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Review

Beef abattoir interventions in a risk-based meat safety assurance system

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ABSTRACT

In risk-based meat safety assurance system, the use of interventions is intended to accomplish the meat safety targets on chilled carcasses, particularly in situations when an abattoir is unable to sufficiently reduce risks arising from specific farms/animal batches by using process hygiene alone. Furthermore, interventions are considered whenever food safety authorities identify meat production processes associated with high risks for consumers. This paper overviews the role of beef interventions in a risk-based, meat safety assurance system. Cattle hide interventions (chemical hide washes and microbial immobilisation treatment with shellac) and beef carcass interventions (pasteurisation treatments with hot water and/or steam and organic (lactic) acid washes), show consistent reduction effects of aerobic bacteria and faecal indicators and reduced prevalences of naturally present VTEC and *Salmonella*. The review also identified interventions where there was a lack of data and further research was needed, and other contextual factors to inform the risk management decisions for further development of risk-based meat safety assurance system.

1. Introduction

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 (RB-MSAS). In such a system, high-risk animal batches are subjected to additional slaughter hygiene control measures complemented with (hide and meat) interventions (Blagojevic et al., 2021; EFSA, 2013a). These recent efforts in the modernisation of meat inspection and its transformation into RB-MSAS integrate both meat inspection procedures and Food Business Operators' (FBO) food safety management systems (FSMS) and other relevant aspects into a coherent whole (Blagojevic & Antic, 2014; Buncic et al., 2014).

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, 2013a). This decision was made through a risk ranking process which was based on the reported incidence and severity of the diseases in humans that were attributed to beef (EFSA, 2013a). *Salmonella* and VTEC are shed by clinically healthy cattle and further spread via various routes on the farms to other cattle, and thus, can be regularly found in their faeces and/or hides. Their total elimination is difficult to achieve at the pre-harvest stage of the meat chain even with the numerous farm risk mitigation measures available (Nørrung, Andersen, & Buncic, 2009). Nevertheless, it is indubitable that large differences exist between cattle farms regarding husbandry practices and risk factors for *Salmonella* and VTEC (EFSA, 2013a). Hence, it is expected that the colonisation status of cattle entering abattoirs as related to these two high-priority hazards, but also hazards of low priority, might differ significantly. Information on the risk category of each animal, batch and/or farm (i.e. being supplied from the farm of origin through the food chain information; FCI) would, in that case, be very useful for abattoir risk managers, enabling them to handle different risk categories appropriately (Blagojevic et al., 2021).

The control of pathogens in the beef chain requires use of Good Manufacturing Practice/Good Hygienic Practice (GMP/GHP) and Hazard Analysis and Critical Control Point (HACCP) based procedures. 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). In some countries, e.g. the USA, decontamination treatments of hides and carcasses are regularly used and integrated within an

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intervention-based HACCP system (Koohmaraie et al., 2005, 2007, Buncic & Sofos, 2012, Wheeler, Kalchayanand, & Bosilevac, 2014); such interventions have not been commonly used under commercial conditions in Europe. There is, however, provision for the use of decontamination strategies in abattoirs in the EU. The EU Regulation 853/2004 allows, in principle, the use of decontamination treatments during slaughter, following appropriate consideration and a risk assessment by EFSA and approval of such treatments by the regulatory authorities (EC., 2004; EFSA, 2010). 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 EU abattoirs (EC, 2013). 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 (Bosilevac, Arthur, et al., 2004). Therefore, interventions must not be a substitute for GHP, but only an additional measure.

Abattoirs differ in their capacities, technology and equipment, hygiene training, staff and management motivation (Alvseike et al., 2019; Djekic et al., 2016), which can be correlated with their different hygienic performances and potentially with *Salmonella*/VTEC presence on dressed carcasses (Alvseike et al., 2019; Blagojevic, Antic, Ducic, & Buncic, 2011). This can be a challenge; therefore, risk categorisation of beef abattoirs appears to be essential when developing RB-MSAS. It is currently based on verification of their HACCP system performance through Process Hygiene Criteria (PHC), i.e. testing for aerobic colony count, *Enterobacteriaceae* count and *Salmonella* presence (EC, 2005) on pre-chilled beef carcasses. There are no PHC set for VTEC in the EU legislation; however, process hygiene measures utilised to control *Salmonella* are expected to have effect on VTEC as well (EFSA, 2013a).

In the RB-MSAS (Fig. 1), the regulatory authority will set meat safety targets for chilled carcasses in abattoirs. The targets (Performance Objectives; POs) need to be clear and measurable and need to contribute to the Food Safety Objective and Appropriate Level of Protection. The system is supposed to be coordinated by a risk manager who will be responsible for adjusting control options in the farm-to-abattoir meat chain, ultimately ensuring that the hazard-based targets for chilled carcasses are achieved. Therefore, the risk manager's task will be to analyse FCI and balance between animals/farms and abattoirs based on their risk categorisations. This can lead to different decisions (Fig. 1), e.g. whether high or low risk animals will be sent to high or low risk abattoirs; and to use additional abattoir interventions when these are the only available solution to meet the microbiological targets in carcasses (Blagojevic et al., 2021; EFSA, 2013a; EFSA, 2013b).

To lower the abattoir risk category and to accomplish the targets on chilled carcasses, various control measures (interventions) are available to be applied at the harvest stage of the meat chain (Nørrung et al., 2009). They are usually GHP- and hazard-based measures (FAO, 2016). GHP-based measures are founded on empirical knowledge and experience (e.g., hide removal methods, rodding, bunging, knife-trimming, chilling, equipment sanitation). Such measures serve as pre-requisites to, and complement, the hazard-based interventions that are evidence-based, i.e., developed from scientific research to control certain hazard(s). The examples include a range of skin and carcass interventions mostly aiming at microbial removal, immobilisation or elimination, and they provide demonstrable and quantifiable reductions in hazard loads (FAO, 2016). 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, to reduce microbial load further, 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 decontamination of resulting beef trimmings (Antic et al., 2018; Blagojevic et al., 2015). 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 (Antic, 2018).

The key events and decisions that have contributed to the development of interventions in the USA beef industry, mainly in relation to the

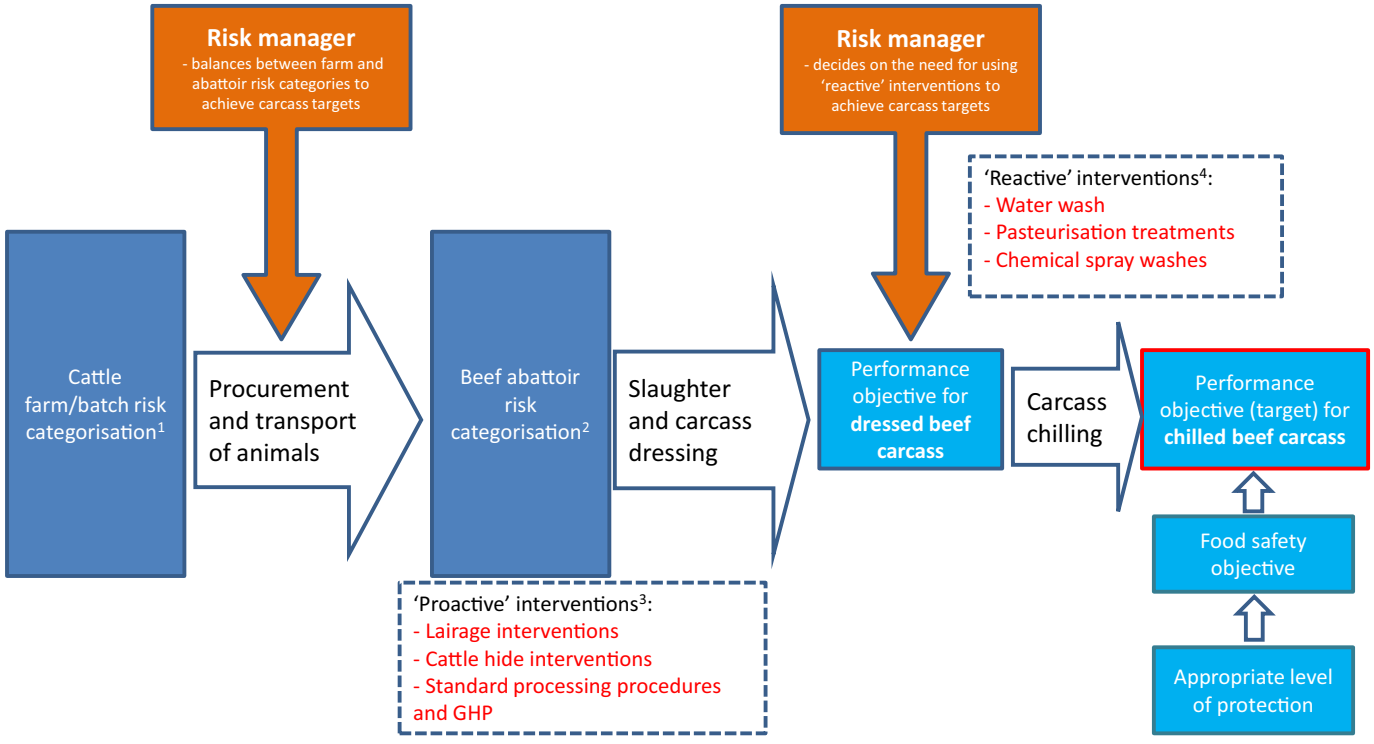

Fig. 1. Generic outline of the risk-based meat safety assurance system in beef abattoirs highlighting the role of interventions, adapted from Blagojevic et al. (2021).

presence and control of *E. coli* O157:H7 and *Salmonella* in ground beef, were discussed in details in previous reviews on the topic (Koohmaraie et al., 2007, 2005, Wheeler et al., 2014). This paper aims to overview the role of beef interventions as an essential component of a RB-MSAS, with a focus on interventions used or investigated under commercial conditions in abattoirs, and provide an update with the most recent findings. For that purpose, a broad critical review of the literature on the contribution of beef abattoir interventions for the reduction of bacterial load on beef carcasses was conducted, with a focus on the pre- and post-slaughter production processes in abattoirs, up to and including primary chilling.

2. Beef abattoir interventions in the risk-based meat safety assurance system

The review considered evidence on beef intervention efficacy available in the public domain, but only primary research studies were used for data extraction and reporting. The populations included cattle produced for meat consumption, including their carcasses at primary processing, but also sources of beef contamination during processing (i.e. cattle hides). Relevant outcome measures for interventions were the effectiveness of each intervention in reducing logarithm transformed counts of indicator bacteria (aerobic colony count (ACC), *Enterobacteriaceae* count (EBC) and generic *E. coli* count) and logarithm transformed counts or prevalences of foodborne pathogens (primarily *E. coli* O157 and other non-O157 VTEC serogroups and *Salmonella*). The outcome measures were analysed as: i) reduction on a treated substrate (i.e. cattle hide and carcass meat surfaces); and ii) reduction in transfer to a substrate (usually carcass meat) from the contamination source (i.e. cattle hide). Any GHP- and hazard-based interventions applied from cattle received in abattoirs up to and inclusive of primary chilling in abattoirs were considered relevant. A full description of the reviewed interventions is presented in Table 1 (Antic, 2018). All experimental and observational study designs were considered for data extraction (controlled, challenge and before-and-after trials, and cross-sectional studies), from studies conducted under laboratory and pilot plant conditions (i.e. in a more controlled environment, often using artificially inoculated microbiota), and commercial abattoir conditions (i.e. a less controlled environment).

Database searches were implemented in the bibliographic databases Scopus, CAB Direct, Agricola and PubMed searching for a literature published from 1996 to 2018. An updates search was also conducted to identify and include relevant articles published in 2019 and 2020. Only articles published after 1996 were included 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 the USA in 1996 and was followed with later requirements for in-plant validation on interventions, with many research studies published after this date (FSIS, 1996). For the purposes of this review, we report analysis of the results from studies conducted under commercial conditions in tabulated form. Results on quantitative log reductions from challenge trials (usually those conducted under the laboratory/pilot plant conditions) are available in the technical report (Antic, 2018).

2.1. Lairage interventions

2.1.1. Cattle handling in lairage

Lairage can be an important risk factor for cattle hide (and subsequently carcass) microbial contamination. Prolonged holding times in lairage can lead to increased contamination of the animals' coats. Change in *Salmonella* and *E. coli* O157:H7 prevalences in cattle between pens on-farm and at the abattoir was shown in the study of Arthur et al. (2008), with increases from 0.7% and 66% on farm to 74.2% and 76.8% in the abattoir lairage, respectively. Application of pulsed field gel electrophoresis methodology demonstrated that 46.9% and 65.1% of

Table 1
Description of GHP- and hazard-based beef abattoir interventions.

Intervention stage, category and subcategory	Definition
Lairage interventions	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
Cattle handling in lairage	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	The scoring and categorisation of hide cleanliness before cattle slaughter according to the established objective system, and actions taken when animals are too dirty to be processed hygienically
Cattle hide interventions (live animal, pre- exsanguination)	All procedures in place which are available for use ante mortem to deal with animals that are excessively soiled, but that do not compromise animal welfare
Hide clipping	Clipping or shaving hair from hide surface to physically remove contamination from hides
Bacteriophage treatment	Treatment with bacteriophages, viruses that infect and kill bacteria
Cattle hide interventions (post-exsanguination)	Cattle hide treatments after animal stunning and bleeding, before dehiding
Chemical washes	i) Oxidisers (electrolysed oxidised (EO) and ozonated water, peroxyacetic acid (PAA), hypobromous acid and hydrogen peroxide); ii) Quaternary ammonium compounds (QAC) (different proprietary sanitisers); iii) Other chemicals (chlorine solutions, cetylpyridinium chloride (CPC), sodium hydroxide, sodium metasilicate, trisodium phosphate (TSP))
Thermal interventions for cattle hides	Various heat treatment washes, rinses and sprays to destroy microbial cells. Examples include scalding hide-on bobby calf carcasses (usually >60 °C), hot water (usually >74 °C) and treatments with steam (usually >82 °C)
Hide chemical dehairing	Process of applying successive water and chemical washes (sodium sulphide followed by a neutralizing solution of hydrogen peroxide) in a cabinet to remove hair and improve visible cleanliness and reduce microbial loads on animal hides
Microbial immobilisation treatments ('shellac hide coating')	Spray treatment of cattle hides with natural resin shellac, to form a protective coating as a barrier to microorganisms, resulting in the reduction in their transfer to beef carcasses
Beef carcass interventions	Beef carcass treatments after dehiding (washes, rinses and sprays aimed at microbial removal and/or killing, applied pre- and/or post- evisceration (pre-chilling))
Standard processing procedures and GHP	A range of different practices that are pre-requisites to hazard-based interventions, are qualitative in nature and based on empirical knowledge and experience and may have a pathogen-reduction effect
Bung bagging (bunging)	Closing off the rectum by cutting around the anus, placing a bag over the rectum and securing it in place with an elastic band or similar during evisceration, to minimise the spread of contamination on a carcass
Trimming	Physical removal of visible contamination from carcasses with knife
Steam vacuuming	Spot application of steam and/or hot water (usually >82 °C) to loosen contamination and kill bacteria, followed by vacuuming
Water wash	Ambient or cold-temperature wash to physically remove contamination from carcass surface. Warm water washes (usually <60 °C)

(*continued on next page*)

Table 1 (*continued*)

Intervention stage, category and subcategory	Definition
	have a similar effect in removing bacteria (depending on the pressure used), and when applied for a short time do not have a microbicidal effect
Organic acid washes	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	Include 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 oxidised (EO) water (acidic, alkaline or neutral), ozonated water, peroxyacetic acid (PAA), acidified sodium chlorite (ASC), hydrogen peroxide, trisodium phosphate (TSP))
Hot water wash	Washing carcasses with water at temperatures >74 °C, up to 85 °C
Steam pasteurisation	Steam (usually >82 °C, up to 105 °C) applied to a whole beef carcass in a closed cabinet. Method involves: i) removal of water from carcass side surfaces, which remains after post-evisceration washing, using air blowers or vacuum; ii) surface "pasteurisation" with pressurized steam (6.5–10 s); and iii) a cold-water spray to cool down carcass surfaces before they are moved to chillers
Chilling	Reducing the carcass temperature to prevent microbial growth, immediately after slaughter and dressing process
Dry chilling	Chilling following all dressing procedures on the slaughterline without the use of any additional spray (acid or water spray)
Spray chilling	Intermittent spraying beef carcass with water during the first several hours of the whole cooling process
Multiple interventions	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 may involve use of interventions on cattle hides, followed by skinned carcass knife trimming, steam vacuuming, pre-evisceration washing, washing, thermal decontamination with water or steam, organic acid rinsing, chilling, and chemical spraying before carcass fabrication

E. coli O157:H7 and *Salmonella* hide strains were attributable solely to the lairage environment, whereas 67% and 30% of the carcass *E. coli* O157:H7 and *Salmonella* strains, respectively, could be attributed solely to the lairage environment (Arthur et al., 2008). 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 hides at slaughter compared with cattle held in culture-negative pens (RR = 8.0; 95% CI (1.6–38.8)). Furthermore, a batch of cattle that was held in lairage pens contaminated with faeces had three times greater risk of positive hides 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)).

Extensive hide and carcass cross-contamination from the lairage environment was found in a study by Collis et al. (2004). They found an increase in the prevalence of a hide marker inoculated onto the hides of 11% of cattle at unloading, to 100% (hide before skinning) and 88.8% (skinned carcass) of examined surfaces. 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) of examined surfaces. The extensive spread of microbial contamination between animals from different holding pens was likely mediated by post-pen environmental surfaces, races and stunning boxes. All these findings highlight the importance of lairage in transmission of hide-level contamination.

2.1.2. Cattle hide cleanliness assessment

Scoring of hide cleanliness before cattle slaughter is a measure commonly implemented in only a few countries, mainly in Europe, such as in the UK, Ireland and Norway (Anon, 2016; Hauge, Nafstad, Røtterud, & Nesbakken, 2012; Hughes, 2001; McEvoy et al., 2000). The UK scoring system is based on a five-category scale. Categories 1 and 2 are visually clean hides with minor quantities of dirt; category 3 are moderately dirty hides (with (dry) dirt covering cutting lines) and categories 4 and 5 are very dirty hides, usually with damp or wet dirt (covering large areas of hide). The Norwegian scoring system is based on a simplified three-category scale (i.e. category 0 (clean), category 1 (moderately dirty) and category 2 (dirty animals)). Similarly, the Irish scoring system is based on a three-category scale: satisfactory, acceptable and unacceptable (Anon, 2016).

In the study of Blagojevic, Antic, Ducic, and Buncic (2012), the mean ACC and EBC on hides and final carcases differed significantly between very dirty cattle (categories 4 and 5) and less dirty or clean cattle (categories 1, 2 and 3 scored according to the UK scoring system). The increase in carcass bacterial load was 1.1 and 0.7 logs, respectively, with increased hide dirtiness. Hauge et al. (2012) reported a 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 in ACC or *E. coli* counts between clean and very dirty animal groups. A similar observation was made in their later study conducted in two commercial abattoirs, wherein carcasses after dehiding showed no significant difference in the number of generic *E. coli* and *Enterobacteriaceae* between clean and very dirty cattle (Hauge et al., 2015). The authors hypothesised that this finding could be plant dependant and due to more careful dehiding of very dirty animals.

Serraino et al. (2012) also showed 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 ACC, 0.7–1.5 logs for *Enterobacteriaceae* and 0.6–0.8 logs for generic *E. coli*. In most cases, the contamination levels decreased with decreasing cattle hide dirtiness, 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. An earlier study 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 previous Irish scoring system used at the time) compared to dirty animals (categories 3 and 5) (McEvoy et al., 2000).

2.1.3. Cattle hide interventions (pre-exsanguination)

Very little research was identified on cattle hide interventions pre-slaughter. The results from studies conducted under commercial conditions are presented in Tables 2 and 3.

The effect of live animal washing was investigated in a study by Mies et al. (2004). They found that a single or double water wash and a lactic acid or 50 ppm chlorine solution wash resulted in an increase in ACC and *E. coli* from 0.1 to 0.8 log CFU/cm^2, and, mostly, increased *Salmonella* prevalence on the hides. The reason for this was likely 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).

Two controlled studies investigated washing cattle hides with water and cetylpyridinium chloride (CPC), which, applied under pilot plant

Table 2
Summary of findings for cattle hide interventions: studies under commercial conditions measuring bacteria counts.

Intervention‡	No. studies/ design	Intervention/outcome surface	Microorganism	Log_{10} CFU reduction[a]	Reference
Pre-exsanguination interventions					
Water wash & CPC (1%)	1/CT	Live animal hide/ carcass*	Aerobic bacteria	1.5	Bosilevac, Arthur, et al. (2004)
			Enterobacteriaceae	1.1	
Post-exsanguination washing/clipping					
Water wash/manual curry comb	1/BA	Veal calf hide	Aerobic bacteria	0.8	Wang et al. (2014)
			Enterobacteriaceae	3.5	
			E. coli	1.6	
Warm water wash	1/BA	Hide cut lines	Aerobic bacteria	0.1	Scanga et al. (2011)
Hide clipping (dirty hides)	2/CT	Hide/carcass*	Aerobic bacteria	0.1–0.3	Van Donkersgoed et al. (1997), McCleery et al. (2008)
			E. coli	0.3	
Organic acids					
Acetic acid (5%)	1/BA	Hide cut lines	Aerobic bacteria	2.6	Scanga et al. (2011)
			E. coli	3.7	
Lactic acid (6%)	1/BA	Hide cut lines	Aerobic bacteria	2.3	
			E. coli	3.7	
Other chemicals					
Chlorine/ASC (200 ppm)	1/BA	Veal calf hide	Aerobic bacteria	1.3	Wang et al. (2014)
			Enterobacteriaceae	1.5	
			E. coli	1.0	
Water wash & sodium hydroxide (1.5%)	1/CT	Hide/carcass*	Aerobic bacteria	0.8	Bosilevac, Nou, et al. (2005)
			Enterobacteriaceae	0.8	
Water wash & sodium hydroxide (1.5%)	2/BA	Hide	Aerobic bacteria	1.5–2.1	Bosilevac, Nou, et al. (2005), Yang, Badoni, Tran, and Gill (2015)
			Enterobacteriaceae	3.4	
Sodium hydroxide (3%)	1/BA	Hide cut lines	Aerobic bacteria	1.6	Scanga et al. (2011)
			E. coli	3.5	
TSP (20%)	1/BA	Hide	Aerobic bacteria	1.8	Çalicioğlu et al. (2010)
Ethanol (75%)	1/BA	Hide	Aerobic bacteria	1.2	
A proprietary QAC sanitiser & vacuuming	1/CT	Hide/carcass*	Aerobic bacteria	1.0	Antic et al. (2011)
			Enterobacteriaceae	1.3	
			E. coli	1.2	
Chemical dehairing and thermal interventions					
Chemical dehairing	1/CT	Hide/carcass*	Aerobic bacteria	2.0	Nou et al. (2003)
			Enterobacteriaceae	1.8	
Hot water wash	1/BA	Hide cut lines	Aerobic bacteria	3.6	Çalicioğlu et al. (2010)
Chlorine spray & hot water rinse	1/BA	Veal calf hide	Aerobic bacteria	2.1	Wang et al. (2014)
			Enterobacteriaceae	2.7	
			E. coli	2.6	
Microbial immobilisation treatments					
Shellac in ethanol hide coating	1/CT	Hide/carcass*	Aerobic bacteria	1.7	Antic et al. (2011)
			Enterobacteriaceae	1.4	
			E. coli	1.3	
Aqueous shellac hide coating	1/CT	Hide/carcass*	Aerobic bacteria	0.3–1.1	Antic et al. (2018)
			Enterobacteriaceae	0.1–0.7	

‡ Acidified sodium chlorite (ASC), trisodium phosphate (TSP), cetylpyridinium chloride (CPC), quaternary ammonium compounds (QAC).
* Reduction in hide-to-carcass transfer.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all cases "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials).

conditions, reduced ACC and *Enterobacteriaceae* by 1.9–4.4 and 1.3–3.8 logs on hides (depending on the water pressure used), respectively (Bosilevac, Wheeler, et al., 2004). This treatment, when applied under commercial conditions in an abattoir, yielded promising reductions in hide-to-carcass transfer of both groups of indicator bacteria (by 1.5 and 1.1 logs, respectively) and also reduced the prevalence of naturally present *E. coli* O157 (from 23% to 3%) (Bosilevac, Arthur, et al., 2004). All three studies using chemicals on live animals concluded that the treatments were more appropriate for application post-exsanguination due to animal welfare concerns.

Three studies evaluated the effect of bacteriophage sprayed onto cattle hides with very variable results. One controlled abattoir trial demonstrated that bacteriophage treatment before processing did not produce a significant reduction in *E. coli* O157:H7 prevalence on cattle hides or beef carcasses during processing (Arthur, Kalchayanand, Agga, Wheeler, & Koohmaraie, 2017). Two challenge trials under laboratory conditions reported from 0.5 to 2 log reductions in inoculated *E. coli* O157:H7 on cattle hide sections after 1 h exposure (Coffey et al., 2011; Tolen, Xie, Hairgrove, Gill, & Taylor, 2018).

2.2. Cattle hide interventions

Hide interventions described in the previous section (aside from the bacteriophage treatment) are more appropriate for use after animal stunning and bleeding due to multiple factors (animal welfare, technical requirements, occupational risks, etc.). For the majority of investigated physical and chemical hide interventions at post-exsanguination, no validation under full commercial conditions was provided. 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 can only indicate the potential reduction in transfer of bacteria to resulting beef carcasses (Antic, 2018). Consequently, the only relevant measurement of cattle hide intervention efficacy is microbial status of resulting beef carcasses immediately after dehiding. Moreover, even if 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. Seven controlled trials conducted under commercial conditions post-exsanguination reported hide intervention effects on resulting beef carcass surfaces: one study on hide washing with sodium hydroxide and

Table 3
Summary of findings for cattle hide interventions: studies under commercial conditions measuring prevalence reductions.

Intervention‡	No. studies/ design	Intervention/outcome surface	Microorganism	% of samples positive in study population		Reference
				No treat-ment[a]	Treat-ment	
Pre-exsanguination interventions						
Water wash	1/BA	Live animal hide/hide	*Salmonella*	36–58%	40–72%	Mies et al. (2004)
Lactic acid (0.5%)	1/BA			50.0%	52.2%	
Chlorine	1/BA			60.0%	55.6%	
Water wash & CPC (1%)	1/CT	Live animal hide/hide	*E. coli* O157	56%	34%	Bosilevac, Arthur, et al. (2004)
	1/CT	Live animal hide/carcass*	*E. coli* O157	23%	3%	
Bacteriophage Finalyse® spray	1/CT	Live animal hide/hide	*E. coli* O157:H7	57.6	51.8	Arthur et al. (2017)
	1/CT	Live animal hide/carcass*	*E. coli* O157:H7	17.6	17.1	
Post-exsanguination washing/clipping						
Water wash	1/BA	Hide	*E. coli* O157:H7	62.5%	38.4%	Arthur et al. (2008)
			Salmonella	88.1%	24.3%	
Water wash & chlorine	2/BA	Hide	*E. coli* O157:H7[b]	4–35%	1–13%	Arthur et al. (2007), Bosilevac et al. (2009)
			Salmonella[b]	27–40%	7-13%	
Water wash/manual curry comb	1/BA	Veal calf hide	*E. coli* O103	26%	17%	Wang et al. (2014)
			E. coli O111	23%	17%	
Warm water wash	1/BA	Hide cut lines	*E. coli* O157:H7	78.0%	84.0%	Scanga et al. (2011)
			Salmonella	68.0%	88.0%	
Organic acids						
Acetic acid (5%)	1/BA	Hide cut lines	*E. coli* O157:H7	76%	30%	Scanga et al. (2011)
Lactic acid (6%)	1/BA		*E. coli* O157:H7	84%	56%	
			Salmonella	74%	50%	
Other chemicals						
Water wash/sodium hydroxide (1.5%)	1/CT	Hide/carcass*	*E. coli* O157	17%	2%	Bosilevac, Nou, et al. (2005)
	1/BA	Hide		44%	16%	
Sodium hydroxide (3%)	1/BA	Hide cut lines	*E. coli* O157:H7	94%	41%	Scanga et al. (2011)
			Salmonella	60%	43%	
Chemical dehairing						
Chemical dehairing	1/CT	Hide/carcass*	*E. coli* O157:H7	50%	1%	Nou et al. (2003)

‡ Cetylpyridinium chloride (CPC).
* Reduction in hide-to-carcass transfer.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all cases "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials).
[b] 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.

one using QAC sanitiser & vacuuming; one investigating chemical dehairing; two studies on microbial immobilisation treatments with ethanol and aqueous shellac solutions and; two studies on hide clipping (Table 2).

2.2.1. Hide washing and clipping

Hide washing post-exsanguination with potable, ambient or cold water was investigated either as a main intervention or as a control treatment for chemical washes. Under pilot plant conditions, up to 1 log reduction of ACC, EBC and *E. coli* on washed hides was achieved (Bosilevac, Nou, et al., 2005, Bosilevac, Shackelford, et al., 2005, Carlson et al., 2008). The efficacy increased if high-pressure washing and additional vacuuming (Bosilevac, Nou, et al., 2005) or manual curry comb were used (Wang, Koohmaraie, Luedtke, Wheeler, & Bosilevac, 2014). No increase in efficacy was observed when warm water at 60 °C was used (Bosilevac, Shackelford, et al., 2005). In addition, the VTEC and *Salmonella* prevalence was significantly reduced on washed hides using plant commercial washing systems (Arthur et al., 2007; Arthur et al., 2008; Bosilevac et al., 2009).

With respect to hide clipping, McCleery, Stirling, McIvor, and Patterson (2008) found that carcasses derived from dirty, hide-clipped cattle had comparable bacterial counts to those from non-clipped, but clean animals (ACC reductions of 0.1–0.3 logs were achieved by hide clipping). In the similar study of Van Donkersgoed, Jericho, Grogan, and Thorlakson (1997), the reductions achieved in abattoir conditions were similar, with a decrease of up to 0.3 logs of aerobic bacteria and faecal indicators, so the authors concluded that the clipping is of questionable practical significance. However, it could be a useful pre-treatment to a subsequent hide washes with chemicals.

2.2.2. Hide washing with organic acids

Highly variable and conflicting results were reported in several studies on organic acid sprays/washes on cattle hides, with most studies conducted in pilot plants and laboratory conditions. Spraying/rinsing with lactic and acetic acid under pilot plant conditions achieved 2–2.5 log reductions of aerobic bacteria and generic *E. coli* (Carlson et al., 2008), while similar treatments under laboratory conditions were highly variable (from 0.5 up to 5 logs of inoculated microbiota) (Antic, 2018). 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). 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 microbiota (ACC and *E. coli*), respectively, on treated hides (Scanga et al., 2011).

2.2.3. Hide washing with other chemicals/oxidisers

A range of different oxidisers (electrolysed oxidised (EO) and ozonated water, peroxyacetic acid, hypobromous acid and hydrogen peroxide) have been investigated for use as cattle hide wash/spray treatments post-exsanguination. Under pilot plant conditions, EO and ozonated water and hypobromous acid significantly reduced general and faecal microbiota by 2.0–3.5 and 2.0–4.0 logs, respectively, and reduced prevalences of *E. coli* O157:H7 and *Salmonella* on treated hides by roughly 3-fold (Bosilevac, Shackelford, et al., 2005, Schmidt, Arthur, et al., 2012).

Furthermore, various other chemicals have been used in commercial or laboratory 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). Spraying/rinsing hides with sodium hydroxide and sodium metasilicate under pilot plant conditions achieved 1.5–3 log reductions of ACC and generic *E. coli* (Carlson et al., 2008). Under commercial conditions, automated hide washes with sodium hydroxide achieved significant reduction in transfer to carcasses of both ACC and EBC of 0.8 logs, and the prevalence of *E. coli* O157 reduced from 17% to 2%. Reductions of ACC and EBC achieved on treated hides were 2.1 and 3.4 logs, respectively, and the prevalence of *E. coli* O157 reduced from 44% to 16% (Bosilevac, Nou, et al., 2005). Additional vacuuming increased the ACC and EBC reductions by 1–2 logs (Bosilevac, Nou, et al., 2005).

2.2.4. Chemical dehairing and thermal interventions

Given the fact that the hide is damaged when using harsh treatments, these interventions are more suitable for bobby calf carcasses, which usually stay with the skin-on, or in situations where hides are not used for leather production. Chemical dehairing to remove hairs, dirt, faeces and microbial contamination from cattle hides comprises treatments using strong acids or alkali (usually sodium sulphide and hydrogen peroxide). In one controlled trial in an abattoir, chemical dehairing treatment significantly reduced *E. coli* O157 prevalence and ACC and *Enterobacteriaceae* counts on pre-evisceration carcasses (Nou et al., 2003). It was demonstrated as the most effective intervention to reduce hide to carcass transfer of contamination, however a feasible approach for its commercial implementation is yet to be determined (Nou et al., 2003).

One challenge study conducted in a pilot plant investigated different single or multiple treatments for bobby calf carcasses, which stay with the hide-on throughout the dressing process. Scalding at temperatures >60 °C reduced inoculated *E. coli* by 2–4 logs and the treatment efficacy was significantly improved when using an additional hot water wash (82 °C) and/or lactic acid (4.5%) spray, with reductions from 4.5–6.3 logs on treated hides (Hasty et al., 2018). In commercial conditions, hot water (under 80 °C) alone (Çalicioğlu, Buege, & Luchansky, 2010) or in combination with chlorine spray (Wang et al., 2014) also significantly reduced both ACC and EBC by 2–3.5 logs.

Two studies investigated the application of steam for the decontamination of cattle hides (McEvoy et al., 2003; McEvoy, Doherty, Sheridan, Blair, & McDowell, 2001). 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, reductions being 1.9–6.0 logs. However, hide quality was severely damaged by this thermal intervention, making it unsuitable for practical application in commercial settings.

2.2.5. Microbial immobilisation treatments

An innovative method of immobilising bacteria on cattle hides was developed using shellac, a natural, food-grade resin, used in ethanol or aqueous solution and sprayed on hides (Antic et al., 2010; Antic et al., 2018; Antic, Blagojevic, & Buncic, 2011). In a laboratory model system, spraying hides with the shellac solution in ethanol markedly reduced the counts of all indicator bacteria (by up to 6.6 logs) and prevalences 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 in transfer of up to 3.6 logs of all indicator bacteria (Antic et al., 2011). Post-slaughter but pre-skinning treatment of hides with a shellac solution in a commercial abattoir significantly reduced (up to 1.7 logs) ACC, EBC and generic *E. coli* counts on beef carcasses (Antic et al., 2011). Overall, the shellac hide coating treatment significantly reduced the risk of cross-contamination from hide to carcass, but 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 ACC and EBC under laboratory conditions were achieved, respectively (Antic et al., 2018). Validation of the treatment under commercial abattoir 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).

2.3. Beef carcass interventions

2.3.1. Standard processing procedures and GHP

In two abattoir trials, the procedure of tying the rectum (bung bagging) to prevent faecal spillage reduced both ACC and EBC by around 1 log (Saleh, El-Maghraby, & El-Morabit, 2012) and significantly reduced the prevalences of VTEC and *Salmonella* (Stopforth, Lopes, Shultz, Miksch, & Samadpour, 2006). Improved hide removal practices appear to reduce transfer of ACC, EBC and generic *E. coli* from hide to carcasses by up to 1 log (Table 4). However, in the only controlled trial available, there was no improvement in the microbial status of beef carcasses regarding aerobic bacteria after hide removal when a supposedly better downward hide removal technique was used and compared to upward hide pulling (Kennedy, Giotis, & McKevitt, 2014).

2.3.2. Pre-chilling carcass treatments

Studies investigating beef carcass interventions post-dehiding and pre-chilling reported a range of different conditions among physical and chemical interventions (temperature, contact time, pressure, mode of application (wash, spray, rinse, dip, deluge, manual or automated), number of samples and sampling method used), and large variations in magnitude of effect are seen across studies. Therefore, the results on intervention efficacy are not directly comparable. Overall, hot water wash and lactic acid, as a standalone intervention or in combination, were by far the most commonly investigated interventions under commercial conditions (Tables 4 and 5).

Water wash with ambient or cold water to remove microorganisms was largely ineffective, with up to 0.5 log reductions of ACC and generic *E. coli* achieved, and dependant on washing time and pressure used. However, in combination with organic acids, the reduction effect for aerobic bacteria appears to increase by 0.5 logs. Trimming of visually contaminated sites with knife reduced ACC and generic *E. coli* by 1–2 logs (Table 4). Furthermore, two abattoir challenge trials used non-pathogenic food-grade organisms to artificially inoculate carcasses. Knife trimming in combination with water and/or hot water rinsing reduced *E. coli* and coliforms by 1.3–1.8 logs (Graves Delmore, Sofos, Reagan, & Smith, 1997; Reagan et al., 1996).

Hot water washing consistently reduced all indicator bacteria by 1–2.5 logs, with further 0.5–1 log reductions if organic acids were used sequentially. The temperatures of carcass surfaces pasteurised with hot water were usually more than 70 °C. The time-temperature combinations required to achieve significant microbiota reductions were typically specific to an individual commercial abattoir. Furthermore, both spot steam vacuuming and whole carcass steam pasteurisation reduced all indicator bacteria by around 1–1.5 logs (Table 4).

Organic acid carcass washes, alone (lactic, acetic or citric) or as a mixture, were effective on-line interventions. Greater reductions were reported for lactic acid (1–2 logs of all indicator bacteria) than other acids (usually up to 1 log). Mixtures of organic acids did not provide any added beneficial effect and reductions were around 1 log for all indicator bacteria (Algino, Ingham, & Zhu, 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 further 1 log reductions for all indicator bacteria (Table 4).

The majority of reported studies were challenge trials under pilot plant and laboratory conditions. 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

Table 4
Summary of findings for beef carcass interventions: studies under commercial conditions measuring bacteria counts.

Intervention‡	No. studies/ design	Intervention/ outcome surface	Microorganism	Log_{10} CFU reduction[a]	Reference
Standard processing procedures and GHP					
Improved hide removal	2/BA 1/CT	Beef/veal carcass*	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.2–1.1 0.0–0.7 0.0–1.0	Bosilevac, Wang, Luedtke, Wheeler, and Koohmaraie (2016), Gill and McGinnis (1999), McEvoy et al. (2000)
Downward hide pull	1/CT	Carcass*	Aerobic bacteria	0.0	Kennedy et al. (2014)
Bung bagging & rodding	1/CT	Carcass*	Aerobic bacteria *Enterobacteriaceae*	1.3 1.3	Saleh et al. (2012)
Physical interventions aimed at removing microorganisms					
Trimming	2/BA 1/CT	Carcass	Aerobic bacteria *E. coli*	0.0–2.2 0.0–2.0	Gill and Landers (2004), Gill, Badoni, and Jones (1996), Kochevar, Sofos, Bolin, Reagan, and Smith (1997)
Water wash	4/BA 1/CT	Carcass	Aerobic bacteria *E. coli*	−0.8-0.3 0.0–0.3	Carranza et al. (2013), De Martinez, Ferrer, and Salas (2002), Gill and Landers (2003b), Hajmeer, Marsden, Crozier-Dodson, Basheer, and Higgins (1999), McEvoy, Sheridan, Blair, and McDowell (2004)
Thermal interventions					
Hot water	6/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.8–2.7 0.6–2.7 0.4–1.4	Algino et al. (2007), Bosilevac, Nou, Barkocy-Gallagher, Arthur, and Koohmaraie (2006), Gill, Bryant, and Bedard (1999), Gill and Bryant (2000), Signorini et al. (2018), Wright (2011)
Steam vacuuming	2/BA 2/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.3–2.0 0.7–1.1 0.2–0.7	Gill and Bryant (1997b), Hochreutener, Zweifel, Corti, and Stephan (2017), Kochevar et al. (1997), Trivedi, Reynolds, and Chen (2007)
Steam pasteurisation	4/BA 1/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.1–1.6 0.6–1.5 0.1–0.8	Corantin et al. (2005), Minihan, Whyte, O'Mahony, and Collins (2003), Nutsch et al. (1997), Nutsch et al. (1998), Retzlaff, Phebus, Kastner, and Marsden (2005)
Organic acid washes					
Lactic acid	5/BA 2/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.9–3.8 0.4–1.0 0.1–1.8	Bosilevac et al. (2006), De Martinez et al. (2002), Dormedy, Brashears, Cutter, and Burson (2000), Rodriguez (2007), Ruby and Ingham (2007), Signorini et al. (2018), Wright (2011)
Acetic acid	2/BA 1/CT	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.4–0.6 1.0 0.5–0.7	Algino et al. (2007), Carranza et al. (2013), Signorini et al. (2018)
Citric acid	1/BA	Carcass	Aerobic bacteria *E. coli*	0.8 0.4	Signorini et al. (2018)
Organic acid mixtures	2/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	0.2 0.6 0.1–0.9	Algino et al. (2007), Signorini et al. (2018)
Multiple interventions applied at one step					
Trimming/steam vacuuming	1/CT	Carcass	Aerobic bacteria *Enterobacteriaceae*	1.2 0.7	Ramish (2011)
Water wash/LA	1/BA	Carcass	Aerobic bacteria	0.4–0.8	Gill and Landers (2003b)
Water wash/AA	1/CT	Carcass	Aerobic bacteria	0.1–0.8	Carranza et al. (2013)
Hot water/LA	4/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	1.1–2.8 1.1–2.5 1.6	Bosilevac et al. (2006), Gill and Landers (2003b), Ruby and Ingham (2007), Wright (2011)
Steam pasteurisation/LA	1/BA	Carcass	Aerobic bacteria	1.6	Gill and Landers (2003b)
PAA/steam pasteurisation	1/BA	Carcass	Aerobic bacteria	1.0	
Multiple interventions applied at multiple steps					
Water wash/thermal/LA/ PAA	2/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	1.1–1.9 1.8 0.6	Gill and Landers (2003b), Wang, King, Koohmaraie, and Bosilevac (2013)
Steam vacuum, PAA & organic acid washes, thermal interventions	6/BA	Carcass	Aerobic bacteria *Enterobacteriaceae* *E. coli*	1.0–3.9 1.2–1.5 0.8–4.1	Arthur et al. (2004), Bacon et al. (2000), Bosilevac et al. (2016), Gill, Bryant, and Landers (2003), Ruby and Ingham (2007), Scott et al. (2015)
Chilling					
Dry chilling (≤3 days)	8/BA	Carcass	Aerobic bacteria *E. coli*	−1.2-2.0 0.0–1.4	Hajmeer et al. (1999), Hauge et al. (2015), Kinsella et al. (2006), Liu et al. (2016), McEvoy et al. (2004), Sampaio et al. (2015), Trivedi et al. (2007), Yang et al. (2017)
Dry chilling (≤3 days) followed on multiple interventions	1/BA	Carcass	Aerobic bacteria *E. coli*	2.1 0.6	Bacon et al. (2000)
Water spray chilling	6/BA	Carcass	Aerobic bacteria *E. coli*	−1.8-2.0 −1.4-1.3	Corantin et al. (2005), Gill and Bryant (1997b), Gill and Bryant (1997a), Gill and Landers (2003a), Jericho, O'Laney, and Kozub (1998), Kinsella et al. (2006)

‡ Lactic acid (LA), acetic acid (AA), peroxyacetic acid (PAA).
* Reduction in transfer to carcass.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all but one case "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials); In the "downward hide pull" a comparison group was "upward hide pull".

Table 5
Summary of findings for beef carcass interventions: studies under commercial conditions measuring prevalence reductions.

Intervention‡	No. studies/ design	Intervention/ outcome surface	Microorganism	% of samples positive in study population		Reference
				No treatment[a]	Treatment	
Standard processing procedures and GHP						
Downward hide pulling	1/CT	Carcass*	*Enterobacteriaceae*	83%	94%	Kennedy et al. (2014)
Bung bagging	1/CT	Carcass*	VTEC non-O157	58%	35%	Stopforth et al. (2006)
			E. coli O157:H7	5%	1.7%	
			Salmonella	8.3%	0.0%	
Thermal interventions						
Hot water	2/BA	Carcass	*Enterobacteriaceae*	19–27%	12–15%	Algino et al. (2007), Bosilevac et al. (2006)
			E. coli	18–24%	3%	
			E. coli O157:H7	27%	5%	
Steam pasteurisation	2/BA	Carcass	*Enterobacteriaceae*	46%	3%	Corantin et al. (2005), Nutsch et al. (1997)
			E. coli	14–16%	0–1.8%	
			Salmonella	0.7%	0%	
Organic acid washes						
Lactic acid	3/BA	Carcass	*E. coli* O157:H7	31%	20%	Bosilevac et al. (2006), Chaves et al. (2013), Ruby and Ingham (2007)
			VTEC non-O157	6.7%	0%	
			Salmonella	45%	28%	
Acetic acid	1/BA	Carcass	*Enterobacteriaceae*	58%	30%	Algino et al. (2007)
			E. coli	47%	13%	
Organic acid mixtures	1/BA	Carcass	*Enterobacteriaceae*	28%	22%	
			E. coli	24%	7%	
Hot water/LA	2/BA	Carcass	*E. coli* O157:H7	19%	4%	Bosilevac et al. (2006), Ruby and Ingham (2007)
			Salmonella	28%	2.3%	
Multiple interventions applied at multiple steps						
Steam vacuum, PAA & organic acid washes, thermal interventions	5/BA	Carcass	*E. coli* O157:H7	4–43%	0–17%	Arthur et al. (2004), Elder et al. (2000), Kanankege et al. (2017), Rekow et al. (2011), Ruby and Ingham (2007)
			VTEC non-O157	70–79%	14–62%	
			Salmonella	45%	2%	

‡ Lactic acid (LA), peroxyacetic acid (PAA).
* Reduction in transfer to carcass.
CT: Controlled trial; BA: Before-and-after trial.
[a] The comparison group was in all but one case "no treatment" (controlled trials) and "pre-treatment" (before-and-after trials); In the "downward hide pulling" a comparison group was "upward hide pulling".

physical (water washes and thermal treatments) and chemical interventions (organic acids and other chemicals) alone or in various combinations, produced a large variation of reductions, between 2 and 5 logs. Recent studies on beef carcass interventions, investigated under pilot plant conditions, evaluated a range of chemical interventions against inoculated O157 and non-O157 VTEC and *Salmonella* (Kalchayanand et al., 2018, 2012, 2015). In these studies, reductions of VTEC and *Salmonella* reported for lactic acid ranged from 2 to 3 logs and 1–1.5 logs, respectively, while citric acid-based antimicrobials reduced VTEC and *Salmonella* by 1–2 logs and 1–1.5 logs, respectively. Similarly, spray treatments with ASC and PAA reduced VTEC by 1–2 logs. The reported reductions should be viewed with caution and only as relative and indicative of the potential intervention effect, because these trials often analysed a small number of samples challenged with a high number of pathogens, which exaggerates the efficacy of the interventions.

2.3.3. Multiple on-line interventions

Sequential application of interventions between pre-evisceration and chilling stages usually started with knife trimming and steam vacuuming, which reduced bacteria on beef surfaces by targeting potentially contaminated areas following the dehiding process (usually along the cattle hide opening lines). This was followed with a pre-evisceration wash of hot water or organic acid that further eliminated pathogens. After evisceration and splitting, carcasses passed through a thermal pasteurisation chamber, where heated water (>74 °C) or steam (>85 °C) was 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 was applied before carcasses entered the chilling room (Tables 4 and 5).

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 (Table 4). 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, reductions of aerobic bacteria and *E. coli* after chilling were 1.5–2.5 logs compared to untreated (only chilled) carcasses (Van Ba et al., 2018). Furthermore, prevalences of naturally present VTEC and *Salmonella* following sequential application of interventions was in most cases significantly reduced, often to levels below detection limits (Table 5).

2.3.4. Chilling

Dry chilling as a means to reduce microbial growth and/or number and presence of bacteria has been investigated as a standalone treatment or following previous multiple, sequential interventions on the slaughterline. Chilling for up to three days under commercial conditions only reduced the counts of indicator bacteria (ACC and generic *E. coli)* in most cases by 0.5 logs (Table 4). Some authors reported reductions of 1–2 logs under similar conditions (Liu, Youssef, & Yang, 2016; Yang, Tran, & Wolters, 2017). Under pilot plant conditions, reductions of inoculated *E. coli* and *Salmonella* were up to 2 logs (Calicioglu, Buege, Ingham, & Luchansky, 1999; Calicioglu, Kaspar, Buege, & Luchansky, 2002; Tittor et al., 2011). Chilling carcasses, previously sprayed with organic acids or treated with hot water or steam on the slaughterline, reduced generic *E. coli* and ACC by 0.6 logs and 2.1 logs under commercial conditions, respectively (Bacon et al., 2000), and *E. coli* by up to 3.5 logs under pilot plant conditions (Calicioglu et al., 2002). The reductions were likely due to a residual effect of the chemical interventions. There is 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.

Spray chilling with water was also investigated under commercial conditions (Table 4) and pilot plant conditions (Kalchayanand, Worlie, & Wheeler, 2019; Tittor et al., 2011). Only two studies investigated spray chilling with chemicals under laboratory (Stopforth et al., 2004) and pilot plant conditions (Kalchayanand et al., 2019). In general, water spray chilling showed very variable effects in reducing ACC and generic *E. coli* on carcasses chilled in abattoir conditions and it appears these were abattoir specific and influenced by other factors (Table 4). Water spray chilling reduced inoculated VTEC and *Salmonella* by up to 2 logs (Kalchayanand et al., 2019; Stopforth et al., 2004; Tittor et al., 2011). Spraying various chemicals (sodium hypochlorite, ASC, ammonium hydroxide, lactic acid and CPC) onto beef carcass tissue during chilling reduced inoculated *E. coli* O157 by 0.7 logs, 2.2 logs, 2.5 logs, 3.2 logs and 4.7 logs, respectively for all chemicals, compared to water spray chilling alone (Stopforth et al., 2004). One novel intervention, spray chilling with aqueous ozone at 12 ppm, provided an additional 0.9 log reduction more than that of spray chilling with cold water alone (Kalchayanand et al., 2019).

3. Discussion

The primary aim of this review was to identify and recommend effective interventions that can be used in abattoirs as a part of the RB-MSAS. Studies conducted in commercial abattoir environments reported here, with naturally present contamination (usually aerobic bacteria and faecal indicators) provide more confidence in the efficacy of interventions. However, there was an overall lack of reported trials conducted under commercial conditions, 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 Fig. 2. They are presented relative to *E*-beam irradiation of carcass surface, which was the only intervention reported to completely eliminate *E. coli* O157:H7 from beef carcass (at a 1 kGy dose, reduction of at least 4 logs and up to 6.6 logs) (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, wherein a range of reduction effects were reported. Furthermore, these derived reductions are based only on observations from across different studies, and statistical analysis was not performed. This graph mainly gives an overview of the relative effectiveness between different available interventions, and its purpose is not to demonstrate exact reduction levels. In previous review on the topic, Wheeler et al. (2014) observed similar trend regarding relative reductions and differences between interventions' efficacies against *E. coli* O157:H7 inoculated on beef surfaces, under controlled laboratory and pilot plant conditions. However, comparisons made in their review did not include cattle hide interventions, and on the other hand, included some other novel interventions more suitable for further beef processing post-abattoir.

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 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. Therefore, cattle hide interventions can be seen as a proactive approach in dealing with sources of contamination, versus carcass treatments that are usually applied after contamination events,

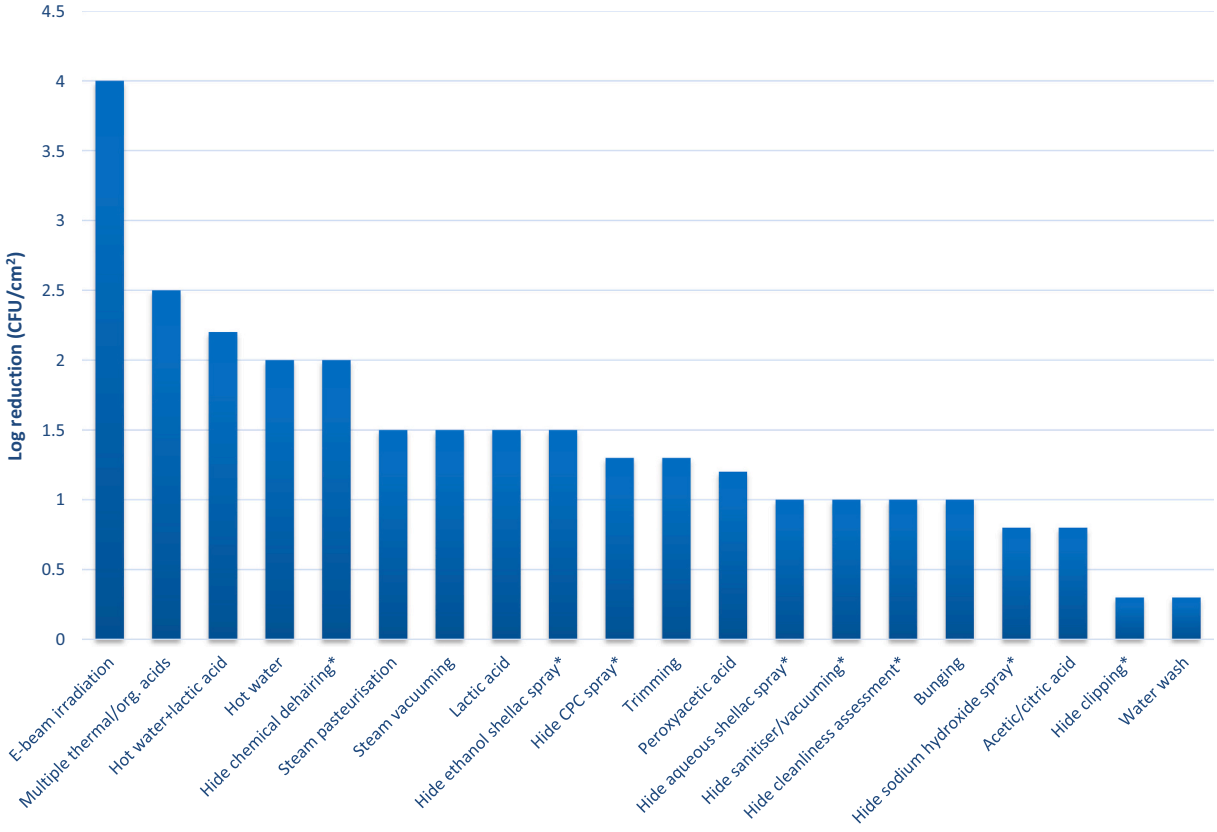

Fig. 2. Relative log reductions for standard processing procedures and interventions reported to reduce indicator bacteria on beef carcass surfaces under commercial abattoir conditions, relative to *E*-beam irradiation (*reduction in hide-to-carcass transfer).

and are, by their nature, reactive (Antic et al., 2018). Nevertheless, both strategies are essential and best applied together in a sequential and coordinated way as a part of the multiple-hurdle approach, which aligns well with the RB-MSAS's longitudinal and integrated nature.

Carcass pasteurisation treatments (with hot water and steam) and organic (lactic) acid washes also produced a consistent reduction effects seen across several studies, from 1 to 2.5 logs, and, when in sequential use, up to a 3 log reduction. Other GHP-based control measures (e.g. hide cleanliness assessment, hide clipping, bunging/rodding, knife trimming and steam vacuuming) are routinely used to assist in overall microbial reduction. For example, cattle hide clipping can enhance the efficacy of hide chemical washes or immobilisation treatment with shellac. It goes without saying that one should not 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 reported studies. They 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 bacterial indicators were higher than when only one single intervention was used, and in most cases, they ranged from 2 to 3 logs of ACC and/or faecal indicators. The prevalences of naturally present VTEC and *Salmonella* were, in most cases, significantly reduced, often to the levels below detection limits.

Taking into consideration the relative efficacies of reported interventions, it could be argued that any intervention that has a 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. Other factors usually taken into consideration are: i) the safety of the intended substance; ii) the development of resistance to therapeutic antimicrobials; and iii) the safety of the substance and its by-products for the environment (EFSA, 2010).

A quantitative microbial risk assessment model was developed to assess the relative impacts of specific interventions on public health risk from consumption of *E. coli* O157:H7 in beef products (Smith, Fazil, & Lammerding, 2013). It was found that the average probability of illness per serving of minced beef and beef cuts following application of single intervention at slaughter (excluding carcass water spray chilling) was reduced by 45%–92% and 44%–96.5%, respectively, relative to the no intervention scenario. 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 use of sequential interventions at slaughter (pre- and post-evisceration hot water wash, steam pasteurisation and acid spray chilling), had the greatest overall impact. They reduced the average probability of illness per serving of minced beef and intact beef cuts by 95.1%–99.6% and 95.1%–99.9%, respectively, relative to the no intervention scenario. The average probabilities of illness per serving when multiple interventions at slaughter were used were calculated as being 8.7×10^{-6} and 2.9×10^{-9} for ground beef and intact beef cuts, respectively (Smith et al., 2013).

When implementing 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 any existing contamination. The actual sources of carcass contamination and, in particular, quantification of their contribution to contamination at the lairage stage and at slaughter and post-slaughter events, are not well-researched areas. There are no or scarce data of the relative contribution of accidental gut spillage, airborne contamination (Schmidt, Wang, et al., 2012) 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; Nou et al., 2003). Studies on quantifying this contamination suggest that up to 1% in commercial and 10% in laboratory conditions of microbial contamination is transferred to carcasses (Antic et al., 2018; Arthur et al., 2004; Bacon et al., 2000). 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 FSMS, it is clear that the first priority should be prevention of microbiological contamination. This priority is in line with the whole chain approach and the need for controls to be implemented in an integrated way, starting from the farm.

The main driver for the implementation of interventions in beef processing premises should be the protection of public health from the high-priority hazards. The USA 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 mandatory implementation of the HACCP system and interventions (FSIS, 1996). 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 technological solutions at this stage of the beef chain, to introduce various interventions such as pasteurisation, lactic and other organic acid treatments, 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, and there must be no measurable chemical residues on the carcasses. These aspects were discussed in details in previous reviews on the topic (Koohmaraie et al., 2007, 2005, Wheeler et al., 2014).

In the RB-MSAS, use of interventions is proposed in high risk situations to accomplish the targets on chilled carcasses (e.g., when an abattoir is unable to sufficiently reduce risks arising from specific farms/animal batches by using process hygiene alone). Furthermore, interventions can be recommended for use whenever food safety authorities identify meat production processes associated with high risks for consumers, e.g. when there is no heat treatment envisaged in further meat processing. For example, the sale and consumption of burgers served less than thoroughly cooked (LTTC) and pink in the middle is a steadily increasing trend in the UK, which prompted concerns that there may be an increased risk of exposure to *E. coli* O157 for consumers (FSA, 2015). The UK Food Standards Agency (FSA) 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 a 6 log reduction will be achieved solely at the catering establishment level. Therefore, the FSA instructed FBOs serving LTTC burgers that they should ensure their suppliers have procedures in place during slaughter, cutting and mincing, to prevent 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 they also must provide advice to consumers at the point of ordering a LTTC burger (FSA, 2015, 2016). Therefore, as the regulatory authority, the FSA has set a clear performance criterion for FBOs supplying meat for LTTC burger production, while also advising that specific interventions may need to be applied to achieve these targets. It is clear from our current review that no single intervention, apart from *E*-beam irradiation, can realistically deliver a 4 log reduction of microbiota on carcasses or beef cuts. However, the sequential application of interventions, based on a multiple-hurdle approach, was able to deliver greater reductions than when only one

single intervention was used, i.e., the overall improvement of the microbiological status of beef was determined by a combination of microbial reductions achieved by all interventions. Therefore, it is expected that the 4 log performance criterion set by the FSA can be achieved by the FBOs which supply meat for LTTC burgers. The multiple-hurdle approach, in this case, would rely on properly implemented prerequisite GHP-based measures in place, for example 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 chilling/post-chilling stage (organic acid washes of carcasses).

4. Summary

This review aimed to provide an overview of the principles for ensuring carcass meat safety in beef abattoirs with particular emphasis on the role of beef interventions as a component of the risk-based meat safety assurance system. A range of different abattoir interventions was discussed. The potential for lairage to moderate amplification and transmission of VTEC and *Salmonella* among cattle was demonstrated. However, although reduced lairage time would be beneficial in reducing cattle contamination with VTEC and *Salmonella*, it is not always practical to minimise the duration in lairage for cattle in commercial settings. Categorisation of cattle based on their cleanliness significantly reduces (by about 1 log) the microbial contamination, including faecal microbiota, of resulting beef carcasses. In contrast, hide water washing of live cattle in lairage with ambient temperature water and hide clipping are both largely ineffective. Chemical decontamination or hide clipping of live cattle are not recommended due to animal welfare concerns and/or practical considerations. Cattle hide interventions, i.e., chemical hide washes and microbial immobilisation treatment with shellac, significantly reduce transfer of aerobic bacteria and *Enterobacteriaceae* to beef carcasses by 1–1.5 logs. Therefore, we recommend that these treatments are applied post-exsanguination and before dehiding, as proactive interventions, to reduce microbial contamination of resulting beef carcasses. We also recommend beef carcass hazard-based interventions to control microbial contamination after dehiding and prechilling. Carcass pasteurisation treatments with hot water and/or steam are efficacious against microorganisms when temperatures of carcass surfaces achieve more than 70 °C. These treatments reduce indicator bacteria by 1–2.5 logs, with an additional reduction of 0.5–1 logs if organic acids are used sequentially. The time-temperature combinations required to achieve significant reductions are specific to an individual commercial abattoir and subject to validation. Chemical washes, particularly with lactic, acetic or citric acids, are efficacious, reducing all indicator bacteria by 1–1.5 logs. Knife trimming and steam vacuuming are also highly efficacious, reducing all indicator bacteria by 1–2 logs. However, the reductions achieved highly depend on the skill and diligence of the operator to spot visible contamination and efficiently remove it, and these interventions' parameters are difficult to optimise to achieve a consistent effect. Carcass water washing to remove microorganisms is largely ineffective, with reductions up to 0.5 log achieved. Use of multiple, sequential carcass interventions has the biggest impact on microbial reduction on beef carcasses; reductions are up to 3 logs, greater than any of these interventions applied alone.

Declaration of Competing Interest

All authors report that they do not have any conflict of interest.

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