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Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on *Salmonella* and Pathogenic *E.coli*

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Abstract

Salmonella and pathogenic *Escherichia coli* are known to be the major bacterial agents responsible for human foodborne infections attributable to meat. A review of the specialized literature was carried out to identify the risk factors for bovine meat contamination by these pathogens from the cattle farm to meat consumption. Animal stress during transport to the slaughterhouse and the duration of the lairage period were identified as the key factors influencing the faecal excretion of *Salmonella* and pathogenic *E. coli* as well as cattle contamination prior to slaughter. At the abattoir level, hides and visceral contents appear to be the main sources of pathogenic bacteria that contaminate carcasses along the meat production chain. Finally, temperature abuses during distribution and meat contamination by infected handlers were found to be important contributors to the post-slaughter contamination of bovine meat. The findings of this study indicate that efficient management of human food borne infections attributable to bovine meat requires an integrated application of control measures involving all actors along the meat chain, namely slaughterhouses, meat processing plants, distributors and consumers.

Keywords: bovine meat, *Salmonella*, pathogenic *E. coli*, safety, risk factors

1. Introduction

Meat is consumed in different parts of the world as a source of animal proteins (Food and Agriculture Organization, 2013) and its chemical composition is favourable for the proliferation of a wide range of microbial populations which makes raw meat to be one of the vehicles of foodborne infections in humans (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Scallan et al., 2011). The actual number of foodborne infections attributable to meat is difficult to assess accurately, principally because only a small proportion of illness cases is officially reported especially in developing countries. On the other hand, even within the reported cases, only a limited number allow identification of the food vehicle. Data from outbreaks constitute an interesting source of information to associate foodborne illness cases to their respective food vehicles and causal agents (Scallan et al., 2011). Greig and Ravel (2009), by using outbreak data published internationally from 1996 to 2005, noted that 12.7 % of reported foodborne outbreaks were attributable to bovine meat while 10.5 and 4.6 % were associated with chicken and pork, respectively. According to the same authors, *Salmonella* and pathogenic *Escherichia coli*, respectively, were identified as the causal agents in 32.9 and 34.6 % of foodborne outbreaks of bacterial origin attributable to beef.

Several studies have addressed the sources and potential control measures of bovine meat contamination by *Salmonella* and pathogenic *E. coli* at different stages of the meat chain i.e. primary production (Barkocy-Gallagher et al., 2003; Millemann, 2008), animal transportation to the slaughterhouse (Arthur et al., 2007; Barham et al., 2002) ; slaughtering operations (Antic et al., 2010); further processing (Carney et al., 2006; Scanga et al., 2000), distribution (Haileselassie, Taddele, Adhana, Kalayou, & Tadesse, 2013); cooking (Juneja, Eblen, & Ransom, 2001); however literature on bovine meat contamination and possible control measures considering the entire meat chain is still limited, probably because of the length and the complexity of the chain.

The contamination of meat by microbial pathogens can occur at any stage of the meat chain (Duffy, Cummins, Nally, O' Brien, & Butler, 2006; Rhoades, Duffy, & Koutsoumanis, 2009). Furthermore, the prevention or mastery of meat contaminations can be carried out at a stage of the chain different from the stages at which the contamination has occurred (Chen et al., 2012). Therefore, the food chain approach constitutes an efficient method to control bacterial contaminations of meat at consumption. The objective of this study was to review the existing knowledge on sources and risk factors for bovine meat bacterial contamination and provide an up to date view on control measures of the same by using a meat chain approach. The focus was put on *Salmonella* and pathogenic *E. coli,* as they are reported to be the leading causes of foodborne bacterial infections attributable to bovine meat (Greig & Ravel, 2009).

The literature search was undertaken first by reviewing literature in databases of peer-reviewed scientific publications, namely Scopus, PubMed and Google Scholar, using the following key words: cattle, bovine, beef, meat, safety, abattoir, slaughter, slaughterhouse, salmonella, salmonellosis, Escherichia coli, microbial (bacterial) contamination, hygiene, risk factors and distribution. Only articles in English or French were retained. On the other hand, books and other official publications dealing with the subject were consulted.

In this paper, an overview of the prevalences of *Salmonella* and pathogenic *E. coli* in bovine meat was carried out before tackling their risk factors along the bovine meat chain and discussing their respective control measures.

2. *Salmonella* and Pathogenic *E. coli* in Bovine Meat

Contaminated bovine meat is considered to be one of the sources of foodborne *Salmonella* and pathogenic *E. coli* infections in humans. The reported prevalence of *Salmonella* and pathogenic *E. coli* in bovine meat and products thereof varies from one product to another, but wide variability is also observed amongst different countries (Tables 1 and 2). The prevalences are globally lower in bovine carcasses at the slaughterhouse level and higher in meat cuts and minced beef at retail (EFSA and ECDC, 2013b; Stevens et al., 2006). This could be associated with bacterial contamination of meat that can occur during the transport of bovine carcasses from the slaughterhouse to the meat processing units, during cutting and mincing operations within meat processing plants and/or during the marketing of bovine meat in retail outlets. Niyonzima et al., (2013) reported a 2.2 log cfu increase in *E. coli* load between the slaughtering and marketing of beef at a commercial abattoir in Kigali city (Rwanda). Similarly, an increase in the prevalence and concentration of *Salmonella* and *E. coli* during the cutting and mincing of bovine meat is generally reported in meat processing plants (Hassanein, Fathi, Ali, & El-malek, 2011; Rhoades et al., 2009; Scanga et al., 2000). The variations in *Salmonella* and pathogenic *E. coli* prevalence amongst different countries could be attributed to a number of factors (including the farming systems and practices, slaughtering practices and post slaughter handling of meat as well as the general hygiene at different stages of the meat chain); which differ from one country to another. Higher prevalences are principally observed in developing countries, where poor hygienic conditions during slaughtering and meat handling are generally reported (Gashe & Mpuchane, 2000; Hassanein et al., 2011; Magwira, Gashe, & Collison, 2005; Stevens et al., 2006), whereas lower prevalence are mostly observed in developed countries where good hygienic practices are reported to be strictly followed and monitored along the meat chain (EFSA and ECDC, 2013; Vipham et al., 2012; Bosilevac et al., 2009).

The reported prevalence in different countries would be, however, not comparable because of differences in the sampling strategy and the analytical methods used. In some studies the number of analyzed samples amounted to thousands (Bosilevac et al., 2009; EFSA and ECDC, 2013b) , whereas in others only a very limited number of samples was analysed (Gashe & Mpuchane, 2000; Temellİ, Eyİgör, & Anar, 2012). Differences were also observed in sampling methodology, where the surface swabbed on bovine carcasses to detect pathogens or the weight of the meat samples analysed varied between different studies. In the studies conducted in European Union countries for example, the surface area covered by a carcass swab was reported to vary from 100 to 600 cm^2, while the weight of the meat sample analysed varied from 1 to 25 g (EFSA and ECDC, 2013b, 2014). Additionally, the analytical methods used to detect *Salmonella* and pathogenic *E. coli* in meat and meat products differed from one study to another. For *Salmonella,* a culturing method including a pre-enrichment phase in buffered peptone water, a selective enrichment and isolation followed by biochemical confirmation of isolates was the predominant method used (Bosilevac et al., 2009; Tafida et al., 2013). However, in other studies other detection methods such as PCR were used alone or in combination with a culturing method (Hassanein et al., 2011; Vipham et al., 2012). The same trend was observed in the methodology used to detect verotoxinogenic *E. coli* in meat and meat products (Temellİ et al., 2012). The prevalence of *Salmonella* or pathogenic *E. coli* in faeces, on hides or on bovine carcasses was reported to be higher when a PCR-based method was used than when the pathogen was detected by conventional culturing methods (Barkocy-Gallagher et al., 2003; Mainil &

Daube, 2005). This would be due to the fact that PCR methods consider the bacterial DNA and take into account all the bacterial cells, whether living or dead; whereas the culture method only consider living bacterial cells (Johansson et al., 2000).

Even if differences in the sampling strategy and analytical methods used in different studies do not allow an accurate comparison of the prevalence of pathogenic *E. coli* and *Salmonella* in meat amongst different countries, it appears that these two pathogens are detectable worldwide in significant proportions in meat in general, and particularly in bovine meat. According to the EFSA and ECDC report (2014) on zoonoses, data collected in 2012 from nine European Union member states showed prevalences of 1.3 and 0.1%, respectively, for verocytotoxigenic *E. coli* (VTEC) and VTEC O157 in fresh bovine meat. The prevalence of VTEC in meat from animal species other than bovines in the EU was not estimated, probably because of the non-representativeness of the data available. However, the prevalence of VTEC in different Member States in 2011 was reported to be higher in bovine meat compared to meat from other animal species.This could be probably due to the fact that the enteric carriage of pathogenic *E. coli* is mostly observed in cattle than in other animal species (Mainil & Daube, 2005). In Ireland, VTEC was detected in 1% of 291 bovine carcass samples, while no positive finding was reported from 134 sheep carcass samples (EFSA and ECDC, 2013b).

Table 1. The prevalence of *Salmonella* in fresh bovine meat

Product	% of positive samples	Number of tested samples	Country	References
Beef carcasses	42.8	236	Senegal	(Stevens et al., 2006)
	0.2	1275	Australia	(Phillips et al., 2001)
	6	250	Mexico	(Narvaez-Bravo et al., 2013)
	0	53	Poland	(EFSA and ECDC, 2014)
Butcher shop beef	20	25	Egypt	(Hassanein et al., 2011)
	9.9	354	Botswana	(Gashe & Mpuchane, 2000)
	2.4	370	Nigeria	(Tafida et al., 2013)
	1.02	2885	USA	(Vipham et al., 2012)
	0.8	274	France	(EFSA and ECDC, 2014)
	0.3	747	Germany	(EFSA and ECDC, 2014)
	1.1	117	Hungry	(EFSA and ECDC, 2014)
	0	26	Italy	(EFSA and ECDC, 2014)
	0.9	649	Netherland	(EFSA and ECDC, 2014)
Ground beef	20	25	Botswana	(Gashe & Mpuchane, 2000)
	11	88	Mexico	(Heredia et al., 2001)
	4.2	4136	USA	(Bosilevac et al., 2009)

A comparable observation was reported in the Czech Republic, where 0.3% of 1159 bovine carcasses were reported to be positive for VTEC while not a single positive sample was found in 1395 pig carcasses (EFSA and ECDC, 2013b). At the retail level, the Netherlands reported 0.3% of 702 bovine meat samples were positive for VTEC while no positive sample was found from 86 sheep meat samples (EFSA and ECDC, 2013b). In contrast, a higher prevalence of VTEC was reported in Spain, where 2.9% of 34 poultry samples were found to be positive for VTEC against a prevalence of 0.0% (n=45) in bovine meat (EFSA and ECDC, 2013b).

Table 2. The prevalence of pathogenic *E. coli* in fresh bovine meat

Product	% of positive samples	Number of tested samples	Country	References
Beef carcasses	0.4	250	Mexico	(Narvaez-Bravo et al., 2013)
	0.9	453	Belgium	(EFSA and ECDC, 2014)
	1.3	622	Czech Rep.	(EFSA and ECDC, 2014)
	5.7	315	Germany	(EFSA and ECDC, 2014)
	0	203	Romania	(EFSA and ECDC, 2014)
Butcher shop meat	10	20	Turkey	(Temellİ et al., 2012)
	11.1	27	Egypt	(Mohammed et al., 2014)
	5.22	134	Botswana	(Magwira et al., 2005)
	1.8	492	Germany	(EFSA and ECDC, 2013b)
	0	45	Spain	(EFSA and ECDC, 2013b)
	3.2	555	Netherland	(EFSA and ECDC, 2014)
Ground beef	3.76	133	Botswana	(Magwira et al., 2005)
	3.85	52	Turkey	(Temellİ et al., 2012)
	16.7	30	Egypt	(Mohammed et al., 2014)
	3.8	479	Germany	(EFSA and ECDC, 2013b)

The prevalence of *Salmonella* in bovine meat has been found to be low compared to meat from other animal species. In the European Union, during 2012, the prevalence of *Salmonella* in bovine meat and products thereof was reported to be 0.2% whereas in pig and broiler meat it was estimated to be 0.7 and 4.1%, respectively (EFSA and ECDC, 2014). The highest *Salmonella* prevalence observed in poultry meat could be attributed to the colonization of the reproductive tract of infected subjects by the pathogen that may increase the probability of *Salmonella* dissemination on carcasses under preparation through cross contamination (Gast, Guraya, Guard-Bouldin, Holt, & Moore, 2007). Although the prevalence of *Salmonella* in bovine meat seems to be relatively low, contaminated bovine meat remains a significant risk for *Salmonella* infection in humans, particularly for people consuming more beef than meat from other animal species. Additionally, the high protein and fat content of foods such as meat was reported to protect the bacterium against the gastric acidity (Birk et al., 2012; Blaser & Newman, 1982; Kothary & Babu, 2001). This suggests that the consumption of contaminated meat, even with a limited number of pathogens, would present a significant risk of infection and/or intestinal colonization in humans.

As for other bacterial pathogens, the minimum number of *Salmonella* capable of causing illness, is difficult to determine as it depends on a number of factors including (but not limited to) the food matrix, the host susceptibility and the virulence factors of the pathogen (McEntire, Acheson, Siemens, Eilert, & Robach, 2014). However, recent studies using outbreak data indicate that doses as low as 36 colony forming units can cause illness in humans (Teunis et al., 2010). This infective dose would be qualified as “low “comparatively to foodborne pathogens such as *Vibrio cholerae* that require doses as high as 10^4-10^8 cells to cause infection in humans (Kothary & Babu, 2001). The infective dose for pathogenic *E coli* is also known to be “low”. Coia (1998) reported contamination levels as low as 2 organisms per 25 grams in food and environmental samples incriminated in VTEC O157 outbreaks. Because of the low infective dose, the contamination limit for these pathogens has been fixed to the absence in 25g of meat preparations intended to be eaten raw (European Commission, 2005).

It is assumed that the level of microbial contamination of meat at the end consumer stage is function of contaminations acquired during different stages of meat preparation. Therefore, reducing the prevalence of foodborne infections such as *Salmonella* and verotoxinogenic *E. coli* attributable to bovine meat in humans requires integrated control measures involving all actors in the bovine meat chain from primary production to the final consumer.

3. Bacterial Contamination of Bovine Meat along the Production Chain

3.1 Preslaughter Contamination of Live Cattle

Salmonella infection is commonly reported in different animal species. Considering their adaptation to hosts, *Salmonella* serotypes are grouped in three categories: namely serotypes only pathogenic for humans like *S.* Typhi and *S.* Paratyphi; serotypes adapted to animal species such as *S.* Gallinarum, *S.* Dublin, *S.* Abortusequi, *S.* Abortusovis and *S.* Choleraesuis which are pathogenic for poultry, cattle, horses, sheep, and pigs respectively; and finally ubiquitous serovars like *S.* Typhimurium and *S.* Enteritidis adapted to humans and other animal species (Jay et al., 2005). In cattle, *Salmonella* infection can be clinically manifested by a wide range of symptoms including diarrhoea and possible dysentery, joint infections, pneumonia as well as abortions (Millemann, 2008). However, bovines may also carry *Salmonella* in their gastro-intestinal tract without any clinical symptom of the disease. In the latter case bovines are called asymptomatic carriers. In both infected and asymptomatic carriers, *Salmonella* can be excreted through the faeces for a relatively long period. Gopinath et al. (2012) reported that the faecal shedding of *Salmonella* in cattle may last up to 400 days.

As with *Salmonella*, asymptomatic carriage and faecal shedding of pathogenic *E. coli* are common in bovines of all ages; but clinical manifestations of the disease are mainly observed in young calves with 2 weeks to 2 months of age with diarrhoea as the main symptom (Alexa, Konstantinova, & Sramkova-Zajakova, 2011; Millemann, 2008). The duration of faecal shedding in cattle can last up to 19 weeks (Khaitsa et al., 2003). On a clinical basis, pathogenic *E. coli* strains are grouped in 3 classes namely those rarely associated to diseases either in animals or in humans (i.e VTEC-2), strains associated to disease in both animals and humans (i.e EHEC-2) and finally strains such as EHEC-1 and VTEC-1 reported to be highly infectious for humans but rarely in animals (Mainil & Daube, 2005).

The faecal shedding of *Salmonella* and pathogenic *E. coli* constitutes an important factor of cattle contamination. In fact, pathogens excreted in the faeces may contaminate the environment through which other cattle can acquire contamination and carry the bacteria in their digestive tract and/or on their hides (Rhoades et al., 2009). The contamination of live cattle destined for slaughter may occur at the farm level, during the transportation of bovines to the slaughterhouse or during the lairage period in the abattoir.

At the farm level, contaminated feed and water have been reported to be the main sources of *Salmonella* and pathogenic *E. coli* infections in cattle (Millemann, 2008). However, dissemination of the infection within the herd is mainly attributable to faecal excretion of the pathogens. The prevalence of pathogenic *E. coli* and *Salmonella* is generally reported to be higher on cattle hides than in the faeces. This is due to the fact that a single animal shedding the pathogen in its faeces may contaminate the hides of many other animals in the herd, either directly or via the ground and lairage fixtures (Small et al., 2002). In a study conducted on 200 steers and heifers in a large feed yard, Barham et al. (2002) reported an *E. coli* O157 prevalence of 18% on hides while its prevalence in faeces was as low as 9.5%. A similar relationship was reported by Barkocy-Gallagher et al. (2003), who detected *E. coli* O157:H7 on 60.6% of cattle presented for slaughter, while the faecal prevalence was 5.9%. As with VTEC, *Salmonella* prevalence was reported to be higher on cattle hides than in faeces. Barkocy-Gallagher et al. (2003) reported a *Salmonella* prevalence of 71% on the hides of feedlot cattle while a prevalence of only 4.3% was recorded from faecal samples of the same group.

The control of pathogenic *E. coli* and *Salmonella* infections on cattle farms includes the treatment of all carriers and infected subjects but also limiting the spread and severity of the disease. When the infection is identified early in the herd and few animals are affected, their isolation is an important measure to consider. Furthermore, faecal dejections from infected animals should be managed in a manner to avoid contamination of feed, water or livestock equipments. Antibiotic therapy, especially in subjects affected by salmonellosis, should be used cautiously as the emergence of *Salmonella* strains resistant to antibiotics commonly used in veterinary medicine is reported to be increasing (EFSA and ECDC, 2013a; Stevens et al., 2006). The treatment of *E. coli* and *Salmonella* infections in cattle herds has been thoroughly reviewed by Millemann (2008) and is not further developed in this paper.

The faecal shedding of pathogens from asymptomatic carriers constitutes a serious obstacle on the control of *Salmonella* and pathogenic *E. coli* infections in cattle; as shedders are not clinically identifiable and in most of times, not subjected to treatment. Traditionally, asymptomatic carriers can be detected through the culture of multiple faecal samples collected from suspected shedders during a relatively long period (Gopinath et al., 2012; Guy, Tremblay, Beausoleil, Harel, & Champagne, 2014). However, this approach presents a disadvantage of being logistically difficult to conduct and inefficient especially in carriers where the faecal shedding of *Salmonella* or pathogenic *E. coli* is intermittent (Edrington et al., 2004; Fitzgerald et al., 2003). As

an alternative to the cultural methods, serological methods that consist in the detection of antibodies specifically directed against some antigens expressed by the pathogen exist. An example is the measurement of immunoglobulins directed against O-antigens from *Salmonella* Dublin in the blood that was reported to be used as an indicator of *Salmonella* infection in cattle (Robertsson, 1984). However, further studies indicate that serological tests are indicative on the current and/or previous infection status of the subjects but not on their shedding status (Olopoenia & King, 2000). Therefore, considering the importance of the detection of shedders in the control of *Salmonella* and pathogenic *E. coli* infections in cattle farms and the weaknesses of the existing methods, it is recommended to develop more sensitive methods to detect shedding animals in the herd. Meanwhile, one should consider an approach consisting of serological screening followed up by a faecal culture of all seropositive animals to detect active carriers (Nielsen, 2013).

Animal stress is known to induce high levels of secretion of *Salmonella* and pathogenic *E. coli* in cattle faeces and increase the probability of contaminating healthy animals (Gopinath et al., 2012; Mainil & Daube, 2005). During their transport to the slaughterhouse cattle may be subjected to a number of stresses, including high stocking densities, long transport duration, abnormal temperatures, noise pollution and changes in the general environment that can significantly increase the number of shedders. Cattle can also be infected by pathogenic microorganisms from a contaminated truck that has not been properly cleaned and disinfected or by direct contact with infected animals embedded in the same truck. Similarly, contaminated transport trucks can be a source of infection for slaughterhouses and farms initially free of *Salmonella* or pathogenic *E. coli*. At the slaughterhouse level, cattle are kept in lairage before killing them. In Europe and the United States, cattle are generally slaughtered on the day of their arrival to the abattoir, while in other countries they are usually slaughtered the day after. In the latter case, the period of lairage allows animals to rest, rehydrate and recover from the stress of transport (Ferguson & Warner, 2008). During the period of lairage, cattle can be subjected to these same stress factors that increase the risk of contamination. On the other hand, in most cases the lairage is only cleaned at the end of the day and is therefore a potential source of contamination for cattle that can acquire an infection from contaminated animals or a soiled environment (Beach, Murano, & Acuff, 2002). Different authors have reported significant increases in pathogen prevalences on cattle hides during their transport and in the lairage period in the slaughterhouse. In a study conducted on 286 cattle, Arthur et al. (2007) reported that the prevalence of *E. coli* O157:H7 on hides increased from 50.3 to 94.4% between the time the cattle were loaded onto tractor-trailers at the feedlot and the time their hides were removed in the slaughterhouse. Similarly, Barham et al. (2002) reported an increase in *Salmonella* prevalence on cattle hides (from 6 to 89%) during the transport and lairage of 200 cattle, whereas the prevalence of *Salmonella* in the faeces of the same group increased from 18 to 46%.

A number of measures could contribute significantly to reducing the risk of bacterial contamination of cattle destined for slaughter in the preslaughter environment. At the farm level, cattle destined for slaughter should be clean and dry with no visible dirt on their hides (Antic et al., 2010). Any practice that can generate animal stress during transport, such as mixing cattle from different farms and over loading trucks should be avoided (Small & Buncic, 2009). Likewise, trucks should be cleaned and disinfected after each transport of cattle (Swanson & Morrow-Tesch, 2001). At the abattoir, the lairage period should be kept to a strict minimum. Heavily contaminated animals must be separated from the others and the lairage pens must be cleaned and disinfected at the end of each slaughtering day and monitored by visual and bacteriological control (Wong et al., 2002).

3.2 Contamination During the Slaughtering Process

In the abattoir, the cattle slaughtering process includes successive steps, namely: stunning, sticking, skinning, evisceration, carcass splitting, refrigeration and eventually cutting and deboning (Figure 1) that can contribute significantly to the overall microbial load of bovine carcasses and meat cuts. In this section, sources, risk factors and control measures for meat contamination by *Salmonella* and pathogenic *E. coli* throughout the cattle slaughtering process are reviewed and discussed.

3.2.1 Cattle Stunning

Stunning is an operation that aims to render animals destined for slaughter unconscious prior to sticking and bleeding. It allows suffering by the animals to be minimised during the slaughtering process, especially the sticking. Beside animal welfare considerations, stunning also makes the throat-slitting less hazardous for the operator (Food and Agriculture Organization, 2006). Although chemical and electrical stunning methods are allowed in domestic ungulates, mechanical stunning is the most commonly used stunning method in cattle (Gregory et al., 2000). The devices used for mechanical stunning can be of a penetrating or non-penetrating type. A number of studies have been conducted to address possible microbial contamination of meat during the stunning process. In one experimental study, Buncic et al. (2002) demonstrated that the use of a penetrating

captive bolt (PCB) in sheep presents a risk of microbial contamination for stunned animals through the stun wound. Marked organisms (*E. coli* K12 or *Ps. fluorescens*) were inoculated into the brains of sheep through the stun wound immediately after stunning by a cartridge-operated, penetrative captive bolt pistol. The marked organisms were found in blood, liver, lungs, spleen and lymph nodes and on the surface of inoculated animals. When the same pistol was then used to stun subsequent healthy sheep, marked organisms were found in the blood of 30% to 40% of the animal carcasses. Similar findings were reported by Daly et al. (2002) after inoculation of a marker strain of *Ps. fluorescens* into the central nervous system of cattle. Prendergast et al. reported dispersion of central nervous system tissues when a PCB was used for animal stunning.

Although the contamination of bovine carcasses by microorganisms introduced into the central nervous system during the stunning process by penetrating devices has been demonstrated experimentally, further studies are needed to assess the risk of such contaminations under commercial conditions. During the mentioned studies (Buncic et al., 2002; Daly et al., 2002), the levels of bacteria inoculated experimentally into the brain were relatively higher comparatively to the levels of bacteria commonly reported in slaughterhouses; suggesting that the risk of transmitting pathogens through the stun wound would be much lower under commercial conditions. However, as it known that *Salmonella* and pathogenic *E. coli* require low infective doses (Blaser & Newman, 1982; Coia, 1998) the risk should be considered as significant. Beside the possible contamination of cattle via the contaminated stunning gun, different authors reported regular cross contamination of hides in the stun box between stunned animals consecutively fallen in the same box via contaminated surfaces (Small & Buncic, 2009; Small et al., 2002) highlighting the need of a proper sanitation of the stun box.

As a control measure for food safety issues associated with the use of penetrating stunning devices in cattle, alternate stunning methods should be considered. The use of non-penetrating guns appears to be a good alternative. Nevertheless, potential problems associated with this type of gun, such as the frequent recovery before sticking, need to be resolved (EFSA, 2004). The use of electrical stunning seems to be another safer option (Anil et al., 2001). This method is used in different countries, namely New Zealand, Australia and the United Kingdom (Wotton, Gregory, Whittington, & Parkman, 2000); however, its high cost and some doubts about animal welfare associated with the ineffective use of this method need to be addressed (Heim, Löpfe, Mumford, & Speedy, 2007). Furthermore, the possibility of cleaning and disinfecting the stun box after stunning each animal should be considered to avoid cross contamination of hides from faecally soiled surfaces during the stunning process.

3.2.2 Cattle Sticking

Sticking is an operation that consists of severing the major blood vessels of the animal in order to extract the maximum amount of circulating blood during bleeding. Two sticking methods are generally used in cattle: thoracic and cervical sticking. Thoracic sticking includes a section of major blood vessels from the heart and allows rapid and complete bleeding, whereas during cervical sticking only vessels in the neck (carotid arteries and jugular veins) are cut and bleeding out is slower (Food and Agriculture Organization, 2006). The stick wound constitutes a channel that can allow the introduction of microbial contaminants into the carcass. The main source of contamination is the sticking knife, which can contaminate carcasses by direct transfer of bacteria from the transpierced skin but also by cross contamination if the knife is not sterilized between successive sticking operations.

In an experimental study (Mackey & Derrick, 1979), it was demonstrated that microbial contamination of bovine carcasses can occur during sticking. Marked strains of *E. coli*, *Cl. perfringens* and *Bacillus thuringiensis* were placed on a sticking knife before use. After the sticking operations, marked organisms were isolated from the internal organs, namely the heart, lung, spleen, liver and kidneys and from muscles. However, even if the potential for meat contamination from the sticking knife has been shown under laboratory conditions, the risk of such transfers, especially for pathogens like *Salmonella* and pathogenic *E. coli*, under commercial conditions seems to be quite low. Mackey and Derrick (1979) reported that in order to induce contamination of the deep tissues of a carcass a large inoculum of 10^{10} to 10^{12} bacteria was required, whereas the actual level of contamination generally encountered in slaughterhouses is many orders of magnitude less. In a study conducted on bovine hides at a beef slaughter plant in Ireland, hide contamination by *E. coli* O 157 was reported to be as low as 100 cfu per 100 cm^2 or less in 90.8% of 109 cattle(O'Brien et al., 2005). Comparable findings were reported in the USA, where 62.7% of 124 cattle were found to carry fewer than 100 cfu/100 cm^2 of *E. coli* O 157 (Rhoades et al., 2009). The concentration of *Salmonella* on cattle hides is also known to be relatively low. In a study conducted on 100 cattle at slaughter, Fegan et al. (2005) reported a prevalence of 68% with the highest concentration being 4.8 MPN per cm^2. Nevertheless, contaminated knives remain an important source of localised microbial contamination of the sticking wound (Rheault et al., 1999). Additionally, the sticking wound

can be contaminated by microorganisms from the environment, especially when exsanguination is performed on animals lying on the ground.

In order to avoid/prevent microbial contamination of bovine carcasses through the sticking wound, cattle should be bled out in a suspended position to prevent contamination from the slaughtering environment (Food and Agriculture Organization, 2006); two separate knives should be used for sticking (one for the skin and another for muscles) and they should be decontaminated in hot water at 82°C or by another method with equivalent effect after being used (Eustace et al., 2007); and finally, the sticking site should be trimmed if any microbial contamination is suspected (Rheault et al., 1999).

3.2.3 Hide Decontamination Treatments

Cattle hides constitute one of the main sources of carcass contamination by bacterial pathogens such as *Salmonella* and pathogenic *E. coli,* whereas the contamination of hides is generally acquired from faeces of colonised animals or indirectly from the soiled environment (Arthur et al., 2010). The contamination of carcasses from soiled hides occurs during the skinning process. A number of intervention strategies to reduce the bacterial load on cattle hides and consequently reduce the risk of carcass contamination during skinning operations, have been addressed by various authors. These include physical, chemical and biological treatments applied alone or in combination. In this section major hide decontamination treatments are reviewed and their effect on reducing the bacteriological load on cattle hides is discussed.

The reported physical decontamination treatments include hide washing with cold or hot water, steam sprayings and cattle dehairing. Washing cattle hides with water has been found to remove dirt from the hides but seemed to have a minimal effect on the bacterial load of treated hides. The study by Mies et al. (2004) showed that washing cattle with cold water for 2 minutes did not permit significant reductions in aerobic bacteria, coliforms and *E. coli* on the hides. However, raising the water temperature from 15 to 60 °C reduced the load of aerobic bacteria by 0.5 logarithmic units (Bosilevac et al., 2005).

The effect of steam sprayings in decontaminating cattle hides was studied under laboratory conditions by McEvoy et al. (2003). These authors, by treating cattle hide pieces with steam at subatmospheric pressure during 1 to 20 seconds, reported reductions in total viable bacteria on treated hides of 2.9 to 3.9 logarithmic units after a treatment at 80°C, while similar treatments at 75°C reduced total viable bacteria counts by only 1.9 to 2.6 log units. In another study, reductions of inoculated *E. coli* O157 by 4.2 to 6.0 log units were reported after spaying cattle hides by steam at 80°C during 10 to 20 seconds (McEvoy, Doherty, Sheridan, Blair, & McDowell, 2001).

Dehairing cattle can be carried out by clipping the hide or using chemicals. The study by Small et al.(2005) showed that dehairing cattle hides with a clipper does not reduce the aerobic bacterial load on the hides, probably because of dust generation and subsequent dispersal of the bacteria. However, treating previously clipped hides with other physical or chemical hide decontamination methods was found to afford bacterial load reductions significantly higher than these obtained on unclipped hides (Baird et al., 2006). The use of chemical dehairing has been studied by Castillo et al. (1998). These authors, using a solution of sodium sulphide, water rinses, and hydrogen peroxide under laboratory conditions, achieved significant reductions in *E. coli* O157:H7 and *S.* Typhimurium previously inoculated on bovine hides (more than 4 logarithmic units). However, in a study conducted on 240 cattle in a commercial beef processing plant, Nou et al. (2003), using a similar method on cattle immediately after stunning, reported a reduction in *E. coli* O157 prevalence on the treated cattle hides from 88 to 67% without any significant reduction in aerobic bacteria or *Enterobacteriacea* populations.

A wide range of chemical antimicrobials have reportedly been used in hide decontamination treatments. These include organic acids (Mies et al., 2004), commercial detergents and disinfectants (Baird et al., 2006; Small et al., 2005), ozonated and electrolysed water (Bosilevac et al., 2005), and combinations of different chemicals (Carlson et al., 2008). However, their efficacy in reducing the bacterial load on cattle hides has been found to be dependent on a number of experimental factors such as the mode of application, the product concentration and temperature, the duration of exposure and the target microbial species. Limited studies have addressed the effect of chemical antimicrobials on pathogens such as *Salmonella* and *E. coli* present on cattle hides destined to slaughter. Nevertheless, organic acids appear to be the most studied group of chemical (Loretz, Stephan, & Zweifel, 2011). The effect of organic acid sprays in reducing *Salmonella* load on cattle hides was studied by Mies et al. (2004). These authors sprayed cattle hides with different concentrations (2 to 6%) of acetic and lactic acids and noted reductions in *Salmonella* Typhimurium previously inoculated on the hides of 2.4 to 4.8 and 1.3 to 5.1 logarithmic units, respectively. However, treating live cattle with a lactic acid solution (0.5%) during 1 minute did not reduce the proportion of *Salmonella*-positive hide samples. In another study, a reduction average

of 2 log units in *Salmonella* and *E. coli* O157 loads was reported on previously inoculated cattle hides using lactic and acetic acid (10%, 55°C) sprays (Carlson et al., 2008).

Although a variety of biological treatments are reported to be used in carcass decontamination, bacteriophages constitute the only biological treatment reported to be used in hide decontamination (Bolder, 1997; Chen et al., 2012). Some bacteriophages targeted to bacteriological pathogens namely *Salmonella* and *E. coli* O157:H7 have been already approved in United States for cattle hide decontamination, however the possibility of their utilization under commercial conditions is still being investigated (Loretz et al., 2011)

Apart from reducing the bacterial load on hides and possibly improving carcass microbiological quality, some hide decontamination interventions were found to present some disadvantages. It has been reported that treatments with water or steam increase the humidity on the surface of the treated hides (Loretz et al., 2011). This makes the skinning operations more difficult for the operator and may increase the risk of carcass contamination from the hide, especially when hide removal is carried out manually. Antic et al. (2010) reported that microbial contamination of bovine carcases during skinning was more likely to occur when the animal hide was wet. Steam treatments were also found to deteriorate the commercial quality of hides (McEvoy et al., 2003). Furthermore, animal and operator welfare problems, namely eye and skin irritation as well as corrosion of slaughtering equipment, have reportedly been associated with the use of chemical antimicrobials, particularly organic acids (Chen et al., 2012; Mies et al., 2004).

Hide decontamination, treatments appear to be an important strategy that can significantly reduce the risk of carcass contamination from soiled hides during the skinning process. However, considering existing data, it is difficult to accurately appraise their effect under normal slaughtering conditions as most of available informations derive from experimental studies. Additionally, very limited number of studied treatments concerned bacterial pathogens such as *Salmonella* or pathogenic *E. coli*. It is therefore imperative to conduct further studies to assess the effects of these interventions on major bacterial pathogens under practical slaughtering conditions. Another issue is to identify the optimal moment in the slaughtering process at which the hide decontamination treatment should be carried out under commercial conditions. The moment between stunning and sticking would be appropriate provided that the animal's unconsciousness lasts until the hide decontamination process ends. Alternatively, the moment after sticking but before hide removal would be used. In the latter case, appropriate measures should be taken so as not to contaminate the sticking wound during the hide decontamination process.

3.2.4 Cattle Skinning

The skinning stage is one of the slaughtering steps where microbial contamination of bovine carcasses is most likely to occur. This is due to the fact that the hide is, in most cases, heavily populated by a wide range of microorganisms that can be transferred to carcasses during skinning operations (Loretz et al., 2011). Bacterial pathogens such as *Salmonella* and *E. coli* O157 are also commonly isolated from hides of cattle destined to slaughter (Barham et al., 2002; Barkocy-Gallagher et al., 2003). During the skinning process, carcass contamination may occur through direct contact between the carcass and the hide or indirectly through equipment or operators contaminated by hides. Carcass contamination by airborne transfer is also possible (Antic et al., 2010).

Cattle hide removal can be carried out either manually or mechanically by means of a hide puller. The advantage of manual hide removal relies mainly in its low financial investment in equipment, but it has been found to present several disadvantages in terms of slaughter productivity and meat hygiene (Food and Agriculture Organization, 2006). These include the requirement for a very high skill level for effective hide removal without damaging both hide and carcass; the difficulty of the task and the time consumed even for a skilled operator; and a high risk of contaminating the carcass with microorganisms from the hide during the skinning process. On the other side, mechanical skinning by means of a hide puller seems to require less manual contact with the hide and consequently minimizes the risk of carcass contamination by microorganisms from operators and slaughtering equipments. Additionally, it increases the productivity of the slaughterhouse and improves the value of the hides by damaging them less. The disadvantages of mechanical skinning include the high cost of the equipment and fractures of the spinal column sometimes associated with the use of a downward hide puller.

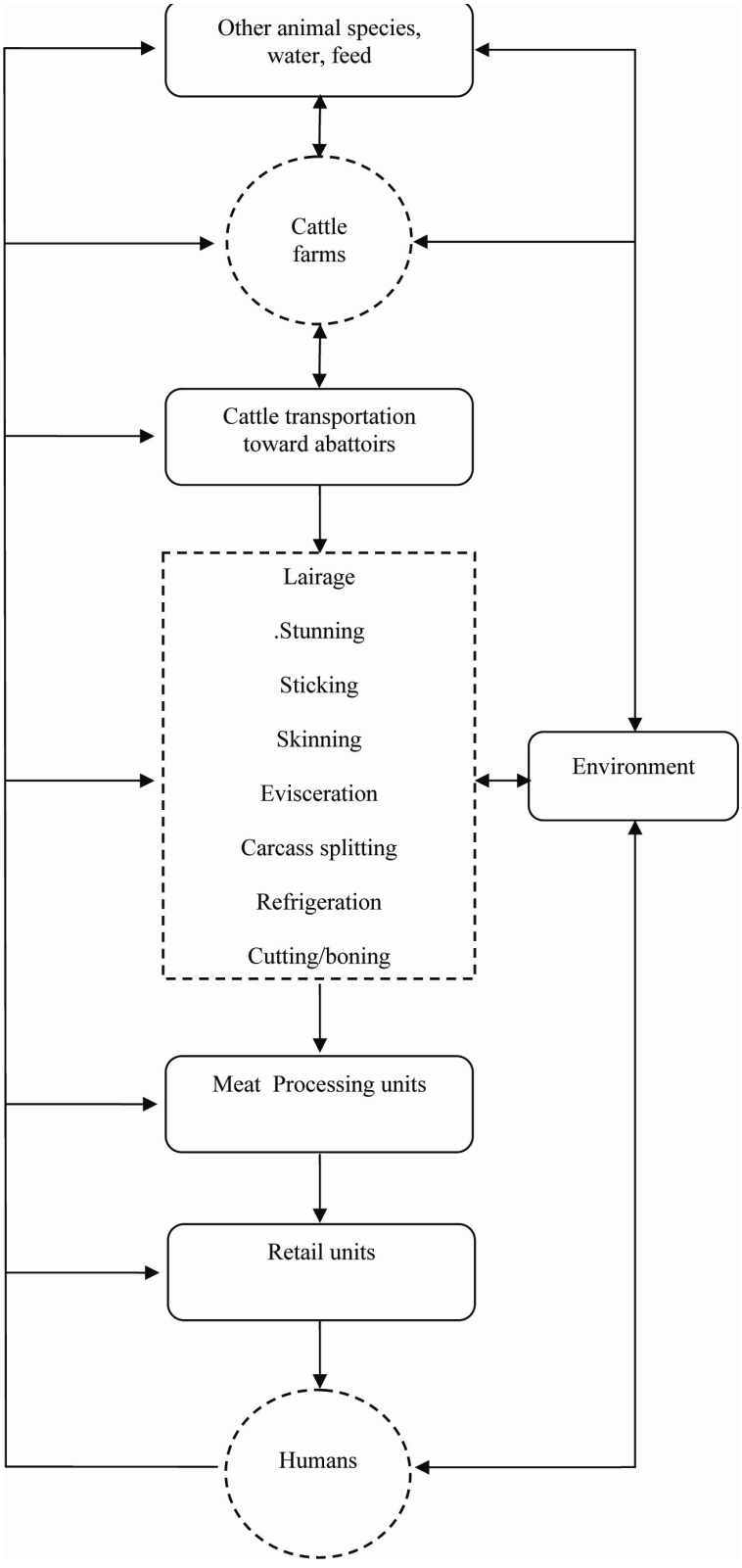

Figure 1. Potential sources and pathways for microbial contamination of bovine meat (Adapted from: Food and Agriculture Organization, 2006 and Millemann, 2008)

Peer-reviewed studies addressing the quantification of microorganisms transferred from hides to carcasses during the skinning indicate that, under commercial conditions, dressed bovine carcasses carry a very small proportion (ranging from 1.6 to 0.003%) of the hide microflora (Arthur et al., 2004; Bacon et al., 2000). Another

study showed that only 0.5 to 0.00002% of the hide microflora is transferred to dressed bovine carcass via direct contact (Antic et al., 2010) highlighting the importance of other transmission pathways such as indirect contamination via knives and/or hands or airborne transfers. Nevertheless, even if the reported hide-to-meat microbial transmission rates appear to be relatively low, it should be noted that the risk associated to these transmissions is still significant. In fact, carcass contamination from hides occurs regularly under commercial slaughtering conditions and the reported bacterial loads on hides are so high that proportions as low as less than 1% would constitute levels of many logarithmic units (Loretz et al., 2011). By summarizing data from numerous studies published internationally, Antic et al. (2010) reported bacterial contamination levels of 6–10 log cfu/cm^2 and 4.5–8 log cfu/cm^2 respectively on visually dirty and clean hides from cattle destined to slaughter.

The control of carcass bacterial contamination from hides during skinning operations consists basically in preventing hide-to-meat contaminations through process hygiene means and/or the elimination microbial contaminants from hides before skinning operations by adequate treatments. Concerning the process hygiene, several studies have reported an association between the hide cleanliness and the microbiological status of dressed carcasses (McEvoy et al., 2000; McCleery et al., 2008). Thus, in many countries (including but not limited to Australia, Ireland, Finland, Norway and United Kingdom) Good Hygienic Practice programs in cattle dressing are based on the cleanliness of cattle hides. In these countries, only cattle with clean hides are slaughtered under normal conditions whereas dirty animals are either cleaned (and allowed to dry before slaughtering) or are slaughtered separately under special conditions as they are considered to present a high risk for cross contaminations (McEvoy et al., 2000). A recent study conducted in Norwegian abattoirs (Hauge, Nafstad, Røtterud, & Nesbakken, 2012) confirmed that, under commercial conditions, carcasses from clean animals present levels of hygiene indicator bacteria (total aerobic bacteria and *E. coli* counts) significantly lower than these from dirty animals. Although the cleanliness of cattle hides prior to skinning presents considerable beneficial effects on the bacteriological status of dressed carcasses, it should be noted however, that these effects are not absolute. In fact, it is known that pathogenic bacteria such as *E. coli* O157 are commonly isolated from visually clean hides (Nastasijevic et al., 2008). Therefore, the selection of cattle with clean hides for slaughter should be combined with other good hygienic practices including hygiene for staff and skinning equipments as well good manufacturing practices particularly an immediate carcass trimming when any carcass contamination is suspected (Kiermeier et al., 2006; J J Sheridan, 1998). The elimination of bacterial contaminants from hides prior to skinning constitutes a promising alternative to consider. However, as presented in previous sections of the present paper, most of the existing informations on the effects of hide decontamination treatments derive from experimental studies. Further studies are therefore still needed to accurately appraise the effects of these treatments under commercial slaughtering conditions.

3.2.5 Evisceration

As the skinning step, evisceration constitutes a critical slaughtering stage where microbial contamination of carcasses is most likely to occur. The gastro-intestinal tract of cattle is naturally colonised by microorganisms that may be transferred to carcasses during the evisceration process (McEvoy et al., 2000). Additionally, bacterial pathogens such as *Salmonella* and *E. coli* are also frequently isolated in faeces of cattle destined to slaughter highlighting their probable presence in the digestive tract of the same animals (Rhoades et al., 2009). During the evisceration process, carcass contamination occurs by direct contact between the carcass and the gastro-intestinal contents or indirectly through soiled slaughtering equipments and staff. Contaminations may also occur during the removal of pharynx, tonsil and tongue as they are reported to be heavily contaminated by various microbial contaminants (Sheridan, 1998; Wheatley, Giotis, & Mckevitt, 2014).

Several peer-reviewed studies indicate a significant increase of bacterial loads on carcasses during the evisceration process; however the degree of increase varies from one study to another. The observed variation could be attributed to a number of factors including the differences in experimental designs and the process hygiene that differ from one slaughterhouse to another. For example, an average increase of 0.7 logcfu/cm^2 in *Enterobacteriaceae* counts was reported during the evisceration of lamb carcasses in 4 Irish abattoirs (Sierra, Sheridan, & McGuire, 1997); whereas in Rwanda increases of 3 and 1.3 log cfu/g were respectively observed in total aerobic bacteria and *E. coli* counts during the evisceration of cattle at a commercial abattoir (Niyonzima et al., 2013). Another Irish study reported an increase of 2-4 log in *Enterobacteriaceae* populations during the evisceration of pork carcasses (Wheatley et al., 2014).

The control of carcass bacterial contaminations during the evisceration process relies mainly on Good Slaughtering Practices. The techniques mostly used include the “bunging” and the “rodding”. The bunging or bung tying consists in sealing the rectum and covering it with a plastic bag in order to reduce the spread of faecal material from the rectum to the carcass; whereas the rodding corresponds to sealing the oesophagus to avoid the

spread of its content onto the carcass (McEvoy, Sheridan, Blair, & McDowell, 2004). These techniques are effective in reducing the risk of bacterial transfers during the evisceration. Nesbakken et al. (1994) reported that bunging reduced significantly the occurrence of *Yersinia enterocolitica* on pig carcasses. Furthermore, the introduction of that technique in Norwegian pork abattoirs resulted in decreasing the incidence of Yersiniosis by 25% in the population (Sheridan, 1998). Similarly, special attention must also be paid to the training of staff on Good Hygienic Practices as well as on the sanitation of slaughtering equipments particularly knives to minimize the risk of cross contaminations. Bolton et al. (2002) recommend sanitizing knives by a two-knife system that consists in the utilization of one knife while the other is being sanitized in hot water at 82°C or above.

Despite the reported increases in bacterial load on carcasses during their evisceration, some authors indicate that the existing measures including rectum and oesophagus sealing, intact removal of visceral contents and an appropriate training of staff in Good Hygienic Practices could reduce the risk of carcass contamination from viscera to the point where they do not contribute significantly to the overall contamination of the carcass (McEvoy et al., 2000; Wheatley et al., 2014).

3.2.6 Carcass Splitting

The carcass splitting stage is not generally considered as a major source of contamination (Wong et al., 2002). However, the splitting saw as well as other slaughtering equipment can be contaminated with pathogenic bacteria such as *Salmonella* and *E. coli* and may contribute to their spread to several carcasses. In a study conducted in 4 European countries, Hald et al. (2003) reported that 9.4% of 384 carcass splitter machines were contaminated with *Salmonella* during the slaughtering process. In addition, Warriner et al. (2002) demonstrated that *E. coli* and potential enteric pathogens can be transferred between pork carcasses through the splitting saw. Therefore, cleaning and disinfection of the splitting saw should be carried out after splitting each carcass in order to reduce the risk of cross contaminations. The European regulations recommend disinfecting the splitting saw after splitting each animal using water at 82°C or above or using another method with an equivalent effect (European Commission, 2004).

Although adherence to Good Hygiene Practices in abattoirs improves the microbiological quality of the meat significantly, it is generally recognized that contamination of meat is unavoidable during the cattle slaughtering process (McCann et al., 2006). Therefore, carcass decontamination before refrigeration appears as a corrective measure to restore the bacterial load of carcasses to the acceptable range.

3.2.7 Carcass Decontamination Treatments

Various treatments including physical, chemical and biological methods applied alone or in combination have been identified to reduce the levels of bacterial load on carcasses. In this section majors carcass decontamination treatments as well as their respective effects on the bacterial load of carcasses are discussed.

The physical decontamination treatments mostly reported for carcasses include hot water washes and application of steam. These treatments are generally carried out in special cabinets where carcasses are splayed with water or steam at controlled pressure and temperature. Carcass sprays with hot water were found to lower significantly the bacterial load on treated carcasses. However, the reduction rates reported were found to be dependent of experimental factors such as the temperature, pressure and the duration of the treatment (Loretz et al., 2011). The effectiveness of carcass decontamination by hot water was demonstrated by Bosilevac et al. (2006). These authors conducted a study in a commercial abattoir and reported 2.7 log reductions in both aerobic plate counts and *Enterobacteriaceae* counts on pre-evisceration bovine carcasses washed in a cabinet with water at 74°C for 5.5 seconds. The prevalence of *E. coli* O157 was also reduced by 81% in treated carcasses. The decontamination of carcasses with steam was reported to yield bacterial reductions comparable to these obtained with hot water spays. However, the treatments with steam presents an advantage of reaching cavities and crevices of carcasses that are generally inaccessible to hot water (Hugas & Tsigarida, 2008). One of the side effects reportedly associated with carcass decontamination treatments by steam or hot water is the change in the carcass colour after a prolonged treatment. McCann et al. (2006) reported a cooked appearance on the surface of carcasses having undergone a steam decontamination treatment of 10 seconds or longer. Furthermore, weight gain resulting from water absorption by treated carcasses generally reported in hot water decontamination treatments may be perceived as a fraud by meat consumers (EFSA, 2010).

Organic acid sprays, namely acetic, citric and lactic acids are the most-reported chemical decontamination methods used on beef carcasses. They are known to reduce the number and prevalence of food borne pathogens and the microbial load on meat carcasses (Huffman, 2002), but their efficacy depends on the type of meat tissue, the type and load of initial microbial contamination, as well as the pH, concentration and temperature of the organic acid solution (Hugas & Tsigarida, 2008). Various studies conducted under laboratory conditions showed

that spraying inoculated bovine carcasses with acetic or citric acid yielded bacterial reductions varying between 0.7 and 4.9 logs for aerobic bacteria, non pathogenic *E. coli*, *E. coli* O157:H7 and *Salmonella* (Loretz et al., 2011). However, lower reductions are generally reported in studies conducted under commercial conditions. This could be due to lower acid concentrations used in respect to meat quality and staff welfare considerations (Chen et al., 2012). By spraying acetic acid (2.5%) to bovine carcasses prior to chilling Algino et al. (2007) reported reductions of coliforms, *Enterobacteiaceae* and *E. coli* levels ranging from 0.6 to 1.4 logs. In a study by Barboza de Martinez et al. (2002) spraying carcasses at the end of slaughter by lactic acid (1.5%) yielded reductions of 0.5, 1.8 and 0.6 logs, respectively, for aerobic bacteria, coliforms and *E. coli*. In another study, lactic acid (2%; 42°C) spraying of pre-eviscerated bovine carcasses was reported to reduce the prevalence of *E. coli* O157:H7 by 35% as well as aerobic bacteria and *Enterobacteriaceae* counts by respectively 1.6 and 1.0 logs (Bosilevac et al., 2006). Other chemicals such as chlorine, trisodium phosphate, acidified sodium chlorite and peroxyacids are also used for meat decontamination but to a lesser extent. Generally, the use of these substances leads to 1–1.5 log reductions in foodborne pathogens such as *Salmonella* and *E. coli* O157 (Hugas & Tsigarida, 2008). A number of drawbacks have however been reportedly associated to the chemical decontamination of carcases especially by organic acids. These include staff welfare problems such as eye or skin irritations and the corrosion of slaughtering equipments (Chen et al., 2012; Mies et al., 2004).

Reported biological treatments for meat decontamination include the use of bacteriocins and bacteriophages. Bacteriocins are anti-microbial proteinaceous compounds produced by some bacteria. The most widely known bacteriocin is nisin, which is produced by *Lactobacillus lactis* subsp. *lactis* and is effective against Gram-positive bacteria. Nisin is used as a preservative agent in foods like cheese but its use in carcass decontamination has been limited by a number of factors, namely its deficient inhibitory effect on Gram-negative bacteria, low level of production *in vivo* and likely inactivation of its effect due to interactions with other food components (Bolder, 1997; Chen et al., 2012). Nevertheless, combinations of nisin and other treatments have been reported to reduce microbial contamination on carcasses. Barboza de Martinez et al. (2002) reported that a combination of nisin and lactic acid sprays under commercial conditions reduced aerobic bacteria, coliforms and *E. coli* populations on carcasses by 2.0, 2.2, and more than 1.0 log, respectively, whereas treatment with nisin alone reduced bacterial levels by less than 0.2 log. The use of bacteriophages has also been reported to present a number of benefits as an alternative biocontrol method. These include their high host specificity and lack of effect on the organoleptic qualities of the food as well as their ability to survive under commercial processing procedures (Hugas & Tsigarida, 2008). However, their use in food decontamination is still limited by factors such as the potential development of resistance in targeted bacteria (Chen et al., 2012). The use of *E. coli* O157:H7 and *Salmonella* targeted bacteriophages for cattle hide decontamination has already been approved in the USA. However, further investigations to address their efficacy under long-term commercial conditions and their possible utilization for carcass decontamination are still required (Loretz et al., 2011).

Although obtaining bovine carcasses free of pathogenic bacteria and with low microbial contamination appears to be a shared goal of all countries, carcass decontamination policies vary from one country to another. In the USA, for example, a number of carcass decontamination treatments are allowed and commonly used in cattle slaughterhouses. These include physical interventions such as hot water or steam spraying and chemical treatment with organic acids, namely lactic and acetic acids (Chen et al., 2012). Contrary to this, in Europe important efforts have been put into the application of Good Manufacturing Practices throughout the entire meat production line, and for many years carcass decontamination treatments in the European Union were limited to the use of clean or potable water. The current European regulation (European Commission, 2004) allows the use of substances other than water for the removal of surface bacterial contamination from meat; however, the European Food Safety Authority (EFSA) must provide a chemical and microbiological risk assessment before the European commission authorizes the use of such substances (Hugas & Tsigarida, 2008). Currently, no chemical substance has yet been approved for decontamination of beef carcasses within the European Union (EFSA, 2014a).

Carcass decontamination treatments constitute a potential control measure to reduce the levels of bacterial and pathogen loads on carcasses. However, available informations indicate a wide variability in bacterial reduction yields as most of data results from studies conducted under different conditions. Thus, studies to compare the effectiveness of different treatments under the same conditions would be of valuable importance to identify the cost-effective interventions to be used in cattle slaughterhouses. Furthermore, the risk, in some abattoir, to rely only on the carcass decontamination step and abandon existing good hygienic and manufacturing practices in previous slaughtering stages need to be considered before the adoption of such interventions.

3.2.8 Carcass Refrigeration

Apart from meat maturation purposes, refrigeration of carcasses after the slaughtering process is performed to inhibit the growth of spoilage and/or pathogenic bacteria that could still be on the carcasses and consequently increase their shelf-life (Dave & Ghaly, 2011). Carcass refrigeration is generally carried in two phases including the rapid chilling phase consisting in rapidly reducing the carcass temperature and a second phase of cold storage intended to maintain the low temperature of carcasses. Different methods of carcass chilling were thoughtfully reviewed by Savell et al. (2005) and are not further developed in this paper. In commercial slaughterhouses, carcasses are generally chilled for 48-72 hours before their transfer in the boning hall. Nevertheless, the duration of carcass chilling may be extended beyond 72 hours to improve the quality of meat. This process is referred to as aging (EFSA, 2014b). The inhibition of bacterial growth on carcasses at refrigeration temperatures is a consequence of low-temperature stress undergone by microorganism. In fact, as the temperature decreases, the bacterial lag phase extends whereas the growth rate decreases and the ultimate cell numbers may decrease (Beales, 2004; N J Russell et al., 1995).

Although bacterial growth on carcasses is known to be inhibited at refrigeration temperatures (Korsak, Clinquart, & Daube, 2004; Russell, 2002), several published studies indicate increases in levels of bacterial loads on refrigerated carcasses. In a study conducted in a commercial abattoir, Bolton et al. (2002) reported an increase in total viable bacterial counts from 3.8 to 4.5 log cfu/cm^2 on carcasses at the refrigeration stage. Another study conducted on poultry carcasses showed that after 9 days of storage, *Salmonella* loads were slightly reduced (by less than 1 log unit) on carcasses refrigerated at 2 and 6°C whereas in carcasses refrigerated at 8°C *Salmonella* number increased by 1.5 log units (Jiménez, Tiburzi, Salsi, Moguilevsky, & Pirovani, 2009).

Microbial growth on carcasses under refrigeration is mainly attributable to the temperatures of chilling equipments that are not sufficiently low to inhibit the microbial growth and/or to intermittent rupture of the cold chain. In fact, bacterial pathogens such as *Salmonella* may multiply to hazardous levels during periods of temperature abuse (Delhalle et al., 2009; Wong et al., 2002). Thus, slaughterhouses should be equipped with chilling equipments capable to rapidly decrease and maintain low the temperature of carcasses during the entire refrigeration period. In European Union countries for example, the carcass temperature must be decreased to maximum 7°C in the first 24 hours of refrigeration (European Commission, 2004). Additionally, trough cross-contaminations, microorganisms present on carcasses under refrigeration or on chilling equipments may get disseminated to other carcasses and proliferate when the environment become favourable to their growth (for example during temperature abuses). Published studies indicate that bacterial pathogens can survive on surfaces of refrigerators (Jackson, Blair, McDowell, Kennedy, & Bolton, 2007) or on chilling evaporators (Evans, Russell, James, & Corry, 2004) and pose a cross-contamination risk to the refrigerated foods. This highlights the need for a regular cleaning and disinfection of chilling rooms and/or equipments in the slaughterhouse.

The refrigeration of carcasses constitutes a determinant slaughtering stage influencing the final bacterial load on carcasses. However, despite the exiting control measures (including adequate chilling equipments, the regular monitoring of the temperature of carcasses and the mastery of cross-contaminations through an effective appliance to Good Hygienic Practices) that have proven their effectiveness in significantly reducing the risk of bacterial/pathogen growth on carcasses under refrigeration (Delhalle et al., 2009); in some slaughterhouses, bacterial growth is still being reported on refrigerated carcasses probably due to the failure in applying adequate control measures. It is thus imperative for slaughterhouses to deploy all material, technical and financial means required to control the bacterial growth on carcasses at this critical stage of slaughter.

3.3 Post Slaughter Contamination of Bovine Meat

The post slaughter section of the meat chain comprises a series of sub-stages (including cutting/boning, transportation of carcasses or meat cuts, meat storage, manufacture of meat products, retail, and eventually cooking) at which contamination of meat may occur. In this section majors sources of post slaughter meat contamination by pathogenic bacteria and their respective control measures are reviewed.

At the end of the slaughtering process carcasses are generally cut in special meat pieces (cutting) and separated from bones (boning) for industrial and commercial utilisations. The cutting and boning of carcasses may take place in the slaughterhouse or in specialised plants. The cutting and boning operations are generally performed on refrigerated carcasses however boning of non refrigerated carcasses (hot boning) is also possible (Røtterud et al., 2006). Even if hot boning presents a number of advantages including a reduced cost and fewer requirements in chilling equipments and space (Pinto Neto, Beraquet, & Cardoso, 2013) it is rarely used in European countries. This is mainly due to a possible proliferation of pathogenic bacteria on processed meat and a reduced shelf life of subsequent vacuum packed meat (Yang, Balamurugan, & Gill, 2011). A recent report from the European Food

Safety Authority indicate that the surface temperature of boned beef cuts from chilled carcasses decreases to 8°C in few hours whereas the temperature of hot boned and vacuum packed meat pieces may remain at 25°C for many hours; creating favourable conditions for the proliferation of spoilage and pathogenic microorganisms (EFSA, 2014b). Data from published studies indicate that bacterial/pathogen loads on carcasses may significantly increase during the cutting/boning operations even in slaughterhouses where cold boning is practicised. In a study conducted in an Irish beef abattoir, McEvoy et al. (2004) reported increases of 2.3 and 2.1 logcfu/cm^2 respectively for total viable bacteria and *Enterobacteriaceae* counts on the inside round of carcasses during the cutting/boning operations. Similar increases were also reported in *E. coli* numbers during the boning of beef carcasses (Gill, McGinnis, & Bryant, 2001). Increases in bacterial/pathogen numbers following the cutting and boning operations could be associated to cross-contaminations. During these operations, carcasses or meat pieces of various origins and different contamination levels are handled in close proximity, creating numerous opportunities for cross-contamination or spread of pathogenic bacteria (Wong et al., 2002). Various origins of microbial contamination during cutting/boning were reported in literature. These include carcasses or meat pieces to be processed (McEvoy et al., 2004); meat cutting/boning equipments such as knives, meat conveyors or cutting boards (Gill, Badoni, & McGinnis, 1999; Gill et al., 2001; Jiménez et al., 2009) and soiled surfaces or operators (Sheridan, Lynch, & Harrington, 1992). One of the measures to prevent cross contaminations resulting from contaminated carcasses or meat pieces would be to identify the most contaminated raw materials and to process them separately preferably at the end of the production (Koohmaraie et al., 2012). Contaminations from equipments can be mastered through a regular cleaning and disinfection of equipments and surfaces whereas effective training of staff on Good Hygienic Practices could help in preventing contaminations from personnel (Delhalle et al., 2009). The working temperature constitutes another factor influencing bacterial growth on meat during the cutting/boning operations. In fact, during these operations the temperature of processed meat increases and this would favour the growth of existing microbial populations. It is therefore recommended to carry out boning operations under refrigerated conditions. In some commercial abattoirs, the boning hall is refrigerated at 10-12°C (EFSA, 2014b; McEvoy et al., 2004).

Although temperature abuse is reportedly the main factor associated to bacterial load increases on carcasses or meat pieces during transportation and storage (Delhalle et al., 2009; Wong et al., 2002), cross-contaminations originating from chilling equipments or personnel are also significant contributors to the ultimate bacterial load of meat (Evans et al., 2004; Jackson et al., 2007; Sheridan et al., 1992). Thus, it is imperative to prevent cross contamination through an effective and regular sanitization of chilling rooms and meat transportation vehicles. Additionally, personnel involved in the loading of meat transportation vehicles should be educated in good hygiene practices. On the other side, the regular monitoring of temperatures in chilling rooms and meat transportation vehicles could contribute significantly in reducing the risk of temperature abuses occurring during meat storage and transportation (Savell et al., 2005). Recent published studies indicate that meat transportation vehicles with a chilling capacity comparable to the one of conventional chilling rooms are commercially available. Most of these vehicles are designed for longer distances and can decrease the core temperature of bovine carcasses from approximately 20°C at the loading time to 7°C or lower after 48 hours. Additionally, these vehicles are equipped with apparatus to continuously monitor the temperature of meat during the transportation (EFSA, 2014b).

As in previous stages of the meat chain, cross-contamination from equipments, personnel or the working environment are likely to occur during the manufacture of meat products if appropriate control measures are not effectively applied (Roels et al., 1997; Wong et al., 2002). Nevertheless, microbial contaminants from incorporated non-meat ingredients as well as contaminations associated to the manufacture method used, appear to be specific to this particular stage of the meat chain. The grinding of meat for example, would result in the dissemination of microorganisms previously localised at the surface of meat pieces in the entire batch of minced meat (Gould et al., 2011). As the meat temperature increases during grinding operations due to friction movements, these microorganisms may proliferate in relatively short period resulting in bacterial number increases in minced meat (Heinz & Hautzinger, 2007). A number of preservative treatments (including thermal interventions, smoking, curing etc.) are commonly used in meat processing to enhance the bacteriological stability of meat products and consequently increase their shelf-life. These interventions, generally based on the control of the temperature, pH, water activity, microbial competition/interaction and oxido-reduction potential, were found to significantly reduce the bacterial/pathogen load in meat (Chen et al., 2012; Hugas & Tsigarida, 2008; Loretz et al., 2011). However, as their preservative effects depend also on the initial bacterial numbers in meat; it is crucial for meat processors to assure that the used raw materials are of good microbiological quality (Wong et al., 2002). Another important measure would be to decide the fate of raw materials according to their microbiological quality. In some commercial meat processing plants, heavily contaminated raw materials are

generally reserved for the manufacture of meat products destined to undergo a heat treatment (Koohmaraie et al., 2012; McCleery et al., 2008; McEvoy et al., 2000; Nastasijevic et al., 2008).

At the retail level, temperature conditions are reportedly an important factor influencing the final microbiological quality and safety of meat products (Delhalle et al., 2009). Several published studies indicate a wide variation in meat product temperatures within retail cabinets but most of the data are simply indicative of the product temperature at the time and place of the study and do not address conditions that would dynamically influence the temperature changes (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Nevertheless, it is recognised that displayed meat products in retail cabinet must be at temperatures sufficiently low (generally below 4°C) to inhibit the growth of spoilage or pathogenic microorganisms (Wong et al., 2002). It is therefore imperative to regularly monitor the temperature of display cases to prevent temperature abuses during meat storage. Furthermore, appropriate control measures must be applied to prevent cross contaminations from equipments, personnel or working environment in retail establishment where meat processing activities such as cutting or grinding are carried out (Gould et al., 2011). The retail level represents an important stage of the meat chain in regard with the final quality and safety of meat products as it constitutes the last "check point" where contaminated products can be identified before their consumption particularly for ready-to-eat meat products (Wong et al., 2002).

The last section of the meat chain related to the transportation, storage and cooking by the consumer appears to be less studied although it is the most important in regards with the food safety aspects of meat products. This is due to the difficulties in collecting data concerning the mode and the duration of meat transportation toward the consumer's household, temperature conditions in domestic refrigerator and freezers, durations of storage before consumption as well as consumer's cooking habits (Nychas et al., 2008). However, it is recognised that the cooking stage is the last line of defence of consumers against *Salmonella* and pathogenic *E. coli* infections attributable to bovine meat (Korsak et al., 2004; Mainil & Daube, 2005). These pathogens are generally destroyed in foods at conventional pasteurisation temperatures. In beef, *Salmonella* is reported to have a decimal reduction time (D-value) of 0.53 minutes (z=5°C) at 65°C (Juneja et al., 2001; Korsak et al., 2004) whereas *E. coli* O157:O7 presents a D-value of 0.39 minutes (z=6°C) at the same temperature (Juneja, Snyder, & Marmer, 1997). It is therefore recommended to cook meat until the internal temperature reaches a minimal temperature of 70°C to assure a thermal destruction of these pathogens in meat as most of the reported *Salmonella* and *E. coli* O157:H7 outbreaks attributable to meat were found to be associated to the ingestion of raw or undercooked meat products (Abong'o & Momba, 2009; Greig & Ravel, 2009; Roels et al., 1997).

4. Conclusion

Despite a large number of control measures along the meat chain, meat contaminations by *Salmonella* and pathogenic *E-coli* remain a serious public health problem in humans. In the pre-slaughter stages of the meat chain, difficulties in identifying asymptomatic shedders constitute the main obstacle to the control of the infection spread in live animals. Further studies are therefore needed to identify cost-effective techniques and approaches to diagnose asymptomatic carriers in cattle herd before animal transportation to abattoirs. During the slaughtering process, the skinning and evisceration operations appears to be the most critical stages for carcass contamination. Thus Good Manufacturing Practices in accordance with HACCP principles must be strictly applied in commercial slaughterhouses to reduce the risk of carcass contamination at those specific stages. The decontamination of carcasses has also shown a potential in reducing pathogen numbers on carcasses prior to chilling, even if its utilisation in some countries is still limited by a number of factors including the cost of installations, the commercial quality of treated carcasses as well as the risk of relying only on the carcass decontamination step and reduce efforts devoted to Good Hygiene and Manufacturing Practices in previous slaughtering stages. Along post-slaughter stages of the chain, handling, time and temperature are the main factors influencing the microbial contamination of meat. Therefore application of appropriate GMP and GHP by meat processing plants is of great importance to prevent cross-contaminations during cutting/boning, processing, transportation and retail of meat products. Similarly, the cold chain must be respected at all stages of meat distribution. Although all stages of the bovine meat chain are of significant relevance in regard to the ultimate bacterial contamination of meat, the cooking step constitutes the most important stage to assure the safety of beef at consumption. In fact, the cooking step is the last stage of the meat chain at which *Salmonella* and pathogenic *E. coli* can be completely destroyed.

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