of this product at catering establishments is likely to be significantly reliant on controls and/or interventions applied at the beef processing facilities previously in the chain, particularly slaughterhouses and cutting plants. Implementation of appropriate additional interventions is required through primary production and beef processing to maintain the overall level of protection the 6 log reduction provides. This would allow LTTC burgers to be served with the same level of protection as fully cooked burgers.

Microbial contamination of beef carcasses occurs regularly in commercial abattoir conditions through direct or indirect routes from a number of sources. Consequently, hazard-based intervention/decontamination measures have been considered, and widely used in beef abattoirs in some countries, as a means to prevent or reduce microbial contamination of beef carcasses and to reduce microbiological hazards further than what is achievable solely by adhering to the Good Hygiene Practices (GHP).

Currently, only potable water (i.e. thermal treatment with hot water and steam pasteurisation) and lactic acid beef carcass washing have been permitted for use in the EU. The integrated and coordinated use of multiple interventions in the minced beef production chain may be able to reduce microbial loads sufficiently to offer the same level of protection to consumers from burgers, which are produced with these interventions and are served LTTC, as that of thoroughly cooked burgers originating from conventional minced beef production chain.

The main aim of the proposed study is to perform a broad critical review of available literature on the scientific research in intervention measures for beef, to obtain quantitative information on the reduction of bacterial load in minced beef production chain. The review covers a range of GHP-based and hazard-based interventions at the abattoir stage (from receive and unload of animals to chilled carcasses) and post-abattoir stage (further processing of raw beef and packaging), looking at the outcome of interventions on a range of bacterial indicators and foodborne pathogens.

Objectives

There were two objectives of this study:

- To perform a broad critical review of the literature of a contribution of interventions applied in a minced beef production chain for the reduction of bacterial load, with a focus on the pre-slaughter, slaughter, and post-slaughter production processes
- To make recommendations on the effectiveness (the quantifiable level of bacterial reduction) of specific interventions for beef and other contextual factors that will inform the risk management decisions for further work

Approach

The review considered evidence on beef intervention efficacy available in the public domain, including primary research, previously published systematic reviews, risk assessments and stochastic models. Only primary research studies were used for detailed data extraction and reporting. The population of interest included all cattle produced for domestic UK meat consumption, including their carcasses at processing and finished products (beef trim and ground/minced beef). Also, population of interest included potential sources of beef contamination during processing (i.e. cattle hides, environment surfaces and tools/knives/ equipment).

Relevant outcome measures for interventions were the effectiveness of each intervention in reducing log levels of indicator bacteria (aerobic colony counts (ACC), *Enterobacteriaceae* counts (EBC), total coliform counts and generic *E. coli* counts) and log levels of foodborne pathogens (primarily *E. coli* O157 and other VTEC and *Salmonella*, but also other foodborne pathogens). Where quantitative data on pathogen reduction were not available for specific intervention, data on prevalence outcomes were used.

Any interventions applied from cattle received in abattoir up to and inclusive of finished product packaging and storage (minced beef production chain) were considered relevant. The interventions can be described as GHP-based and hazard-based control measures.

Pre-slaughter beef interventions

Several interventions were identified at the lairage stage, from cattle received to the stunning and bleeding steps. Good hygiene practices such as lairage cleaning, proper cattle handling to prevent hide cross-contamination and hide cleanliness assessment, are recommended for use. It has been shown that categorisation of cattle based on their cleanliness can statistically significantly reduce the microbial contamination of resulting beef carcasses including with faecal microbiota, but no such evidence exists in relation to bacterial pathogens. Only one potential hazard-based intervention that was identified, bacteriophage application to cattle hides at least one hour before slaughter, have been shown to have promising results in reducing levels of *E. coli* O157:H7 and *Salmonella* spp.,

but is not commercially used at present. Other hide treatments of live cattle, such as chemical decontamination or hide clipping, are not recommended due to animal welfare concerns and/or practical considerations.

Beef interventions at slaughter

Cattle hide interventions, such as chemical hide washes and microbial immobilisation treatment with shellac, are recommended for consideration as potential hazard-based interventions when applied post-exsanguination and before dehiding for reducing microbial contamination of resulting beef carcasses. It has been shown that these hide treatments, can deliver statistically significant reduction in microbial transfer effect to carcasses of 1-1.5 logs.

Beef carcass hazard-based interventions are recommended for consideration for control of microbial contamination after dehiding and pre-chill. Carcass pasteurisation treatments with hot water and/or steam are efficacious against microorganisms when temperatures of carcass surfaces achieve more than 70^{o}C, with reductions of 1-2.5 logs. The time-temperature combinations required to achieve statistically significant reductions are usually specific to an individual commercial abattoir and subject to validation.

Chemical washes, particularly with lactic acid and other organic acids (acetic and citric) have also been efficacious, delivering 1-1.5 logs reductions. Some other treatments, such as knife trimming and steam vacuuming are also highly efficacious when properly applied, delivering statistically significant reduction effect. However, reduction effects highly depend on the skill and diligence of the user to spot visible contamination and efficiently remove it, therefore interventions' parameters are difficult to optimise to achieve consistent effect in reducing microbial hazards. Standard processing procedures, such as improved hide removal and bunging/rodding, have not been well researched but can have statistically significant effect in preventing carcass contamination, so are recommended for use as GHP-based measures.

Multiple use of carcass interventions (knife trimming, steam vacuuming, pasteurisation treatments and organic acid washes) was shown to have the biggest impact on microbial reduction on beef carcasses, up to 3 logs, more than any of these interventions applied alone.

Carcass chilling had a limited and inconsistent effect in reducing microbial contamination but was found to be efficacious in inhibiting further bacterial growth. Water spray chilling showed very variable effects and was largely ineffective in reducing natural microbiota on carcasses in commercial conditions. There was insufficient evidence of the efficacy of spray chilling with various chemicals, but lactic acid washes during chilling delivered up to 1.5 logs reduction.

Post-slaughter beef interventions

Good hygiene practices during carcass fabrication are necessary to prevent and minimise carcass cross-contamination post-chill. Various interventions for beef primals, subprimals and trim with physical (hot water) or chemical substances have shown good reduction effects on microbiota, often statistically significant. However, these treatments can only be used if properly optimised so to retain acceptable sensory quality of the final products.

Packaging-based interventions for beef cuts and minced beef had very variable effects in reduction of microbiota. Modified atmosphere packaging (MAP) and vacuum packaging are considered useful to extend the shelf life of beef trim and minced beef, but they had very limited and not statistically significant reduction effect on *E. coli* O157:H7. However, the reduction effect can be increased up to 2 logs by adding lactic acid to the packaging which would make this intervention worth considering as a hazard-based.

Irradiation can be considered a very efficacious, hazard-based intervention for final products and delivers complete elimination of potentially present bacterial pathogens. Other emerging non-thermal technologies (such as high-pressure processing, cold atmospheric plasma and UV light irradiation) have not been well researched but under laboratory conditions have shown promising reduction effects on microorganisms. However, the commercial uptake of all these hazard-based interventions for final beef products will highly depend on consumer acceptance.

Recommendations and future work

This review identified a number of options for delivering the required level of protection to consumers of LTTC burgers. They are summarised below.

- Cattle hide interventions, such as chemical hide washes and microbial immobilisation treatment with shellac, have been identified as efficacious and able to deliver 1-1.5 logs reduction in transfer of bacteria to carcasses. They can be recommended for consideration as hazard-based interventions when applied post-exsanguination and before dehiding for reducing microbial contamination of resulting beef carcasses.

- Beef carcass interventions, such as pasteurisation treatments with hot water and/or steam, have been identified as efficacious and able to deliver 1-2.5 logs reduction. Also, organic (lactic) acid washes can deliver 1-1.5 logs reduction. When both interventions are in in sequential use, they can deliver up to a 3 logs reduction. Both carcass pasteurisation treatments and organic (lactic) acid washes can be recommended for consideration as hazard-based interventions when applied after dehiding and pre-chill.

- Organic (lactic) acid washes have also been identified as efficacious when applied on beef carcasses during chilling and at post-chill, pre-fabrication stage, and able to deliver around 1.5 logs reduction. They can be recommended for consideration as hazard-based interventions when applied on carcasses at these stages.

- Interventions for beef cuts and minced beef at the post-slaughter stage, such as organic acid washes, MAP and vacuum packaging of meat (with added lactic acid), have been identified as efficacious and able to deliver up to 2 logs reduction. They can be recommended for consideration as hazard-based interventions when applied at the final product, but only if properly optimised to retain the quality of the product.

- There are certain interventions for which there is a lack of evidence (e.g. hide removal practices, bunging/rodding); that have shown inconsistent results in reducing microbial contamination (particularly in respect to pathogens, e.g. hide cleanliness assessment, hide clipping, chilling); or where no processing parameters in reducing carcass contamination can be clearly established (e.g. environment, equipment and tools sanitation, knife trimming and steam vacuuming). These interventions can be recommended for use as GHP-based control measures, alongside hazard-based interventions, to assist in overall microbial reduction.

- The sequential use of beef carcass interventions as a part of 'multiple-hurdle approach' (knife trimming, steam vacuuming, pasteurisation treatments and organic acid washes) delivered higher reductions than any of the interventions applied alone, from 2 to 3 logs. The sequential use of GHP- and hazard-based carcass interventions can be recommended for consideration, particularly when they are used alongside other recommended interventions at pre-slaughter, slaughter and post-slaughter.

- In order to address differences in study designs and results on the intervention efficacies between multiple studies identified in this review, further meta-analysis of data generated in this study is needed. This, coupled with subsequent use of data in quantitative risk modelling can enhance the confidence of the contribution of beef interventions in the reduction of microbial load to meet required performance criteria, and would provide a more evidence-based model for public health analyses.

- The review identified certain interventions where there was a relative lack of data and further research is needed. These are: i) the interventions in the pre-slaughter stage, particularly cattle handling in the lairage and hazard-based bacteriophage treatment for cattle hides; ii) cattle hide interventions post-exsanguination and carcass interventions during chilling and at post chill, pre-fabrication stage; iii) novel emerging technologies for beef cuts and minced beef, such as electron beam and gamma irradiation, high-pressure processing and bacteriophage treatments; and iv)

generally controlled trials conducted under commercial conditions, particularly investigating multiple interventions applied at slaughter, prior to dehiding to pre-fabrication stage.

1 Background and rationale

The most relevant bovine meat-borne biological hazards categorised as of high-priority for control in the beef chain by the European Food Safety Authority (EFSA) are *Salmonella* and verocytotoxin-producing *Escherichia coli* (VTEC) (EFSA, 2013). This decision was made through a risk ranking process which was based on the assessment of: (i) the magnitude of the human health impact based on reported incidence, (ii) the severity of the disease in humans based on fatalities among reported cases, and (iii) the strength of evidence that meat from bovine animals is an important risk factor for the disease in humans, including carcass/animal prevalence (EFSA, 2013).

Salmonella and VTEC can be harboured in and excreted from the gastrointestinal tract of cattle. They are subsequently transferred from cattle to humans (leading to beef-borne illness), most often through faecal contamination or cross-contamination of meat, and/or their growth during production, handling and consumption of beef and products thereof (Buncic *et al.* 2014). The control of these pathogens in the beef chain requires use of Good Manufacturing Practice/Good Hygienic Practice (GMP/GHP) and Hazard Analysis and Critical Control Point (HACCP) principles. In many cases under commercial conditions, this is not sufficient to control microbial contamination and therefore must be accompanied by implementation of appropriate additional intervention measures, taking into account considerations regarding resources and technical possibilities, consumers' attitude and behaviours, and cost-benefit (Buncic *et al.* 2014).

Microbial contamination of beef carcasses occurs regularly in commercial abattoir conditions through direct or indirect routes from a number of sources. The main sources are: i) faecal material and rumen/gut contents; ii) hide of slaughtered cattle; and iii) slaughterline environment (machinery, equipment, workers and aerosols). However, while in modern abattoirs leakage/spillage of gut contents onto the meat occurs rarely (with some estimations of 1 in 1,000 carcasses), and the slaughterline environment as a contamination source is efficiently controlled through the pre-requisite programmes (GMP/GHP), the contamination of carcasses from the cattle hides is a key and inevitable event (Antic *et al.* 2011, Blagojevic *et al.* 2012).

Most often, bacterial counts obtained from carcasses after dehiding are correlated with those on hides (Blagojevic *et al.* 2011) and are strongly dependent on cattle hide cleanliness (Blagojevic *et al.* 2012). It was found that cattle hides can carry up to 11 log CFU/cm^2 of aerobic bacteria (Antic *et al.* 2010), including pathogens such as *E. coli* O157 and other VTEC and *Salmonella*, which consequently can contaminate carcass meat (Reid *et al*. 2002). The proportion of microbiota transferred from hides onto beef carcasses via all routes, commercially, was found to be between 1.6% and 0.003% (Bacon *et al.* 2000, Arthur *et al.* 2004). More recently, it was shown that microbial counts on beef after direct contact with cattle hides can reach up to 7.7 log CFU/cm^2 of aerobic bacteria and 4.0 log CFU/cm^2 of

Enterobacteriaceae, with up to 10% of artificially inoculated *E. coli* O157 on cattle hides being transferred to beef (Antic *et al.* 2018).

Results obtained in Scotland revealed that 55% of cattle had *E. coli* O157 contaminated hides after bleeding (Mather *et al.* 2007). A quantitative microbial risk assessment (QMRA) model developed for *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland indicated that the initial prevalence and numbers of *E. coli* O157:H7 on the bovine hide had the greatest impact on the overall probability of illness from this pathogen, and that the cross-contamination at the hide removal stage impacted on predicted risk (Duffy *et al.* 2006).

Another related quantitative simulation model indicated that risk reduction measures should be directed towards reducing the hide to carcass transfer during dehiding and the initial *E. coli* O157:H7 prevalence and counts on bovine hides (Cummins *et al.* 2008). These conclusions highlight the necessity for the development and implementation of effective intervention strategies to control foodborne pathogens (particularly *E. coli* O157) at slaughter. This is of particular relevance because of the recent and growing preference by some consumers for less than thoroughly cooked (LTTC) burgers in the UK, which increases the risk of exposure to *E. coli* O157 for those individuals (FSA, 2015).

Interventions are used in most countries with the aim to reduce microbiological risks further than what is achievable solely by adhering to GHP. Some aspects of these control strategies are pathogen- and meat chain stage-specific. Thus, some pathogens in beef and the products thereof (e.g. VTEC, *Salmonella*) are most efficiently controlled by the main measures applied during primary production (on-farm) combined with optimization of the slaughter hygiene (at-abattoir), whilst some others (e.g. *Listeria monocytogenes*) are most efficiently controlled at the processing–storage stages (Buncic *et al.* 2014).

Interventions can be GHP-based measures applied throughout slaughter and dressing process (i.e. cleaning and disinfection of lairage-to-stunning areas, hide cleanliness assessment, bunging, oesophagus tying, hide removal methods, trimming, chilling, equipment sanitation, etc) and hazard-based intervention measures (i.e. a range of different interventions for cattle hides and carcass meat mostly aimed at microbial removal, immobilisation and/or killing). Interventions are also applied at post-fabrication (processing–storage) stages aimed at microbial killing or inhibiting their growth. In some countries, e.g. USA, decontamination treatments of hides and carcasses are regularly used and integrated within a intervention-based HACCP system (Byelashov & Sofos, 2009; Koohmaraie *et al.* 2005, 2007; Wheeler *et al*. 2014); such interventions have not yet been used under commercial conditions within the EU (including the UK). There is, however, provision for the use of decontamination strategies in abattoirs in the EU. The EU Food Hygiene Regulations (EC, 853/2004) allow, in principle, the use of decontamination treatments during slaughter, following appropriate consideration and approval of such treatments by the regulatory authorities (EC, 2004).

Currently, only potable water (i.e. thermal treatment with hot water and steam pasteurisation) and lactic acid beef carcass washing (Regulation EC 101/2013) have been permitted for use in European abattoirs. However, no intervention strategy can be expected to sufficiently reduce the microbiological load of a highly contaminated carcass. The ultimate effectiveness of antimicrobial treatments, when assessed through the levels of surviving microbiota remaining on a treated substrate, depends primarily on the initial microbial load (Sofos & Smith, 1998). Therefore, interventions must not be a substitute for GHP, but only an additional measure.

Implementation of successful interventions against relevant microbial hazards in the meat chain up to and including the chilled carcass stage is now recognised as an essential component of a risk-based meat safety assurance system in which high-risk animal batches should be subjected to additional slaughter hygiene control measures complemented with (hide and meat) decontamination treatments (Blagojevic and Antic, 2014; EFSA, 2013). These recent efforts in the modernisation of meat inspection and its transformation into a risk-based meat safety assurance system integrate both meat inspection procedures and FBO's food safety management systems (FSMS) and other relevant aspects into a coherent whole (Buncic *et al.* 2014).

Interventions can routinely be used either alone or applied at multiple points as a 'multiple hurdle strategy' in a coordinated way, in order to ultimately achieve an acceptable reduction in the residual microbiological safety risk associated with beef (Buncic *et al*. 2014). For example, cattle hide interventions can be used as a part of a multiple-hurdle strategy in combination with the beef carcass interventions (spot or whole dressed carcass decontamination) and with the resulting beef trimmings decontamination to reduce microbial load further (Koohmaraie *et al*. 2007; Antic *et al*. 2018).

Where multiple interventions are applied, it is reasonable to expect that the overall improvement of the microbiological status of beef would be determined by a combination of microbial reductions achieved by all interventions, and be greater than the individual effect of each intervention in isolation. Therefore, the integrated and coordinated use of multiple interventions in the minced beef production chain may be able to reduce microbial loads sufficiently to offer the same level of protection to consumers from burgers, which are produced with these interventions and are served LTTC as that of thoroughly cooked burgers originating from conventional minced beef production chain.

The recent growing preference by some consumers in the UK for LTTC burgers prompted concerns that there may be an increased risk of exposure to *E. coli* O157 for those consumers (FSA, 2015). The sale and consumption of burgers served LTTC and pink in the middle is a steadily increasing trend and a number of catering chains and outlets now offer this option to customers. The safe production of LTTC burgers at catering establishments is likely to be significantly reliant on controls and/or interventions applied at the beef processing facilities previously in the chain, particularly slaughterhouses and cutting plants.

The Food Standards Agency Board concluded that burgers served LTTC should be delivered to the same level of protection as thorough cooking provides the consumer (a 6 log reduction in microbial load).

However, given the reduced cooking procedures, it is highly unlikely that 6 log reduction will have been achieved solely at the catering establishment level. Therefore, implementation of appropriate additional interventions is required through primary production and beef processing to maintain the overall level of protection achieved by the 6 log reduction thorough cooking provides. This ensures LTTC burgers, produced with these additional interventions in primary production and beef processing, to be served with the same level of protection as fully cooked burgers produced without such interventions (FSA, 2015).

The FSA's position is that the Food Business Operators (FBOs) serving LTTC burgers should ensure that their suppliers have procedures in place during slaughter, cutting and mincing, which are as hygienic as possible, with the specific intention of preventing meat surface contamination with pathogens. Furthermore, FBOs must have documented and validated evidence of procedures throughout the supply chain, that can achieve at least a 4 log reduction before the burger is served to the final consumer, and also an advice to consumers at the point of ordering a burger (FSA, 2015, 2016).

2 Scope and objectives of the study

The main aim of this study was to perform a broad critical review of available scientific literature on intervention measures for beef, and to obtain quantitative information on the reduction of bacterial load in the minced beef production chain achieved via interventions applied at pre-slaughter, slaughter and post-slaughter.

More specific objectives of this study were twofold:

- To perform a broad critical review of the literature of a contribution of interventions applied in a minced beef production chain for the reduction of bacterial load, with a focus on the pre-slaughter, slaughter, and post-slaughter production processes
- To make recommendations on the effectiveness (the quantifiable level of bacterial reduction) of specific interventions for beef and other contextual factors that will inform the risk management decisions for further work

The review considered evidence on beef intervention efficacy available in the public domain, including primary research, previously published systematic reviews, risk assessments and stochastic models. However, only primary research studies were used for detailed data extraction and reporting. The population of interest included all cattle produced for domestic UK meat consumption, including their carcasses at processing and finished products (beef trim and ground/minced beef). Also, population of interest included potential sources of beef contamination during processing (i.e. cattle hides, environment surfaces and tools/knives/ equipment).

Meat from cattle is primarily destined for consumption as minced beef or beef cuts. Beef cuts are whole muscle cuts commonly consumed as steaks or roasts and are derived from subprimal cuts subdivided from primal cuts fabricated (initially separated from the carcass) during cutting and deboning of cattle carcasses. Minced beef is derived from boned beef that has been minced into fragments and contains less than 1% salt. In the case of beef minced meat produced from chilled meat, the requirements specified in the hygiene regulations are that it must be prepared: i) within no more than six days of animal slaughter or ii) within no more than 15 days from the date of slaughter of the animals in the case of boned, vacuum-packed beef and veal (EC, 2004).

Relevant outcome measures for interventions were the effectiveness of each intervention in reducing log levels of indicator bacteria (aerobic colony counts, *Enterobacteriaceae* counts, total coliform counts and generic *E. coli* counts) and log levels of foodborne pathogens (primarily *E. coli* O157 and other non-O157 VTEC serogroups and *Salmonella*, but also other foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium perfringens*, where data were available). Where quantitative data on pathogen reduction were not available for specific intervention, data on prevalence outcomes were used.

Any interventions applied from cattle received in abattoir up to and inclusive of finished product packaging and storage (minced beef production chain) were considered relevant. The interventions can be described as GHP-based and hazard-based control measures.

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

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

i) at abattoir level:

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

ii) at post-abattoir level for fabricated beef (primals and subprimals, trimmings and minced meat):

- Thermal washes (hot water) and chemical washes (organic acids and other chemicals), electron beam and gamma irradiation, ultraviolet (UV) light, use of bacteriophages, cold atmospheric plasma and high-pressure processing, modified packaging and preservation techniques (including active and bioactive packaging systems).

The concentration and prevalence outcomes (intervention efficacy results) are presented as log reductions and prevalence reductions in the intervention compared with the control group. They are analysed as: i) reduction on a treated substrate (i.e. surfaces, hide, carcass meat, fabricated beef); and ii) reduction in transfer to a substrate (usually carcass meat) from the contamination source. The review also distinguished between study trials

conducted under laboratory and pilot plant conditions (often using artificially inoculated microbiota[1]), as well as those investigated under commercial conditions.

More details regarding methodology used in this study can be found in Annex 1.

[1] Artificially inoculated microorganisms are often used in challenge trials where subjects are artificially challenged or exposed to the disease agent and then allocated to the intervention groups for evaluation of the outcome. It is a study method of choice when the presence and levels of microorganisms of interest in given population are naturally low.

3 Beef chain intervention assessment

3.1 Lairage interventions

Four observational studies investigating lairage cleaning and disinfection found consistent presence of foodborne pathogens, such as *Salmonella*, *E. coli* O157 and *Campylobacter* on lairage surfaces, even after routine cleansing operations, sometimes containing up to 10^4 organisms per sampled area (2,500 cm^2). Up to a 5 log-cycles of microbial reduction can be achieved on lairage surfaces using pressure water wash with quaternary ammonium sanitisers and/or steam under pressure. No evidence for specific interventions against foodborne pathogens applied at the lairage stage in cattle was identified in this review.

Seven observational and molecular studies, as well as one study using marker organisms, suggested the potential for lairage to be an area of amplification and transmission of VTEC and *Salmonella* among cattle. Although reduced lairage time can be beneficial to reducing cattle contamination with VTEC and *Salmonella*, it is not always practical to minimise the duration in lairage for cattle in commercial settings.

There was a direct correlation between visual hide cleanliness and microbial contamination of resulting beef carcasses with microbiological indicators of general (Aerobic Colony Counts (ACC)) and faecal contamination (*Enterobacteriaceae* (EBC) and generic *E. coli*). A steady decrease in carcass microbial load by 0.5-3 log-cycles of ACC, 0.7-1.5 logs of EBC and 0.4-0.8 logs of generic *E. coli* was found with the increase in hide cleanliness (as measured according to the hide cleanliness scoring systems) in four reviewed studies. Therefore, this GHP measure could be efficacious in reducing bacterial transfer from dirty hides to resulting carcasses by about 1 log-cycles.

Hide water wash of live cattle in lairage with ambient temperature water was ineffective in reducing microbial load in three reviewed studies. Washing with cetylpyridinium chloride (CPC) yielded promising reductions in hide-to-carcass transfer of 1.5 and 1.1 logs of ACC and EBC, respectively, and reduced prevalence of naturally present *E. coli* O157 (from 23% in control to 3% in carcasses whose hides had been washed). Hide clipping was found to be largely ineffective, with very moderate reductions in transfer of ACC to carcass of up to 0.3 logs in one reviewed study. Bacteriophage spray applications with 1 h contact time are suitable for use on live cattle and were reported to achieve up to 2 log reduction of inoculated *E. coli* O157:H7 on cattle hide sections in lab conditions (bacteriophages e11/2 and e4/1c).

However, only one study conducted under commercial conditions found no reductions in *E. coli* O157:H7 prevalence (a proprietary bacteriophage formulation Finalyse®). Apart from the phage treatment, for which a certain contact time with the hide is required for the full intervention effect, other interventions (washing and clipping) are more appropriate for use post-exsanguination where harsher treatments can also be applied.

3.2 Cattle hide interventions

The review found a relative lack of published information on cattle hide interventions (33 studies reviewed on different interventions). Cattle hide is a major source of resulting beef carcass microbial contamination, and therefore there were some attempts to control microbial contamination on the hides with the aim to remove, kill or immobilise bacteria, and ultimately prevent their transfer to derived carcasses during dehiding.

Most studies investigated intervention efficacy on hides only, without measuring actual efficacy in reducing microbial transfer to the meat. Therefore, the efficacies achieved on hides can be referred to as 'relative efficacies' and only as an indication of the potential reduction in transfer of bacteria to resulting beef carcasses. Consequently, the only relevant measurement of cattle hide intervention efficacy is microbial status of resulting beef carcasses immediately after dehiding. Hence, even when some of these interventions showed promising efficacy in reducing microbiota on hides, it is largely expected that the effect in reducing carcass meat surface contamination would be much smaller.

There were only six controlled trials conducted under commercial conditions post-exsanguination that reported hide intervention effects on resulting beef carcass surfaces: one study on hide wash with sodium hydroxide, one investigating chemical dehairing, two studies on microbial immobilisation treatments with ethanol and aqueous shellac solutions, and two on hide clipping.

Hide washing post-exsanguination with ambient or warm water under pilot and commercial conditions was found to reduce indicator bacteria by up to 1 log-cycles on hides and also decrease the prevalence of VTEC and *Salmonella* in eight reviewed studies. Increased efficacy of water washing was achieved when additional vacuuming or manual curry comb were used, often by 1 log-cycle.

On the other hand, four studies that investigated hide clipping found very moderate reductions in transfer of ACC to beef carcasses of up to 0.3 logs of indicator bacteria. It was noted in several studies that hide clipping could be useful as a GHP pre-treatment to subsequent hazard-based hide interventions.

One study under commercial conditions found that localised application of lactic and acetic acids yielded reductions on cattle hides of 2.3-2.6 and 3.7 logs, respectively, of general and faecal microbiota.

Under pilot plant conditions, oxidisers reduced general and faecal microbiota by 2.0-3.5 and 2.0-4.0 log cycles on treated cattle hides. Under commercial conditions, automated hide washes with sodium hydroxide achieved statistically significant reduction of 0.8 logs in the transfer to carcasses of aerobic and enteric bacteria and 17% to 2% in the prevalence of *E. coli* O157. Vacuuming following hide washing with chemicals appears to further decrease bacterial levels on hides by 1-2 log-cycles.

Harsher treatments such as chemical dehairing and thermal interventions were reported to be highly efficacious, but with questionable practical use because of hide damage and difficulties of waste disposal. Chemical dehairing was the most successful treatment under commercial conditions achieving reduction in the transfer to carcasses of aerobic and enteric bacteria of 2 logs and 1.8 logs and the prevalence of *E. coli* O157 from 50% to 1%. Hot water washes of hides and steam treatments achieved reductions on treated hides of up to 6 log-cycles.

Three studies investigated a novel approach to immobilise rather than eliminate bacteria on hides, using natural resin shellac sprayed onto cattle hides. Reductions in transfer to meat of general microbiota of up to 3.6 logs under lab and 1.7 logs under commercial conditions were reported when shellac in ethanol was used. Comparable results were also observed when using aqueous shellac solutions, with reductions in transfer to meat of up to 3 logs and 2.4 logs of aerobic and enteric bacteria, respectively, under lab conditions and to resulting beef carcasses of up to 1.1 logs and 0.7 logs of ACC and EBC, respectively, under commercial conditions.

3.3 Beef carcass interventions

3.3.1 Standard processing procedures and GHP

There was a lack of published studies describing the efficacy of standard processing procedures and good hygiene practices (hide removal methods, bung bagging and overall process hygiene) in reducing beef carcass microbial contamination (13 studies reviewed in total).

An assessment of hide removal practices in four studies indicated statistically significant reduction in transfer of indicator bacteria from hides to carcasses by 1 log-cycle and reduced prevalence of VTEC and *Salmonella* on beef carcasses when practices were improved (measured by subjective assessment). In relation to this, one study in commercial conditions didn't find any benefit of implementing downward vs. upward hide pulling method, but some differences were noted on specific carcass sites, often in favour of upward technique. Bung bagging appears to be efficacious in the three studies where reductions of indicator bacteria by around 1 log-cycle and of the prevalence of VTEC were reported.

Alternative methods for knives sanitation were in most cases shown to be equivalent to the current sanitation procedures in water at 82°C for one second, in 11 reviewed studies. These include methods suitable for use on the slaughterline with contact times up to 1 minute, such as dipping knives in water for longer times at lower temperatures (60-70^{o}C), use of ultrasound combined with organic acids, and use of chemicals (sanitisers, peroxyacetic and organic acids).

3.3.2 Pre-chill carcass treatments

A relatively large number of studies have been published on beef carcass interventions post dehiding but pre-chill (the review identified 90 such studies). Most of these were conducted under laboratory conditions using inoculated microbiota. Studies reported on water washes, thermal treatments (hot water wash, spot steam vacuuming and whole carcass steam pasteurisation), chemical washes with organic acids and other chemicals. There were large variations in the magnitude of reduction effect seen in studies investigating the same intervention, due to different intervention conditions used, and therefore the results on intervention efficacy are not directly comparable.

Water wash with ambient or cold water to remove microorganisms was largely ineffective with up to 0.5 log reduction achieved, but also dependant on washing time and pressure used. Very often, washing carcasses appeared to have increased contamination and/or redistributed bacteria.

Trimming of visually contaminated sites reduced levels of natural microbiota by 1-2 logs. Steam-vacuum uses steam to loosen contamination and kill bacteria, followed by the application of a vacuum to remove contaminants, and it was shown to have similar effects to trimming. Steam vacuum cleaning of visible carcass contamination is often used before evisceration and is considered as effective as carcass trimming in removal of bacterial contamination, with the additional effect of killing bacteria with heat. However, the effectiveness of steam vacuum often depends on the skill and diligence of the user and is reliant on spotting visible contamination so there is no guarantee that all contamination will be removed.

Hot water washing provided consistent reduction effects (i.e. seen across a number of studies) of 1-2.5 logs, with an additional reduction of 0.5-1 log-cycles if organic acids were used concurrently. Hot water wash was usually efficacious against microorganisms when temperatures of carcass surfaces achieve more than 70^{o}C. The time-temperature combinations required to achieve statistically significant reductions are usually specific to an individual commercial abattoir and subject to validation.

The whole carcass steam pasteurisation effect in reducing natural microbiota was most often around 1-1.5 log-cycles. Generally, the process of steam pasteurisation should allow the carcass surface temperature to reach at least 90°C for a sufficient time in order to achieve bacterial reduction, which is then followed by rapid cooling.

Organic acid carcass washes (lactic, acetic and citric) were effective on-line interventions with higher reductions reported for lactic acid (1-2 logs of natural microbiota) than for acetic and citric acid or their mixtures (usually up to 1 log). Therefore, based on the large amount of data generated on lactic acid efficacy, an average reduction of 1.5 log from lactic acid treatment of carcasses can be expected.

A large number of studies conducted under pilot and laboratory conditions investigated various physical (water washes and thermal treatments) and chemical interventions (organic acids and other chemicals) alone or in combinations, reporting a large variation of reduction effects, very often between 2-5 logs. This must be taken with caution and only as an indication of the potential intervention effect, because of the artificial nature of inoculated microorganisms, controlled study conditions and often low number of samples investigated.

3.3.3 Chilling

The primary reason for chilling is inhibition of further bacterial growth and it is widely assumed not to have a significant reduction effect against bacteria. The review identified limited and inconsistent effects of chilling at reducing microbial contamination. There is also a likely overestimation of reported lethal effects of chilling on some pathogens (particularly mesophiles such as VTEC and *Salmonella*), which sometimes have a poor recovery from an injured state induced by the chilling; this could influence the interpretation of efficacy.

In all reported studies (34 reviewed in total), the temperatures investigated were within regulatory limits (i.e. from 0^{o}C -5^{o}C). Chilling for up to three days reduced levels of indicator bacteria in most cases up to only 0.5 logs under commercial conditions and up to 2 logs of inoculated *E. coli* and *Salmonella* under pilot and lab conditions. Chilling for one day of carcasses previously sprayed with organic acids or treated with hot water or steam on the slaughterline reduced indicator bacteria by 0.6-2.1 logs under commercial conditions and up to 3.5 logs of *E. coli* under pilot and lab conditions. This is likely due to a residual effect of chemical interventions.

An average 0.1-0.2 log reduction per day of inoculated *Salmonella* during 14-day dry aging of beef cuts was observed, leading to overall reductions of up to 2 logs of faecal indicators in the first four days of dry aging or around 1-3 logs of inoculated enteric pathogens after seven days of dry aging.

Water spray chilling showed very variable effects and was largely ineffective in reducing natural microbiota on carcasses in commercial conditions. However, reduction effects of up to 2 logs were observed on inoculated VTEC and *Salmonella*, which increased when various chemicals were sprayed onto beef carcass cuts during chilling, producing reductions from 1-4.5 logs comparing to water spray chilling alone in only one reported study.

3.3.4 Post-chill and pre-fabrication carcass treatments

The review identified only nine studies that investigated interventions for beef carcasses at this stage. Lactic acid spray of carcasses following the completion of chilling and prior to carcass fabrication was shown to statistically significantly reduce aerobic bacteria up to 3 log-cycles and faecal bacteria up to 1.5 logs in two studies conducted under commercial conditions, with reductions increasing to up to 7 logs of inoculated VTEC and *Salmonella* in five studies conducted under laboratory conditions.

One novel non-thermal intervention, electron beam (E-beam) irradiation, was reported in only one study to be highly efficacious at a 1 kGy dose, and when applied to chilled beef primals reduced *E. coli* O157:H7 numbers by up to 6.6 log-cycles.

3.3.5 Multiple on-line interventions and HACCP

Sixteen studies investigated the sequential application of interventions after dehiding but before chilling, based on a 'multiple-hurdle approach' under commercial abattoir conditions.

The interventions usually started with knife trimming and steam vacuuming which achieve reduction of bacteria on beef surfaces by targeting potentially contaminated areas following the dehiding process (usually along the cattle hide opening lines). This is followed with a pre-evisceration wash of hot water or organic acid that further eliminates pathogens. After evisceration and splitting, carcasses pass through a thermal pasteurisation chamber, where heated water (>74^{o}C) or steam (>85^{o}C) is applied. This treatment is lethal to most bacteria on the carcass surface and further cleanses the carcass. Finally, a heated organic acid or peroxyacetic acid rinse is applied before carcasses enter the chilling room.

Consistent reductions of naturally present bacterial indicators were achieved across a number of studies and were higher than when only one single intervention was used. In most cases they ranged from 2-3 logs of ACC and/or faecal indicators. The prevalence of naturally present VTEC and *Salmonella* following sequential application of interventions was in most cases statistically significantly reduced, often to levels below detection limits. In one controlled trial in a pilot plant where cattle hides were washed with lactic and acetic acid followed by carcass organic acid washes prior to chilling, the reductions obtained and measured after chilling were in the range 1.5-2 logs comparing to untreated (only chilled) carcasses.

No overall effect of HACCP implementation on pathogen (VTEC and *Salmonella*) reduction was reported in eight before-and-after studies. However, levels of ACC's and faecal indicator bacteria were reduced on carcasses by 0.5-1 log-cycle after HACCP implementation.

3.4 Post- carcass fabrication interventions

3.4.1 Standard processing procedures and GHP

Three studies found an inconsistent effect of the carcass fabrication procedures, with trimming off potentially contaminated carcass sites showing some bacterial reduction, but also with an increased possibility for microbial cross-contamination. One study investigating post HACCP implementation in beef cutting plants indicated a reduction effect of 1-2 logs of ACCs compared to before HACCP implementation. Regular sanitation with detergents and sanitisers is highly efficacious against residual microbiota with up to 3 log reductions achieved on food contact surfaces. Overall, adherence to GHP-based control measures is important to reduce bacterial contamination during the carcass fabrication process.

3.4.2 Interventions for beef primals, subprimals and trim

Post-fabrication hazard-based interventions involve treatments of beef primals, subprimals and trim with various physical (hot water) or chemical substances. There is a limit to how high temperature and/or concentration of chemicals can be used in this final product so as to retain acceptable sensory quality. However, these treatments can be used if properly optimised. The review identified 51 study that investigated interventions at this stage.

Hot water wash and steam treatment of beef primals and trim had reduction effects of up to 2 logs of inoculated VTEC and *Salmonella*, whereas reductions of 0.5-1 log were reported for ACC and faecal microbiota in seven reviewed studies. Using dry heat with a hot air gun at temperatures up to 100^{o}C increased efficacy to 4-6 logs reductions in inoculated VTEC and *Salmonella* in one study. Nevertheless, all these thermal treatments could have a detrimental effect on product quality if intervention parameters are not optimised.

Research investigating various organic acids and other chemicals demonstrated large variations in the magnitude of the effect. Lactic acid and other organic acids, alone or in a combination with other chemicals or hot water, were shown to have an efficacy of around 1-2 logs reduction of inoculated pathogens or natural microbiota in 29 reviewed studies. Multiple treatments reported in only one study (hot water spray, hot air, lactic acid spray), followed by vacuum storage, gave better reductions of natural aerobic and faecal microbiota which ranged from 1.6-3.7 logs. Phage treatment were efficacious against inoculated *E. coli* O157:H7 and *Salmonella* in the range of 1-2 logs in two studies.

3.4.3 Packaging and storage

Packaging-based interventions for beef trim or minced beef are subject to many factors such as naturally present microbiota, temperature, storage time, pH and type of packaging. These were reviewed from a total of 43 studies.

Cold aerobic storage up to seven days reduced inoculated *E. coli* O157:H7 by 1.5 logs and natural aerobic microbiota by up to 0.5 logs in five reviewed studies. Modified atmosphere packaging (MAP) and vacuum packaging are considered useful for extending the shelf life of beef trim and minced beef. However, it had limited and not statistically significant reduction effect on inoculated *E. coli* O157:H7 of up to 0.4 logs, but in combination with lactic acid the effect increased to 2 logs in seven reviewed studies.

The use of lactic acid bacteria (*Lactobacillus* spp.) to control pathogens in the final products resulted in variable reductions of inoculated *E. coli* O157:H7 of up to 3 logs in minced beef in four reviewed studies. Nisin was mostly found to be effective against inoculated *E. coli* O157:H7 and *L. monocytogenes* (1-2 logs); similarly, phages achieved up to 1 log reduction in *E. coli* O157:H7 numbers.

Irradiation appears to be one of the most effective interventions able to deliver complete elimination of inoculated pathogens, with reduction effects exceeding 6 logs (as reported in

seven reviewed studies). Other emerging technologies such as high-pressure processing produced highly variable reductions depending on the study conditions, ranging from 3-5 logs, in nine reviewed studies.

3.5 Risk management considerations

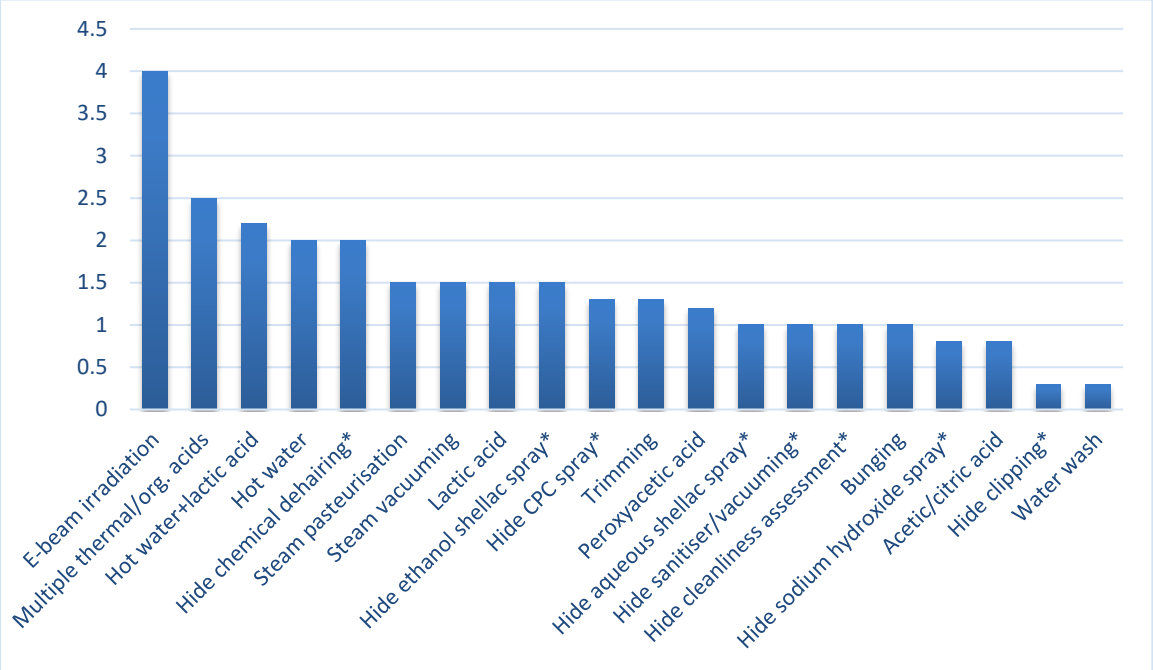
The primary objective of this study was to identify and recommend effective interventions in minced beef production chain. Most studies identified in the course of this review were conducted under laboratory conditions that often reported an exaggerated intervention efficacy in comparison with what would be expected in practice (i.e. often 1-2 log-cycles better reduction effect of the same intervention than studies performed under commercial conditions).

Studies on industrial scale and pilot scale, with naturally contaminated products, provide more confidence in the efficacy of interventions. Therefore, where sufficient number of these studies were reported per intervention, the reductions achieved were used to draw the conclusions. There was an overall lack of reported controlled trials conducted under commercial conditions (only eight on cattle hide and fourteen on beef carcass interventions, out of 316 studies identified), which hampers a proper estimation of the true effect of interventions.

The relative log reductions of indicator bacteria for standard processing procedures and interventions reported to reduce microbial contamination on beef carcass surfaces under commercial abattoir conditions are shown in Figure 1. They are presented as relative to E-beam irradiation of carcass surface, which was the only intervention reported to completely eliminate *E. coli* O157 from beef carcass (Arthur *et al.* 2005). These reductions include data from controlled and before-and-after trials investigating cattle hide interventions with the effect measured as reduction-in-transfer to resulting carcasses, as well as post-dehiding carcass interventions up to the carcass fabrication stage.

Caution must be exercised when interpreting the efficacies of interventions, because some data are derived from multiple studies using different study designs where a range of reduction effects were reported. Furthermore, these reductions are based only on the observations from across different studies and the statistical analysis was not performed. A systematic literature review coupled with meta-analysis is one method that can be used to address differences between experimental methods and results within a body of literature (Greig et al. 2012, Zhilyaev et al. 2017). Then, the data obtained in this way can be used in quantitative risk modelling which enhances the confidence in risk predictions and provides a more evidenced-based model for public health analyses (Dodd et al. 2011, Smith et al. 2013).

Figure 1. Relative log reductions for standard processing procedures and interventions reported to reduce indicator bacteria on beef carcass surfaces under commercial abattoir conditions, relative to E-beam irradiation (*reduction in hide-to-carcass transfer)



Taking into consideration the relative efficacies of reported interventions, it could be argued that any intervention that has a statistically significant and consistent effect in reducing carcass microbial contamination can be considered as hazard-based and recommended for use, dependant on other contextual factors as well. According to EFSA (2010), the use of substance(s) for decontaminating treatments is considered efficacious when any reduction of the prevalence and/or numbers of pathogenic target microorganisms is statistically significant when compared to the control and, at the same time, this reduction has a positive impact on reduction of human illness cases.

One way of assessing the latter aspect is to conduct a QMRA on the effects of interventions for given microbiological risk, such as a stochastic QMRA model for *E. coli* O157:H7 in ground beef and beef cuts discussed in the section below (Smith *et al.* 2013). Other factors usually taken into consideration are: i) the safety of the intended substance; ii) the effect as to the development of resistance to therapeutic antimicrobials; and iii) the safety of the substance and its by-products for the environment (EFSA, 2010).

With respect to the efficacy of reviewed beef interventions, cattle hide interventions such as chemical washes with vacuuming and immobilisation treatments with shellac, had a statistically significant and consistent reduction effect reported in several studies (1-1.5 logs). The use of these interventions could have the greatest effect on an overall reduction of carcass bacterial load as it reduces the risk of hide to carcass cross-contamination, thus preventing major carcass contamination problems before even they occur. Furthermore, carcass pasteurisation treatments and organic (lactic) acid washes also produced a

consistent reduction effects seen across several studies, from 1-2.5 logs, and, when in sequential use, up to a 3 logs reduction.

Other interventions for which there is a lack of evidence (e.g. hide removal practices, bunging/rodding); that have shown inconsistent results in reducing microbial contamination (particularly in respect to pathogens, e.g. hide cleanliness assessment, hide clipping, chilling); or where no processing parameters in reducing carcass contamination can be clearly established (e.g. environment, equipment and tools sanitation, knife trimming and steam vacuuming) can be used as GHP-based control measures to assist in overall microbial reduction.

All these measures are necessary in beef production premises and their use can often increase the efficacy of subsequently applied hazard-based intervention. For example, cattle hide clipping can enhance the efficacy of hide chemical washes or immobilisation treatment with shellac. It goes without saying that one shouldn't rely on the interventions' efficacy to counteract previous inadequate hygiene.

The sequential application of interventions after dehiding but before chilling, based on a 'multiple-hurdle approach' under commercial abattoir conditions, delivered the highest reductions consistent across seven reported studies. Multiple interventions following the dehiding process usually involved some or all of the following: knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam and organic acid (or peroxyacetic acid) rinsing before chilling.

The reductions of naturally present bacterial indicators were higher than when only one single intervention was used and in most cases they ranged from 2-3 logs of ACC and/or faecal indicators. Also, the prevalence of naturally present VTEC and *Salmonella* was in most cases statistically significantly reduced, often to the levels below detection limits, in twelve reviewed studies. In only one study where cattle hide organic acid washes were investigated as a part of 'multiple-hurdle approach' (concurrently with beef carcass organic acid washes) under pilot plant conditions, the reductions obtained and measured on beef carcasses after chilling were in the range 1.5-2 logs compared to untreated (only chilled) carcasses (Van Ba *et al*. 2018).

In relative terms, the reductions shown in Figure 1 correlate to some extent to the ones reported in systematic reviews and meta-analyses on interventions in beef for *Salmonella* and *E. coli* (Greig *et al.* 2012, FAO 2016, Zhilyaev *et al.* 2017). In the meta-analysis on the effect of interventions used in cattle processing plants to reduce *E. coli* contamination, Zhilyaev *et al.* (2017) analysed data both from studies performed under commercial conditions and from pilot and laboratory studies. They found least-squares mean reductions of *E. coli* (log CFU/cm^2) on beef surfaces of 1.44 [95% CI: 0.73–2.15] for acetic acid, 2.07 [1.48–2.65] for lactic acid, 3.09 [2.46–3.73] for steam vacuum and 1.90 [1.33–2.47] for water wash. There is a certain discrepancy between their results and those presented in

Figure 1 which might be due to an exaggerated intervention efficacy from the pilot plant and laboratory studies these authors analysed.

In another systematic review and meta-analysis performed by Greig *et al*. (2012), a stochastic simulation model was used to evaluate combined effects of carcass water wash, steam or hot water pasteurisation and a 24 h dry chilling on *E. coli*. The authors analysed only studies conducted under commercial conditions to reduce *E. coli* numbers or prevalence on beef carcasses.

The study found that final wash using potable water, pasteurisation with steam or hot water with or without an acid treatment, and dry chilling are effective interventions for reducing generic *E. coli* contamination of finished beef carcasses. Pasteurisation had the single largest impact on decreasing the prevalence of *E. coli* contaminated carcasses, as well as the concentration of *E. coli* on the carcasses. It was reported that the steam pasteurisation was as effective as hot water pasteurisation.

Further decrease in prevalence of *E. coli* was noticed after application of lactic acid (no data on the effect on *E. coli* levels were available). Retzlaff *et al.* (2005) recommended optimum operating temperature in a steam chamber of 87.8^{o}C and a minimum temperature of 85^{o}C for 10 sec as a critical limit, when steam pasteurisation is employed as a critical control point in a HACCP-based system. In a similar systematic review and meta-analysis performed by Young *et al.* (2016), it was reported that prechill hot water washes and steam pasteurisation are effective for reducing *Salmonella* contamination on beef carcasses (FAO, 2016; Young *et al.* 2016).

One QMRA model was developed in Canada and used to quantitatively assess the relative impacts of specific interventions on public health risks from consumption of *E. coli* O157:H7 in beef products (Smith *et al.* 2013). This QMRA model provides a useful tool to compare relative efficacies of different interventions to determine their potential impact on public health risks. To quantify the impacts of various interventions applied at processing level on concentrations of *E. coli* O157:H7 on cattle carcasses, the authors used data from a systematic review and meta-analysis published by Greig *et al*. (2012). They found that any intervention (excluding carcass water spray chill) applied at processing level significantly reduced the probability of illness from *E. coli* O157:H7 consumed in undercooked minced beef and beef cuts, compared to applying no interventions.

The average probability of illness per serving of minced beef and beef cuts following application of single intervention at slaughter (excluding carcass water spray chill) was reduced by 45%-92% and 44%-96.5%, respectively. Generally, single processing interventions reduced risks more than single pre-harvest interventions (use of probiotics and/or vaccine). Combinations of interventions, such as the use of pre-harvest interventions followed by sequential use of interventions at slaughter (pre-evisceration hot water wash, post-evisceration hot water wash, steam pasteurization and acid spray chill), had the

greatest impact and reduced the average probability of illness per serving of minced beef and non-intact beef cuts by 95%-99.6% and 95%-99.9%, respectively, relative to the no intervention scenario (Smith *et al*. 2013).

The authors also concluded that the scenarios investigated that related to the current practices in Canada (i.e. pre-evisceration hot water wash followed by post-evisceration hot water wash, steam pasteurization and acid spray chill) were effective at reducing risks from consumption of *E. coli* O157:H7 in beef products, with average probabilities of illness per serving of 8.7×10^{-6}, 3.3×10^{-8}, and 2.9×10^{-9} for ground beef, non-intact beef cuts, and intact beef cuts, respectively.

In another QMRA model, Dodd *et al.* (2011) evaluated the effects of multiple concurrent pre-harvest interventions and interventions at slaughter for *E. coli* O157 on the risk of beef carcass contamination. In this model, beef interventions were not individually evaluated but rather as a part of larger intervention category (i.e. grouped as cattle hide and carcass interventions). The authors used prevalence parameters and estimated that the risk of *E. coli* O157 carcass contamination was conditional, among various pre-harvest factors, on the transport and lairage effects, hide interventions, and carcass interventions.

Sensitivity analyses revealed that faecal prevalence, faecal-to-hide transfer, hide-to-carcass transfer, and carcass intervention efficacy significantly affected the risk of carcass contamination (correlation coefficients of 0.37, 0.56, 0.58, and 20.29, respectively). The results indicated that combinations of pre-harvest interventions are important for supplementing interventions at slaughter, but also emphasise the importance of lairage, cattle hide and beef carcass interventions for controlling *E. coli* O157 (Dodd *et al*. 2011).

When implementing such interventions, various factors should be taken into account. Interventions during processing should be designed to minimise the introduction of additional contamination and to reduce or eliminate the existing one. The sources of overall carcass contamination and, in particular, the quantification of their contribution to the contamination at the lairage stage and at slaughter and post-slaughter events is not a well-researched area.

There are no data of the relative contribution of accidental gut spillage, airborne contamination and contamination from other indirect sources (workers, equipment), but it can be assumed that these events are highly likely plant specific and would differ in various environments. Cattle hide is the only constant and frequent contamination source for which sufficient research data has been generated. Even in the abattoirs performing at the best standards, contamination from hides occurs regularly (Antic *et al*. 2011). Studies on the quantification of this contamination suggest that up to 1% in commercial and 10% in lab conditions of microbial contamination is transferred to carcasses (Bacon *et al*. 2000, Arthur *et al*. 2004, Antic *et al*. 2018). Also, the resulting microbiological status of the carcasses often mirrors that of the hides prior to dehiding (Blagojevic *et al.* 2011). Given the proactive

nature of current FSMS, it is clear that the first priority should be prevention of microbiological contamination. This also should be in line with the whole chain approach and controls implemented in an integrated way, starting from the farm. One molecular study has shown, through prevalence determination and isolate genotyping with pulsed-field gel electrophoresis, that survival of *E. coli* O157:H7 on the hides of live cattle is relatively short, with an approximate duration of 9 days or less (Arthur *et al.* 2011).

The results of this study suggest that any pre-harvest interventions that are to be administered at the end of the finishing period will achieve the maximum effect in reducing *E. coli* O157:H7 levels on cattle hides if given nine days before the cattle are presented for processing in the lairage. However, any contamination events during lairaging due to poor lairage cleaning practices or inadequate cattle handling, would give rise to additional hide contamination and negate effects of pre-harvest interventions (Small *et al.* 2002, Small *et al.* 2003).

The main driver for the implementation of interventions in beef processing premises should be the protection of public health from the most significant microbial hazards. The United States food safety policy of declaring *E. coli* O157:H7 an adulterant (i.e., a prohibited contaminant) in raw ground beef has resulted in substantial changes in the approach to FSMS implemented at the beef processing stage, including requirement for mandatory implementation of the HACCP system (FSIS, 1993; 1996).

The improved hygienic slaughter practices and implementation of additional controls are designed to reduce the likelihood of pathogen presence at detectable levels. The implementation of such controls was based on the preference of some consumers in the USA for lightly cooked ground beef. The adulterant policy was fundamental in forcing a technological solutions at this stage of the beef chain, to introduce various interventions such as pasteurisation treatments, lactic and other organic acids, and other suitable chemicals as treatment options for decontaminating carcasses and beef trim. Due to their temporary effect, such decontaminants are not considered to be food additives but rather processing aids.

These chemical treatments are used with the understanding that there must be no measurable chemical residue on the carcass and that the treatment effect in reducing microbial contamination is temporary.

The FSA's position is that the FBOs serving LTTC burgers should ensure that their suppliers must have documented and validated procedures in place throughout the supply chain (during slaughter, cutting and mincing), that can achieve at least a 4 log reduction before the burger is served to the final consumer (FSA, 2015, 2016). When considering all available evidences generated in this review, no single intervention, apart from E-beam irradiation, can realistically deliver 4 logs reduction of microbiota on carcasses or beef cuts. However, the sequential application of interventions, based on a 'multiple-hurdle approach', was able

to deliver the highest reductions which were consistent across seven reviewed studies conducted under commercial abattoir conditions.

The reductions in numbers of naturally present bacterial indicators, when multiple beef carcass interventions from post-dehiding to pre-chill stage were used, in most cases ranged from 2-3 logs of ACC and/or faecal indicators, and in some studies up to 4 logs. Also, the intervention effects against naturally present VTEC and *Salmonella*, measured in prevalence estimates in twelve reviewed studies, were in most cases statistically significant, and the presence of these pathogens was often reduced to the levels below detection limits. Therefore, the reductions were higher than when only one single intervention was used and the overall improvement of the microbiological status of beef was determined by a combination of microbial reductions achieved by all interventions. Nevertheless, apart from one study conducted in pilot plant (that investigated sequential use of one hide and one carcass intervention), no other studies investigating cattle hide interventions, interventions during chilling and in post-chill stage as a part of an overall 'multiple hurdle approach' alongside beef carcass interventions, were identified in this review.

Some of the reviewed hazard-based interventions in abovementioned stages (for example chemical hide washes and microbial immobilisation treatment with shellac, and organic acid washes of carcasses and beef cuts post-chill) were often able to deliver additional 1-2 logs of microbial reductions. Therefore, it can be expected that the 4 logs performance criterion can be achieved in the minced beef production chain, at the FBOs which supply meat for LTTC burgers. This is possible if sequential application of the interventions is utilised, in an integrated and coordinated way. The 'multiple-hurdle approach' in this case would rely on properly implemented prerequisite GHP-based measures in place, for example lairage cleaning, proper cattle handling in the lairage, hide cleanliness assessment, carcass knife trimming and steam vacuuming alongside careful hide removal and bunging/rodding. This can then extend to the hazard-based cattle hide interventions (chemical hide washes or microbial immobilisation treatment), beef carcass interventions at slaughter (pasteurisation treatments with hot water and/or steam and organic acid washes) and carcass interventions at chill/post-chill stage (organic acid washes of carcasses); concluding with interventions for beef cuts post-chill (organic acid washes), and also interventions in packaging stage (MAP and vacuum packaging of meat with added lactic acid).

The comprehensive use of interventions within this 'multiple-hurdle approach', may be able to reduce microbial loads sufficiently to offer the same level of protection to consumers from burgers, which are produced with these interventions and are served LTTC as that of thoroughly cooked burgers originating from conventional minced beef production chain.

3.6 Recommendations and future work

On the basis of the work undertaken during this review, the options for delivering the required level of protection to consumers of LTTC burgers have been identified and are summarised below. Recommendations are made on areas that merit further research efforts.

- Cattle hide interventions, such as chemical hide washes and microbial immobilisation treatment with shellac, have been identified as efficacious and able to deliver 1-1.5 logs reduction in transfer of bacteria to carcasses. They can be recommended for consideration as hazard-based interventions when applied post-exsanguination and before dehiding for reducing microbial contamination of resulting beef carcasses.

- Beef carcass interventions, such as pasteurisation treatments with hot water and/or steam, have been identified as efficacious and able to deliver 1-2.5 logs reduction. Also, organic (lactic) acid washes can deliver 1-1.5 logs reduction. When both interventions are in in sequential use, they can deliver up to a 3 logs reduction. Both carcass pasteurisation treatments and organic (lactic) acid washes can be recommended for consideration as hazard-based interventions when applied after dehiding and pre-chill.

- Organic (lactic) acid washes have also been identified as efficacious when applied on beef carcasses during chilling and at post-chill, pre-fabrication stage, and able to deliver around 1.5 logs reduction. They can be recommended for consideration as hazard-based interventions when applied on carcasses at these stages.

- Interventions for beef cuts and minced beef at the post-slaughter stage, such as organic acid washes, MAP and vacuum packaging of meat (with added lactic acid), have been identified as efficacious and able to deliver up to 2 logs reduction. They can be recommended for consideration as hazard-based interventions when applied at the final product, but only if properly optimised to retain the quality of the product.

- There are certain interventions for which there is a lack of evidence (e.g. hide removal practices, bunging/rodding); that have shown inconsistent results in reducing microbial contamination (particularly in respect to pathogens, e.g. hide cleanliness assessment, hide clipping, chilling); or where no processing parameters in reducing carcass contamination can be clearly established (e.g. environment, equipment and tools sanitation, knife trimming and steam vacuuming). These interventions can be recommended for use as GHP-based control measures, alongside hazard-based interventions, to assist in overall microbial reduction.

- The sequential use of beef carcass interventions as a part of 'multiple-hurdle approach' (knife trimming, steam vacuuming, pasteurisation treatments and organic acid washes) delivered higher reductions than any of the interventions applied alone, from 2 to 3 logs. Therefore, the sequential use of GHP- and hazard-based carcass interventions can be recommended for consideration, particularly when they are used alongside other recommended interventions at pre-slaughter, slaughter and post-slaughter stage.

- In order to address differences in study designs and results on the intervention efficacies between multiple studies identified in this review, further meta-analysis of data generated in this study is needed. This, coupled with subsequent use of data in quantitative risk modelling can enhance the confidence of the contribution of beef interventions in the reduction of microbial load to meet required performance criteria, and would provide a more evidence-based model for public health analyses.

- The relative lack of data was found on the interventions in the pre-slaughter stage, particularly cattle handling in the lairage and hazard-based bacteriophage treatment for cattle hides. Also, more data are needed on cattle hide interventions post-exsanguination and carcass interventions during chilling and at post chill, pre-fabrication stage. Novel emerging technologies for beef cuts and minced beef, such as electron beam and gamma irradiation, high-pressure processing and bacteriophage treatments, merit further investigation. There was an overall lack of large controlled trials conducted under commercial conditions, particularly investigating multiple beef interventions at slaughter, prior to dehiding to pre-fabrication stage. These are the areas where further research is needed to fill the knowledge gaps.

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Annex 1: Efficacy of interventions in minced beef production chain

1 Methods

1.1 Review approach, question and scope

The review considered all evidence on beef intervention efficacy available in the public domain, including primary research, previously published systematic reviews, risk assessments and stochastic models. Only primary research[2] studies were used for detailed data extraction and reporting. Previously published systematic reviews and narrative literature reviews on similar topics were used to define specific intervention categories and to cross check data (where similar interventions were reviewed) (Loretz *et al.* 2011, FAO 2016, Young *et al.* 2016). All main research literature types were included in review: peer reviewed articles published in journals, conference papers, government and industry reports and theses.

The review question was: "What is the efficacy of all possible interventions to control microbiological contamination in beef and beef processing environment at any stage in minced beef production chain from cattle received in abattoir to the packaging and storage inclusive?"

The population of interest included all cattle produced for domestic meat consumption, including their carcasses at processing and finished products (beef trim and ground/minced beef). Also, population of interest included potential sources of beef contamination during processing (i.e. cattle hides, environment surfaces and tools/knives/equipment). Any interventions[3] applied from cattle received in abattoir up to and inclusive of finished product packaging and storage (minced beef production chain) were considered relevant. Relevant outcome measures for interventions were the effectiveness of each intervention in reducing log levels of indicator bacteria (aerobic colony counts, *Enterobacteriaceae* counts, total coliform counts and generic *E. coli* counts, where data was available) and log levels of foodborne pathogens (primarily *E. coli* O157 and other non-O157 VTEC serogroups and *Salmonella*, but also other foodborne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium perfringens*, where data was available).

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

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

1.2 Search strategy and information sources

A comprehensive search algorithm was developed and used for the search of peer-reviewed literature (Appendix A). The algorithm was developed by extracting key words from a selection of 20 known relevant articles on beef interventions (different articles per intervention category), and by reviewing and adapting search strategies and key terms of previously published reviews and risk assessments on this and similar topics (Wilhelm *et al.* 2011, Greig *et al.* 2012, FAO 2016).

Key terms were combined using the Boolean operator "OR" into categories for Pathogen/Outcome (microorganism terms), Intervention (intervention terms) and Population (beef/hide/tools terms), and the categories were combined using the "AND" operator (Appendix A). Algorithms were pre-tested in Scopus and CAB Direct to ensure that a known list of 25 relevant articles (five per broad intervention categories) could be sufficiently identified (Appendix A).

Final searches were implemented in the bibliographic databases Scopus, CAB Direct, Agricola and PubMed on 14 September 2018. Updated search was also conducted on 05 December 2018 to check for any literature published after the first search so to include all relevant articles published in 2018 (the updated search did not retrieve any eligible articles for the review). No language restriction was imposed, but only literature from 1996 to date was searched. The reason only articles published after 1996 were included was because it was considered that the evidence on interventions published prior to this period was not reflective enough of current industry conditions and practices. Also, mandated HACCP regulation came into force in 1996 and was followed with later requirements for in-plant validation on interventions with many research studies published after this date.

Search verification was conducted by reviewing the reference lists of a selection of relevant review and primary research articles (22 in total, Appendix A), reviewing relevant conference proceedings and through targeted searches in Google to identify potential grey literature (e.g. government and industry reports and theses). All details of internet searches for relevant grey literature citations are presented in Appendix A.

1.3 Relevance screening and eligibility criteria

The relevance of each unique citation was assessed at the title and abstract level using form developed and modified from FAO (2016) (Appendix B). Citations describing research evaluating the efficacy and/or effectiveness of interventions to control microbiological contamination in beef and beef processing environment at any stage in minced beef production chain from cattle received in abattoir to the packaging and storage inclusive, were considered relevant and passed to the next stage. As potentially relevant for this review, citations describing interventions in sheep/lambs/goats, narrative reviews and studies on microbiological contamination in beef processing environment, were retained to

be used for search verification and/or to describe contextual factors relevant for this review. The data on the intervention efficacy from sheep/lambs/goats intervention studies were not further analysed as these were considered not reflective enough of beef interventions.

1.4 Relevance confirmation and prioritisation

Citations passing the previous relevance-screening step were procured as full articles and confirmed for relevance using another pre-specified form (Appendix B). This form was used to characterize articles according to the document type, region, study design and setting, stage in food chain and intervention categories investigated and outcomes investigated.

All experimental and observational study designs[4] were considered for detailed data extraction (these include controlled trials, challenge trials and before-and-after trials, and cross-sectional studies). Therefore, all study designs measuring intervention efficacy through concentration (e.g. colony forming units 'CFU'/sample) and/or prevalence (presence or absence) of indicator or pathogenic microorganisms were considered.

Intervention application settings were described as commercial (large or small) abattoirs and pilot plants, as well as research conducted under laboratory conditions as long as it was applied on specific target population (i.e. cattle hides, carcass meat, beef trim, ground/minced beef, tools/knives). The interventions were categorised into the three main stages of minced beef production chain: i) abattoir (pre-slaughter); ii) abattoir (slaughter and post-slaughter); and iii) post-abattoir. Also, they were presented as per four main intervention categories: i) lairage interventions; ii) cattle hide interventions; iii) beef carcass interventions; and iv) post- carcass fabrication interventions.

[4] **Experimental study**: Each subject is assigned to a treated group or a control group before the start of the treatment. Lab trials are executed under highly controlled conditions. Field/commercial (abattoir) trials are executed under less controlled and more "real" conditions.
Observational study: Assignment of subjects into a treated group versus a control group is outside the control of the investigator.
Controlled trial: Subjects are allocated to intervention/comparison groups and evaluated for outcomes (natural pathogen exposure).
Challenge trial: Similar to controlled, but subjects are artificially challenged or exposed to the disease agent and then allocated to the intervention groups for evaluation of the outcome (artificial pathogen exposure).
Before-and-after trial: Observations (for intervention outcome) are made on a population before and after receiving an intervention.
Cross-sectional study: Examines the relationship of a risk factor and outcome (disease) at a point in time on representative samples of the target population.

'In vitro' studies and/or trials (model broth system experiments) were excluded because this setting does not reflect specific target population and/or commercial processing conditions. Also, in the post- carcass fabrication stage, interventions on beef subjected to mechanical tenderization, moisture enhancement, marination or restructuring, as well as other processes that would make beef unsuitable for use in minced beef production[5], were excluded from the review. Investigated outcomes other than previously mentioned (e.g. spoilage) were also excluded. Articles written in language other than English where there wasn't sufficient information presented in English language to extract, were also excluded. Where information in articles were presented only in visual form, such as graphs, and no other extractable data were present in the text, data on microbial reduction were not considered due to reduced precision and articles were excluded.

1.5 Data extraction

Detailed data extractions were conducted for prioritised articles using pre-specified tools (Appendix B). The data extraction tool included targeted questions about intervention and population descriptions, outcomes measured, comparison group(s) and intervention efficacy results.

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

1.6 Data analysis and reporting

Results of primary research studies were summarised narratively and shown in tabular form, per stage in the minced beef production chain and intervention categories. For studies that measured concentration outcomes (e.g. log CFU/cm^2), intervention efficacy results are presented as mean log reductions in the intervention compared with the control group. For studies measuring prevalence outcomes (positive vs negative), intervention efficacy results are presented as the change in a microorganism prevalence due to the intervention in the included studies.

1.7 References

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2 Results of review

A flow chart below shows the flow of studies through the review process. Key characteristics of 316 relevant articles for beef interventions are shown in table 2.1.

Figure 2.1. Review flow chart

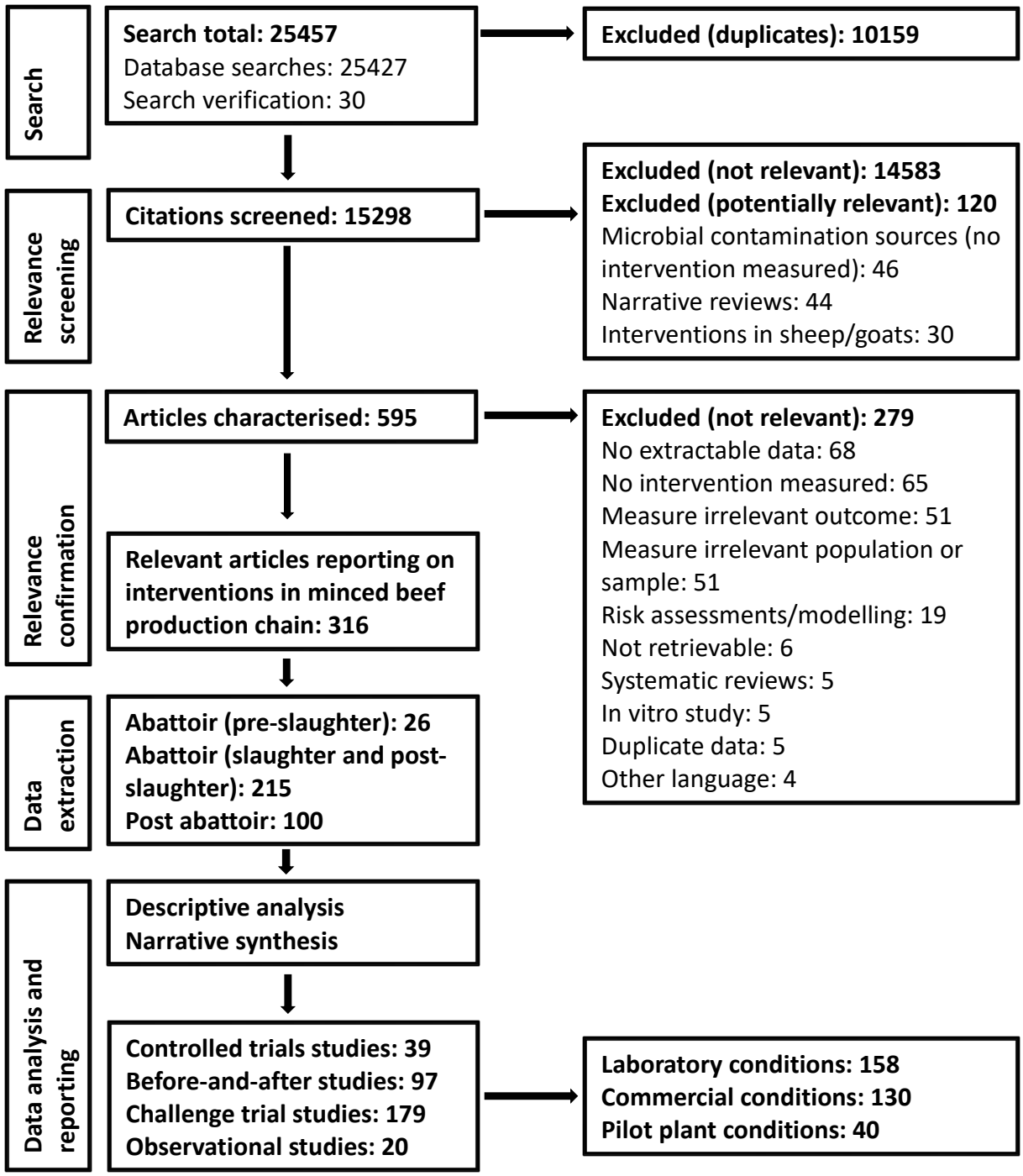

Table 2.1. Key characteristics of relevant primary research articles on beef interventions in minced beef production chain

Article characteristic	Number of articles*	%
Region		
North America	212	67.0%
Europe	69	21.8%
Australia/South Pacific	13	4.2%
Asia/Middle East	11	3.6%
Central and South America/Caribbean	9	2.8%
Africa	2	0.6%
Document type		
Journal article	302	95.6%
Thesis	8	2.6%
Conference paper	3	0.9%
Government or research report	3	0.9%
Study design		
Challenge trial	179	53.4%
Before-and-after trial	97	29.0%
Controlled trial	39	11.6%
Cross-sectional study	20	6.0%
Study conditions		
Laboratory conditions	158	48.3%
Commercial/field conditions	130	39.7%
Research/pilot plant	40	12.0%
Intervention stage/category		
Abattoir (pre-slaughter, lairage interventions):	**26**	**7.7%**
Lairage cleaning	5	1.5%
Cattle handling in lairage	8	2.3%
Hide cleanliness assessment	5	1.5%
Cattle hide interventions (pre- exsanguination)	8	2.3%
Abattoir (slaughter and post-slaughter):	**215**	**63%**
Cattle hide interventions (post- exsanguination)	33	9.7%
Cleaning/disinfection of tools/knives	11	3.2%

Article characteristic	Number of articles*	%
Standard processing procedures/GHP	13	3.8%
Carcass interventions (pre- and post- evisceration, pre-chill)	90	26.4%
Chilling and spray chilling	34	10.0%
Post chill and pre-fabrication carcass treatments	9	2.6%
Multiple interventions/HACCP	25	7.3%
Post abattoir:	**100**	**29.3%**
Standard processing procedures/GHP	6	1.8%
Post fabrication interventions (trim/ground beef)	51	15.0%
Packaging and storage	43	12.6%
Outcomes investigated		
Pathogenic *E. coli*	169	22.2%
Aerobic colony counts	157	20.6%
Salmonella	126	16.6%
Generic *E. coli* counts	116	15.2%
Total coliform counts	85	11.2%
Enterobacteriaceae counts	54	7.1%
Other	29	3.8%
Listeria monocytogenes	25	3.3%

* The total number of articles per category not necessarily equals to 316 as one article often reports on the study conducted in more than one study condition, intervention stage/category, using different study designs and investigating different outcomes.

In total, 316 relevant articles were used for data extraction and reporting in this review. More articles were identified in the abattoir (slaughter and post-slaughter) stage (63%), with significantly less in the pre-slaughter stage (lairage interventions, only 7.7%). Around 2/3 of studies were conducted in North America (USA and Canada), and roughly half of them in laboratory conditions (these predominantly challenge trials). This is not surprising because the focus of microbial hazards control in USA and Canada has been at the processing level through the implementation of HACCP-pathogen reduction programmes. Controlled, before-and-after trials and cross-sectional studies in commercial conditions were reported in around 40% of articles. The most researched population were beef carcasses in pre-chill stage (90 articles, 25%) followed by interventions in post-carcass fabrication stage (beef primals, subprimals, trim and minced beef, 51 articles, 15%). Cattle hide interventions pre- and post- exsanguination were reported in 39 articles in total (2 articles reporting on both stages). There was a striking disproportion of published studies on lairage interventions and

standard processing practices comparing to hazard-based interventions for hides, carcasses and meat (i.e. 48 vs 293, respectively).

The results on the intervention efficacies are presented in the following sections grouped as per different Intervention Category (IC), and then subdivided into intervention subcategories. A short summary of key findings (brief synopsis of the information covered in the section, including key take-home messages and overall implications) and intervention descriptions are also provided in each section, concluding each section with the list of references cited in each intervention category.

The results on the intervention effects are presented in the tables and/or as a narrative description, per intervention stage/category/subcategory and presented separately for challenge trials (those conducted under the lab/pilot conditions) and controlled/before-and-after trials (those usually conducted under commercial/pilot conditions). Each table indicates information regarding: study setting and design; number of studies; intervention and outcome sample; comparison group; outcome/microorganism, quantitative intervention effect and references.

Study setting can be either in commercial conditions (abattoir) or in more controlled environments (research/pilot plant or laboratory). Study design can be controlled trial (natural pathogen exposure), challenge trial (artificial pathogen exposure) and before-and-after trial (effect measured before and after receiving an intervention).

The number of studies indicates the number of studies where the respective intervention is investigated and reported.

The intervention sample indicates the sample type to which the intervention was applied (hide, beef, processing environment, tools). The outcome sample indicates the sample type that was subsequently measured for microbial contamination. In most cases these two samples were the same, but sometimes they differ, e.g. cattle hide interventions where the effect is measured on resulting beef carcass surfaces (reduction-in-transfer) or carcass interventions where the effect is measured in the resulting product (cuts, trim, mince).

The comparison group refers to the control group to which the intervention is compared and is usually: i) no treatment (controlled trials and challenge trials); ii) a reference treatment, usually water (again controlled and challenge trials); and iii) the ‘before’ or pre-intervention sample for ‘before-and-after’ trials.

The intervention effect for the studies that measured concentration outcomes are presented as a range of values of mean log reductions in the intervention compared with the control group. Log reduction (short for logarithmic reduction) is a ten-fold reduction of number of bacteria (e.g. 1 log reduction = 90% reduction; 4 log reduction = 99.99% reduction; 6 log reduction = 99.9999% reduction). For the studies measuring prevalence

outcomes (positive vs negative), intervention efficacy results are presented as the change in a microorganism prevalence due to the intervention in the included studies.

IC 1: Lairage interventions

IC 1.1 Summary of key findings

IC 1.1.1 Lairage cleaning

Several observational studies of lairage cleaning and disinfection practices found that the lairages in the UK were washed commonly with cold water only, with no detergents and/or disinfectants. Foodborne pathogens, such as *Salmonella*, *E. coli* O157 and *Campylobacter* are regularly found on lairage surfaces, even after routine cleansing operations, sometimes containing numbers of up to 10^4 organisms per sampled area (2,500 cm^2). Up to a 5 log-cycles of microbial reduction can be achieved on lairage surfaces using pressure water wash with quaternary ammonium sanitisers and/or steam under pressure.

IC 1.1.2 Cattle handling in lairage

No investigations on specific interventions applied at this stage were identified. In total, eight observational and molecular studies investigated the importance of lairage as a risk factor for cattle hide (and subsequently carcass) microbial contamination. A study of *E. coli* O157:H7 and *Salmonella* in cattle conducted in USA found an increase in prevalence of both pathogens between pen on-farm and at the abattoir and that the majority of isolates from both hides and carcasses at slaughter genotypically matched those from abattoir lairage, and not those from the farm of origin. In another two USA studies, risk factors identified for increased odds of hide contamination with *Salmonella* and *E. coli* O157 were holding cattle in lairages contaminated with cattle faeces and positive for these pathogens. On the other hand, three studies conducted in the UK, Ireland and Australia did not find that the lairage lead to an increase in the number or isolation rate of VTEC and *Salmonella* from cattle hides or carcasses.

Extensive hide and carcass cross-contamination from the lairage environment was found in one study using marker organism inoculated on hides and lairage surfaces. One observational study reported on the opportunities for hide cross-contamination during lairaging and found that the important risk factors were the number of animals in the lot and the animals' stocking density.

IC 1.1.3 Hide cleanliness assessment

The relationship between cattle hide cleanliness and microbiological status of derived beef carcasses have been investigated in only five cross-sectional studies conducted under commercial condition in Ireland, Italy, Norway (two studies) and Serbia. Scoring of hide cleanliness was performed according to the similar scoring systems that are used in the UK, Ireland and Norway. In all but one study, a direct correlation between visual hide cleanliness category and microbial contamination of resulting beef carcasses were found for microbiological indicators of general (ACC) and faecal contamination (EBC and *E. coli*). There

was a steady trend of decrease in carcass microbial load by 0.5-3 log-cycles of ACC, 0.7-1.5 logs of EBC and 0.4-0.8 logs of generic *E. coli* with the increase in hide cleanliness. Therefore, this GHP measure appears to be efficacious in reducing bacterial transfer from dirty hides to resulting carcasses.

IC 1.1.4 Cattle hide interventions (pre-exsanguination)

In total, eight studies were identified describing research on live animal hide interventions. Four studies that investigated live animals hide washes, with or without chemicals (cetylpyridinium chloride (CPC), lactic acid and chlorine), found that hide water wash with ambient temperature water was ineffective at reducing microbial load and had highly variable efficacy. On the other hand, washing with CPC yielded promising reductions in hide-to-carcass transfer of ACC and EBC by 1.5 and 1.1 logs, respectively, and reduced prevalence of naturally present *E. coli* O157 (from 23% in control to 3% in carcasses whose hides had been washed). The use of chemicals for cattle hide treatments was suggested to be more appropriate on hides post-exsanguination due to animal welfare concerns. Only one study that investigated hide clipping found very moderate reductions in transfer of ACC to carcass of up to 0.3 logs. Two lab studies on bacteriophage spray application reported up to 2 log reduction of inoculated *E. coli* O157:H7 on cattle hide sections after 1 h contact time, whereas one study under commercial condition found no reductions in *E. coli* O157:H7 prevalence. Apart from the phage treatment for which certain contact time with the hide is required for the full intervention effect, other interventions (washing and clipping) are more appropriate for use post-exsanguination.

IC 1.2 Intervention description

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

Lairage cleaning: refers to cleaning and sanitation practices of the lairage surfaces.

Cattle handling in lairage: refers to the time animals are held in lairage before slaughter and other handling practices. There is an increasing opportunity for cross-contamination between animals and animals and surfaces, particularly due to prolonged lairage time and/or increased stress.

Hide cleanliness assessment: refers to the scoring and categorisation of hide cleanliness before cattle slaughter according to the established objective system, and actions taken in case animals are too dirty to be processed hygienically.

Cattle hide interventions (pre-exsanguination): refers to all procedures in place which are available for use ante mortem to deal with animals that are excessively soiled, but not to compromise animal welfare.

- **Hide water wash**: refers to an ambient or cold-temperature wash to physically remove contamination from hides.
- **Hide clipping**: refers to clipping or shaving hair from hide surface to physically remove contamination from hides.
- **Bacteriophage treatment**: Treatment with bacteriophages (phages), which are viruses that infect and kill bacteria.

IC 1.3 Lairage cleaning

Several observational studies of lairage cleaning and disinfection practices were found and one challenge study applied at the lairage stage.

In the study of Small *et al.* (2003), the cleaning practices in 17 UK abattoirs slaughtering cattle were investigated using questionnaires and validated through subsequent visits. The authors report that bedding was used in the majority of lairages and was changed either between animal batches, daily, weekly or monthly. Approximately, one quarter of lairages investigated were washed daily, commonly with cold water with no detergents and/or disinfectants. The authors concluded that the cleaning and disinfection protocols employed, in general, were unlikely to eliminate the microbial load.

Small *et al.* (2002) reported the overall prevalence of *E. coli* O157 and *Salmonella* spp. in UK cattle lairages of 7.2 and 6.1%, respectively, and an increase *in E. coli* O157 and *Salmonella* prevalence in environmental samples from 6.7% and 1.1%, before work in abattoir started, to 7.8% and 11.1%, during working hours, respectively, for both pathogens. In another study, they found 6.5% of lairage samples positive for *Salmonella* (after routine cleansing operations at the end of the previous day's processing), containing estimated numbers of up to 10^4 organisms per sampled area (50 by 50 cm) (Small *et al.* 2006).

In the study of Small *et al.* (2007a), authors showed that microbial contamination often remains in UK lairage holding pens after routine cleaning operations, with up to 2.8 log CFU/cm^2 of *E. coli* remaining at some sites. In their subsequent study authors investigated cleaning methods for concrete surfaces under various conditions using pressure water with or without sanitising agent and/or steam (Small *et al.* 2007b). The reductions achieved on surfaces of inoculated *E. coli* and *Enterobacteriaceae* ranged from 0.9-5.2 log CFU/cm^2 and 0.9-5.8 log CFU/cm^2, respectively, depending on treatment applied. Pressure wash followed by steam and sanitiser appeared to have had the greatest reduction effect.

IC 1.4 Cattle handling in lairage

The importance of lairage as a risk factor for cattle hide (and subsequently carcass) microbial contamination has been investigated in a few observational and molecular studies, but no specific intervention has been applied at this step. It has been speculated that prolonged holding times in lairage leads to increased contamination of the animals' coats.

Change in *Salmonella* and *E. coli* O157:H7 prevalence in cattle between pen on-farm and at the abattoir was shown in the study of Arthur *et al.* (2008a), with increases from 0.7% and 66% on farm to 74.2% and 76.8% in the lairage, respectively for each pathogen. Also, application of pulsed field gel electrophoresis methodology demonstrated that 46.9% and 65.1% of *E. coli* O157:H7 and *Salmonella* hide isolates were attributable solely to the lairage environment, whereas 67% and 30% of the carcass *E. coli* O157:H7 and *Salmonella* isolates, respectively, could be attributed solely to the lairage environment (Arthur *et al.* 2008a).

Dewell *et al.* (2008a) reported that lots of cattle held in *E. coli* O157-positive lairage pens had eight times greater risk of having positive slaughter hide samples compared with cattle held in culture-negative pens (RR=8.0; 95% CI (1.6-38.8)). Furthermore, a lot of cattle that were held in lairage pens contaminated with faeces had three times greater risk for positive slaughter hide samples compared with cattle held in clean pens (RR=3.1; 95% CI (1.2-7.9)). The same authors reported similar findings regarding *Salmonella* (Dewell *et al.* 2008b), where it was found that slaughter cattle spending time in dirty lairage had greater risk of *Salmonella*-positive hides at slaughter relative to those in clean lairage (RR = 1.83, 95% CI (0.7–3.14)). All these findings highlight the importance of lairage in transmission of hide-level contamination. This can be reduced by minimising the duration in lairage for cattle in commercial settings, which is not always a practical measure.

On the other hand, Fegan *et al.* (2009) did not find that lairage lead to an increase in the number or isolation rate of *E. coli* O157 from cattle, which was supported by the study of Minihan *et al.* (2003a). Furthermore, time in lairage was a non-significant predictor for *Salmonella* or VTEC contamination of beef carcasses, reported in a cross-sectional study of *Salmonella* in carcasses in UK abattoirs (Milnes *et al.* 2009).

Extensive hide and carcass cross-contamination from the lairage environment was found in one small study by Collis *et al.* (2004). The authors found an increase in the presence of a hide marker inoculated onto the hides of 11% of cattle at unloading, to 100% (hide before skinning) and 88.8% (skinned carcass) samples. Also, the environmental surface marker inoculated onto lairage pens, races, and stunning box was detected on 83.3% (hide before skinning) and 88.8% (skinned carcass) samples. The extensive spread of microbial contamination between animals from different holding pens in that study was likely mediated by post-pen environmental surfaces, races and stunning boxes.

Small and Buncic (2009) investigated the opportunities for hide cross-contamination to occur during lairage of cattle. At unloading, there was a statistically significant association between the number of animals in the lot and the number of contacts they made with the unloading bay structures and other animals. Also, the frequency of contacts increased as the animals' stocking density increased. Animals at lower stocking densities were much less likely to suffer incidents of cross-contamination by direct contact than the animals at high stocking densities. On average there were more contacts per animal per minute in the first 10 minutes of holding, while the cattle explored their new surroundings.

IC 1.5 Hide cleanliness assessment

The relationship between cattle hide cleanliness and microbiological status of derived beef carcasses has been investigated in several studies. Scoring of hide cleanliness before cattle slaughter in practice varies in different countries such as the UK, Ireland, Norway and Australia (McEvoy *et al.* 2000, Hughes 2001, Kiermeier *et al.* 2006, Hauge *et al.* 2012). Most studies shown that visually dirty cattle produce carcasses with higher microbial counts than clean cattle.

In the study of Blagojevic *et al.* (2012), the mean aerobic colony counts (ACC) and *Enterobacteriaceae* load of hides and final carcases differed statistically significantly between very dirty cattle (category 4) and less dirty or clean cattle (categories 1, 2 and 3 scored according to the UK scoring system), with an increase in carcass bacterial load by 1.1 and 0.7 log, respectively, with increased hide dirtiness.

Hauge *et al.* (2012) reported a statistically significant difference in ACC between carcasses derived from clean animals and moderately dirty animals (on a three-category scale). The reduction in ACC was 0.5-0.9 logs and in generic *E. coli* 0.4-0.7 logs. There was no statistical difference for ACC and *E. coli* counts between clean and very dirty animal groups. This was partly explained by the fact that very dirty cattle were dehided more carefully. Similar observation was made in their later study conducted in two commercial abattoirs where carcass swabs after dehiding showed no statistically significant difference in the number of generic *E. coli* and *Enterobacteriaceae* between clean and very dirty cattle (Hauge *et al.* 2015). Authors hypothesised that this finding could be plant dependant and due to more careful dehiding of very dirty animals, thus an indication that there was no hygienic reason for diverting the carcasses derived from very dirty cattle into a separate processing line.

Serraino *et al.* (2012) also showed statistically significant reduction of bacterial counts on carcasses produced from clean animals compared to dirty animals (on a five-category scale according to the UK scoring system). The microbial reductions ranged from 0.9-2.9 logs for ACCs, 0.7-1.5 logs for *Enterobacteriaceae* and 0.6-0.8 logs for generic *E. coli*. In most cases the reductions increased as the cattle hide dirtiness decreased, i.e. there was a direct correlation between visual hide cleanliness category and microbial contamination of beef carcasses for all three groups of microbiological indicators.

Some earlier studies also showed a similar trend, with ACC reduced by 0.4 logs in clean animals (category 2 on a five category scale according to the Irish scoring system) compared to dirty animals (categories 3 and 5) (McEvoy *et al.* 2000).

IC 1.6 Cattle hide interventions (pre-exsanguination)

In total, eight studies were identified describing research on cattle hide interventions pre-slaughter, four investigating live animal washing, three investigating bacteriophages use in lairaged cattle and one ante- and post-mortem online cattle hide clipping.

IC 1.6.1 Live animal washing and clipping

Three studies conducted under commercial conditions were identified evaluating the effect of live animal hide washes (Byrne *et al.* 2000, Bosilevac *et al.* 2004a, Mies *et al.* 2004). One study found that a single or double water wash and a lactic acid or 50 ppm chlorine solution wash resulted in an increase in ACC, coliforms and *E. coli* from 0.1 to 0.8 log CFU/cm^2, as well as an increase in *Salmonella* prevalence of the hide (only 50 ppm chlorine solution slightly decreased *Salmonella* prevalence). It was speculated that the reason for this was that washing released bacteria encapsulated in dirt, mud and faeces on the hide, thus enabling them to more evenly contaminate the hide (Mies *et al.* 2004).

In another study conducted under commercial conditions (Byrne *et al.* 2000), it was reported that washing of cattle for 3 min using a power hose removed all visible faecal materials on the live animals and reduced inoculated *E. coli* O157:H7 by 1.7 log, whereas washing for 1 min showed hardly any effect in removing the pathogen. Nevertheless, after washing for 3 min, *E. coli* O157:H7 was not detected on three of the four areas of the resulting carcasses sampled, but the reduction was not statistically significant due to the high degree of variation.

Two controlled studies investigated treatment of cattle hides with water wash and cetylpyridinium chloride (CPC), which, applied under pilot plant conditions, reduced ACC and *Enterobacteriaceae* by 1.9-4.4 and 1.3-3.8 logs (depending on the water pressure used) (Bosilevac *et al.* 2004c). This treatment, when applied under commercial conditions in an abattoir, yielded promising reductions in hide-to-carcass transfer of both groups of indicator bacteria and also reduced prevalence of naturally present *E. coli* O157 (Bosilevac *et al.* 2004a). All three studies using chemicals (CPC, lactic acid and chlorine) on live animal hides concluded that the treatments were more appropriate for application post-exsanguination due to animal welfare concerns.

Only one study was identified which was conducted under commercial abattoir conditions and investigated ante- and post-mortem online cattle hide clipping (McCleery *et al.* 2008). The results are grouped with two other identified studies on hide clipping post-exsanguination in the section IC 2.3.1.

Table IC 1.6.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Water wash	1/ChT	Live animal hide/ hide	No treatment	*E. coli* O157:H7	0.3-1.7	(Byrne *et al.* 2000)
		Live animal hide/ carcass*	No treatment	*E. coli* O157:H7	0.5-0.6	
Water wash and CPC (1%)	1/CT	Live animal hide/carcass*	No treatment	Aerobic bacteria	1.5	(Bosilevac *et al.* 2004a)
				Enterobacteriaceae	1.1	
Warm water and CPC (1%)	1/CT‡	Hide	No treatment	Aerobic bacteria	1.9-4.4	(Bosilevac *et al.* 2004c)
				Enterobacteriaceae	1.3-3.8	

* Reduction in hide-to-carcass transfer
‡ Pilot
ChT: Challenge trial
CT: Controlled trial

Table IC 1.6.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ micro-organism	% Samples positive in study population		References
					No treatment	Treatment	
Water wash	1/BA	Live animal hide/hide	No treatment	*Salmonella* spp.	36-58%	40-72%	(Mies *et al.* 2004)
Lactic acid (0.5%)	1/BA	Live animal hide/hide	No treatment	*Salmonella* spp.	50.0%	52.2%	(Mies *et al.* 2004)
Chlorine	1/BA	Live animal hide/hide	No treatment	*Salmonella* spp.	60.0%	55.6%	(Mies *et al.* 2004)
Water wash and CPC (1%)	1/CT	Live animal hide/hide	No treatment	*E. coli* O157	56%	34%	(Bosilevac *et al.* 2004a)
	1/CT	Live animal hide/carcass*	No treatment	*E. coli* O157	23%	3%	

* Reduction in hide-to-carcass transfer
BA: Before-and-after-trial

IC 1.6.2 Bacteriophage application to cattle hides in lairage

There were three studies evaluating the effect of bacteriophage sprayed onto cattle hides (Coffey *et al.* 2011, Arthur *et al.* 2017, Tolen *et al.* 2018). The results obtained from these experiments were variable, with one controlled trial demonstrating that the treatment with bacteriophages before processing did not produce a statistically significant reduction in *E. coli* O157:H7 numbers on cattle hides or beef carcasses during processing (Arthur *et al.* 2017), whereas two challenge trials under lab conditions reported up to 2 log reductions in inoculated *E. coli* O157:H7 on cattle hide sections after 1 h exposure (Coffey *et al.* 2011, Tolen *et al.* 2018).

Table IC 1.6.3. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Bacteriophage Finalyse® spray	1/CT	Live animal hide/hide	No treatment	*E. coli* O157:H7	57.6	51.8	(Arthur *et al.* 2017)
	1/CT	Live animal hide/carcass*	No treatment	*E. coli* O157:H7	17.6	17.1	

* Reduction in hide-to-carcass transfer

Table IC 1.6.4. Challenge trial studies

Intervention	No. studies	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Bacteriophages e11/2 and e4/1c	1	Hide	No treatment	*E. coli* O157:H7	2.0	(Coffey *et al.* 2011)
	1	Hide	Water wash	*E. coli* O157:H7	1.5	
Bacteriophages	1	Hide	No treatment	*E. coli* O157:H7	0.5	(Tolen *et al.* 2018)
	1	Hide	No treatment	VTEC *O103:H2* & *O121:H19*	0.4-0.7	
	1	Hide	No treatment	VTEC *O111:H-* & *O45:H2*	<0.1	

IC 1.7 References cited in IC 1

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IC 2: Cattle hide interventions (post-exsanguination)

IC 2.1 Summary of key findings

Interventions for cattle hides as a main source of beef carcass microbial contamination have been investigated in the post-exsanguination stage in a total of 33 studies. The hide interventions described in the previous section (apart from the phage treatment) are more appropriate for use after animal stunning and bleeding due to multiple factors (animal welfare, technical requirements, risk for workers, etc). For the majority of physical and chemical interventions for cattle hides post-exsanguination used, no validation under full commercial conditions was provided. Hence, even when some of these interventions showed promising efficacy in reducing microbiota on hides, it is largely expected that the effect in reducing carcass meat surface contamination would be much smaller. Only five controlled trials conducted under commercial conditions reported hide intervention effects on resulting beef carcass surfaces (one intervention using hide wash with sodium hydroxide, two microbial immobilisation treatments with ethanol and aqueous shellac solutions, one on chemical dehairing and two on hide clipping).

IC 2.1.1 Hide washing and clipping

Hide washing with ambient or warm water under pilot and commercial conditions was found to reduce indicator bacteria of up to 1 log-cycles. Also, the prevalence of VTEC and *Salmonella* in studies conducted under pilot and commercial conditions was statistically significantly reduced. The increased efficacy of water washing was achieved when additional vacuuming or manual curry comb was used, often by 1 log-cycle.

On the other hand, four studies that investigated hide clipping found very moderate reductions in transfer of ACC to beef carcasses of up to 0.3 logs of indicator bacteria. It was noted in several studies that hide clipping could be useful as a GHP pre-treatment to subsequent hazard-based hide interventions.

IC 2.1.2 Hide washing with organic acids

A limited number of studies describing investigations on organic acids as hide treatments reported highly variable results. One study under commercial conditions found that localised application of lactic and acetic acids yielded reductions of 2.3-2.6 and 3.7 logs of general and faecal microbiota.

IC 2.1.3 Hide washing with other chemicals/oxidisers

More studies have investigated a range of different chemicals, including oxidisers. Under pilot plant conditions, oxidisers reduced ACC and faecal microbiota by 2.0-3.5 and 2.0-4.0 log cycles on treated hides. Under commercial conditions, an automated hide wash with sodium hydroxide achieved statistically significant reduction in transfer to carcasses of both

aerobic and enteric bacteria of 0.8 logs and prevalence of *E. coli* O157 from 17% to 2%. Vacuuming following hide washing with chemicals appears to increase efficacy in removing bacteria by 1-2 log-cycles.

IC 2.1.4 Chemical dehairing and thermal interventions

Harsh treatments involving chemical dehairing and heat treatments of hide appear to be the most efficacious treatments, however with questionable practical use. Chemical dehairing was the most successful treatment under commercial conditions, achieving reduction in transfer to carcasses of aerobic and enteric bacteria of 2 logs and 1.8 logs and prevalence of *E. coli* O157 from 50% to 1%. Hot water washes of hides and steam treatments achieved reductions on treated hides of up to 6 log-cycles.

IC 2.1.5 Microbial immobilisation treatments

This novel approach, with the purpose to coat cattle hides, thus preventing microbial transfer onto meat, was investigated in three studies using natural resin shellac in ethanol or aqueous solution. Reductions of up to 3.6 logs and 1.7 logs in transfer to meat of general microbiota under lab and under commercial conditions, respectively, were reported when shellac in ethanol was used. Comparable reductions in transfer of microbiota to meat were also observed when using aqueous shellac solutions, with reductions of up to 3 logs and 2.4 logs of aerobic and enteric bacteria, respectively, under lab conditions and to resulting beef carcasses of up to 1.1 logs and 0.7 logs of ACC and EBC, respectively, under commercial conditions.

IC 2.2 Intervention description

Hide water wash: refers to an ambient or cold-temperature wash to physically remove contamination from hides. Warm water washes (usually <60^{o}C) have a similar effect in removing bacteria (depending on the pressure used), and when applied for a short time don't have a microbicidal effect.

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

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

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

Thermal treatments: refers to various heat treatment washes to destroy microbial cells. Examples include scalding bob-veal hide-on carcasses (usually >60^{o}C), hot water (usually >74^{o}C), treatments with steam (usually >82^{o}C) and naked flame/singeing (>300^{o}C).

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

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

IC 2.3 Hide washing and clipping

Hide washing post-exsanguination with potable, ambient or cold water was investigated in several studies, either as a main intervention (Small *et al.* 2005, Arthur *et al.* 2007, Arthur *et al.* 2008a, Bosilevac *et al.* 2009, Wang *et al.* 2014) or a control treatment for chemical washes (Bosilevac *et al.* 2005a, Bosilevac *et al.* 2005b, Baird *et al.* 2006, Carlson *et al.* 2008a, Carlson *et al.* 2008b, Scanga *et al.* 2011). There is conflicting evidence on the efficacy of water washes as a standalone intervention, with higher microbial reductions reported in laboratory studies using artificially inoculated microbiota which do not reflect the real life conditions (Carlson *et al.* 2008b). Baird *et al.* (2006) reported that bacterial reductions obtained on clipped hides after water wash were generally higher than on un-clipped hides, and concluded that hide clipping could be a useful pre-treatment to subsequent hide washes with chemicals.

Under pilot plant conditions, up to 1 log reduction of ACC, EBC and *E. coli* on washed hides was achieved (Bosilevac *et al.* 2005a, Bosilevac *et al.* 2005b, Carlson *et al.* 2008a), with increased efficacy if high-pressure washing and additional vacuuming (Bosilevac *et al.* 2005a) or manual curry comb were used (Wang *et al.* 2014). Also, the VTEC and *Salmonella* prevalence was statistically significantly reduced on washed hides using plant commercial washing systems (Arthur *et al.* 2007, Arthur *et al.* 2008a, Bosilevac *et al.* 2009).

With respect to hide clipping, Small *et al.* (2005) observed an increase in aerobic bacterial load by 0.3 logs after hide clipping, attributed to the generation of dust and subsequent spread of bacteria during the process. In the study of McCleery *et al.* (2008), carcasses derived from dirty, hide-clipped cattle, showed comparable bacterial levels with those from non-clipped, but clean animals (a reduction 0.1-0.3 logs of ACC). In the study of Van Donkersgoed *et al.* (1997), the reductions achieved were similar, with a decrease of up to 0.3 logs of aerobic bacteria and faecal indicators, so the author concluded that the clipping is of questionable practical significance. Fisher *et al.* (2009) achieved modest reductions of inoculated *E. coli* K12 on hides (0.9 logs) and carcasses (0.1 logs) following hide clipping in a pilot plant.

Table IC 2.3.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Water wash	1/BA‡	Hide	No treatment	Aerobic bacteria	0.5	(Bosilevac *et al.* 2005b)
				Enterobacteriaceae	0.9	
Water wash	1/CT‡	Hide	No treatment	Aerobic bacteria	1.0	(Carlson *et al.* 2008a)
				Coliforms	0.5-0.7	
				E. coli	0.8-1.0	
Water wash/ manual curry comb	1/BA	Veal calf hide	No treatment	Aerobic bacteria	0.8	(Wang *et al.* 2014)
				Enterobacteriaceae	3.5	
				Coliforms	1.4	
				E. coli	1.6	
Warm water wash	1/BA‡	Hide	No treatment	Aerobic bacteria	1.0	(Bosilevac *et al.* 2005b)
				Enterobacteriaceae	0.9	
Warm water wash	1/BA	Hide cut lines	No treatment	Aerobic bacteria	0.1	(Scanga *et al.* 2011)
				Coliforms	-0.1	
Warm water wash	1/CT‡	Hide	No treatment	Coliforms	1.6	(Bosilevac *et al.* 2005a)
Warm water + vacuum	1/CT‡	Hide	No treatment	Coliforms	3.6	(Bosilevac *et al.* 2005a)
Hide clipping (dirty hides)	2/CT	Hide/carcass*	No treatment	Aerobic bacteria	0.1-0.3	(Van Donkersgoed *et al.* 1997, McCleery *et al.* 2008)
				E. coli	0.3	
				Coliforms	0.3	

* Reduction in hide-to-carcass transfer
‡ Pilot

Table IC 2.3.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies / design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Water wash	1/BA	Hide	No treatment	*E. coli* O157:H7	62.5%	38.4%	(Arthur *et al.* 2008a)
				Salmonella	88.1%	24.3%	
Water wash and chlorine	2/BA	Hide	No treatment	*E. coli* O157:H7[a]	4-35%	1-13%	(Arthur *et al.* 2007, Bosilevac *et al.* 2009)
				E. coli O157:H7	46-98%	34-90%	
				Salmonella [a]	27-40%	7-13%	
				Salmonella	95%	69-83%	
Water wash/ manual curry comb	1/BA	Veal calf hide	No treatment	*E. coli* O103	26%	17%	(Wang *et al.* 2014)
				E. coli O111	23%	17%	
Warm water wash	1/BA	Hide cut lines	No treatment	*E. coli* O157:H7	78.0%	84.0%	(Scanga *et al.* 2011)
				Salmonella	68.0%	88.0%	

[a] Percentage of total samples that had *E. coli* O157:H7 and *Salmonella* spp. counts at or above the detection limit of 40 CFU/100 cm^2 after enumeration.

Table IC 2.3.3. Challenge trial studies

Intervention	No. studies	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Water wash	2	Hide	No treatment	*S.* Typhimurium	0.7	(Mies *et al.* 2004, Carlson *et al.* 2008b)
				Salmonella spp.	1.7	
				E. coli O157:H7	2.3	
Water sponge appl.	1	Hide	No treatment	Aerobic bacteria	0.6-0.9	(Baird *et al.* 2006)
				Coliforms	<0.5	
				E. coli	0.2	
Hide clipping	1‡	Hide/Hide	No treat.	*E. coli* K12	0.9	(Fisher *et al.* 2009)
	1‡	Hide/carcass*	No treat.	*E. coli* K12	0.1	

* Reduction in hide-to-carcass transfer
‡ Pilot

IC 2.4 Hide washing with organic acids

Highly variable and conflicting results were reported in several studies on organic acid sprays/washes on cattle hides. Most studies on lactic and acetic acid were conducted under simulated environments in pilot plants and lab conditions (challenge trials using inoculated microbiota). Spraying/rinsing or sponge rubbing hides with lactic and acetic acid under pilot plant conditions achieved 2-2.5 log reductions of indicator bacteria (Baird *et al.* 2006, Carlson *et al.* 2008a), while similar treatments under lab conditions were highly variable (from 0.5 up to 5 logs of inoculated microbiota) (Mies *et al.* 2004, Baird *et al.* 2006, Carlson *et al.* 2008a, Carlson *et al.* 2008b, Fisher *et al.* 2009, Elramady *et al.* 2013, Jadeja and Hung 2014). It was inconclusive whether the increase in lactic acid concentration led to increased microbial reduction, as this was noted only in one study (Mies *et al.* 2004). In one small study in a pilot plant, promising reductions of inoculated *E. coli* K12 were achieved on hides and resulting beef carcasses after lactic acid spray (2.4 and 1.7 logs respectively) (Fisher *et al.* 2009). Only one before-and-after study under full commercial conditions investigating localised application of lactic and acetic acid found reductions of 2.3-2.6 and 3.7 logs of general and faecal naturally present microbiota, respectively, on treated hides (Scanga *et al.* 2011).

Table IC 2.4.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Acetic acid (10%)	1/CT‡	Hide	No treatment	Aerobic bacteria	2.4-2.6	(Carlson *et al.* 2008a)
				Coliforms	2.6-2.7	
				E. coli	2.5-2.8	
Acetic acid (5%)	1/BA	Hide cut lines	No treatment	Aerobic bacteria	2.6	(Scanga *et al.* 2011)
				Coliforms	3.7	
				E. coli	3.7	
Lactic acid (6%)	1/BA	Hide cut lines	No treatment	Aerobic bacteria	2.3	(Scanga *et al.* 2011)
				Coliforms	3.6	
				E. coli	3.7	
Lactic acid (10%)	1/CT‡	Hide	No treatment	Aerobic bacteria	2.1-2.3	(Carlson *et al.* 2008a)
				Coliforms	2.7	
				E. coli	2.7	

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Lactic acid (2%), sponge appl.	1/BA	Clipped hide	No treatment	Aerobic bacteria	2.3	(Baird *et al.* 2006)
				Coliforms	2.6	
				E. coli	2.1	

‡ Pilot

Table IC 2.4.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies / design	Intervention/ outcome sample	Comparison group	Outcome/ micro-organism	% Samples positive in study population		References
					No treat.	Treatment	
Acetic acid (5%)	1/BA	Hide cut lines	No treatment	*E. coli* O157:H7	76%	30%	(Scanga *et al.* 2011)
Lactic acid (6%)	1/BA	Hide cut lines	No treatment	*E. coli* O157:H7	84%	56%	(Scanga *et al.* 2011)
				Salmonella	74%	50%	

Table IC 2.4.3. Challenge trial studies

Intervention	No. studies	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Acetic acid (2-6%)	1	Hide	Water wash	*S.* Typhimurium	2.4-4.8	(Mies *et al.* 2004)
Acetic acid (10%)	1‡	Hide	No treatment	*E. coli* O157:H7	2.6	(Carlson *et al.* 2008b)
				Salmonella spp.	2.0	
Acetic acid (10%)	1	Hide	No treatment	*E. coli* O157:H7	0.7-2.1	(Carlson *et al.* 2008a)
Lactic acid (1%)	1	Hide	No treatment	*E. coli* O157:H7	0.3	(Elramady *et al.* 2013)
Lactic acid (1%)	1‡	Hide	No treat.	*E. coli* K12	2.4	(Fisher *et al.* 2009)
Lactic acid (1%)	1‡	Hide/carcass*	No treat.	*E. coli* K12	1.7	(Fisher *et al.* 2009)

Intervention	No. studies	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Lactic acid + SDS (1%)	1	Hide	No treat.	*E. coli* O157:H7	4.6	(Elramady *et al.* 2013)
Lactic acid (2%), sponge appl.	1	Hide	No treatment	Aerobic bacteria	2.7	(Baird *et al.* 2006)
				Coliforms	2.8	
				E. coli	3.3	
Lactic acid (2%), sponge appl.	1	Clipped hide	No treatment	Aerobic bacteria	4.1	(Baird *et al.* 2006)
				Coliforms	4.1	
Lactic acid (2-6%)	1	Hide	Water wash	*S.* Typhimurium	1.3-5.1	(Mies *et al.* 2004)
Lactic acid (5%)	1	Hide	No treatment	*E. coli* O157:H7	2.7	(Jadeja and Hung 2014)
				S. Typhimurium	3.0	
Lactic acid (10%)	1‡	Hide	No treatment	*E. coli* O157:H7	3.4	(Carlson *et al.* 2008b)
				Salmonella spp.	2.8	
Lactic acid (10%)	1	Hide	No treatment	*E. coli* O157:H7	0.8-4.3	(Carlson *et al.* 2008a)

* Reduction in hide-to-carcass transfer
‡ Pilot

IC 2.5 Hide washing with oxidisers/other chemicals

A range of different oxidisers (electrolyzed oxidized (EO) and ozonated water, peroxyacetic acid, hypobromous acid, and hydrogen peroxide) have been investigated for use as cattle hide wash/spray treatments post-exsanguination (Bosilevac *et al.* 2005b, Baird *et al.* 2006, Schmidt *et al.* 2012, Jadeja and Hung 2014). Under pilot plant conditions, they statistically significantly reduced general and faecal microbiota by 2.0-3.5 and 2.0-4.0 log cycles on treated hides (Bosilevac *et al.* 2005b, Schmidt *et al.* 2012).

Furthermore, various other chemicals have been used in commercial or lab studies for hide treatments (surfactants, sanitisers, chlorine solutions, cetylpyridinium chloride, sodium hydroxide, sodium metasilicate, trisodium phosphate, alcohols, phosphoric acid, caprylic acid, B-resorcylic acid, chloroform and carvacrol) (Sultemeier 2003, Mies *et al.* 2004, Bosilevac *et al.* 2005a, Small *et al.* 2005, Baird *et al.* 2006, Carlson *et al.* 2008a, Carlson *et al.* 2008b, Çalicioğlu *et al.* 2010, Antic *et al.* 2011, Scanga *et al.* 2011, Baskaran *et al.* 2012, McDonnell *et al.* 2012, Baskaran *et al.* 2013, Wang *et al.* 2014, Yang *et al.* 2015, Long *et al.*

2018). Under commercial conditions, automated hide washes with sodium hydroxide achieved statistically significant reduction in transfer to carcasses of aerobic and enteric bacteria of 0.8 logs and prevalence of *E. coli* O157 from 17% to 2%, as well as reductions on treated hides of 2.1 and 3.4 logs and 44% to 16% respectively (Bosilevac *et al.* 2005a). Across all chemicals used, the reductions highly depended on the study design and nature of microbiota used, as well as different treatment conditions (chemical concentration, application method and contact time). Additional vacuuming increased efficacy in removing bacteria by 1-2 log-cycles.

Table IC 2.5.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Oxidiser chemicals						
Ozonated water wash	1/BA‡	Hide	No treatment	Aerobic bacteria	2.1	(Bosilevac *et al.* 2005b)
				Enterobacteriaceae	3.4	
EO water wash	1/BA‡	Hide	No treatment	Aerobic bacteria	3.5	(Bosilevac *et al.* 2005b)
				Enterobacteriaceae	4.3	
Hypobromous acid (200-500 ppm)	1/BA‡	Hide	No treat.	Aerobic bacteria	2.2-3.3	(Schmidt *et al.* 2012)
				Coliforms	2.2-3.8	
				E. coli	2.3-3.8	
Hydrogen peroxide (3%), sponge appl.	1/BA	Clipped hide	No treatment	Aerobic bacteria	2.2	(Baird *et al.* 2006)
				Coliforms	2.6	
				E. coli	3.0	
Other chemicals						
Water wash and chlorine (200 ppm)	1/CT‡	Hide	No treat.	Coliforms	2.9	(Bosilevac *et al.* 2005a)
Chlorine/ ASC (200 ppm)	1/BA	Veal calf hide	No treatment	Aerobic bacteria	1.3	(Wang *et al.* 2014)
				Enterobacteriaceae	1.5	
				Coliforms	1.2	
				E. coli	1.0	
Water wash and sodium hydroxide (1.5%)	1/CT	Hide/carcass*	No treatment	Aerobic bacteria	0.8	(Bosilevac *et al.* 2005a)
				Enterobacteriaceae	0.8	
Water wash and sodium hydroxide (1.5%)	2/BA	Hide	No treatment	Aerobic bacteria	1.5-2.1	(Bosilevac *et al.* 2005a, Yang *et al.* 2015)
				Enterobacteriaceae	3.4	
Sodium hydroxide (1.5%)	1/CT‡	Hide	No treat.	Coliforms	1.5-3.7	(Bosilevac *et al.* 2005a)

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Sodium hydroxide (1.5%) and vacuum	1/CT‡	Hide	No treat.	Coliforms	3.8-3.9	(Bosilevac *et al.* 2005a)
Sodium hydroxide (3%)	1/CT‡	Hide	No treat.	Aerobic bacteria	1.3-1.6	(Carlson *et al.* 2008a)
				Coliforms	2.8-2.9	
				E. coli	2.8	
Sodium hydroxide (3%) + lactic acid (10%)	1/CT‡	Hide	No treat.	Aerobic bacteria	2.0-2.4	(Carlson *et al.* 2008a)
				Coliforms	2.1-2.9	
				E. coli	2.3-3.0	
Sodium hydroxide (3%)	1/BA	Hide cut lines	No treat.	Aerobic bacteria	1.6	(Scanga *et al.* 2011)
				Coliforms	3.5	
				E. coli	3.5	
TSP (4%)	1/CT‡	Hide	No treat.	Coliforms	1.5	(Bosilevac *et al.* 2005a)
TSP (4%) and vacuum	1/CT‡	Hide	No treat.	Coliforms	2.5	(Bosilevac *et al.* 2005a)
TSP (20%)	1/BA	Hide	No treat.	Aerobic bacteria	1.8	(Çalicioğlu *et al.* 2010)
Ethanol (75%)	1/BA	Hide	No treat.	Aerobic bacteria	1.2	(Çalicioğlu *et al.* 2010)
Chloroform (4%)	1/CT‡	Hide	No treat.	Coliforms	2.7-3.9	(Bosilevac *et al.* 2005a)
Chloroform (4%) and vacuum	1/CT‡	Hide	No treat.	Coliforms	3.6-4.4	(Bosilevac *et al.* 2005a)
Phosphoric acid (4%)	1/CT‡	Hide	No treat.	Coliforms	2.5-4.1	(Bosilevac *et al.* 2005a)
Phosphoric acid (4%) and vacuum	1/CT‡	Hide	No treat.	Coliforms	3.5-5.4	(Bosilevac *et al.* 2005a)

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Sodium metasilicate (4%)	1/CT‡	Hide	No treat.	Aerobic bacteria	1.6-1.7	(Carlson *et al.* 2008a)
				Coliforms	2.4-2.9	
				E. coli	2.3-2.9	
CPC (1%), sponge appl.	1/BA	Clipped hide	No treatment	Aerobic bacteria	3.8	(Baird *et al.* 2006)
				Coliforms	3.3	
				E. coli	3.0	
A proprietary QAC sanitiser and vacuuming	1/CT	Hide/carcass*	No treatment	Aerobic bacteria	1.0	(Antic *et al.* 2011)
				Enterobacteriaceae	1.3	
				E. coli	1.2	

* Reduction in hide-to-carcass transfer
‡ Pilot

Table IC 2.5.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Oxidiser chemicals							
Ozonated water wash	1/BA‡	Hide	No treatment	*E. coli* O157:H7	89%	31%	(Bosilevac *et al.* 2005b)
EO water wash	1/BA‡	Hide	No treatment	*E. coli* O157:H7	82%	35%	(Bosilevac *et al.* 2005b)
Hypobromous acid (200-500 ppm)	1/BA‡	Hide	No treatment	*E. coli* O157:H7	21-25%	10%	(Schmidt *et al.* 2012)
				Salmonella	28-33%	7-8%	
Other chemicals							
Water wash and sodium hydroxide (1.5%)	1/CT	Hide/carcass*	No treatment	*E. coli* O157	17%	2%	(Bosilevac *et al.* 2005a)
Water wash and sodium hydroxide (1.5%)	1/BA	Hide	No treatment	*E. coli* O157	44%	16%	(Bosilevac *et al.* 2005a)
Sodium hydroxide (3%)	1/BA	Hide cut lines	No treatment	*E. coli* O157:H7	94%	41%	(Scanga *et al.* 2011)
				Salmonella	60%	43%	
Trichloromelamine (200 ppm)	1/BA	Hide	No treatment	*E. coli* O157:H7	10%	2%	(Sultemeier 2003)
				Salmonella	61%	39%	

* Reduction in hide-to-carcass transfer
‡ Pilot

Table IC 2.5.3 Studies under laboratory conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Oxidiser chemicals						
EO water wash (alkaline/neutral)	1/ChT	Hide	No treatment	Aerobic bacteria	1.3-1.5	(Jadeja and Hung 2014)
				Enterobacteriaceae	1.5-1.8	
				E. coli O157:H7	0.6-1.7	
				S. Typhimurium	1.1-2.1	
PA acid (0.02%)	1/ChT	Hide	No treatment	Aerobic bacteria	1.1	(Jadeja and Hung 2014)
				Enterobacteriaceae	0.5	
				E. coli O157:H7	0.3	
				S. Typhimurium	0.7	
Hydrogen peroxide (3%), sponge appl.	1/ChT	Hide	No treatment	Aerobic bacteria	1.5	(Baird *et al.* 2006)
				Coliforms	2.2	
				E. coli	2.9	
Hydrogen peroxide (3%), sponge appl.	1/ChT	Clipped hide	No treatment	Aerobic bacteria	4.4	(Baird *et al.* 2006)
				Coliforms	3.9	
Oxidiser chemicals						
Chlorine (100-400 ppm)	1/ChT	Hide	Water wash	*S.* Typhimurium	0.6-1.3	(Mies *et al.* 2004)
Ethanol (70%-90%)	1/ChT	Hide	Water wash	*S.* Typhimurium	5.0-5.5	(Mies *et al.* 2004)
Ethanol (95%)	1/ChT	Hide	No treatment	*E. coli* O157:H7	1.5-1.9	(Baskaran *et al.* 2012, Baskaran *et al.* 2013)
				S. Typhimurium	0.9	
				L. monocytogenes	1.4	
Carvacrol	1/ChT	Hide	No treatment	*E. coli* O157	1.6-2.4	(McDonnell *et al.* 2012)
Octenidine hydrochloride in ethanol (0.05-0.25%)	1/ChT	Hide	No treatment	*E. coli* O157:H7	5.2-5.2	(Baskaran *et al.* 2012)
				S. Typhimurium	4.9	
				L. monocytogenes	5.3-5.4	
Caprylic acid (1%)	1/ChT	Hide	No treatment	*E. coli* O157:H7	3.0-3.9	(Baskaran *et al.* 2013)

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
B-resorcylic acid (1%)	1/ChT	Hide	No treatment	*E. coli* O157:H7	2.9-3.6	(Baskaran *et al.* 2013)
CPC (1%), sponge appl.	1/ChT	Hide	No treatment	Aerobic bacteria	4.1	(Baird *et al.* 2006)
				Coliforms	5.3	
				E. coli	4.5	
CPC (1%), sponge appl.	1/ChT	Clipped hide	No treatment	Aerobic bacteria	4.6	(Baird *et al.* 2006)
				Coliforms	4.5	
Sodium metasilicate (4-5%)	2/ChT	Hide	No treatment	*E. coli* O157:H7	1.9-4.7	(Carlson *et al.* 2008a, Carlson *et al.* 2008b)
				Salmonella spp.	2.6	
Sodium hydroxide (1.5%)	1/ChT	Hide	No treatment	*E. coli* O157:H7	5.0	(Carlson *et al.* 2008b)
				Salmonella spp.	4.4	
Sodium hydroxide (3%)	2/ChT	Hide	No treatment	*E. coli* O157:H7	2.4-5.1	(Carlson *et al.* 2008a, Carlson *et al.* 2008b)
				Salmonella spp.	2.6	
Citric/hydrochloric acid	1/CT	Hide	No treatment	Aerobic bacteria	2.4	(Long *et al.* 2018)
				Enterobacteriaceae	3.5	
QAC sanitisers	1/CT	Hide	No treatment	Aerobic bacteria	3.9	(Long *et al.* 2018)
				Enterobacteriaceae	2.1	
A proprietary QAC sanitisers and vacuuming	2/CT	Hide	No treatment	Aerobic bacteria	2.0-4.9	(Small *et al.* 2005, Antic *et al.* 2010)
				Enterobacteriaceae	3.4	
				E. coli	2.7	
A proprietary QAC sanitisers and vacuuming	1/CT	Hide/beef cuts*	No treatment	Aerobic bacteria	0.2-2.3	(Antic *et al.* 2011)
				Enterobacteriaceae	1.4-2.2	
				E. coli	1.4-1.7	

* Reduction in hide-to-meat transfer

‡ Pilot

IC 2.6 Chemical dehairing and thermal interventions

Several harsh hide treatments have been investigated, mostly in lab conditions. Given the fact that the hide is damaged during the process, these harsh interventions are more suitable for bob veal calves which usually stay with the skin-on, or in situations where hides are not used for leather production.

Some studies evaluated the efficacy of chemical dehairing for removing hairs, dirt, faeces and microbial contamination from cattle hides (Castillo *et al.* 1998a, Nou *et al.* 2003, Carlson *et al.* 2008b). Chemical dehairing comprised treatment using sodium sulphide, hydrogen peroxide (H_2O_2) and/or potassium cyanate applied under laboratory conditions, which statistically significantly reduced inoculated bacteria by >4 logs (Castillo *et al.* 1998a, Carlson *et al.* 2008b). In one controlled trial, chemical dehairing treatment statistically significantly reduced *E. coli* O157 prevalence and ACC and *Enterobacteriaceae* counts on pre-evisceration carcasses (Nou *et al.* 2003).

One challenge study investigated different single or multiple treatments for bob veal calves which stay with the hide-on throughout the dressing process. Scalding at temperatures >60^{o}C reduced inoculated *E. coli* by 2-4 log cycles and the treatment efficacy was statistically significantly improved when using an additional hot water wash (82^{o}C) and/or lactic acid (4.5%) spray with reduction ranging from 4.5-6.3 logs on treated hides (Hasty *et al.* 2018). Hot water (under 80^{o}C) alone (Fisher *et al.* 2009, Çalicioğlu *et al.* 2010) or in combination with chlorine spray (Wang *et al.* 2014) also was shown to statistically significantly reduce aerobic and enteric bacteria by 2-3.5 logs.

Two studies investigated the application of steam for the decontamination of cattle hides (McEvoy *et al.* 2001, McEvoy *et al.* 2003). Under laboratory conditions, steam treatment reduced aerobic bacteria by 1.9-4.0 logs whereas the reduction effect on inoculated *E. coli* O157:H7 was even greater, 1.9-6.0 logs. However, hide quality was severely damaged by this thermal intervention, making it unsuitable for practical application in commercial settings.

Naked flame and singeing (>300^{o}C) was highly effective with reductions from 2-5 log-cycles on treated hides (Small *et al.* 2005, Fisher *et al.* 2009). However, the downside of this treatment, beside the hide damage, is generation of smoke and ash, which can present occupational hazard.

Table IC 2.6.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Chemical dehairing	1/CT	Hide/carcass*	No treat.	Aerobic bacteria	2.0	(Nou *et al.* 2003)
				Enterobacteriaceae	1.8	
Hot water wash	1/BA	Hide cut lines	No treat.	Aerobic bacteria	3.6	(Çalicioğlu *et al.* 2010)
Chlorine spray and hot water rinse	1/BA	Veal calf hide	No treat.	Aerobic bacteria	2.1	(Wang *et al.* 2014)
				Enterobacteriaceae	2.7	
				Coliforms	2.7	
				E. coli	2.6	

* Reduction in hide-to-carcass transfer

Table IC 2.6.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Chemical dehairing	1/CT	Hide/carcass*	No treat.	*E. coli* O157:H7	50%	1%	(Nou *et al.* 2003)

* Reduction in hide-to-carcass transfer

Table IC 2.6.3. Studies under laboratory conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Chemical dehairing	1/ChT	Hide	No treatment	Aerobic bacteria	3.4	(Castillo *et al.* 1998a)
				Coliforms	3.9	
				E. coli	4.3	
				E. coli O157:H7	4.8	
				S. Typhimurium	4.6	
Chemical dehairing	1/ChT‡	Hide	No treat.	*E. coli* O157:H7	4.8-5.1	(Carlson *et al.* 2008b)
				Salmonella spp.	0.7-4.2	
Scalding	1/ChT‡	Hide-on bob veal	No treat.	*E. coli*	2.2-4.1	(Hasty *et al.* 2018)
Hot water wash	1/ChT‡	Hide	No treat.	*E. coli* K12	3.2	(Fisher *et al.* 2009)
Hot water wash	1/ChT‡	Hide/carcass*	No treat.	*E. coli* K12	1.5	(Fisher *et al.* 2009)
Hot water wash	1/ChT‡	Hide-on bob veal	No treat.	*E. coli*	4.5	(Hasty *et al.* 2018)
Hot water wash and lactic acid	1/ChT‡	Hide-on bob veal	No treat.	*E. coli*	6.1	(Hasty *et al.* 2018)
Multiple (Scalding, hot water and lactic acid)	1/ChT‡	Hide-on bob veal	No treat.	*E. coli*	5.1-6.3	(Hasty *et al.* 2018)
Steam treatment	1/ChT	Hide	No treat.	*E. coli* O157:H7	1.9-6.0	(McEvoy *et al.* 2001)
Steam treatment	1/BA	Hide	No treat.	Aerobic bacteria	1.9-4.0	(McEvoy *et al.* 2003)
Naked flame	1/ChT‡	Hide	No treat.	*E. coli* K12	4.9	(Fisher *et al.* 2009)
Naked flame	1/ChT‡	Hide/carcass*	No treat.	*E. coli* K12	2.3	(Fisher *et al.* 2009)

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Clipping and singeing	1/CT	Hide	No treat.	Aerobic bacteria	2.1	(Small *et al.* 2005)

* Reduction in hide-to-carcass transfer
‡ Pilot

IC 2.7 Microbial immobilisation treatments

A number of physical methods of immobilising bacteria on the hide along the cut lines have been investigated in a small study commissioned by the FSA, with various and inconsistent antimicrobial effects (Fisher *et al.* 2009). However, better and more consistent microbial immobilising effect has been achieved using the innovative treatment of cattle hides with shellac, a natural, food-grade resin, used in ethanol or aqueous solution and sprayed on hides (Antic *et al.* 2010, Antic *et al.* 2011, Antic *et al.* 2018).

In a laboratory model system, spraying hides with the shellac solution in ethanol markedly reduced the levels of general microbiota (up to 6.6 $\log_{10}$ CFU/cm^2) and the prevalence of *E. coli* O157 (up to 3.7-fold) recoverable from hide by swabbing (Antic *et al.* 2010). The reductions were primarily due to the bacterial immobilisation effect of the shellac component, whilst the bactericidal effect of the solvent (ethanol) itself played a comparably smaller role in the overall reduction. Laboratory experiments, involving the direct contact of treated hides with meat, achieved reductions of up to 3.6 $\log_{10}$ CFU/cm^2 of general microbiota (Antic *et al.* 2011). Post-slaughter but pre-skinning treatment of hides with a shellac solution, examined during the operation of a commercial abattoir, statistically significantly reduced (up to 1.7 logs) the levels of general microbiota found on beef carcasses (Antic *et al.* 2011). Therefore, the shellac-based hide-coating treatment was demonstrated to statistically significantly reduce the risk of cross-contamination from hide to carcass, and also reduced the potential for airborne contamination of the skinned carcass from dust and dirt that detach from non-treated hides during hide removal.

In a subsequent study using a range of aqueous shellac solutions, reductions in transfer to meat of up to 3 logs and 2.4 logs of aerobic and enteric bacteria under lab conditions were achieved. Validation of the treatment under commercial conditions reported reductions in transfer to resulting beef carcasses of up to 1.1 logs and 0.7 logs of ACC and EBC respectively, on different carcass sites (Antic *et al.* 2018).

Table IC 2.7.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Shellac in ethanol hide coating	1/CT	Hide/carcass*	No treat.	Aerobic bacteria	1.7	(Antic *et al.* 2011)
				Enterobacteriaceae	1.4	
				E. coli	1.3	
Aqueous shellac hide coating	1/CT	Hide/carcass*	No treat.	Aerobic bacteria	0.3-1.1	(Antic *et al.* 2018)
				Enterobacteriaceae	0.1-0.7	

* Reduction in hide-to-carcass transfer

Table IC 2.7.2. Studies under laboratory conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Shellac in ethanol hide coating	1/CT	Hide	No treat.	Aerobic bacteria	6.6	(Antic *et al.* 2010)
				Enterobacteriaceae	4.8	
				E. coli	2.9	
Shellac in ethanol hide coating	1/ChT	Hide	No treat.	*E. coli* O157	2.1	(Antic *et al.* 2010)
Shellac in ethanol hide coating	1/CT	Hide/beef cuts*	No treat.	Aerobic bacteria	2.3-3.5	(Antic *et al.* 2011)
				Enterobacteriaceae	1.0-2.5	
				E. coli	1.0-1.7	
Aqueous shellac hide coating	1/ChT	Hide/beef cuts*	No treat.	*E. coli* O157	0.9-1.3	(Antic *et al.* 2018)
Aqueous shellac hide coating	1/CT	Hide/beef cuts*	No treat.	Aerobic bacteria	0.8-3.0	(Antic *et al.* 2018)
				Enterobacteriaceae	1.6-2.4	

* Reduction in hide-to-meat transfer

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IC 3: Beef carcass interventions

IC 3.1 Summary of key findings

IC 3.1.1 Standard processing procedures and GHP

There was a lack of published studies describing the efficacy of standard processing procedures and GHP in reducing beef carcass microbial contamination. Subjective assessment of improved hide removal practices in four studies indicated statistically significant reduction in transfer of indicator bacteria from hides to carcasses by 1 log-cycle and reduced prevalence of VTEC and *Salmonella* on beef carcasses. Only one study in commercial conditions didn't find benefit of implementing downward vs. upward hide pulling method, but some differences were noted on specific carcass sites, often in favour of upward technique. Hence, it was concluded that the differences could be due to possible deficiencies in the implementation of the HACCP pre-requisite programmes and were not necessarily associated with the skinning method per se. Bung bagging appear to have been efficacious in the three studies where reductions of indicator bacteria by around 1 log-cycle and prevalence of VTEC were reported. Overall better processing hygiene represented by better hygiene scores between abattoirs were associated with improved carcass microbial status in five observational studies.

Alternative methods for knives sanitation were in most cases shown to be equivalent to the current sanitation procedures in water at 82°C for one second duration. Methods suitable for use on the slaughterline with contact times up to 1 minute such as dipping knives in water for longer times at lower temperatures, use of ultrasound combined with organic acids, and use of chemicals (sanitisers, peroxyacetic and organic acids) produced equivalent reductions of bacteria comparing to current procedures using water at 82°C for one second.

IC 3.1.2 Pre-chill carcass treatments

Large number of studies have been published on beef carcass interventions post dehiding but pre-chill. There were large variations in magnitude of reduction effect across studies within single intervention, because of different intervention conditions used, therefore the results on intervention efficacy are not directly comparable.

Water wash with ambient or cold water to remove microorganisms was largely ineffective with up to 0.5 log reduction achieved, but dependant on washing time and pressure used. Very often, washing carcasses appeared to have increased contamination and/or redistribute bacteria. On the other hand, trimming of visually contaminated sites reduced levels of natural microbiota (ACC and faecal indicators) from 1-2 logs, whereas spot steam vacuuming had similar effect of 1-2 logs.

Hot water washing provided consistent reduction effect from 1-2.5 logs (seen across a number of studies), increasing by 0.5-1 log-cycles if organic acids were used concurrently.

The whole carcass steam pasteurisation effect in reducing natural microbiota was most often around 1-1.5 log-cycles.

Organic acid carcass washes (lactic, acetic and citric) were effective on-line interventions with higher reductions reported for lactic acid (1-2 logs of natural microbiota) than for acetic and citric acid or their mixtures (usually up to 1 log).

A large number of studies conducted under pilot and laboratory conditions investigated various physical (water washes and thermal treatments) and chemical interventions (organic acids and other chemicals) alone or in combinations. They reported large variation of reduction effects, but very often between 2-5 logs. This must be taken with caution and only as relative and indication of the potential intervention effect, because of artificial nature of inoculated microorganisms, controlled study conditions and often low number of samples investigated.

IC 3.1.3 Chilling

Chilling for up to three days reduced levels of indicator bacteria in most cases up to only 0.5 logs under commercial conditions and up to 2 logs of inoculated *E. coli* and *Salmonella* under pilot and lab conditions. Chilling carcasses for one day previously sprayed with organic acids or treated with hot water or steam on the slaughterline reduced indicator bacteria from 0.6-2.1 logs under commercial conditions and up to 3.5 logs of *E. coli* under pilot and lab conditions, likely due to a residual effect of chemical interventions.

Dry aging of carcasses up to two weeks reported reductions of up to 2 logs of faecal indicators in first four days of dry aging. Reductions of around 1 log after six days or around 3 logs of inoculated enteric pathogens after seven days of dry aging have been also reported with on average 0.1-0.2 log reduction per day of inoculated *Salmonella* during 14-day dry aging of beef cuts.

Water spray chilling showed very variable effects in reducing natural microbiota on carcasses in commercial conditions and it appears it was plant specific and influenced by various different factors. On inoculated VTEC and *Salmonella* reductions effects of up to 2 logs were observed, which increased when various chemicals were sprayed onto beef carcass cuts during chilling producing reductions from 1-4.5 logs comparing to water spray chilling alone.

IC 3.1.4 Post-chill and pre-fabrication carcass treatments

Following the completion of chilling and prior to carcass fabrication, only a few studies reported intervention for carcasses at this stage. Lactic acid spray was shown to statistically significantly reduce aerobic bacteria up to 3 log-cycles and faecal bacteria up to 1.5 logs, with reductions increasing to up to 7 logs of inoculated VTEC and *Salmonella* under laboratory conditions.

One novel non-thermal intervention, electron beam (E-beam) irradiation, was reported to be highly efficacious at a 1 kGy dose, and when applied to chilled beef primals reduced *E. coli* O157:H7 numbers by up to 6.6 log-cycles.

IC 3.1.5 Multiple on-line interventions and HACCP

Sequential application of interventions after dehiding but before chilling based on a 'multiple-hurdle approach' was investigated in a total of 16 studies under commercial abattoir conditions. The interventions usually involved some or all of the following: knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam and organic acid (or peroxyacetic acid) rinsing before chilling. Consistent reductions of naturally present bacterial indicators were achieved across a number of studies and were higher than when only one single intervention was used. In most cases they ranged from 2-3 logs for ACC and/or faecal indicators. Furthermore, the prevalence of naturally present VTEC and *Salmonella* following sequential application of interventions was in most cases statistically significantly reduced, often to levels below detection limits. In one controlled trial in a pilot plant where hides were washed with lactic and acetic acid followed by carcass organic acid washes prior to chilling, the reductions obtained and measured after chilling were in the range 1.5-2 logs compared to untreated (only chilled) carcasses.

No overall effect of HACCP implementation on pathogen (VTEC and *Salmonella*) reduction was reported in eight before-and-after studies, but levels of indicator aerobic and faecal bacteria were reduced on carcasses from 0.5-1 log-cycle after HACCP implementation.

IC 3.2 Intervention description

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

Tool: an implement that is used in the dressing/processing of carcasses and coming into contact with a carcass/meat.

Cleaning and/or disinfection: Removal of dirt and organic substances from and sanitation of meat processing plant equipment and environment.

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

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

Water wash: refers to an ambient or cold-temperature wash to physically remove contamination from carcass surface. Warm water wash (usually <60^{o}C) has similar effect in removing bacteria (depending on the pressure used) and when applied for a short time doesn't have microbicidal effect.

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

Washes containing other chemicals and oxidizers: includes washes containing other miscellaneous products that destroy bacteria through various actions, such as oxidation and disruption of cellular functions, or that prevent bacterial attachment to meat. Examples include: electrolyzed oxidized (EO) water (acidic, alkaline or neutral), ozonated water, peroxyacetic acid, acidified sodium chlorite, hydrogen peroxide, sodium hypochlorite, hydrobromous acid, trisodium phosphate).

Thermal interventions: refers to various heat treatment washes to destroy microbial cells.

Non-thermal interventions: refers to non-chemical and non-thermal interventions that aim to reduce microbial contamination while preserving product quality and nutrients that can be affected by thermal treatments (electron beam irradiation and ultraviolet (UV) light).

Hot water wash: refers to washing carcasses with water at temperatures >74^{o}C, up to 85^{o}C.

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

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

Dry heat: refers to non-hydrating thermal interventions such a forced-air heating.

Dry chilling: refers to chilling following all dressing procedures on the slaughterline without the use of any additional spray (acid or water).

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

Dry aging: refers to multiday refrigeration of carcasses.

Multiple interventions: refers to an application of interventions based on the 'multiple hurdle approach', where chemical and/or physical interventions are applied in sequence or simultaneously, inflicting concurrent and variable injuries to bacterial cells. Sequential

application of interventions involves use of interventions on cattle hides, followed by knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam, organic acid rinsing, chilling, and chemical spraying before carcass fabrication.

HACCP: Hazard Analysis Critical Control Point (system that identifies, evaluates, and controls hazards significant for the safety of food produced in the given process).

IC 3.3 Standard processing procedures and GHP

Standard processing procedures and GHP were investigated in 13 studies, with another 11 studies reporting on knives sanitation interventions. In the three studies conducted under commercial conditions, the procedure of tying the rectum (bung bagging) to prevent faecal spillage reduced levels of indicator bacteria by around 1 log-cycle (Saleh *et al.* 2012) and statistically significantly reduced presence of enteric marker bacteria and pathogens (Hudson *et al.* 1998, Stopforth *et al.* 2006). Improved hide removal practices appear to reduce transfer of indicator bacteria from hide to carcasses by up to 1 log (Gill and McGinnis 1999, McEvoy *et al.* 2000, Bosilevac *et al.* 2016) and also statistically significantly reduce the transfer of enteric pathogens (Bosilevac *et al.* 2017). However, there was no improvement in the microbial status of beef carcasses after hide removal when a supposedly better downward hide removal technique was used and compared to upward technique in only one controlled trial (Kennedy *et al.* 2014).

Five observational cross-sectional studies compared process hygiene between abattoirs (Hudson *et al.* 1996, Rahkio and Korkeala 1996, Alegre and Buncic 2004, Muluneh and Kibret 2015, Nastasijevic *et al.* 2016). When structured UK food hygiene assessment scoring systems were used (HAS or MOC) (Hudson *et al.* 1996, Alegre and Buncic 2004, Nastasijevic *et al.* 2016), it was observed that abattoirs assessed as 'better' in terms of hygienic practices employed were associated, in most cases, with final beef carcasses carrying a lower microbial load, sometimes with up to 2 log difference.

Knives sanitation has been researched in a total of 11 studies (Midgley and Eustace 2003, Uradziński *et al.* 2005, Eustace *et al.* 2007, Taormina and Dorsa 2007, Goulter *et al.* 2008, Rajkovic *et al.* 2010, Heres and Verkaar 2011, Leps *et al.* 2013, Tapp Iii *et al.* 2013, Musavian *et al.* 2015, Brasil *et al.* 2017). Dipping knives in water for shorter times at higher temperatures or longer times at lower temperatures produced equivalent reductions of bacteria compared to current procedures in water at 82°C for one second (Midgley and Eustace 2003, Eustace *et al.* 2007, Goulter *et al.* 2008, Leps *et al.* 2013). The benefits of using alternative system are: i) saving on energy consumption required to heat the water; ii) saving on the water consumption in a through-flow system; iii) reduced incidents of scalding of personnel; iv) reduced condensation and fogging in the slaughter hall; and v) reduced maintenance costs in the long term (Midgley and Eustace 2003, Eustace *et al.* 2007).

Other procedures investigated as alternative to the current hot water knife sanitation included various chemicals such as detergents (Brasil *et al.* 2017), organic acids (Heres and Verkaar 2011, Leps *et al.* 2013), sanitisers and peroxyacetic acid (Taormina and Dorsa 2007, Tapp Iii *et al.* 2013), prolonged exposure to ozone (Uradziński *et al.* 2005), ultrasound with or without steam or detergent (Leps *et al.* 2013, Musavian *et al.* 2015, Brasil *et al.* 2017) and UV light (Rajkovic *et al.* 2010). With respect to procedures that don't require prolonged contact time with knives and hence are suitable for use on the slaughterline (contact time of up to 1 minute with knives rotation), use of warm water for longer times in combination with organic acids and/or ultrasound, appears to be comparably effective as the current hot water knife sanitation at 82°C (Heres and Verkaar 2011, Leps *et al.* 2013). Other sanitation procedures that require prolonged contact time with knives (ultrasound in combination with detergents, UV light and ozone) are more suitable for knives sanitation during breaks or after the work has been finished (Uradziński *et al.* 2005, Rajkovic *et al.* 2010, Brasil *et al.* 2017).

Table IC 3.3.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Improved hide removal	2/BA 1/CT	Beef/veal carcass*	No treatment	Aerobic bacteria	0.2-1.1	(Gill and McGinnis 1999, McEvoy *et al.* 2000, Bosilevac *et al.* 2016)
				Enterobacteriaceae	0.0-0.7	
				Coliforms	0.0-1.0	
				E. coli	0.0-1.0	
Downward hide pulling	1/CT	Carcass*	Upward hide pulling	Aerobic bacteria	0.0	(Kennedy *et al.* 2014)
Bung bagging and rodding	1/CT	Carcass*	No treatment	Aerobic bacteria	1.3	(Saleh *et al.* 2012)
				Enterobacteriaceae	1.3	
Knives sanitation						
	3/BA	Knives		Aerobic bacteria	0.2-1.2	

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Current hot water (82°C, 1 s)			No treatment	*Enterobacteriaceae*	0.2-0.3	(Midgley and Eustace 2003, Heres and Verkaar 2011, Brasil *et al.* 2017)
Alternative hot water (72°C, 15 s)	1/BA	Knives	No treatment	Aerobic bacteria	1.3	(Midgley and Eustace 2003)
Alternative warm water (60°C, 30 s)	1/CT	Knives	Current hot water	Aerobic bacteria	0.3-0.4	(Eustace *et al.* 2007)
Steam/ ultrasound	1/BA	Knives	No treatment	Aerobic bacteria	5.3-6.1	(Musavian *et al.* 2015)
				Enterobacteriaceae	2.5	
Inspexx© 200	1/BA	Knives	No treatment	Aerobic bacteria	1.0-1.8	(Heres and Verkaar 2011)
				Enterobacteriaceae	0.6-0.7	

* Reduction in transfer

Table IC 3.3.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Improved hide removal	1/BA	Veal carcass*	No treatment	*E. coli* O157:H7	12%	1%	(Bosilevac *et al.* 2017)
				VTEC non-O157	5-64%	2-25%	
Downward hide pulling	1/CT	Carcass*	Upward hide pulling	*Entero-bacteriaceae*	83%	94%	(Kennedy *et al.* 2014)
Bung bagging	1/ChT	Carcass*	No treatment	*E. coli* K12	30-83%	13-70%	(Hudson *et al.* 1998)
Bung bagging	1/CT	Carcass*	No treatment	VTEC non-O157	58%	35%	(Stopforth *et al.* 2006)
				E. coli O157:H7	5%	1.7%	
				Salmonella spp.	8.3%	0.0%	

* Reduction in transfer

Table IC 3.3.3. Studies under laboratory conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Knives sanitation						
Current hot water (82°C, 1 s)	3/ChT	Knives	No treat.	Aerobic bacteria	4.0	(Taormina and Dorsa 2007, Goulter *et al.* 2008, Leps *et al.* 2013)
				E. coli	1.2	
				E. coli O157:H7	0.8	
				S. Typhimurium	1.1	
Alternative hot water (70-75°C)	3/ChT	Knives	No treat.	Aerobic bacteria	3.2-4.0	(Midgley and Eustace 2003, Goulter *et al.* 2008,
				E. coli	1.8-5.1	

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
						Leps *et al.* 2013)
Alternative warm water (60-65°C)	2/ChT	Knives	No treat.	Aerobic bacteria	0.8-4.0	(Goulter *et al.* 2008, Leps *et al.* 2013)
				E. coli	1.4-3.7	
Warm water/ ultrasound (40-65°C)	1/ChT	Knives	No treat.	Aerobic bacteria	0.2-4.0	(Leps *et al.* 2013)
Lactic acid (40°C)	1/ChT	Knives	No treat.	Aerobic bacteria	2.3-4.0	(Leps *et al.* 2013)
Warm water + LA (40°C)/ultrasound	1/ChT	Knives	No treat.	Aerobic bacteria	4.0	(Leps *et al.* 2013)
Sanitiser, peroxyacetic acid, sodium metasilicate, lactic acid (20°C)	2/ChT	Knives	No treat.	Aerobic bacteria	0.6-2.9	(Taormina and Dorsa 2007, Tapp Iii *et al.* 2013)
				E. coli O157:H7	0.7-3.5	
				S. Typhimurium	0.6-3.4	

IC 3.4 Pre-chill carcass treatments

Beef carcass interventions post-dehiding and pre-chill have been investigated in 90 studies., A range of different conditions have been reported among different physical and chemical interventions (temperatures, contact time, pressure, mode of application (wash, spray, rinse, dip, deluge, manual or automated), number of samples and sample method used), and there were large variations in magnitude of effect across studies. Therefore, the results on intervention efficacy are not directly comparable.

Overall, 35 controlled and before-and-after trial studies conducted under commercial conditions have been reported (Gill *et al.* 1996a, Gill *et al.* 1996b, Bell 1997, Gill and Bryant 1997b, Kochevar *et al.* 1997, Nutsch *et al.* 1997, Nutsch *et al.* 1998, Gill *et al.* 1999, Hajmeer *et al.* 1999, Dormedy *et al.* 2000, Gill and Bryant 2000, De Martinez *et al.* 2002, Gill and Landers 2003b, Minihan *et al.* 2003b, Gill and Landers 2004, McEvoy *et al.* 2004, Corantin *et al.* 2005, Retzlaff *et al.* 2005, Bosilevac *et al.* 2006, Algino *et al.* 2007, Rodriguez 2007, Ruby *et al.* 2007, Trivedi *et al.* 2007, Ramish 2011, Trairatapiwan *et al.* 2011, Wright 2011, Thomas *et al.* 2012, Carranza *et al.* 2013, Chaves *et al.* 2013, Narváez-Bravo *et al.* 2013, Wang *et al.* 2013, Dong *et al.* 2014, Dong *et al.* 2015, Hochreutener *et al.* 2017, Signorini *et al.* 2018). Hot water wash and lactic acid, as a standalone intervention or in combination, were by far the most often investigated interventions under commercial conditions.

Water wash with ambient or cold water to remove microorganisms was largely ineffective, with up to 0.5 log reduction achieved, and dependant on washing time and pressure used. Higher reductions were reported only in the study by Gill *et al.* (1996b) on more contaminated sites. However, in combination with organic acids, the reduction effect appears to increase by 1 log-cycle (Gill and Landers 2003b, Carranza *et al.* 2013). Trimming of visually contaminated sites reduced levels of natural microbiota by 1-2 logs (Gill *et al.* 1996a, Kochevar *et al.* 1997, Gill and Landers 2004). Furthermore, two challenge trials conducted under commercial conditions reported using permitted artificial microbiota to inoculate carcasses and investigate the effects of trimming, water and hot water wash, as well as chemicals (hydrogen peroxide and ozone) (Reagan *et al.* 1996, Graves Delmore *et al.* 1997). Trimming in combination with water and/or hot water rinsing removed inoculated coliform bacteria by 1.3-1.8 logs.

Hot water washing provided consistent reduction effect by 1-2.5 logs, increasing by 0.5-1 log-cycles if organic acids were used concurrently (Bosilevac *et al.* 2006, Algino *et al.* 2007, Wright 2011, Signorini *et al.* 2018). The temperatures of carcass surfaces pasteurised with hot water usually achieved more than 70^{o}C. The time-temperature combinations required to achieve statistically significant reductions were usually specific to an individual commercial abattoir. Furthermore, both spot steam vacuuming and whole carcass steam pasteurisation reduced natural microbiota by around 1-1.5 log-cycles (Kochevar *et al.* 1997, Nutsch *et al.* 1997, Nutsch *et al.* 1998, Minihan *et al.* 2003b, Corantin *et al.* 2005, Retzlaff *et al.* 2005, Trivedi *et al.* 2007, Hochreutener *et al.* 2017).

Organic acid carcass washes, alone (lactic, acetic and citric) or as a mixture, were effective on-line interventions with higher reductions reported for lactic acid (1-2 logs of natural microbiota) (Dormedy *et al.* 2000, De Martinez *et al.* 2002, Bosilevac *et al.* 2006, Rodriguez 2007, Ruby *et al.* 2007, Wright 2011, Signorini *et al.* 2018) than other acids (usually up to 1 log) (Algino *et al.* 2007, Carranza *et al.* 2013, Signorini *et al.* 2018). Mixtures of organic acids did not provide any added beneficial effect and reductions achieved were around 1 log-cycles (Algino *et al.* 2007, Signorini *et al.* 2018). If more than one wash was applied at a single step, often combining a thermal effect with an organic acid, this produced additional reduction effects of 1 log-cycles (Gill and Landers 2003b, Bosilevac *et al.* 2006, Ruby *et al.* 2007, Wright 2011, Carranza *et al.* 2013, Wang *et al.* 2013).

Challenge trials under pilot plant conditions have been reported in 14 articles (Castillo *et al.* 1998c, Castillo *et al.* 1998b, Castillo *et al.* 1999a, Castillo *et al.* 1999b, Castillo *et al.* 2001b, Castillo *et al.* 2003, Marshall *et al.* 2005, Kalchayanand *et al.* 2008, Niebuhr *et al.* 2008, Cabrera-Diaz *et al.* 2009, Kalchayanand *et al.* 2009, Davidson 2010, Sevart *et al.* 2016, Krug 2017). The conditions in pilot plants are considered to mimic those in commercial abattoirs, and in most cases, researchers used whole carcasses or large beef primals to investigate intervention efficacy in commercial washing/spraying cabinets. Various physical (water washes and thermal treatments) and chemical interventions (organic acids and other chemicals) alone or in combinations, have been shown to produce large variation of reduction effects, very often between 2-5 logs. However, this must be viewed with caution and only as relative and indicative of the potential intervention effect.

Most often, intervention studies were conducted under laboratory conditions using artificially inoculated microbiota (challenge trials). A total of 39 lab trials (most often challenge trials) were identified that investigated one or several interventions on pre-rigor carcass meat to generate data on their relative efficacy and their suitability for commercial on-line application (Cabedo *et al.* 1996, Dorsa *et al.* 1996a, Dorsa *et al.* 1996b, Bell *et al.* 1997, Cutter *et al.* 1997a, Cutter *et al.* 1997b, Dorsa *et al.* 1997a, Dorsa *et al.* 1997b, Gorman *et al.* 1997, Phebus *et al.* 1997, Tinney *et al.* 1997, Delazari *et al.* 1998a, Delazari *et al.* 1998b, Dorsa *et al.* 1998, Graves Delmore *et al.* 1998, Cutter 1999a, Cutter *et al.* 2000, Cutter and Rivera-Betancourt 2000, Hajmeer *et al.* 2004, Retzlaff *et al.* 2004, McCann *et al.* 2006b, Penney *et al.* 2007, Arthur *et al.* 2008b, Pearce and Bolton 2008, Sawyer *et al.* 2008, Ingham *et al.* 2010, Yoder *et al.* 2010, Carpenter *et al.* 2011, Njongmeta *et al.* 2011, Kalchayanand *et al.* 2012, McDonnell *et al.* 2012, Yoder *et al.* 2012, Youssef *et al.* 2012, Kalchayanand *et al.* 2015, Scott *et al.* 2015, Rodríguez-Melcón *et al.* 2017, Scott-Bullard *et al.* 2017, Woerner 2017, Yang *et al.* 2017a). The reductions reported should be viewed with caution and only as relative and indicative of the potential intervention effect because these trials often used a small number of samples challenged with a high number of pathogens, which exaggerates the efficacy of interventions.

Table IC 3.4.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies / design	Intervention / outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Physical interventions aimed at removing microorganisms						
Trimming	2/BA 1/CT	Carcass	No treatment	Aerobic bacteria	0.0-2.2	(Gill *et al.* 1996a, Kochevar *et al.* 1997, Gill and Landers 2004)
				Coliforms	1.6-1.8	
				E. coli	0.0-2.0	
Water wash	6/BA 1/CT	Carcass	No treatment	Aerobic bacteria	-1.2-1.3	(Gill *et al.* 1996b, Bell 1997, Hajmeer *et al.* 1999, De Martinez *et al.* 2002, Gill and Landers 2003b, McEvoy *et al.* 2004, Carranza *et al.* 2013)
				Coliforms	-0.8-1.9	
				E. coli	0.1-1.9	
Thermal interventions						
Hot water	6/BA	Carcass	No treatment	Aerobic bacteria	0.8-2.7	(Gill *et al.* 1999, Gill and Bryant 2000, Bosilevac *et al.* 2006, Algino *et al.* 2007, Wright 2011, Signorini *et al.* 2018)
				Enterobacteriaceae	0.6-2.7	
				Coliforms	0.4-2.6	
				E. coli	0.4-1.4	
Steam vacuuming	2/BA 2/CT	Carcass	No treatment	Aerobic bacteria	0.3-2.0	(Gill and Bryant 1997b, Kochevar *et al.* 1997, Trivedi *et al.* 2007, Hochreutener *et al.* 2017)
				Enterobacteriaceae	0.7-1.1	
				Coliforms	0.2-2.2	
				E. coli	0.2-0.7	
Steam pasteurisation	4/BA 1/CT	Carcass	No treatment	Aerobic bacteria	0.1-1.6	(Nutsch *et al.* 1997, Nutsch *et al.* 1998, Minihan *et al.* 2003b, Corantin
				Enterobacteriaceae	0.6-1.5	
				Coliforms	0.1-1.6	

Intervention	No. studies / design	Intervention / outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
				E. coli	0.1-0.8	*et al.* 2005, Retzlaff *et al.* 2005)
Organic acid washes						
Lactic acid	5/BA 2/CT	Carcass	No treatment	Aerobic bacteria	0.9-3.8	(Dormedy *et al.* 2000, De Martinez *et al.* 2002, Bosilevac *et al.* 2006, Rodriguez 2007, Ruby *et al.* 2007, Wright 2011, Signorini *et al.* 2018)
				Enterobacteriaceae	0.4-1.0	
				Coliforms	0.3-2.7	
				E. coli	0.1-1.8	
Acetic acid	2/BA 1/CT	Carcass	No treatment	Aerobic bacteria	0.4-0.6	(Algino *et al.* 2007, Carranza *et al.* 2013, Signorini *et al.* 2018)
				Enterobacteriaceae	1.0	
				Coliforms	0.6-0.8	
				E. coli	0.5-0.7	
Citric acid	1/BA	Carcass	No treatment	Aerobic bacteria	0.8	(Signorini *et al.* 2018)
				Coliforms	0.4	
				E. coli	0.4	
Organic acid mixtures	2/BA	Carcass	No treatment	Aerobic bacteria	0.2	(Algino *et al.* 2007, Signorini *et al.* 2018)
				Enterobacteriaceae	0.6	
				Coliforms	0.2-0.8	
				E. coli	0.1-0.9	
Multiple interventions applied at one step						
Trimming / steam vac.	1/CT	Carcass	No treatment	Aerobic bacteria	1.2	(Ramish 2011)
				Enterobacteriaceae	0.7	

Intervention	No. studies / design	Intervention / outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Water wash / lactic acid	1/BA	Carcass	No treatment	Aerobic bacteria	0.4-0.8	(Gill and Landers 2003b)
Water wash / acetic acid	1/CT	Carcass	No treatment	Aerobic bacteria	0.1-0.8	(Carranza *et al.* 2013)
				Coliforms	1.3-1.5	
Hot water / lactic acid	4/BA	Carcass	No treatment	Aerobic bacteria	1.1-2.8	(Gill and Landers 2003b, Bosilevac *et al.* 2006, Ruby *et al.* 2007, Wright 2011)
				Enterobacteriaceae	1.1-2.5	
				Coliforms	2.1	
				E. coli	1.6	
Steam past. / lactic acid	1/BA	Carcass	No treatment	Aerobic bacteria	1.6	(Gill and Landers 2003b)
Peroxyacetic acid / steam pasteurisation	1/BA	Carcass	No treatment	Aerobic bacteria	1.0	(Gill and Landers 2003b)
Multiple interventions applied at multiple steps						
Water wash /thermal/lactic acid/PAA	2/BA	Carcass	No treatment	Aerobic bacteria	1.1-1.9	(Gill and Landers 2003b, Wang *et al.* 2013)
				Enterobacteriaceae	1.8	
				Coliforms	0.5	
				E. coli	0.6	

Table IC 3.4.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Water wash	5/BA	Carcass	No treatment	*E. coli* O157:H7	0.7%	0.7%	(Trairatapiwan *et al.* 2011, Thomas *et al.* 2012, Narváez-Bravo *et al.* 2013, Dong *et al.* 2014, Dong *et al.* 2015)
				E. coli non-O157	0.5-5.5%	0-2%	
				Salmonella spp.	1.5-10%	0-4.5%	
Hot water	2/BA	Carcass	No treatment	*Enterobacteriaceae*	19-27%	12-15%	(Bosilevac *et al.* 2006, Algino *et al.* 2007)
				Coliforms	19-26%	8-9%	
				E. coli	18-24%	3%	
				E. coli O157:H7	27%	5%	
Steam pasteurisation	2/BA	Carcass	No treatment	*Enterobacteriaceae*	46%	3%	(Nutsch *et al.* 1997, Corantin *et al.* 2005)
				Coliforms	34-38%	1.5-15%	
				E. coli	14-16%	0-1.8%	
				Salmonella spp.	0.7%	0%	
Lactic acid	3/BA	Carcass	No treatment	*E. coli* O157:H7	31%	20%	(Bosilevac *et al.* 2006, Ruby *et al.* 2007, Chaves *et al.* 2013)
				E. coli non-O157	6.7%	0%	
				Salmonella spp.	45%	28%	
Acetic acid	1/BA	Carcass	No treatment	*Enterobacteriaceae*	58%	30%	(Algino *et al.* 2007)
				Coliforms	50%	15%	
				E. coli	47%	13%	

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Organic acid mixtures	1/BA	Carcass	No treatment	*Enterobacteriaceae*	28%	22%	(Algino *et al.* 2007)
				Coliforms	26%	13%	
				E. coli	24%	7%	
Hot water/ lactic acid	2/BA	Carcass	No treatment	*E. coli* O157:H7	19%	4%	(Bosilevac *et al.* 2006, Ruby *et al.* 2007)
				Salmonella spp.	28%	2.3%	

IC 3.5 Chilling

Chilling efficacy in reducing microbial growth and/or number and presence of bacteria has been reported in a total of 34 studies. Dry chilling effects on carcass microbial load have been investigated in 17 studies under commercial conditions, on its own or following previous multi sequential interventions on the slaughterline (Hajmeer *et al.* 1999, Sofos *et al.* 1999, Bacon *et al.* 2000, McEvoy *et al.* 2004, Fegan *et al.* 2005a, Fegan *et al.* 2005b, Carney *et al.* 2006, Kinsella *et al.* 2006, Trivedi *et al.* 2007, Trairatapiwan *et al.* 2011, Dong *et al.* 2014, Dong *et al.* 2015, Hauge *et al.* 2015, Sampaio *et al.* 2015, Fontcuberta *et al.* 2016, Liu *et al.* 2016, Yang *et al.* 2017b). In addition, nine challenge trials in pilot or lab conditions were reported on dry chilling (Calicioglu *et al.* 1999, Calicioglu *et al.* 2002, Crowley *et al.* 2009, Kinsella *et al.* 2009, Ingham *et al.* 2010, Tittor *et al.* 2011, Hudson *et al.* 2013, Sevart *et al.* 2016, Reid *et al.* 2017)

Chilling for up to three days only reduced the levels of indicator bacteria in most cases by only 0.5 logs under commercial conditions (Hajmeer *et al.* 1999, McEvoy *et al.* 2004, Kinsella *et al.* 2006, Trivedi *et al.* 2007, Hauge *et al.* 2015, Sampaio *et al.* 2015), but some authors reported reductions of 1-2 logs under similar conditions (Liu *et al.* 2016, Yang *et al.* 2017b). Under pilot and lab conditions, reductions of inoculated *E. coli* and *Salmonella* were up to 2 logs (Calicioglu *et al.* 1999, Calicioglu *et al.* 2002, Crowley *et al.* 2009, Kinsella *et al.* 2009, Tittor *et al.* 2011, Sevart *et al.* 2016, Reid *et al.* 2017). Chilling carcasses previously sprayed with organic acids or treated with hot water or steam on the slaughterline for one day reduced indicator bacteria from 0.6-2.1 logs under commercial conditions (Bacon *et al.* 2000) and up to 3.5 logs of *E. coli* under pilot and lab conditions (Calicioglu *et al.* 2002, Ingham *et al.* 2010), likely due to a residual effect of the chemical interventions.

Effects of cold temperatures after completed chilling, during dry aging of carcasses for up to two weeks, have been reported in four studies, one before-and-after trial under commercial conditions (Algino *et al.* 2007), one challenge trial in pilot conditions (Calicioglu *et al.* 2002) and two in lab conditions (Ingham *et al.* 2010, Knudsen *et al.* 2011). Algino *et al.* (2007) reported reductions of up to 2 logs of faecal indicators in the first four days of dry aging. Reductions of around 1 log after six days or around 3 logs of inoculated enteric pathogens after seven days of dry aging have also been reported (Calicioglu *et al.* 2002, Ingham *et al.* 2010). Knudsen *et al.* (2011) reported 0.1-0.2 logs reduction per day of inoculated *Salmonella* during a 14-day dry aging of beef cuts.

Spray chilling with water was investigated in six studies under commercial conditions (Gill and Bryant 1997b, Gill and Bryant 1997a, Jericho *et al.* 1998, Gill and Landers 2003a, Corantin *et al.* 2005, Kinsella *et al.* 2006); two challenge trials under pilot and lab conditions reported on water spray chilling (Tittor *et al.* 2011) and spray chilling with chemical solutions (Stopforth *et al.* 2004). In general, water spray chilling showed very variable effects in reducing natural microbiota on carcasses in commercial conditions and it appears these were plant specific and influenced by other factors. On inoculated VTEC and

Salmonella, water spray chilling achieved up to 2 logs reduction (Stopforth *et al.* 2004, Tittor *et al.* 2011). Spraying various chemicals onto beef carcass cuts during chilling (sodium hypochlorite, acidified sodium chlorite, ammonium hydroxide, lactic acid and cetylpyridinium chloride) increased effectiveness by 0.7 logs, 2.2 logs, 2.5 logs, 3.2 logs and 4.7 logs, respectively for all chemicals, comparing to water spray chilling alone (Stopforth *et al.* 2004).

Table IC 3.5.1. Studies on chilling measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Dry chilling (≤3 days)	8/BA	Carcass	Before treatment	Aerobic bacteria	-1.2-2.0	(Hajmeer *et al.* 1999, McEvoy *et al.* 2004, Kinsella *et al.* 2006, Trivedi *et al.* 2007, Hauge *et al.* 2015, Sampaio *et al.* 2015, Liu *et al.* 2016, Yang *et al.* 2017b)
				Coliforms	-0.4-1.9	
				E. coli	0.0-1.4	
Dry chilling (≤3 days)	7/ChT‡	Subprimals and cuts	Before treatment	Aerobic bacteria	-3.5-0.0	(Calicioglu *et al.* 1999, Calicioglu *et al.* 2002, Crowley *et al.* 2009, Kinsella *et al.* 2009, Tittor *et al.* 2011, Sevart *et al.* 2016, Reid *et al.* 2017)
				Coliforms	0.3	
				E. coli	0.4-2.1	
				E. coli O157:H7	0.1-2.3	
				S. Typhimurium	0.1-1.5	
Dry chilling (≤3 days) followed on single or multiple interventions	1/BA	Carcass	Before treatment	Aerobic bacteria	2.1	(Bacon *et al.* 2000)
				Coliforms	1.2	
				E. coli	0.6	
	2/ChT‡	Subprimals	Before treatment	*E. coli*	0.5-2.6	(Calicioglu *et al.* 2002, Ingham *et al.* 2010)
				E. coli O157:H7	0.5-3.4	
Dry aging (3-14 days)	1/BA	Carcass	Before treatment	*Enterobacteriaceae*	0.4-2.1	(Algino *et al.* 2007)
				Coliforms	0.7-2.1	

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
				E. coli	0.6-2.0	
Dry aging (3-14 days)	2/ChT‡	Subprimals	Before treatment	Coliforms	0.9	(Calicioglu *et al.* 2002, Ingham *et al.* 2010)
				E. coli	0.6-3.7	
				E. coli O157:H7	0.8-4.4	
Water spray chilling	6/BA	Carcass	Before treatment	Aerobic bacteria	-1.8-2.0	(Gill and Bryant 1997b, Gill and Bryant 1997a, Jericho *et al.* 1998, Gill and Landers 2003a, Corantin *et al.* 2005, Kinsella *et al.* 2006)
				Coliforms	-1.4-1.4	
				E. coli	-1.4-1.3	
Water spray chilling	2/ChT‡	Carcass cuts	Before treatment	*E. coli* O157:H7	0.0-1.9	(Stopforth *et al.* 2004, Tittor *et al.* 2011)
				Salmonella spp.	1.3-2.0	
Spray chilling chemicals	1/ChT‡	Carcass cuts	Water spray chilling	*E. coli* O157:H7	0.7-4.7	(Stopforth *et al.* 2004)

‡ Pilot or lab conditions

IC 3.6 Post-chill and pre-fabrication carcass treatments

Two studies under commercial conditions investigated interventions for carcasses after completion of chilling but before fabrication. Lactic acid spray was shown to statistically significantly reduce aerobic bacteria by up to 3 log-cycles and faecal bacteria by up to 1.5 logs (Castillo *et al.* 2001a, Ruby *et al.* 2007). Highly variable reductions with lactic acid were achieved in lab conditions on inoculated VTEC and *Salmonella*, varying from 1-7 logs (Castillo *et al.* 2001b, King *et al.* 2005, Sevart *et al.* 2016, Acuff 2017, Krug 2017). Reductions of around 1 log-cycle were achieved when peroxyacetic acid was sprayed onto beef subprimals (King *et al.* 2005, Acuff 2017, Krug 2017).

One novel non-thermal intervention, electron beam (E-beam) irradiation, was reported to be highly efficacious at a 1 kGy dose, and when applied to chilled beef primals, reduced *E. coli* O157:H7 numbers by at least 4 logs and up to 6.6 log-cycles (Arthur *et al.* 2005).

Table IC 3.6.1. Studies on post-chill interventions measuring concentration outcomes

Intervention	No. studies / design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Lactic acid	2/BA	Carcass	No treatment	Aerobic bacteria	0.6-3.3	(Castillo *et al.* 2001a, Ruby *et al.* 2007)
				Coliforms	0.3-1.6	
				E. coli	0.2	
Lactic acid	5/ChT‡	Subprimals	No treatment	*Salmonella*	1.6-6.8	(Castillo *et al.* 2001b, King *et al.* 2005, Sevart *et al.* 2016, Acuff 2017, Krug 2017)
				E. coli O157:H7	2.4-7.2	
				E. coli non-O157	0.5-1.5	
				E. coli	4.0-5.7	
Peroxyacetic acid	3/ChT‡	Subprimals	No treatment	*E. coli* O157:H7	0.5-1.3	(King *et al.* 2005, Acuff 2017, Krug 2017)
				E. coli non-O157	0.6-1.3	
Steam vacuuming	1/ChT‡	Carcass	No treatment	*Salmonella*	0.6	(Bacon *et al.* 2002b)
Electron beam irradiation	1/ChT‡	Primals	No treatment	*E. coli* O157:H7	4.0-6.6	(Arthur *et al.* 2005)

‡ Pilot or lab conditions

IC 3.7 Multiple on-line interventions and HACCP

Sixteen before-and-after trial studies and one controlled trial study evaluated the effect of multiple interventions applied between pre-evisceration and chilling stage under commercial conditions (Bacon *et al.* 2000, Elder *et al.* 2000, Arthur *et al.* 2002, Bacon *et al.* 2002a, Barkocy-Gallagher *et al.* 2003, Gill *et al.* 2003, Arthur *et al.* 2004, Rivera-Betancourt *et al.* 2004, Ruby *et al.* 2007, Brichta-Harhay *et al.* 2008, Brichta-Harhay *et al.* 2011, Rekow *et al.* 2011, Koohmaraie *et al.* 2012, Scott *et al.* 2015, Bosilevac *et al.* 2016, Kanankege *et al.* 2017, Van Ba *et al.* 2018). Sequential application of interventions after dehiding usually involved some or all of the following: knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam and organic acid (or peroxyacetic acid) rinsing before chilling. Consistent reductions were achieved, which were higher than when only one single intervention was used, and in most cases reductions ranged from 2 to 3 logs of aerobic or faecal indicators (Bacon *et al.* 2000, Arthur *et al.* 2004, Ruby *et al.* 2007, Bosilevac *et al.* 2016). In one controlled trial in a pilot plant where hide organic acid washes were investigated concurrently with carcass washes, the reduction obtained after chilling was in the range of 1.5-2 logs compared to untreated (only chilled) carcasses (Van Ba *et al.* 2018). Furthermore, the prevalence of naturally present VTEC and *Salmonella* following sequential application of interventions was in most cases statistically significantly reduced, often to levels below detection limits (Elder *et al.* 2000, Arthur *et al.* 2002, Bacon *et al.* 2002a, Barkocy-Gallagher *et al.* 2003, Arthur *et al.* 2004, Rivera-Betancourt *et al.* 2004, Ruby *et al.* 2007, Brichta-Harhay *et al.* 2008, Brichta-Harhay *et al.* 2011, Koohmaraie *et al.* 2012).

The effect of HACCP implementation on overall improvement of microbial status of beef carcasses was investigated in eight before-and-after studies (Phillips *et al.* 2001, Rose *et al.* 2002, Sumner *et al.* 2003, Sumner *et al.* 2004, Ghafir *et al.* 2005, Phillips *et al.* 2006, Tergney and Bolton 2006, Nastasijevic *et al.* 2009). It appears that there is no overall effect of HACCP on pathogen (VTEC and *Salmonella*) reduction, but the levels of indicator aerobic and faecal bacteria were reduced on carcasses by 0.5-1 log-cycles after HACCP implementation.

Table IC 3.7.1. Studies under commercial conditions measuring concentration outcomes

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Multiple (steam vacuum, peroxyacetic and organic acid washes, thermal treatments)	7/BA	Carcass	Before treatment	Aerobic bacteria	1.0-3.9	(Bacon *et al.* 2000, Gill *et al.* 2003, Arthur *et al.* 2004, Ruby *et al.* 2007, Brichta-Harhay *et al.* 2008, Scott *et al.* 2015, Bosilevac *et al.* 2016)
				Enterobacteriaceae	1.2-1.5	
				Coliforms	0.4-3.9	
				E. coli	0.8-4.1	
Multiple (acetic acid hide spray, lactic/ acetic acid carcass spray, chill)	1/CT‡	Hide and carcass/ carcass	No treatment	Aerobic bacteria	1.7-2.5	(Van Ba *et al.* 2018)
				Coliforms	1.0-1.6	
				E. coli	1.5-1.7	
				Salmonella spp.	0.6-1.2	
HACCP	6/BA	Carcass	Before HACCP	Aerobic bacteria	0.6-1.4	(Phillips *et al.* 2001, Sumner *et al.* 2003, Sumner *et al.* 2004, Phillips *et al.* 2006, Tergney and Bolton 2006, Nastasijevic *et al.* 2009)
				Enterobacteriaceae	0.1-0.8	
				Coliforms	0.9	
				E. coli	0.6	

‡ Pilot

Table IC 3.7.2. Studies under commercial conditions measuring prevalence reductions

Intervention	No. studies/ design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	% Samples positive in study population		References
					No treat.	Treatment	
Multiple (steam vacuum, peroxyacetic and organic acid washes, thermal treatments)	12/BA	Carcass	No treatment	*E. coli* O157:H7	7-43%	0.0-1.8%	(Elder *et al.* 2000, Arthur *et al.* 2002, Bacon *et al.* 2002a, Barkocy-Gallagher *et al.* 2003, Arthur *et al.* 2004, Rivera-Betancourt *et al.* 2004, Ruby *et al.* 2007, Brichta-Harhay *et al.* 2008, Brichta-Harhay *et al.* 2011, Rekow *et al.* 2011, Koohmaraie *et al.* 2012, Kanankege *et al.* 2017)
				E. coli non-O157	54-58%	8-9%	
				Salmonella spp.	10-67%	0-7.5%	
HACCP	6/BA	Carcass	Before HACCP	*Salmonella*	0-2.5%	0.0-0.6%	(Phillips *et al.* 2001, Rose *et al.* 2002, Sumner *et al.* 2003, Sumner *et al.* 2004, Ghafir *et al.* 2005, Phillips *et al.* 2006)
				E. coli	2.5-22%	8-11%	
				E. coli O157:H7	0.5%	0.0%	

IC 3.8 References cited in IC 3

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IC 4: Post-carcass fabrication interventions

IC 4.1 Summary of key findings

IC 4.1.1 Standard processing procedures and GHP

Three studies found inconsistent effects of carcass fabrication procedures, with some reduction seen after trimming potentially contaminated carcass sites, but increased possibility for microbial cross-contamination. HACCP implementation appeared to reduce ACC by 1-2 logs compared to pre-HACCP implementation levels in beef cutting plants. Regular sanitation with detergents and sanitisers is highly efficacious against residual microbiota with up to 3 log reductions achieved on food contact surfaces.

IC 4.1.2 Interventions for beef primals, subprimals and trim

A large number of studies investigated various thermal and chemical interventions post-carcass fabrication of beef primals, subprimals and trim. Hot water wash and steam treatment of beef primals and trim had a reduction effect of up to 2 logs in numbers of inoculated VTEC and *Salmonella,* whereas reductions of 0.5-1 logs were reported on natural aerobic and faecal microbiota. Dry heat at temperatures of up to 100^{o}C from a hot air gun increased efficacy to a reduction in inoculated VTEC and *Salmonella* by 4-6 logs. However, these thermal and chemical interventions post-carcass fabrication could have detrimental effects on product quality if intervention parameters are not optimised. Studies that investigated various organic acids and other chemicals reported large variations in the magnitude of effect. Lactic acid and other organic acids, alone or in a combination with other chemicals or hot water, were shown to have had efficacies of around 1-2 logs on inoculated pathogens or natural microbiota. Novel treatments such as phages were efficacious against inoculated *E. coli* O157:H7 and Salmonella in the range of 1-2 logs.

IC 4.1.3 Packaging and storage

Studies that described research on various chemical, physical and biological interventions for the final product (beef trim and minced beef) found variable efficacies dependant on intervention conditions. Cold aerobic storage for up to seven days reduced inoculated *E. coli* O157:H7 by 1.5 logs and natural aerobic microbiota by up to 0.5 logs, whereas MAP and vacuum packaging had limited and not statistically significant reduction effects on inoculated *E. coli* O157:H7 of up to 0.4 logs, which in combination with lactic acid increased to 2 logs. The use of lactic acid bacteria (*Lactobacillus* spp.) to control pathogens in the final product reported variable reductions of inoculated *E. coli* O157:H7 of up to 3 logs in minced beef. Nisin was mostly found to be effective against inoculated *E. coli* O157:H7 and *L. monocytogenes* (1-2 logs); similarly, phages achieved up to 1 log reduction of *E. coli* O157:H7.

Irradiation appears to be one of the most effective interventions and is able to deliver the complete elimination of inoculated pathogens, with reduction effects >6 logs, whereas high-pressure processing produced highly variable reductions depending on the study conditions, ranging from 3-5 logs.

IC 4.2 Intervention description

Packaging-based interventions: interventions that can be applied to prevent spoilage and inhibit microbial growth during final product distribution and storage.

Modified atmosphere and vacuum packaging: refers to the packaging where natural composition of air is altered and replaced by an alternative atmosphere, most often by active displacement of gases in the package and their replacement by a desired mixture of gases (usually a different mixture of oxygen, nitrogen and carbon dioxide, comprising 60–75% CO_2, 10–25% oxygen and 15–30% nitrogen). Vacuum packaging has the air completely removed.

Non-thermal interventions: refers to non-chemical (physical) and non-thermal interventions that aim to reduce microbial contamination while preserving product quality and nutrients that can be affected by thermal treatments (electron beam and gamma irradiation, ultraviolet (UV) light, cold atmospheric plasma and high-pressure processing).

Biological treatments (biopreservation): refers to the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. Some compounds include bacteriocins and bacteriocin-producing bacteria, bacteriophages, chitosan, lactic acid bacteria (LAB), lactoferricin and lysozyme.

IC 4.3 Standard processing procedures and GHP

Two studies that investigated carcass fabrication hygiene found that operations involved in carcass fabrication usually led to an increase in carcass microbial contamination with aerobic bacteria, and also increased cross-contamination from operators/environment that led to an increase in faecal indicators in the resulting beef trimmings (Gill and Jones 1999, Gill and McGinnis 2000). One challenge trial in pilot plant conditions found that knife trimming of adipose and potentially contaminated sites after inoculation of *E. coli* was partially effective for up to 3 logs (Laster *et al.* 2012). However, trimming also led to cross-contamination of sites that were previously not inoculated.

After HACCP implementation, meat cutting plants were shown to have a reduced microbial load on food contact surfaces and the processing environment by 1-2 logs of aerobic bacteria compared to levels before HACCP implementation (Tomasevic *et al.* 2016).

Two before-and-after studies that investigated cleaning and sanitation procedures in beef cutting plants found statistically significant reductions of aerobic and faecal indicators by 0.5-3 logs on food contact surfaces after the application of different combinations of detergents and sanitisers (Yang *et al.* 2017c, Wang *et al.* 2018).

IC 4.4 Interventions for beef primals, subprimals and trim

A total of 51 laboratory and pilot plant trials were identified that investigated the efficacy of post-carcass fabrication interventions on beef primals, subprimals and trimmings. Compared with no treatment or water wash, most interventions tended to reduce natural or inoculated microbiota.

Three challenge studies investigated the physical removal of inoculated bacteria by trimming and washing with water at ambient temperature (Kang *et al.* 2001a, Lemmons *et al.* 2011, Liao *et al.* 2015). Trimming removed inoculated *E. coli* O157:H7 by 2.4 logs and washing only by 2 logs.

Thermal treatments (hot water, steam, hot air) were investigated in nine studies (Gill and Badoni 1997, Ellebracht *et al.* 1999, Delmore Jr *et al.* 2000, Gill *et al.* 2001, Stivarius *et al.* 2002c, Logue *et al.* 2005, Purnell *et al.* 2005, McCann *et al.* 2006a, Özdemir *et al.* 2006, Schmidt *et al.* 2014). A hot water wash and steam had statistically significant reduction effects of up to 2 logs on inoculated *E. coli* O157:H7 and *Salmonella* (Ellebracht *et al.* 1999, Logue *et al.* 2005, Schmidt *et al.* 2014), whereas reductions of 0.5-1 logs were reported on natural aerobic and faecal microbiota (Gill and Badoni 1997, Delmore Jr *et al.* 2000, Gill *et al.* 2001, Purnell *et al.* 2005). Dry heat using a hot air gun achieved comparably higher reductions on beef trim of 1-2 logs at lower temperatures (60°C and 75°C) and 4-6 logs at higher temperatures (90°C and 100°C) of inoculated VTEC and *Salmonella* (McCann *et al.* 2006a). Obviously, these thermal treatments can have unwanted detrimental effect on

product quality, therefore intervention parameters should be balanced to meet both safety and quality needs.

Organic acid washes were by far most investigated intervention in the post-fabrication stage with 29 studies reporting on their efficacy (Podolak *et al.* 1996, Prasai *et al.* 1997, Delmore Jr *et al.* 2000, Kang *et al.* 2001a, Pohlman *et al.* 2002b, Pohlman *et al.* 2002a, Stivarius *et al.* 2002a, Stivarius *et al.* 2002c, Ransom *et al.* 2003, Ellebracht *et al.* 2005, Harris *et al.* 2006, Özdemir *et al.* 2006, Laury *et al.* 2009, Fouladkhah *et al.* 2012, Geornaras *et al.* 2012a, Geornaras *et al.* 2012b, Harris *et al.* 2012, Pittman *et al.* 2012, Wolf *et al.* 2012, Pohlman *et al.* 2014, Schmidt *et al.* 2014, Tango *et al.* 2014, Zhao *et al.* 2014, Liao *et al.* 2015, DeGeer *et al.* 2016, Mohan and Pohlman 2016, Dan *et al.* 2017, Kassem *et al.* 2017, Yeh *et al.* 2018). Lactic acid, alone or in a combination with other chemicals or hot water, was shown to have an efficacy of around 1-2 logs for inoculated pathogens or natural microbiota. Other organic acids (acetic, citric, malic, fumaric, gluconic, pyruvic, levulinic, caproic, caprylic and capric acid) exhibited similar reductions but there were large variations in the magnitude of effect across studies.

Washes containing other chemicals and oxidizers were reported in 27 studies (Delmore Jr *et al.* 2000, Pohlman *et al.* 2002b, Pohlman *et al.* 2002a, Pohlman *et al.* 2002c, Stivarius *et al.* 2002b, Stivarius *et al.* 2002a, Ransom *et al.* 2003, Bosilevac *et al.* 2004b, Lim and Mustapha 2004, Harris *et al.* 2006, Pohlman *et al.* 2009, Quilo *et al.* 2010, Coll Cárdenas *et al.* 2011, Geornaras *et al.* 2012a, Geornaras *et al.* 2012b, Harris *et al.* 2012, Mohan *et al.* 2012, Dias-Morse *et al.* 2014, Pohlman *et al.* 2014, Schmidt *et al.* 2014, Tango *et al.* 2014, Liao *et al.* 2015, Mehall *et al.* 2015, DeGeer *et al.* 2016, Kassem *et al.* 2017, Stella *et al.* 2017, Yeh *et al.* 2018). Various chemicals were investigated: acidified sodium chlorate, ozone, sodium metasilicate, trisodium phosphate, chlorine, lauric arginate, cetylpyridinium chloride, peroxyacetic acid, sodium decanoate, hypobromous acid, potassium sorbate, potassium lactate and sodium dodecyl sulfate. They had very variable effects depending on study conditions, but consistent statistically significant bacterial reductions in most studies.

Phages and Lactoferricin B were investigated in four studies (Venkitanarayanan *et al.* 1999, Ransom *et al.* 2003, Tomat *et al.* 2013, Yeh *et al.* 2017). It was reported that the efficacy of phages against inoculated *E. coli* O157:H7 and *Salmonella* was in the range of 1-2 logs. On the other hand, lactoferrricin B achieved reductions of inoculated *E. coli* O157:H7 of 0.7-0.8 logs.

Multiple interventions were investigated in a controlled trial study by Kang *et al.* (2001b). Multiple treatments (hot water spray, hot air, lactic acid spray) followed by vacuum storage gave better reductions of natural aerobic and faecal microbiota which ranged from 1.6-3.7 logs.

Table IC 3.4.1. Studies under laboratory and pilot plant conditions measuring concentration outcomes

Intervention	No. studies / design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
Physical interventions aimed at removing microorganisms						
Trimming	1/ChT	Subprimals	No treat.	*E. coli* O157:H7	2.4	(Lemmons *et al.* 2011)
Water wash	3/ChT	Subprimals, trim	treatment	Coliforms	1.1-2.0	(Kang *et al.* 2001a, Lemmons *et al.* 2011, Liao *et al.* 2015)
				E. coli O157:H7	0.3-0.4	
Thermal interventions						
Hot water	1/BA 2/CT 4/ChT	Trim, cheek meat	No treatment	Aerobic bacteria	0.6-1.3	(Gill and Badoni 1997, Ellebracht *et al.* 1999, Delmore Jr *et al.* 2000, Gill *et al.* 2001, Stivarius *et al.* 2002c, Özdemir *et al.* 2006, Schmidt *et al.* 2014)
				Coliforms	0.6-1.2	
				E. coli	0.6	
				E. coli O157:H7	0.5-2.2	
				Salmonella	0.5-2.3	
Steam	1/BA 1/CT 1/ChT	Primals, trim, cheek meat	No treatment	Aerobic bacteria	0.3-2.1	(Delmore Jr *et al.* 2000, Logue *et al.* 2005, Purnell *et al.* 2005)
				Coliforms	0.5	
				E. coli	0.3	
				E. coli O157:H7	0.9-2.1	
Hot air	1/ChT	Beef cuts	No treatment	*Salmonella*	1.5-5.8	(McCann *et al.* 2006a)
				E. coli O157:H7	1.3-6.1	
Other interventions						
Lactic acid	1/CT 1/BA 18/ChT	Subrimals, trim, cheek meat	No treatment	Aerobic bacteria	1.0-1.5	(Podolak *et al.* 1996, Prasai *et al.* 1997, Ellebracht *et al.* 1999, Delmore Jr *et al.* 2000, Kang *et al.* 2001a, Stivarius *et al.* 2002c, Harris *et al.*
				Coliforms	0.5	
				E. coli	0.2-3.4	
				E. coli O157:H7	0.2-2.8	
				Salmonella	0.7-2.4	

Intervention	No. studies / design	Intervention/ outcome sample	Comparison group	Outcome/ microorganism	Log_{10} CFU reduction	References
						2006, Özdemir *et al.* 2006, Laury *et al.* 2009, Fouladkhah *et al.* 2012, Harris *et al.* 2012, Pittman *et al.* 2012, Wolf *et al.* 2012, Schmidt *et al.* 2014, Zhao *et al.* 2014, Liao *et al.* 2015, DeGeer *et al.* 2016, Dan *et al.* 2017, Kassem *et al.* 2017, Yeh *et al.* 2018)
Phages	2/ChT	Trim	No treatment	*E. coli* O157:H7	1.4-2.6	(Tomat *et al.* 2013, Yeh *et al.* 2017)
				Salmonella	1.2	

IC 4.5 Packaging and storage

In the packaging and storage stage, a total of 43 articles were identified that described research on different chemical, physical and biological interventions for the final product (beef trim and minced beef).

The effect of cold aerobic storage on the survival of bacteria has been reported in five studies (Jericho *et al.* 2000, Barkocy-Gallagher *et al.* 2002, Ashton *et al.* 2006, Mann and Brashears 2006, Crowley *et al.* 2010). Up to seven days of cold aerobic storage was shown to reduce inoculated *E. coli* O157:H7 by 1.5 logs (Barkocy-Gallagher *et al.* 2002, Ashton *et al.* 2006) and natural aerobic microbiota by up to 0.5 logs (Jericho *et al.* 2000, Crowley *et al.* 2010), which then recovered and sharply increased in numbers leading to spoilage. In another study, cold storage appeared not to have had any effect on inoculated *E. coli* O157:H7 over a 3 day cold storage of minced beef (Mann and Brashears 2006).

Modified atmosphere (MAP) and vacuum packaging interventions were reported in seven studies, alone or in combination with various preservatives (Cutter 1999b, Tsigarida *et al.* 2000, Meurehg 2006, Crowley *et al.* 2010, Kudra *et al.* 2011, Miya *et al.* 2014, Salim *et al.* 2018). MAP and vacuum packaging had limited and not statistically significant reduction effects on inoculated *E. coli* O157:H7 of up to 0.4 logs (Kudra *et al.* 2011), but in combination with lactic acid, achieved 2 logs reduction (Salim *et al.* 2018). Both MAP and vacuum packaging had statistically significant reduction effects on *L. monocytogenes* of 1.5-3.5 and 1.0-2.7 logs, respectively (Tsigarida *et al.* 2000).

Four challenge trial studies investigated the use of lactic acid bacteria (*Lactobacillus* spp.) to control pathogens in the final product (Muthukumarasamy *et al.* 2003, Hoyle *et al.* 2009, Ruby and Ingham 2009, Kirsch *et al.* 2017) and reported variable reductions of inoculated *E. coli* O157:H7 of up to 3 logs in minced beef. Other biological interventions include the use of phages, nisin and lactoferricin and were reported in four challenge trial studies (Zhang and Mustapha 1999, Solomakos *et al.* 2008, Cui *et al.* 2017, Stratakos and Grant 2018). Nisin was mostly found to be effective against *E. coli* O157:H7 and *L. monocytogenes* (1-2 logs) as well as phages, with up to 1 log reduction of *E. coli* O157:H7.

Other preservation treatments, such as using various salts, organic acids and other chemical preservatives with or without active packaging films, were investigated in seven studies (Cutter 2000, Ahn *et al.* 2004, Chao and Yin 2009, Ryu and Fung 2010, Marcous *et al.* 2017, Stratakos and Grant 2018, Visvalingam and Holley 2018), with very variable effects depending on the intervention conditions.

Other non-thermal interventions investigated included electron beam and gamma irradiation (Chung *et al.* 2000, Ouattara *et al.* 2002, Turgis *et al.* 2008, Prendergast *et al.* 2009, Ramamoorthi *et al.* 2009, Kundu *et al.* 2014, Li *et al.* 2015), ultraviolet (UV) light irradiation (Kim *et al.* 2014), cold atmospheric plasma (Bauer *et al.* 2017, Stratakos and

Grant 2018) and high-pressure processing (Patel and Solomon 2005, Morales *et al.* 2008, Black *et al.* 2010, Patel *et al.* 2012, Bulut 2014, Hsu *et al.* 2015, Jiang *et al.* 2015, Zhou *et al.* 2016, Chien *et al.* 2017). Irradiation appears to be one of the most effective interventions and is able to deliver complete elimination of inoculated pathogens, with reduction effects >6 logs, whereas UV light was less effective on VTEC, *Salmonella* and *L. monocytogenes* (reductions of up to 1.5 logs after a prolonged period of exposure). High-pressure processing produced highly variable reductions depending on the study conditions, but these reductions were often very high, ranging from 3-5 logs.

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Zhou, Y., Karwe, M. V. and Matthews, K. R. (2016) 'Differences in inactivation of *Escherichia coli* O157:H7 strains in ground beef following repeated high pressure processing treatments and cold storage', *Food Microbiology*, 58, 7-12.

Appendix A: Search strategy details

Full search algorithm used for the search of peer-reviewed literature

Date	14 September 2018
Performed by	Dragan Antic
Databases / Platform	Scopus (1823-2018) / Scopus CAB Direct (1973-2018) / CAB Direct PubMed (1951-2018) / PubMed Agricola (1970-2018) / EBSCO
Search string:	*("Escherichia coli" OR O157 OR shiga* OR STEC OR VTEC OR salmonella OR aerob* OR Enterobacteriaceae) AND (intervention* OR decontaminat* OR contamination OR treatment* OR inactiv* OR reduce* OR reducing OR reduction OR decreas* OR efficacy OR cleaning OR disinfect* OR slaughter* OR hygien* OR HACCP OR dehid* OR dehair* OR skin* OR dress* OR eviscerat* OR bung* OR rodding OR wash* OR rins* OR spray* OR vaccum* OR steam OR pasteuriz* OR pasteuris* OR "hot water" OR chlorine OR "organic acid*" OR "lactic acid" OR irradiat* OR chill* OR cool* OR debon* OR boning OR cut* OR fabricat* OR trim* OR grinding OR mincing OR storage OR packaging OR "modified atmosphere" OR ultraviolet) AND (beef OR veal OR cattle OR bovine OR cow OR cows OR steer OR steers OR heifer* OR bull OR bulls OR calf OR calves OR lairage* OR abattoir* OR slaughterhouse* OR "processing plant*" OR "cutting plant" OR "packing plant" OR knives OR hide* OR carcass*)* in Article title OR in Abstract OR in Key words
Limits	Published since 1996
Hits	Scopus: 13180 CAB Direct: 5223 PubMed: 3695 Agricola: 3329

Details of internet searches for relevant grey literature citations

- scholar.google.co.uk
- www.globalhealthlibrary.net/php/index.php (World Health Organization)
- www.fao.org (Food and Agriculture Organisation of the United Nations)
- www.efsa.europa.eu (European Food Safety Authority)
- www.food.gov.uk/search/research (Food Standards Agency, UK)
- www.vetinst.no/en/reports-and-publications/reports (Norwegian Veterinary Institute, Norway)
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List of search verification articles whose reference lists were hand-searched

Review articles:

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Byelashov, O. A., & Sofos, J. N. (2009). Strategies for on-line decontamination of carcasses. *Safety of meat and processed meat* (pp. 149-182): Springer.

FAO (2016). *Interventions for the control of non-typhoidal Salmonella spp. in beef and pork: Meeting report and systematic review* (Available: http://www.fao.org/3/ai5317e.pdf. Accessed 18 August 2018): Food and Agriculture Organization of the United Nations.

Greig, J., Waddell, L., Wilhelm, B., Wilkins, W., Bucher, O., Parker, S., & Rajić, A. (2012). The efficacy of interventions applied during primary processing on contamination of beef carcasses with *Escherichia coli*: A systematic review-meta-analysis of the published research. *Food Control*, 27(2), 385-397.

Koohmaraie, M., Arthur, T., Bosilevac, J., Brichta-Harhay, D., Kalchayanand, N., Shackelford, S., & Wheeler, T. (2007). Interventions to reduce/eliminate *Escherichia coli* O157: H7 in ground beef. *Meat science*, 77(1), 90-96.

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interventions to control nontyphoidal *Salmonella* in beef and pork. *Journal of food protection*, 79(12), 2196-2210.

Primary research articles:

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List of relevant articles used to pre-test search algorithms in four databases searched to ensure they could be identified

Lairage and hide cleanliness assessment:

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). Source tracking of *Escherichia coli* O157: H7 and *Salmonella* contamination in the lairage environment at commercial US beef processing plants and identification of an effective intervention. *Journal of food protection*, 71(9), 1752-1760.

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Appendix B: Relevance screening, confirmation and data extraction

Relevance screening form

Question	Options
1. Does this citation describe research evaluating the efficacy and/or effectiveness (including costs or practically of implementation) of interventions to control microbiological contamination (with indicator bacteria and pathogens) in beef and beef processing environment at any stage in minced beef production chain from cattle received in abattoir to the packaging and storage inclusive (abattoir and post abattoir level)? **Options 1-3** pass the citation to relevance confirmation stage and the article is procured for this purpose.	1. Yes, primary research 2. Yes, systematic review/ meta-analysis 3. Yes, risk assessment, risk profile, cost-benefit analysis, stochastic modelling 4. No (go to question 2)
2. If no to the above, is the article: i. narrative literature review on beef interventions; or, ii. describing research evaluating the efficacy and/or effectiveness of interventions to control microbiological contamination (with indicator bacteria and pathogens) in sheep/lambs/goats and their processing environment at any stage from their receive in abattoir to the packaging and storage inclusive (abattoir and post abattoir level)? or, iii. describing research on the sources of bacterial contamination of beef and the quantification of their contribution to the cattle hide and beef carcass contamination **Option 1** pass the citation to relevance confirmation stage and the article is procured for this purpose to be used: i. for possible search verification; ii. in case of sparse data for specific beef intervention; iii. to contextualise the relative importance of specific beef intervention.	1. Yes, proceed to the next review stage 2. No (exclude)

Relevance confirmation form

Question	Options	Notes
Relevance confirmation		
Does this article investigate **primary research** on the efficacy and/or effectiveness of interventions to control microbiological contamination (with indicator bacteria and pathogens) in beef and beef processing environment at any stage in minced beef production chain from cattle received in abattoir to the packaging and storage inclusive (abattoir and post abattoir level) and meet the PICOS eligibility criteria?	1. Yes, proceed to data extraction stage 2. No, summarise it narratively – previous systematic reviews, risk assessments and stochastic models 3. No, exclude – measures irrelevant population (study on manufactured, i.e. cured, fermented, dried, tenderised, marinated and ready-to-eat beef) – measures irrelevant outcome (i.e. spoilage) – in vitro study – not primary research – no extractable data – duplicate data – no intervention measured – language other than English – other, specify: _____	"PICOS" elements summarise the population (P), the intervention (I), the comparator (C), the main outcome (O) and the study design chosen (S)
Key primary research article characteristics		
What type of document is this article?	1. Journal article 2. Conference paper 3. Government or research report 4. Thesis 5. Book or book chapter 6. Other, specify _____	
In what regions and country was the study conducted?	1. North America 2. Europe 3. Australia/South Pacific 4. Central and South America/ Caribbean 5. Asia/Middle East 6. Africa 7. Not stated	
Study design:	1. Experimental research: – Controlled trial – Challenge trial	

Question	Options	Notes
	– Before-and-after trial 2. Observational research – Cohort study – Case-control study – Cross-sectional study – Other	
In what setting was the study carried out?	1. Commercial/field conditions 2. Research/pilot plant 3. Laboratory conditions 4. Not reported	
What stage in the minced beef production chain and category of intervention(s) are investigated in this article?	1. Abattoir (pre-slaughter, lairage interventions): – Lairage cleaning – Cattle handling in lairage – Hide cleanliness assessment – Cattle hide interventions (pre-exsanguination) 2. Abattoir (slaughter and post-slaughter): – Cattle hide interventions (post-exsanguination) – Cleaning/disinfection of tools/knives – Standard processing procedures/GHP – Carcass interventions (pre- and post- evisceration, pre-chill) – Chilling and spray chilling – Post chill and pre-fabrication carcass treatments – Multiple interventions/HACCP 3. Post abattoir: – Standard processing procedures/GHP – Post fabrication interventions (trim/ground beef) – Packaging and storage	**Multiple interventions** (multiple-hurdle strategy): usually interventions placed in a single step or (more often) in consecutive steps on a processing line
What outcomes did the study investigate?	1. Aerobic colony counts 2. *Enterobacteriaceae* counts 3. Total coliform counts 4. Generic *E. coli* counts 5. Pathogenic *E. coli* 6. *Salmonella*	

Question	Options	Notes
	7. *Listeria monocytogenes* 8. Other, specify: _____	

Data extraction form

Question	Options
Specify **intervention** category (and subcategory) being extracted and specify stage in the minced beef production chain where intervention is applied	1. Abattoir (pre-slaughter, lairage interventions): – Lairage cleaning – Cattle handling in lairage – Hide cleanliness assessment – Cattle hide interventions (pre- exsanguination) 2. Abattoir (slaughter and post-slaughter): – Cattle hide interventions (post- exsanguination) – Cleaning/disinfection of tools/knives – Standard processing procedures/GHP – Carcass interventions (pre- and post- evisceration, pre-chill) – Chilling and spray chilling – Post chill and pre-fabrication carcass treatments – Multiple interventions/HACCP 3. Post abattoir: – Standard processing procedures/GHP – Post fabrication interventions (trim/ground beef) – Packaging and storage
Intervention description (concentration, temperature, application method, contact time, pressure)	– _____
Specify target (intervention) **population**/sample to which intervention is applied	1. Live animal 2. Cattle hide 3. Carcass 4. Beef primals/subprimals/cuts/trim/variety meats (head, cheek) 5. Ground/minced beef 6. Environment surfaces 7. Tools/knives/equipment
Specify **outcome sample** category	1. Live animal 2. Cattle hide 3. Carcass 4. Beef primals/subprimals/cuts/trim/variety meats (head, cheek) 5. Ground/minced beef 6. Environment surfaces 7. Tools/knives/equipment
What type of sample was measured?	1. Swab (sponge, other) 2. Excised meat sample 3. Ground

Question	**Options**
Specify **comparison** group	1. No treatment 2. Water wash 3. Other: _____
What **outcomes group** did the study investigate?	1. Aerobic colony counts (ACC) 2. *Enterobacteriaceae* (EBC) – *Enterobacteriaceae* counts – Total coliform counts – Generic *E. coli* counts 3. Pathogenic *E. coli* (VTEC) 4. *Salmonella* 5. *Listeria monocytogenes* 6. Other, specify: _____
What **outcomes strains** did the study investigate?	– _____
What outcome data were measured?	1. Concentration (log CFU) 2. Prevalence (presence/absence)
Intervention efficacy results	– log/CFU control – log/CFU treatment – log reduction on an outcome sample – log reduction in transfer to an outcome sample – prevalence in control sample – prevalence in treatment sample
Significant reduction?	– Yes – Not significant – Not provided

Appendix C: Generic flow diagram of beef production processes for application of intervention measures

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

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

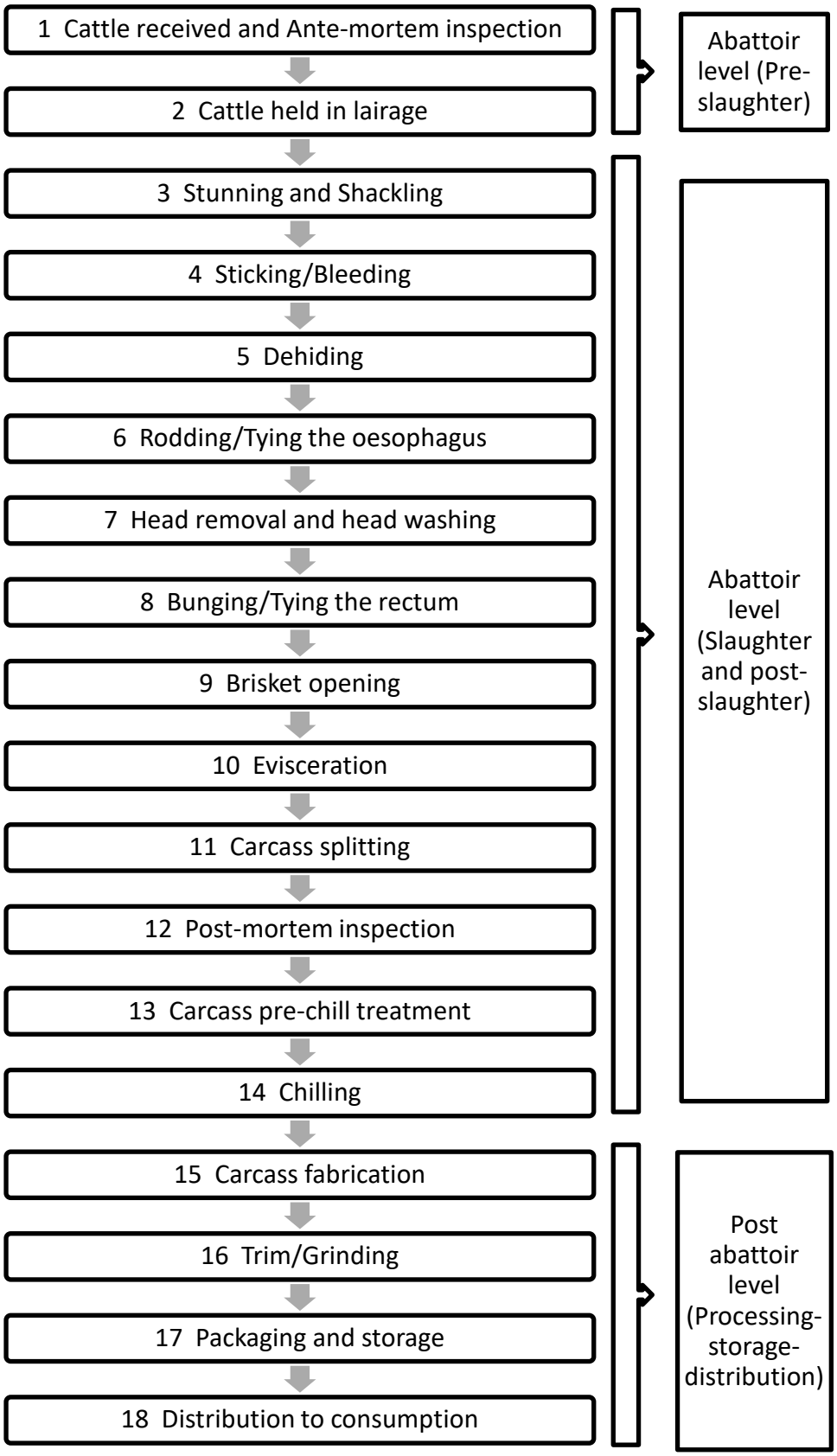
GHP-based measures are pre-requisites to hazard-based measures and are qualitative in nature and based on empirical knowledge and experience. Some examples of GHP-based control measures applied throughout slaughter and dressing process are: cleaning and disinfection of lairage-to-stunning areas, hide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

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

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

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

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



Appendix D: List of intervention measures at abattoir and post abattoir level

Step 1: Cattle received and Ante-mortem inspection

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

GHP-based control measures

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

Step 2: Cattle held in lairage

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

GHP-based control measures

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

Hazard-based intervention measures

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

Step 3: Stunning and Shackling

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

GHP-based control measures

- Frequent cleaning of stunning box and area;

- Hygienic shackling to avoid contact between stick wounds (if sticking is performed in lying position) and contaminated areas.

Hazard-based intervention measures

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

Step 4: Sticking/Bleeding

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

GHP-based control measures

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

Hazard-based intervention measures

- Hide washing with ambient water;
- Hide clipping;
- Thermal interventions;
- Chemical dehairing;
- Organic acid washes;
- Oxidiser chemical washes;
- Other chemical washes;
- Microbial immobilisation treatment of cattle hides with ethanol or aqueous shellac.

Step 5: Dehiding

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

GHP-based control measures

- Using two-knife system with frequent changing knives;
- Knives, equipment and tools sanitation;

- Hide removal methods - mechanical hide pullers used in such way to pull hide away from the carcass (i.e. downward and backward motion).

Hazard-based intervention measures

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

Step 6: Rodding/Tying the oesophagus

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

GHP-based control measures

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

Step 7: Head removal and head washing

Head is severed from the carcass in a hygienic manner.

GHP-based control measures

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

Step 8: Bunging/Tying the rectum

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

GHP-based control measures

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

Step 9: Brisket opening

GHP-based control measures

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

Step 10: Evisceration

GHP-based control measures

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

Step 11: Carcass splitting

GHP-based control measures

- Equipment and tools sanitation.

Step 12: Post-mortem inspection

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

GHP-based control measures

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

Step 13: Carcass pre-chill treatment

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

Hazard-based intervention measures

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

Step 14: Chilling

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

GHP-based control measures

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

Hazard-based intervention measures

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

Step 15: Carcass fabrication

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

GHP-based control measures

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

Hazard-based intervention measures

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

Step 16: Trim/Grinding

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

GHP-based control measures

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

Hazard-based intervention measures

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

Step 17: Packaging and storage

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

GHP-based control measures

- Temperature controls in packaging room.

Hazard-based intervention measures

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

Step 18: Distribution to consumption

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