EXTERNAL SCIENTIFIC REPORT

Escherichia coli and *Enterobacteriaceae* counts on pig and ruminant carcasses along the slaughterline, factors influencing the counts and relationship between visual faecal contamination of carcasses and counts: a review[1]

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ABSTRACT

A literature review was conducted covering the period 2000-2012 to gather information concerning the presence and counts of *E. coli* and *Enterobacteriaceae* on carcasses of main livestock species during different stages of the slaughterline (review question 1); risk factors that could explain the variability of the counts of the indicator organisms (review question 2) and the relationship between the counts of indicator organisms and visual faecal contamination on carcasses (review question 3). In total, 86 papers considering the main livestock species (cattle, pigs, sheep and goats) with the exception of poultry, and providing pertinent data for the scopes of the search, were retrieved. In relation to review question 1, the steps of the processing line where a decrease of indicator bacteria was more evident were: sequential decontamination treatments such as pasteurization and hot water washing applied before chilling for cattle; scalding and also according to some authors, pasteurization and chilling for pigs, plus chilling and pasteurization for small ruminants. Concerning review question 2, most of the retrieved studies investigated risk factors related to slaughtering process. Hot water washing and steam pasteurization were clearly effective in reducing bacterial load on beef carcasses. Hot water treatments were effective also for pig carcasses. The dressing technique and pasteurization treatment were described as factors able to control bacterial contamination of small ruminant carcasses. In relation to review question 3, only studies providing data about ruminants were available and the reported results confirmed that the presence of visible faecal contamination led to higher bacterial loads on carcasses of dirty animals than those obtained from clean animals and the application of additional hygienic measures can be effective in order to reduce bacterial load of contaminated carcasses at the end of the processing line.

KEY WORDS

Escherichia coli, *Enterobacteriaceae*, counts, ruminant carcasses, pig carcasses, slaughterhouse, process hygiene criteria

DISCLAIMER

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SUMMARY

A project entitled "Usefulness of *Escherichia coli* and *Enterobacteriaceae* as Process Hygiene Criteria in poultry" was awarded by EFSA to Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Padova, Italy) with the purpose to collect available data on the indicator organisms *E. coli* or *Enterobacteriaceae* as Process Hygiene Indicators (PHI) for the main livestock species, based on a literature search and an experimental study, in this case in broiler slaughterhouses, located in the EU. The present document is the report on the extensive literature review on *Escherichia coli* and *Enterobacteriaceae* counts on pig and ruminant carcasses. The extensive literature review covering poultry carcasses and the experimental study in broiler slaughterhouses are published as two separate external scientific reports (Barco et al., 2014; Cibin et al., 2014).

The extensive literature review was conducted to gather information concerning the presence of indicator bacteria, *E. coli* and *Enterobacteriaceae* and their counts on beef, small ruminants and pig carcasses during different stages in the slaughterline (review question 1); risk factors that could explain the variability of the counts of the indicator organisms (review question 2) and the relationship between the counts of indicator organisms and visual faecal contamination on carcasses (review question 3).

A worldwide literature search, covering the period 2000-2012, was conducted. Two electronic databases (PubMed and Web of Science) were consulted; in addition web-searching through Google-scholar was also carried out.

The principles of "systematic review methodology" were applied and included the following steps: definition of the review questions and the eligibility criteria, searching for research studies, selecting the studies for inclusion or exclusion in the review, collecting data from the included studies, synthesising data collected from included studies, presenting data, interpreting results and drawing conclusions.

A total of 86 papers satisfied the eligible criteria considered at the different stages of the screening process and were used to collect data for the three review questions. As far as the different meat animal species were concerned, 41 papers provided pertinent data about beef carcasses, 31 papers about swine carcasses and 21 papers about small ruminants.

A high level of variability among the different studies, due to different aspects and to the complexity of the slaughterlines, was evidenced. Some variables, like the sampling and analytical methods used, the area of carcass sampled, the specific step of the slaughterline investigated and the decontamination treatments applied along the slaughterline, render the available data barely comparable and could lead to conflicting conclusions among studies describing counts at the same stage of the slaughterline or investigating the same risk factor.

Among the indicator bacteria used, aerobic plate counts are frequently used as indicators to monitor the hygiene of the entire meat production process, whereas *Enterobacteriaceae* or *E. coli* are two interchangeable indicators used to specifically address the level of faecal contamination. *Enterobacteriaceae* and *E. coli*, the two indicator bacteria investigated in the present review, are generally used to assess enteric contamination in foodstuffs. These are classified as faecal indicators, which can be easily detected and used as markers of pathogenic zoonotic agents present in processing environment or coming from the animals.

In the context of the review, the studies considering both *E. coli* and *Enterobacteriaceae* generally lead to the same conclusions. However, in case that different results were obtained for the two

indicators, it should be pointed out that also additional variables could equally have had a role in explaining the final outcome.

REVIEW QUESTION 1. Presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughterline

Cattle

Ten eligible papers provided pertinent data concerning this issue on beef carcasses. A main challenge to identify the steps of the beef slaughterline that lead to a decrease or an increase of indicator bacteria counts on the carcasses was the difficulty of finding studies that provide data before and after a single stage. Data have been generally collected at distant sampling points, and in between, different decontamination treatments have been used. Hence, it was hard to identify if the effect in terms of change of bacterial loads was due to a specific phase of the slaughterline or a specific treatment applied to the carcasses.

One study described the decrease of *E. coli* counts after carcasses were washed before being eviscerated, whereas another study showed that when this was combined with spraying with lactic acid, no effect in terms of *E. coli* counts reduction was observed. Decontamination treatments applied before evisceration was effective in reducing bacterial load (1 study). Evisceration and trimming led to an increase of microbial load in one study, whereas in five other studies, changes of bacterial loads correlated to these slaughter phases were not observed. Washing treatment after evisceration was considered as an effective strategy to reduce the *E. coli* load of carcasses in one study, whereas three studies demonstrated that washing at this step had no effect on *E. coli* and *Enterobacteriaceae* counts. The application of different sequential decontamination treatments, such as hot water, pasteurization or washing with acids in all retrieved studies (5) led to reductions in numbers of both indicator bacteria. Finally, at the chilling step, conflicting data were collected. In two studies, carcasses after chilling had higher *E. coli* and *Enterobacteriaceae* counts than in the previous phases, in one study, a drop of the *E. coli* counts was reported and for another study, this step did not have any effect on *Enterobacteriaceae* counts. Likely, the possibility of reducing contamination at chilling step relies on the counts of carcasses at the previous steps.

Pigs

Fourteen papers dealing with counts of *E. coli* and/or *Enterobacteriaceae* on pig carcasses at different stages in the slaughterhouse where retrieved. As with cattle, the identification of the steps in the pig slaughterline that lead to a decrease or an increase of indicator bacteria counts on the carcasses is challenging because data have been generally collected at distant sampling points, and in between, different operations have been usually performed. Hence, the identification of stages that can influence bacterial counts strongly relies on authors' conclusions.

Regarding both *E. coli* and *Enterobacteriaceae*, a decrease in microbial contamination was observed during scalding (nine studies), which is generally recognized as an important operation to achieve a reduction in bacterial counts. The planning and managing of this operation, hence, is critical at plant level. The efficiency of high temperature-based stages was demonstrated against *E. coli*. Pasteurization was identified as effective in three retrieved studies; however more studies in commercial abattoirs are needed to confirm this. Evisceration was confirmed as a key contamination point in particular regarding *Enterobacteriaceae*. The washing process was investigated in seven studies (described in five different papers) and never led to significant decrease in bacterial counts justifying its use as decontamination treatment. Regarding chilling, there was no general agreement among the selected studies focusing on the effects of chilling on *E. coli*; in contrast, a reduction of *Enterobacteriaceae* during chilling was observed in three out of four studies. However, the ability to

assess chilling efficacy depends on the study design and the location of sampling points. Moreover, the possibility of reducing contamination relies on the counts before the investigated chilling stage. Other operations along the processing line, such as polishing, scraping and singeing were investigated in different studies with contrasting results for both *E. coli* and *Enterobacteriaceae*.

Small ruminants

Six papers provided information on counts of *E. coli* and/or *Enterobacteriaceae* on small ruminant carcasses at different stages in the slaughterhouse. In five out of six papers, samples were at several stages of the slaughtering process distant to each other; this feature, coupled with the fact that the sampled area varied considerably among studies make the comparison rather difficult. Thus, conclusions could only be drawn according to the authors' observations.

An increasing level of contamination along the slaughterline was recorded in three out of the six retrieved papers; in particular, the skinning and the evisceration steps contributed mostly to the final counts of *E. coli* and *Enterobacteriaceae*. Furthermore, it seems clear that the different steps of the slaughtering process can have an influence on counts.

Along the slaughterline, the chilling step is the most effective point where microbial contamination can be reduced: two papers concluded that this phase seems to be the most effective point in order to reduce the counts; thus, chilling should be regarded as a control point along the slaughterline. Moreover, two studies showed that carcass pasteurization was another important step in reducing bacterial loads.

Finally, concerning the washing step, the results were not clear or unanimous; according to two studies, washing had no effect in reducing the bacterial counts, while another study showed that washing reduced the *E. coli* counts before the chilling step.

REVIEW QUESTION 2. Risk factors that could explain the variability of the counts of the indicator organisms

Cattle

According to the defined search process and the established eligibility criteria, a total of 29 papers dealing with risk factors influencing *E. coli* and *Enterobacteriaceae* counts on beef carcasses were obtained. For papers providing data for review question 2, a level of variability hampering the comparability of data was also clear.

Season emerged as a risk factor which could have a direct impact on indicator bacteria prevalence and counts on beef carcasses and in particular the lowest levels of contamination were observed during dry season (compared to wet season) and coldest months.

Indicator bacteria counts on carcasses showed different values according to the plant where the slaughtering process took place. In some studies, specific aspects related to this point, such as the design of the plant, the throughput, or the surveillance system in place, were evaluated, whereas in some other cases differences among plants in terms of bacterial loads of carcasses were simply reported without attributing these findings to specific reasons. The effect of plants' throughput on indicator bacteria counts was widely investigated. Lower prevalences/counts were generally reported in low-throughput plants, but in only one study the differences between the two types of slaughterhouses were significant. Hence, different slaughterhouses could induce different effects on the bacterial counts of carcasses, but the data collected from the retrieved studies did not clarify

whether any particular aspect related to different slaughterhouses could produce a main effect in this context.

The effect of using physical and chemical decontamination treatments on indicator bacteria counts was another aspect that was frequently investigated. Steam pasteurization was frequently described as an effective treatment to reduce *E. coli* and *Enterobacteriaceae* loads on beef carcasses. The equipment tested was used both in high and low-throughput plants, and all studies reported a clear reduction of indicator bacteria counts on carcasses due to such decontamination treatment. Similarly, hot water pasteurization was demonstrated as an effective way to improve microbiological quality of beef carcasses, but in this last case the improvement of microbiological quality was associated with a worsening of the organoleptic features.

The effect of washing with potable water at environmental temperature was also taken into account by different studies, and produced conflicting results. Some authors demonstrated that the effectiveness of this treatment mainly depends on the bacterial loads of carcasses to be treated.

The effect of chemical decontamination treatments (e.g. washing-spraying with lactic acid, chlorine, peroxyacetic acid, nisin) was unclear, since different studies described opposite results related to their effectiveness in reducing bacterial loads on beef carcasses. These conflicting results could be due to the different chemicals tested, as well as the procedures followed and the steps of the slaughterline where the treatments were applied.

The two studies addressing the effect of the chilling on bacterial load of carcasses investigated extremely different treatments and therefore, their results cannot be compared. Hence, it was not possible to produce a definitive answer about the effect of the chilling on the bacterial load of carcasses.

Finally, only one study investigated batch related risk factors, and in particular it evaluated the effect of feed and water treatment or any possible interactions on numbers of *E. coli* recovered from hide or carcass swabs without evidencing any correlation.

Pigs

Numerous risk factors were investigated in the eighteen selected papers, but comparisons were barely technically feasible since few studies considered the same factors, and also in this case, the operational environments were very often not comparable. Only one paper considered the influence of risk factors at farm level on carcass contamination and observed that feeding/fasting regime (feeding the pigs pelleted five times a day followed by a 24 h fast), resulted in lower *E. coli* counts on the thoracic area than the other regimes examined. Regarding animal management before slaughtering, neither rough handling nor batch size were identified as risk factor. This suggested that the stress condition applied had limited impact on carcass microbial quality.

Plant throughput and features obviously have an influence on bacterial counts, but the level of carcass contamination was not found to be related to the throughput of the slaughterhouse. In low-throughput plants, *Enterobacteriaceae* were considered useful to provide indication of abattoir specific hygienic weak points. However, some authors underline the ineffectiveness of EC-related EU process hygiene criteria, based on daily mean log *E. coli* value for carcasses.

Several managerial factors that could influence microbial conditions of carcasses have been investigated and described in the retrieved papers. The use of water during lairage cleaning and a high frequency of lairage disinfection seemed to be protective against high *E. coli* counts. In contrast, spraying live animals when external temperature was considered by operators as hot was correlated

with an increase in carcass contamination. Moreover, microbial load on carcasses increased proportionally with the length of processing time between killing and scalding. In contrast, protective factors in relation to *E. coli* contamination were a scalding procedure using steam instead of immersion, the disinfection of the splitting machine three times a day and changing the carcass hooks before chilling. As regards the effect of decontamination treatments, both hot water and solution an acidified sodium chlorite water solution (SANOVA) were effective, but since the latter is not approved according to EU legislation, only the use of hot water could currently be an efficient decontamination intervention to reduce *E. coli* levels on slaughtered carcasses in the EU.

Focusing on both *E. coli* and *Enterobacteriaceae*, washing was considered effective, regardless of the temperature used, in one study. However, this result was not confirmed in another paper where the implementation of good manufacturing practices (GMP) during anal plugging at the evisceration stage was recognised as effective. Both the trimming of contaminated sites and the cooling process were found to be effective in decreasing *E. coli* counts on contaminated carcass sites as well as on randomly selected ones. Pasteurization produced significant decreases in both *E. coli* and *Enterobacteriaceae* counts, justifying the possibility of using such a treatment in order to reduce contamination, in agreement with the results of review question 1. Other authors, after testing the efficacy of steam treatment for reducing *Enterobacteriaceae* loads, suggested the possibility of using household domestic steam cleaning systems as a control measure in low and very low throughput meat processing plants.

Small ruminants

Among the retrieved papers, 16 provided data on the risk factors that can affect *E. coli* and *Enterobacteriaceae* counts on small ruminant carcasses. Also for this question, the comparability of data was hampered by the variability of the studies.

Among the factors investigated, the season did not have a significant effect on the counts: in fact, two studies investigating this factor concluded that there was no difference between counts on carcasses during the warm season compared to the cold season. The throughput of the abattoir was taken into account by three studies as a possible factor that could have an impact on counts: low throughput plants recorded lower prevalence and counts of indicator bacteria compared to high throughput slaughterhouses.

The application of treatments along the slaughterline and the effect of the slaughtering technique on indicator bacteria were investigated by eight papers. An effective treatment is represented by hot water pasteurisation of carcasses after dressing; several authors concluded that this treatment led to a significant reduction of the prevalence and counts of *E. coli* and *Enterobacteriaceae* on carcasses. Another step that can have an effect in reducing microbial load is the chilling phase. The use of experimental chilling treatments was effective in the rapid reduction of the carcass temperature and the bacterial load; however, a loss of meat quality was frequently recorded.

Finally, the dressing technique had an effect on indicator bacteria counts on small ruminant carcasses. In fact, inverted carcass dressing, which minimizes the contact between hands and carcass during pelt removal, was considered by several authors as the technique to be adopted in order to limit carcass contamination.

REVIEW QUESTION 3. The potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses

Cattle

Five papers provided pertinent information on the relationship between faecal contamination of carcasses and their *E. coli* and *Enterobacteriaceae* counts.

Clean cattle produced carcasses with better microbiological quality than those derived from visibly dirty animals. However, the identification of the visibly contaminated animals and the application of effective measures either on animals before entering the slaughterhouse and along the slaughterline can lead to a comparable contamination level to clean animals or in some cases, even to a lower bacterial contamination level. Hence, the retrieved studies support the conclusion that the pre-slaughter visual evaluation of the level of animal contamination and the application of proper corrective measures for the initially dirty carcasses can be an effective approach to reduce their bacterial load at the end of the slaughterline.

Pigs

The literature research did not provide any papers dealing with the possible relationship between visual faecal contamination on pig carcasses and counts of indicator bacteria: therefore it has not been possible to provide any information about this topic in this species.

Small ruminants

Three papers investigated the relationship between faecal contamination of carcasses and their *E. coli* and/or *Enterobacteriaceae* counts. The available data suggested that:

1) the distinction between clean and dirty carcasses could be an important starting point in order to improve the hygiene of small ruminant carcasses;

2) additional hygiene measures should be applied for high-risk (dirty) animals (i.e.: slaughtering at the end of the day; reducing line speed; thorough cleaning of operator hands, arms and aprons; the use of the inverted dressing procedure; greater spacing between carcasses);

3) modifications of the pelt removal methods reducing the contact between the carcass and the hands of the slaughterman or the fleece can significantly improve gross visible contamination.

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BACKGROUND AS PROVIDED BY EFSA

Regulation (EC) No 854/2004 of the European Parliament and of the Council lays down specific rules for the organization of official controls on products of animal origin intended for human consumption. Among others, inspection tasks within this Regulation include checks and analysis of food chain information, ante-mortem inspection and post-mortem inspection.

EFSA received a mandate from the Commission in May 2010 on the modernization of meat inspection, requesting a series of scientific opinions. The main scope of these opinions was to identify and rank the most relevant meat safety risks, to assess the strengths/weaknesses of the current meat inspection system, to propose alternative approaches for addressing current meat-safety risks, and to outline a generic framework for inspection, prevention and control (including related methodology) for the prioritized hazards that are not (sufficiently) covered by the current system.

Several species were to be considered. The scientific opinions on the public health hazards to be covered by inspection of swine meat (EFSA-Q-2010-00886) and poultry meat (EFSA-Q-2010-01469) were published in 2011 and 2012. Four more opinions concerning the inspection of meat from bovines/cattle (EFSA-Q-2011-00365), farmed game (EFSA-Q-2011-00366), small ruminants (EFSA-Q-2011-00365) and solipeds (EFSA-Q-2011-00367) were published in 2013.

Current post-mortem visual inspection is not able to detect any of the public health hazards identified as the main concerns for food safety. Visual detection of faecal contamination of carcasses at post-mortem inspection can be an indicator of slaughter hygiene.

The BIOHAZ Panel proposed recommending that the current visual inspection process is replaced by the establishment of targets for the main biological hazards on the carcass and by verification of the food business operators own hygiene management through the use of Process Hygiene Criteria (PHC). A potential approach for the latter is measuring *E. coli* or *Enterobacteriaceae* on carcasses.

SPECIFIC OBJECTIVES AS PROVIDED BY EFSA

The purpose of the Service Contract is to provide EFSA with the available data on the indicator organisms *E. coli* or *Enterobacteriaceae* as Process Hygiene Indicators (PHI) for the main livestock species. Based on this literature search, an experimental study in broiler slaughterhouses located in the EU should be designed and carried out to collect relevant data on these two indicator organisms. The ultimate aim is to support the purpose of potential PHC for evaluating process control in EU broiler slaughterhouses.

According to the Technical Specifications of the Service Contract CFT/EFSA/BIOHAZ/2012/03-CT1, the tasks to be covered are as follows:

- To carry out literature searches for data related to the main livestock species on (i) the presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughterline; (ii) information that could explain the variability of the counts of the indicator organisms and (iii) the potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses;

- To perform an experimental study in broiler slaughterhouses located in the EU in order to (i) collect relevant data on the variability of the counts of *E. coli* and *Enterobacteriaceae* on broiler carcasses after chilling; (ii) collect information that could lead to interpretation of the variability of these counts and (iii) compare *E. coli* and *Enterobacteriaceae* counts on carcasses with and without visual faecal contamination.

The present document is the report on the literature search for available data on *E. coli* and *Enterobacteriaceae* on carcasses of pigs and ruminants. The extensive literature review covering beef, pig and ruminant carcasses and the experimental study in broiler slaughterhouses are published as two separate external scientific reports (Barco et al., 2014; Cibin et al., 2014).

This contract was awarded by EFSA to:

Contractor: Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Contract title: Usefulness of *Escherichia coli* and *Enterobacteriaceae* as Process Hygiene Criteria in poultry

Contract number: CFT/EFSA/BIOHAZ/2012/03

INTRODUCTION AND OBJECTIVES

Routine examination of meat samples for potential pathogens is impractical mainly because of their low level, inconsistent distribution in meat samples and in some cases the need for laborious analytical methods (Schaffner and Smith, 2004). Since indicator bacteria are found at much higher prevalences on foods of animal origin, they are frequently used as indicators of pathogen presence (Matias et al., 2010).

Ideally, an indicator bacterium should meet certain criteria (Jay et al., 2005); in particular, it should be:

- rapidly detectable and easily differentiable from other microorganisms present in the samples;
- strictly associated with the pathogen whose presence it should indicate (e.g. correlate counts, comparable growth rates).

However, controversy still remains over the degree to which the presence/amount of indicator bacteria can be indicative of the presence of pathogens (Schaffner and Smith, 2004). On the contrary, the recognition that indicator bacteria are an effective tool in process hygiene assessment has been well-documented (EFSA, 2012).

Several indicators can be useful to evaluate hygiene levels during meat slaughtering process. Aerobic colony count (ACC) is commonly used to evaluate the hygiene of the entire meat production process. *Enterobacteriaceae* and *E. coli* are more frequently used to assess enteric contamination (Ghafir et al., 2008). Psychrotrophic microorganisms, such as *Pseudomonas*, have great importance as spoilage indicators in products that are stored at low temperatures since they are responsible for the superficial alteration of these products (Gonzalez-Miret et al., 2006).

Enterobacteriaceae are defined as Gram-negative, glucose fermenting, oxidase negative, usually catalase-positive and nitrate reducing organisms. This family includes many bacteria associated with faeces, but also many non-faecal organisms (Schaffner and Smith, 2004). Faecal coliforms are defined as Gram-negative bacilli fermenting lactose within 48 h at 44.5 to 45.5 °C and this group includes several bacteria, such as *E. coli*, *Enterobacter* spp., *Klebsiella pneumoniae* and *Citrobacter fruendii* (Schaffner and Smith, 2004). Within this group, *E. coli* is the most relevant microorganism in relation to faecal contamination of foods; thereby, it is the most widely used indicator of faecal contamination (Smooth and Pierson, 1997). *E. coli* counts are usually highly correlated with *Enterobacteriaceae* counts, which are commonly used in slaughterhouses as indicators of faecal as well as environmental contamination (Ghafir et al., 2008). The proliferation of *Enterobacteriaceae* on livestock carcasses has

been routinely linked to inadequate or unhygienic processing or inappropriate handling or storage conditions (Whyte et al., 2003).

In the European Union, Regulation (EC) No 2073/2005[3] and Regulation (EC) No 1441/2007[4] on microbiological criteria for food-stuffs has established the monitoring of Aerobic colony count and *Enterobacteriaceae* as process hygiene criteria for carcasses of cattle, sheep, goats, horses and pigs.

These regulations introduced two different types of criteria: Food Safety Criteria and Process Hygiene Criteria. An EU Food Safety Criteria (FSC) defines the acceptability of food products placed on the market; if the criterion is not met, the product/batch has to be withdrawn from the market. An EU Process Hygiene Criterion (PHC) is an indicator of the acceptable functioning of Hazard Analysis and Critical Control Points (HACCP) system-based manufacturing handling and distribution processes, so it is applicable at the process level. It sets an indicative contamination value above which corrective actions are required; if the criterion is not met, the process has to be reviewed and improved. Table 1 summarizes the EC-related PHC to be applied in red meat slaughterhouses.

Table 1: Process Hygiene Criteria applied in red meat slaughterhouses as defined by Regulation (EC) No 2073/2005 and Regulation (EC) No 1441/2007

Food category	Microorganism	Limits	Stage where the criterion applies
Carcasses of cattle, sheep, goats and horses	Aerobic colony count	<3.5 log CFU/cm^2: acceptable 3.5-5.0 log CFU/cm^2: marginal > 5.0 log CFU/cm^2: unacceptable	Carcasses after dressing but before chilling
	Enterobacteriaceae	<1.5 log CFU/ cm^2: acceptable 1.5-2.5 log CFU/ cm^2: marginal > 2.5 log CFU/ cm^2 : unacceptable	
	Salmonella	up to 2 out of 50 samples can be positive	
Carcasses of pigs	Aerobic colony count	< 4.0 log CFU/cm^2: acceptable 4.0-5.0 log CFU/cm^2: marginal > 5.0 log CFU/ cm^2: unacceptable	
	Enterobacteriaceae	< 2.0 log CFU/ cm^2: acceptable 2.0-3.0 log CFU/ cm^2: marginal > 3.0 log CFU/ cm^2: unacceptable	
	Salmonella	up to 5 out of 50 samples can be positive	

[3] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26.

[4] Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. OJ L 322, 7.12.2007, p. 12–29.

In Europe, Regulation (EC) No 853/2004[5], which lays down specific rules on the hygiene of food of animal origin, provides that food business operators should not use any substance other than potable water to remove surface contamination from products of animal origin, unless use of the substance has been approved in accordance with that Regulation. In this context, on July 2011 the European Food Safety Authority (EFSA) adopted a Scientific Opinion on the evaluation of the safety and efficacy of lactic acid for the removal of microbial surface contamination from beef carcasses, cuts and trimmings. It was concluded that the use of lactic acid (from 2 to 5 %, at temperatures of up to 55 °C and applied either by spraying or misting) for decontamination is not a safety concern and although variable, the microbial reductions achieved by treatment of beef are generally significant and that it is unlikely that such treatments would contribute to the development of microbial resistance.

This opinion is the scientific basis for the Regulation (EC) No. 101/2013[6], which lays down that food business operators are allowed to use lactic acid to reduce microbiological surface contamination on domestic bovine carcasses or half carcasses or quarters at the level of the slaughterhouse in compliance with the conditions set out in the Annex of the Regulation and when the use is integrated into good hygienic practices and HACCP-based systems.

A literature search considering all the main livestock species (poultry, pigs and ruminants) was conducted to obtain data on:

- the presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughterline;
- factors that could lead to the variability of the counts of these indicator organisms, such as the design of the slaughterhouses, the throughput of the slaughterhouse, the processing techniques, and any other batch specific information;
- any potential relationship between the counts of indicator organisms and visual faecal contamination on carcasses.

The present report specifically considers data related to the main livestock species other than poultry (cattle, pigs, small ruminants). The review considered studies performed worldwide.

[5] Commission Regulation (EC) No 853/2004 of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

[6] Commission Regulation (EC) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses. OJ L 34, 5.2.2013, p. 1-3.

1. MATERIALS AND METHODS

The principles of "systematic review methodology" (EFSA, 2010) were applied to the present literature search. This involved the following steps:

- defining the review questions and developing the eligibility criteria for studies;
- searching for research studies;
- selecting studies for inclusion or exclusion in the review;
- collecting data from the included studies and creating evidence tables;
- assessing validity and quality of included studies;
- synthesising data from included studies;
- presenting data and results;
- interpreting results and drawing conclusions.

1.1. Defining the review questions and developing the eligibility criteria for studies

The first step of the literature review process consisted of the analysis of the three review questions in order to identify the key elements and to clarify their scopes.

Review question 1 is related to the presence of the indicator organisms, *E. coli* and *Enterobacteriaceae*, and their counts on carcasses during different stages of the slaughterline. The key elements of the question are:

- the population of interest, represented by the main livestock species (ruminants and pigs);
- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses;
- the setting, represented by the slaughterline.

Review question 2 is related to the identification of the factors which could explain the differences in terms of amount of the indicator bacteria on carcasses. The key elements of the question are:

- the population of interest, represented by the main livestock species (ruminants and pigs);
- the intervention strategies/scenarios that could influence the counts, which could be represented by structural/managerial characteristics of the slaughterhouse or pre-slaughter handling of animals (e.g. transport time and lairaging before slaughter, diet and feed withdrawal period before slaughter);
- the scenarios against which the interventions/strategies/scenarios have been compared (e.g. batches slaughtered in different ways, in slaughterhouses with different processing characteristics, animals handled in different ways before being slaughtered);
- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses;

- the setting, represented by the slaughterline.

Review question 3 is related to the potential relationship between the counts of indicator organisms and visual faecal contamination of carcasses. The key elements of the question are:

- the population of interest, represented by the main livestock species (ruminants and pigs);
- the outcome, represented by the presence and amounts of indicator organisms (*E. coli* and *Enterobacteriaceae*) on carcasses in relation to their visual faecal contamination;
- the setting, represented by the slaughterline.

The three key elements (population, outcome and setting) identified for review question 1 remain unchanged also for review questions 2 and 3, although for these last two review questions additional aspects, such as variables influencing the outcome of interest and the relationship between the outcome of interest and other factors, have to be considered respectively. Since the review questions shared these main key elements it was decided to combine the three review questions in a unique literature search.

1.2. Searching for research studies

Electronic databases (search A) and Web-searching (search B) were used to retrieve pertinent studies.

1.2.1. Search A: electronic databases

For search A, the search terms used in relation to the specific key elements of the three review questions related to “outcome”, “setting” and “intervention strategy /scenarios”, are listed in Table 2.

Terms related to the “population” were omitted from the search string in order to get as many papers as possible and then to select the relevant ones in terms of species of interest in the following steps of the screening process.

Regarding the “intervention strategy /scenarios” related to review question 2, not all plausible terms were included in the search string. Conversely, for “outcome” and “setting” the terms used were selected in order to include as many synonymous terms as possible, since these two elements were considered the most important ones to retrieve relevant papers.

More specifically, the search string used was:

(E. coli) OR (Coliform*) OR (Escheric*) OR (Enterobacter*) OR (Indicator) OR (Hygien*) OR (Microbi*) AND (Slaughter) OR (Slaughterhouse*) OR (Abattoir*) OR (Carcas*) OR (HACCP) OR (Chill*) OR (Eviscerat*) OR (Defeathering) OR (De-feathering) OR (Post-harvest) OR (Post harvest) OR (Pre harvest) OR (Pre-harvest) OR (Holding pen*)

Before identifying the definitive search terms some other terms, such as “coli” and “process” were tested, but they were not included in the final search string since it was verified that they did not result in the retrieval of any additional relevant papers.

After having adjusted the search string for minor differences in syntax, it was applied to two electronic bibliographic databases: PubMed and Web of Science.

The search covered the period January 2000 - December 2012 (01.01.2000 - 24.06.2013).

The bibliographic software RefWorks was used to collect and manage the references downloaded from the electronic databases.

Table 2: List of the terms included in the search string in relation to the specific key elements of the three review questions

Outcome	Setting	Intervention strategy /scenarios
E. coli	Slaughter	HACCP
Escheric*	Slaughterhouse*	Chill*
Enterobacter*	Abattoir*	Eviscerat*
Coliform*	Carcas*	Defeathering
Indicator		De-feathering
Hygien*		Post-harvest
Microbi*		Post harvest
		Pre harvest
		Pre-harvest
		Holding pen*

1.2.2. Search B: Web-searching

Search B was conducted by using the Internet search engine Google Scholar.

Since Google Scholar looks for the search terms in the entire document the search process was conducted by using a few very specific terms, related to the "outcome" (*Escherichia coli* – *Enterobacteriaceae*) and the "setting" (slaughterhouse). Moreover, to limit the retrieval of non-pertinent articles the terms O157 and resistance were excluded from results.

The search string used was:

((Escherichia coli) OR Enterobacteriaceae) AND (slaughterhouse)) NOT (O157) NOT (resistance).

The titles of the first 500 returns were assessed in order to identify the pertinent documents.

1.3. Selecting studies for inclusion or exclusion in the review

For the purpose of the present review, primary research studies performed at the slaughterhouse and providing data on the presence/counts of *E. coli* and *Enterobacteriaceae* on carcasses of the livestock species of interest were considered.

Moreover, the relevant studies were defined as the ones:

- referring to slaughtering process from the point at which animals enter the slaughterhouse up to the conclusion of the chilling phase;
- providing data on the carcasses collected at the slaughterhouses.

For both searches A and B, papers/documents in English, French, Spanish or Italian were considered. Geographical restrictions were not imposed.

The screening process was independently carried out by three veterinarians.

In the following paragraphs the eligibility criteria used to select relevant papers at abstract and full text level are described. Moreover, in Appendix A the checklists developed to identify relevant papers at

the different steps of the screening process are reported. These checklists were validated beforehand by the three reviewers involved in the screening process using a subset of 50 retrieved papers.

1.3.1. Screening of the titles and abstracts for the relevance to the study questions

For Search A, the first level assessment was conducted considering the title and the abstract of the papers. For Search B, the first level assessment was conducted considering title and, if the document was pertinent, also abstract (when available), or in the case of evaluating directly, the full text.

The first level assessment consisted of two steps. Papers that did not fulfil one or more criteria considered in these steps were discarded and considered ineligible.

For the first step, it was defined that if the two initial criteria were not fulfilled (the papers were written in languages different from English, French, Spanish or Italian, or were review papers) it was not necessary to proceed with the screening and the article was considered ineligible.

This first step consisted of selecting papers that:

1) were written in English, French, Spanish or Italian;
2) described data provided by primary research (review articles were excluded);
3) provided data related to the main livestock species;
4) provided data on the presence and counts of generic *Escherichia coli* and/or *Enterobacteriaceae*;
5) did not have the investigation of antimicrobial resistance as their main purpose.

The second step consisted of selecting papers that:

1) provided data on more than one stage of the slaughterline or data on risk factors influencing the loads of indicator bacteria on carcasses;
2) provided data on carcasses (papers considering parts of the carcasses obtained after a secondary process were excluded);

When the abstract screening did not identify precise information concerning a specific eligibility criterion, the reviewers provided an inconclusive reply (e.g. unknown), that did not lead to the exclusion of the paper.

Each retrieved paper was individually evaluated by two independent reviewers (parallel review). In the case of disagreements between them, the paper was discussed to reach a consensus before proceeding to the next step of the screening process.

1.3.2. Examining full-text for the eligibility of studies

All retrieved articles (both from searches A and B) related to livestock species other than poultry were submitted to the second level assessment, conducted examining the full-text of the papers.

At this stage some eligibility criteria already taken into account in the first level assessment were included since, in some cases, the abstract analysis did not allow precise information to be obtained, and a final decision could not be made.

In particular, the following eligibility criteria were used to select the papers at second level assessment. Relevant papers:

1) described data provided by primary research (review articles were excluded);
2) considered main livestock species (cattle, sheep, goats, pigs);
3) provided data obtained from carcasses;
4) provided data on the presence and counts of *E. coli* and/or *Enterobacteriaceae*;
5) did not provide data about counts of *E. coli* and/or *Enterobacteriaceae* from carcasses that had been artificially contaminated with these indicator bacteria;
6) reported *E. coli* and/or *Enterobacteriaceae* counts at more than one stage of the slaughterline, or described factors influencing the counts of *E. coli* and/or *Enterobacteriaceae*, or considered the relationship between visual faecal contamination and *E. coli* and/or *Enterobacteriaceae* counts.

Moreover, at this stage, further data (e.g. the country where the study was done, the setting of the study, at which stage of the slaughterline samples were collected, the procedure used to get samples, which type of risk factor was investigated) were gathered from the screened papers. The collection of these data was also useful to develop forms for the collection of pertinent data from the relevant papers (Appendix B).

1.4. Collecting data from the included studies and creating evidence tables

Two standardized forms were designed to extract pertinent data from the selected papers (Appendix B).

The first form was aimed at gathering general information about the materials and methods of the studies. It included information on the type of reference (e.g. article, technical report, meeting proceeding), the aim of the study, where and when the study was conducted, the type of study (observational or experimental), and the number of plants involved in the study. Moreover, specifications on the sampling method and sample size (number of samples collected, sampled area on the carcass, significance and power of the sample size) were collected. The second form was aimed at collecting pertinent analytical data for the scope of the review. It included information concerning the species considered, the indicator bacteria investigated, the unit of enumeration, data on prevalence and counts of indicator bacteria at each step of the slaughterline. Finally, it included an evaluation of the steps of the slaughterline where the counts decreased or increased, or the effect of the investigated risk factors on the counts of the indicator bacteria or the relationship between visual faecal contamination and the counts.

Data related to multiple slaughterhouses or data from different visits at the same slaughterhouses or presenting different scenarios were considered separately.

Data were extracted from each paper by one reviewer and then verified by a second person (sequential method). In cases where inconsistencies between the data reported in the paper and those included in the forms were observed, the reviewers again verified the paper, but together this time, and if appropriate, they modified data in the form accordingly.

In order to avoid double counting of the studies published more than once, the papers were compared for author names, geographic area where the study was conducted, sample size and data reported. The duplicates were discarded.

The extraction forms were used to minimise the transcription errors and to obtain a record of all the collected data. The management of references, the screening and the data extraction processes were done through the web-based software DistillerSR (Evidence Partners, Ottawa, Canada).

2. RESULTS

2.1. Literature search and relevance screening

Figure 1 shows the flow chart of the screening processes. Search A provided 2148 papers from PubMed and 5345 papers from Web of Science. A total of 578 papers were excluded before starting the screening since they were review papers and/or were written in languages different from English, French, Spanish or Italian. Google Scholar search (search B) resulted in 73 potentially pertinent documents out of the first 500 returns. After having eliminated the duplicates, a total of 5500 papers remained.

Of the 5500 retrieved papers, 4646 were excluded at step 1 (thus 854 remaining) and 591 at step 2 (thus 263 remaining) of the first level relevance screening assessment since they did not fulfil one or more eligibility criteria considered at that level.

Of the 263 papers that passed both steps of the first level assessment 145 reported data on main livestock species different from poultry.

After having examined the full-text, 57 out of 145 papers were excluded at the second level assessment because one or more eligibility criteria were not fulfilled and 88 main livestock-related papers were selected since they provided appropriate data for the scope of the review and were assessed in detail in order to collect pertinent data.

Two out of the 88 selected papers were eliminated since in one case the study described a hygienic situation that was hardly comparable with the European situation, whereas in the second case it was not possible to get the full-text paper.

As a result, 86 papers moved forward for data extraction; in particular 41 papers provided pertinent data related to bovine slaughterhouses, 31 papers to swine slaughterhouses and 21 papers to small ruminant slaughterhouses**.**

2.2. Discussion of relevant studies

The studies described in the retrieved papers differ notably in study design, number of samples but overall on the choice of effect sizes. Effect sizes used were prevalence, mean with standard errors or mean with standard deviations. Moreover the unit of enumeration varied from logCFU, CFU, log of the sum of counts recovered from different sampling sites or also from different carcasses and counts were based on cm^2 or 100 cm^2 or on the total area sampled.

Authors' conclusions about significance of results, in particular in the case of risk factors, were mainly based on p-values. However, often, important data to interpret the quality of statistical evaluation are lacking. It's known that the p-value alone is an unobjective and inadequate measure of evidence when statistically testing hypotheses (Hubbard and Lindsay, 2008).

For these reasons studies were mainly compared qualitatively (increase or decrease) basing the judgement about significance of results on authors conclusions. It has been assumed that authors knowledge about all relevant information, also if not reported, could support their conclusion.

In any case, all the pertinent data extracted from the selected studies are available in the appendices and are available for further evaluations.

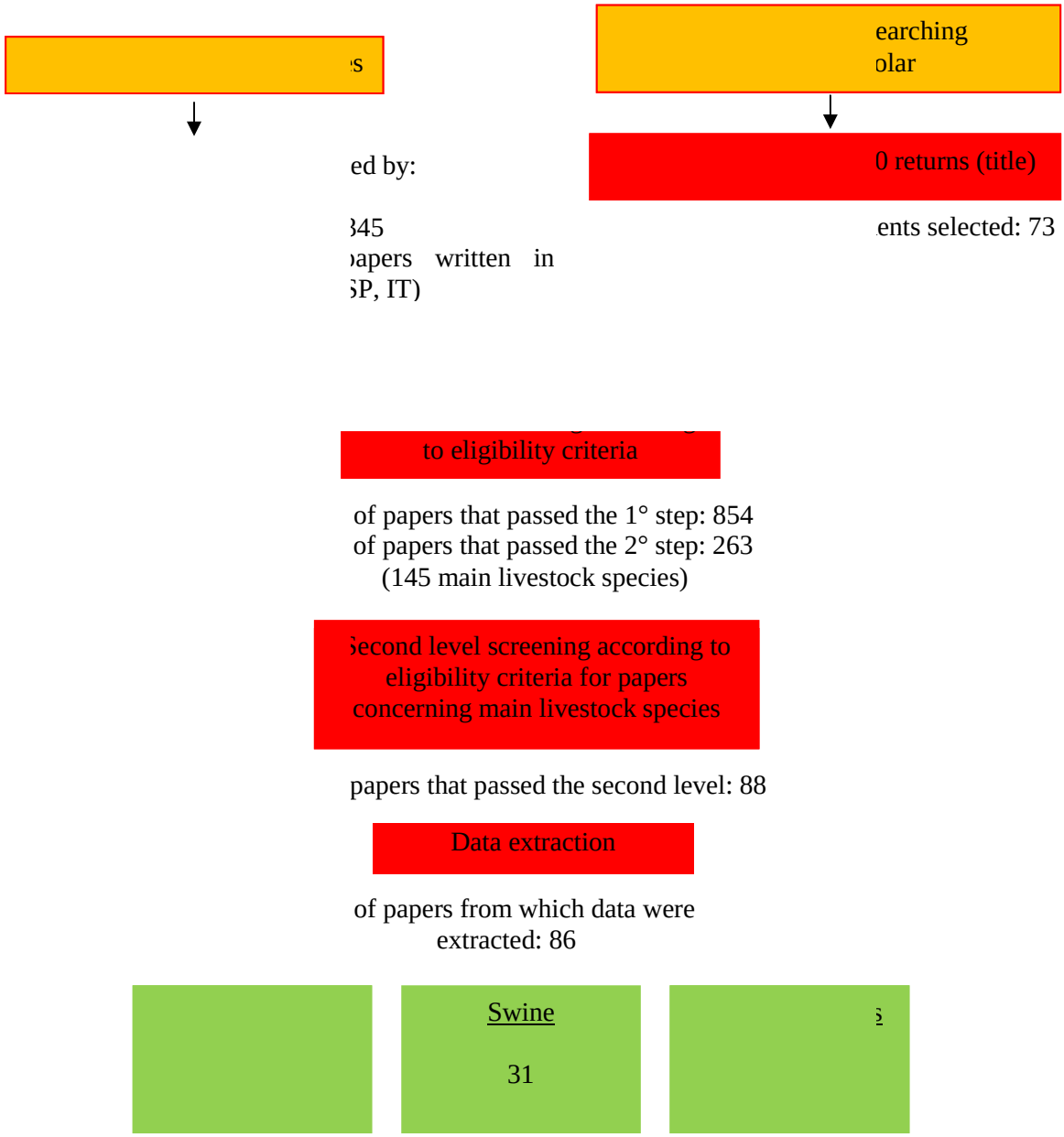

Figure 1: Flow-chart summarizing the results of the literature search – the screening and the data extraction process

The number of retrieved papers (86) was lower than the sum of the papers of the three species since some papers provided data about more than one species.

2.3. Bovine

2.3.1. General information about the considered papers

Among the retrieved papers related to bovine slaughterhouses, 41 papers provided pertinent data for the three review questions.

Fifteen papers corresponded to studies conducted in Europe, while the other 26 were conducted outside Europe. All papers described observational studies with the exception of seven presenting results obtained from an experimental design.

Twenty papers provided data on *E. coli*, thirteen papers on *Enterobacteriaceae* and eight papers considered both the indicator bacteria.

Seven papers provided information about review question 1, twenty-six papers addressed review question 2, three papers dealt with both review question 1 and 2 and five papers provided data for review question 3.

The most common sampling method was swabbing (36 papers), skin excision was used in three cases and meat excision was used in the last three papers. The unit of enumeration most commonly used was log cfu/cm^2. The sampling site on carcasses varied greatly among the retrieved studies as well as the analytical method used to culture the indicator organisms, which was most commonly the Petrifilm plating system.

More details concerning the general information about the retrieved papers are available in Table 6 (Appendix C).

2.3.2. General information about the slaughtering process

In beef slaughterhouses, the animals are received and kept in lairage facilities. The animals are given water, but in most cases are not fed unless they are kept more than one day. Then the animals are driven from the lairage to the slaughtering area, where the activities summarized in Figure 2 take place. Moreover, along the slaughterline, different decontamination treatments can be applied. In the graph below (Figure 2), the treatments described in the retrieved papers are listed, while the step in the slaughterline where they are applied is also specified, in order to clarify the specific situations described.

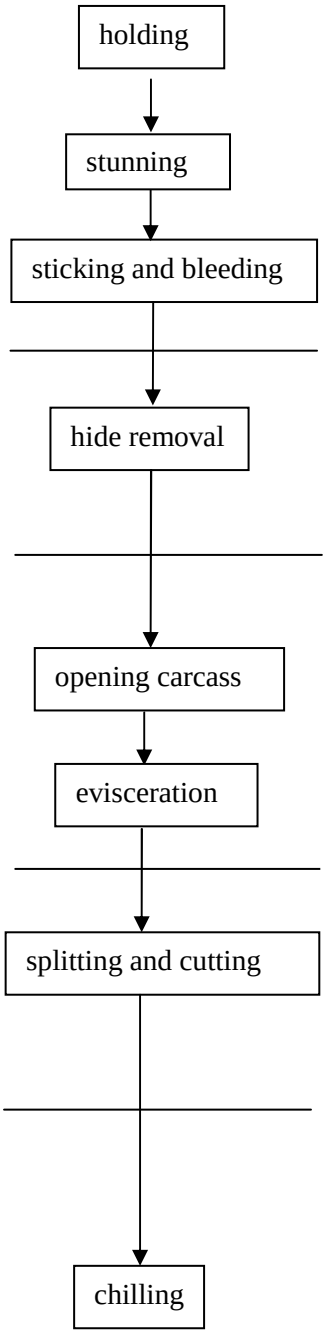

Figure 2: Flow-chart summarizing the activities carried out along the beef slaughterline. The decontamination treatments described in the retrieved papers are also reported.

Since it has been demonstrated that the application of decontamination treatments has great potential to reduce the presence of microorganisms, especially when multiple approaches are sequentially used (Ruby et al., 2007), in modern slaughterhouses different decontamination treatments, such as water washes, organic acids washes (only lactic acid in EU slaughter houses), steam vacuuming and pasteurization, can be applied at different steps of the slaughterline.

The retrieved studies described the application of different decontamination treatments and different technologies. Hence, the data reported in the different studies at the same steps of the slaughterline cannot be directly compared. The effect of decontamination treatments applied along the process is one of the variables that should be taken into consideration before drawing conclusions about the effect of each step of the slaughterline on the bacterial loads of carcasses.

Other variables, such as the sampling methods, the unit of enumeration and the area sampled on the carcass further hamper the comparability of the data among the studies.

2.3.3. Review question 1

As regards the presence and amount of *E. coli* and *Enterobacteriaceae* on beef carcasses during different stages of the slaughterline, among the ten eligible papers, five papers provided information on *E. coli*, three papers on *Enterobacteriaceae* and two papers on both. All the papers provided data obtained through observational studies obtained in commercial slaughterhouses.

The main challenge in identifying the steps of the slaughterline that led to a decrease or an increase of indicator bacteria counts on the carcasses was the paucity of studies that provided data obtained before and after a single stage. Data have generally been collected at distant sampling points and in between, different decontamination treatments have usually been used. As a consequence, the effective role of the single stages in a change in counts was not always evident. For this reason, it has been very often necessary to draw conclusions according to authors' opinions instead of simply comparing the data among studies.

In order to identify the steps of the slaughterline that could influence the variation of carcasses bacterial loads, as reported in literature (Gill et al., 2003), differences of less than 0.5 log unit between two sampling points were considered of no practical importance.

Gill et al. (2003) examined the trend of *E. coli* counts along the slaughterline in a high-throughput beef plant in Canada. These authors reported that after hide removal, indicator bacteria were recovered from less than 15 out of the 25 sampled carcasses, at log total number of 3.16 log cfu/2,500 cm^2. The bacterial load after pre-evisceration washing and spraying with 2 % lactic acid solution was similar to the numbers recovered after hide removal, and the counts reported after evisceration, splitting, vacuum/hot water cleaning and trimming still remained very similar. The number of *E. coli* was reduced by the washing of the carcass sides (about 1 log unit less), and the pasteurisation followed by spraying with lactic acid led to a further reduction (about 2 log unit less). Finally, after cooling for 24 hours *E. coli* counts increased about 2 log units. Hence, all operations on carcasses between hide removal and trimming had only trivial effects on the microbiological condition of meat. Then, washing and steam pasteurization of eviscerated carcasses appeared to be effective in reducing *E. coli* counts. Finally, after chilling, the number of bacteria recovered was similar to the counts recorded before applying the decontamination treatments and authors hypothesised that this increase was due to re-growth of injured bacterial cells rather than new contaminations of carcasses.

Another study conducted in Canada (Yang et al., 2012) recovered less than 2 log cfu/2,500 cm^2 of *E. coli* from carcasses after hide removal. According to the authors, this low level of initial contamination was due to the decontamination treatments applied on hide-on carcasses (washing with 1.5 % sodium hydroxide at 55 °C and rinsing with chlorinated water). Then, when uneviscerated carcasses were washed, a reduction of bacterial load of more than 1 log unit was reported, but after evisceration and trimming operations the trend was reversed. Finally, the counts were again reduced by spraying carcasses with lactic acid and pasteurization and after these last treatments, no *E. coli* were recovered from the sampled carcasses.

Another study (McEvoy et al., 2004), conducted in Ireland, aimed to estimate *E. coli* and *Enterobacteriaceae* contamination at different sites (hock, brisket, cranial back and bung) of carcasses and at different steps along the processing line. After hide removal, comparable *E. coli* counts were obtained from the hock, bung and brisket samples, whereas the indicator bacteria were not detected from the cranial back. After evisceration, bacterial loads remained similar to the numbers recovered in the previous step in all the sampled sites apart from the cranial back where an increase of 1 log unit was reported. Trimming, splitting and washing with warm water led to a reduction of *E. coli* of about 0.5 log unit for all sites apart from the cranial back where a further increase (of about 1 log unit) was reported. Finally, chilling caused a reduction of the *E. coli* counts of about 1 log unit for all sites apart from the bung samples where a bacterial load comparable to the previous step was reported. In the same study, *Enterobacteriaceae* counts were also registered at the same sampling sites of the carcass and at the same points of the slaughterline. After hide removal, *Enterobacteriaceae* counts at the cranial back were more than 1 log unit lower than at the other sampling sites. An increase of 1.45 log units was reported after splitting at the cranial back site. After washing, similar numbers were recovered from all sites, and after chilling, clear reductions of the *Enterobacteriaceae* counts were obtained for all sites apart from cranial back, where an opposite trend was reported. Both *E. coli* and *Enterobacteriaceae* counts were reduced or remained unchanged after cooling and the authors attributed the reductions to the injury of bacterial cells due to stresses from low water activity (a_w) and temperature.

The effectiveness of applying sequential decontamination treatments along the slaughterline was investigated in different retrieved papers.

Bacon et al. (2000) conducted a study involving eight different plants, which applied different decontamination technologies. At the beginning of the process, after hide removal, in the eight plants sampled, *E. coli* counts were in the range 2.6 – 5.3 log cfu/100 cm^2. Along the slaughtering line, after the application of multiple decontamination treatments (including steam vacuuming, pre-evisceration carcass water and organic acid washing, hot water washing, post-evisceration final water and organic acid washing) a reduction of the *E. coli* counts ranging from 1.3 to 3.0 log cfu/100 cm^2 was reported in all plants. Finally, at the end of the process, after a 24-36 h chilling period, the *E. coli* load was always lower than 1 log cfu/100 cm^2. These authors demonstrated the effectiveness of using different decontamination treatments along the slaughterline.

Arthur et al. (2004) also evaluated the effectiveness of applying sequential antimicrobial interventions in reducing the load of indicator bacteria on hides and carcasses at two commercial processing plants. The authors collected samples a) after hide removal, b) after evisceration and carcass trimming, c) after the application of all antimicrobial treatments and d) at chilling. The carcasses were treated with the following decontamination treatments along the processing line:

- after hide removal the cut lines were steam vacuumed,
- before evisceration the carcasses were steam vacuumed again and washed with lactic acid,
- after evisceration two other washing steps were carried out with hot water and peroxyacetic acid and a steam pasteurisation treatment was also used,
- at chilling, carcasses were sprayed for 29 hours before the collection of the final sample.

At the beginning of the slaughterline *Enterobacteriaceae* counts on hides were equal to 6.2 log cfu/100 cm^2. After hide removal the bacterial load on the carcasses resulted in a level of 1.4 log cfu/100 cm^2 and a slight increase (0.3 log cfu/100 cm^2) was reported at post-evisceration. After having washed carcasses with hot water and peroxyacetic acid and after steam pasteurisation, the *Enterobacteriaceae* counts were equal to 0.2 log cfu/100 cm^2, then, after cooling the bacterial load remained substantially unchanged.

The same sampling points were selected by Ruby et al. (2007), who estimated the *Enterobacteriaceae* loads on carcasses to verify the effectiveness of the different decontamination treatments used in three high-throughput beef abattoirs. In this case the treatments used along the processing line were:

- before hide removal, carcasses were steam vacuumed,
- after hide removal, carcasses were sprayed with lactic acid,
- after evisceration, carcasses were steam vacuumed, then sprayed with ambient temperature water, hot water and lactic acid.

Also in this study, as reported by Arthur et al. (2004), the initial load of *Enterobacteriaceae* on hide was quite high (5.28 log cfu/100 cm^2), and after hide removal bacterial load on carcasses was equal to 1.04 cfu/cm^2. A slight reduction down to an *Enterobacteriaceae* load of 0.8 log cfu/100 cm^2 after evisceration was described and a further reduction was reported at the end of the decontamination treatments (-0.38 log cfu/100 cm^2). Finally the chilling step led to an increase of the *Enterobacteriaceae* counts (0.2 log cfu/100 cm^2).

Bacon et al. (2000) reported that the chilling step was an effective phase of the processing line for reducing or blocking the bacterial load. These findings were confirmed also by Arthur et al. (2004), whereas Ruby et al. (2007) described an increase of bacterial load during this stage; however, in this last study, the bacterial load of carcasses before starting chilling were extremely low. In these last three studies, authors demonstrated the effectiveness of using sequential decontamination treatments in reducing bacterial loads on carcasses, especially when these treatments are applied after evisceration and before chilling. These studies showed that the overall intervention processes from hide-on to post-chilled samples effectively reduced microbiological contamination of carcasses.

Two other studies (Rigobelo et al., 2008; Nero et al., 2012), conducted in Brazil, estimated the frequency of recovery and the counts of indicator bacteria at different points of the slaughterline. No detailed information about the techniques applied along the processing line as well as the decontamination treatments used were available, so it was not possible to infer if specific treatments had an effect on the reported reduction. Nero et al. (2012) presented the results of a study involving three plants. *E. coli* and *Enterobacteriaceae* counts were estimated after bleeding, after skinning, after evisceration and after washing. A progressive decrease of the microbiological counts during the slaughter process was described. In the second study (Rigobelo et al., 2008), only one plant was investigated and samples were collected immediately after hide removal (pre-evisceration), after splitting and trimming (post-evisceration) and after washing carcasses hanging in the cooler (post-processing). The frequency of *E. coli* positive carcasses progressively decreased from the pre-evisceration step (58 %) to the post-processing step (32 %).

An opposite trend was described by Barboza et al. (2002), who estimated *E. coli* counts on neck, brisket and renal sites of carcasses after hide removal, after trimming and splitting, and after washing. In this case also, detailed information about the stages of the processing line and the decontamination treatments used were not available. A clear increase of the *E. coli* counts was reported after splitting, then after washing a marginal decrease of the bacterial load was registered, and the final counts were about 1 log unit higher than those recovered after hide removal. Then, during the washing phase the bacterial load remained essentially unchanged and the authors concluded that at best, the washing of carcass with cold water produces a redistribution of bacteria over the entire carcasses, rather than removing the contamination.

The same sampling points along the slaughterline were investigated by Madden et al. (2004), who estimated the *Enterobacteriaceae* counts on brisket samples collected after hide removal, after carcass splitting and after washing-before chilling in different plants in Northern Ireland. The counts reported

after hide removal were comparable to the numbers obtained after carcass splitting, whereas in at least one of the sampled plants, the washing step led to an increase of the bacterial load. The authors concluded that washing with potable water cannot be considered an effective treatment to reduce bacterial load on carcasses. They remarked that during the washing process, the heavy bacterial load at the posterior region (mainly faecal contamination) may not only be removed, but also redistributed to the anterior areas of the carcasses.

In order to identify the steps of the slaughterline that could influence the variation of carcasses bacterial loads, as reported in literature (Gill et al., 2003), differences of < 0.5 log unit between two sampling points were considered of no practical importance. Therefore, values differing by < 0.5 log unit were regarded as similar, whereas values differing by > 0.5 log unit were regarded as different.

In the following graph (Figure 3), trends of *E. coli* and *Enterobacteriaceae* counts at different stages of the slaughterline are summarized. Only studies providing quantitative data were considered.

It should be pointed out that in some cases it was very hard to identify if the effect in terms of increase or decrease of bacterial loads was due to a specific phase of the slaughterline or a specific treatment applied on carcasses since, as reported above, in only very few cases did the retrieved studies provide data before and after a specific point of the processing line. Moreover, samples were usually collected at distant points on the slaughterline. The trends summarised in Figure 3 were defined considering the data collected in the retrieved studies as well as the considerations reported by their authors.

One study described the decrease of *E. coli* counts after carcasses were washed before being eviscerated, whereas another study showed that when this was combined with spraying with lactic acid, no effect in terms of *E. coli* counts reduction was observed. Decontamination treatments applied before evisceration was effective in reducing bacterial load (1 study). Evisceration and trimming led to an increase of microbial load in one study, whereas in five other studies, changes of bacterial loads correlated to these slaughter phases were not observed. Washing treatment after evisceration was considered as an effective strategy to reduce the *E. coli* load of carcasses in one study, whereas three studies demonstrated that washing at this step had no effect on *E. coli* and *Enterobacteriaceae* counts. The application of different sequential decontamination treatments, such as hot water, pasteurization or washing with acids in all cases (five) led to reductions in numbers of both indicator bacteria. Finally, at the chilling step, conflicting data were collected. In two studies, carcasses after chilling had higher *E. coli* and *Enterobacteriaceae* counts than in the previous phases, in one study, a drop of the *E. coli* counts was reported and for another study, this step did not have any effect on *Enterobacteriaceae* counts.

The counts of *E. coli* on beef carcasses described in the selected papers at the different stages of the slaughterline are reported in Table 9 (Appendix D), while the counts of *Enterobacteriaceae* are presented in Table 10 (Appendix D). In these tables, the number of samples analysed at each step, the increase or decrease of the counts for each step, the mean and the standard deviation are reported when these data were available.

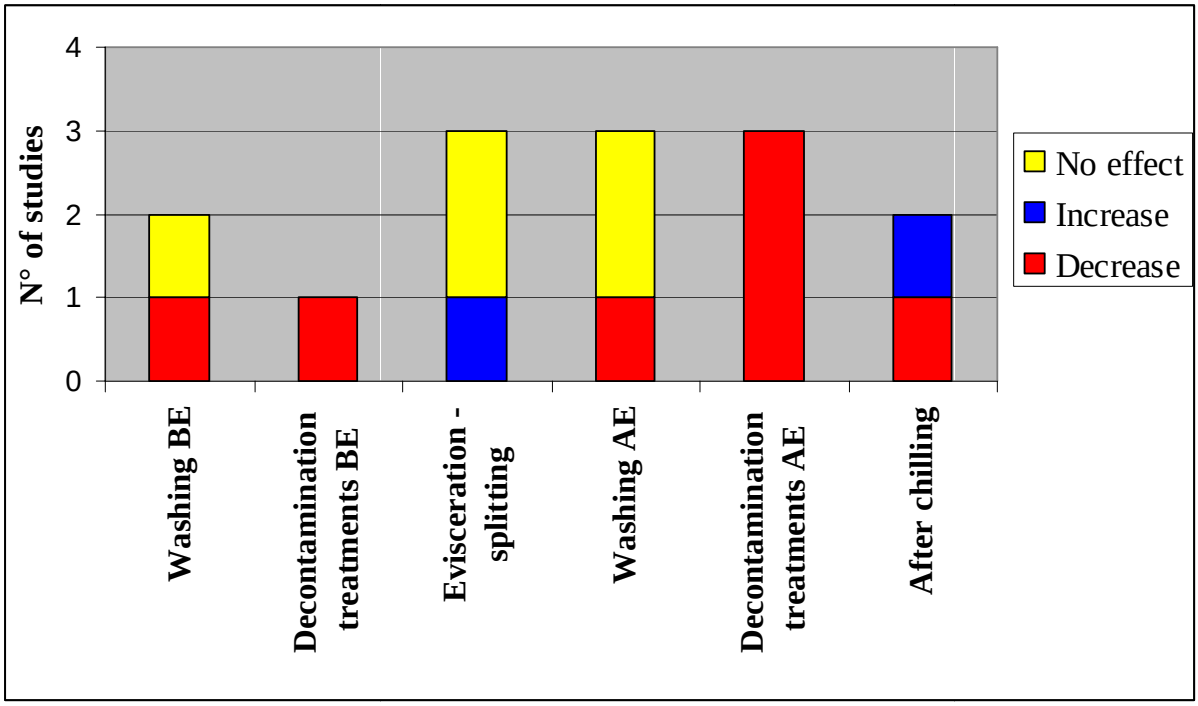

Figure 3: Trends (increase and decrease) of *E. coli* counts at different stages of the slaughterline in beef slaughterhouses (BE: before evisceration - AE: after evisceration)**.**

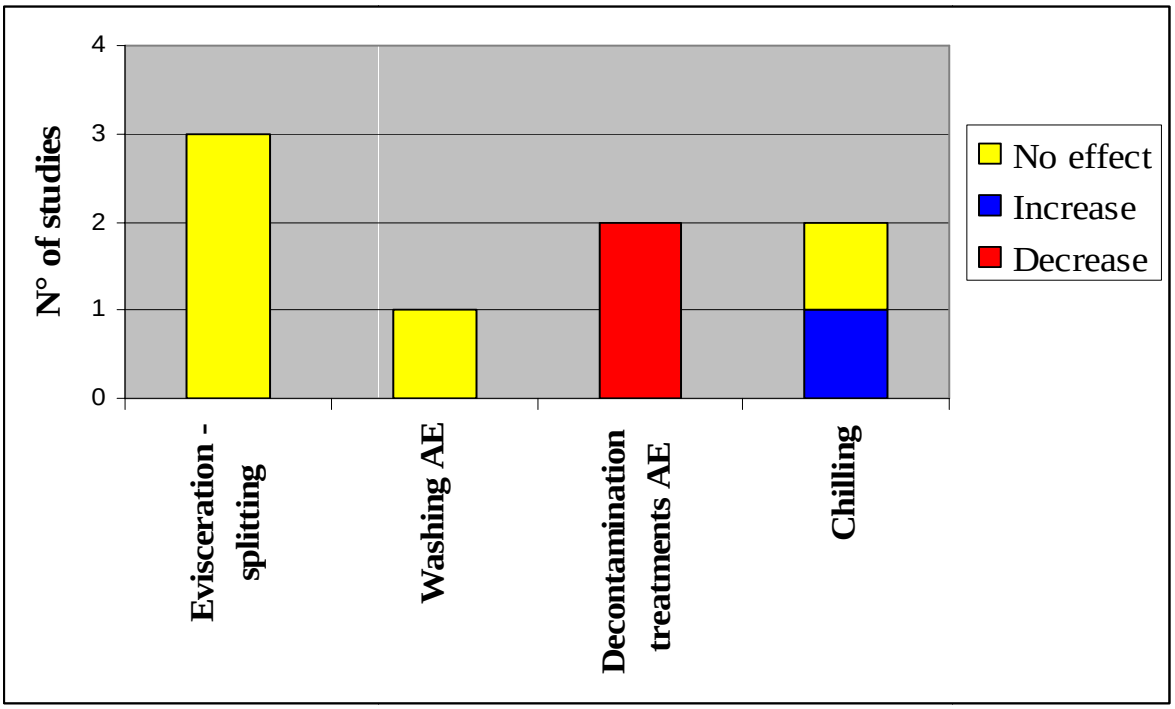

Figure 4: Trends (increase and decrease) of *Enterobacteriaceae* counts at different stages of the slaughterline in beef slaughterhouses (BE: before evisceration - AE: after evisceration).

2.3.4. Review question 2

According to the defined search process and the established eligibility criteria, a total of 29 papers dealing with risk factors influencing *E. coli* and *Enterobacteriaceae* counts on beef carcasses were obtained. Fifteen papers described studies that reported *E. coli* counts, nine papers reported *Enterobacteriaceae* counts and five papers considered both types of indicator bacteria.

As already mentioned for the review question 1, the comparability of data provided by different studies was hampered by different aspects, such as the sampling method, the unit of enumeration, the area sampled on the carcass and the decontamination treatments applied, which were not consistent across studies. Hence, different studies considering the same factor were compared in terms of conclusions drawn and not in terms of counts reported. However, many other aspects apart from the specific risk factors investigated could influence the final results presented in the retrieved papers.

The effect of the annual season on the prevalence of *E. coli* on beef carcasses was addressed by three different studies (Rigobelo et al., 2006; Ruby et al., 2007; Rigobelo et al., 2008), concluding that season could have a direct impact on indicator bacteria prevalence and counts on beef carcasses. In particular, it was demonstrated that there is a higher probability of finding *E. coli* positive carcasses during the rainy season (Rigobelo et al., 2006; Rigobelo et al., 2008), and that warmer months (June, July) could lead to higher *Enterobacteriaceae* counts on carcasses than colder months (November, December and March) (Ruby et al., 2007). These last authors investigated the influence of other risk factors and also concluded that the geographical position of the plant and the year of collection of samples significantly influence the bacterial loads of beef carcasses (Ruby et al., 2007).

E. coli and *Enterobacteriaceae* counts on carcasses varied, perhaps according to the slaughterhouse where the slaughtering took place. In some studies, specific aspects related to this point, such as the design of the plant, the throughput, or the surveillance system in place were evaluated, whereas in some other cases differences among plants in terms of bacterial loads of carcasses are simply reported without attributing these findings to some specific reasons.

Zweifel et al. (2008) conducted a survey in low-throughput abattoirs in Switzerland (about 220 animals slaughtered annually). *Enterobacteriaceae* prevalence on cattle carcasses ranged from 0 % to 55 % and some differences among the slaughterhouses in terms of counts were reported. The other study dealing with this point was conducted in France and reported significant differences in terms of *Enterobacteriaceae* loads on carcasses collected at four different plants (Collobert et al., 2002). In both cases it was not possible to identify which main features of the plants involved were responsible for such differences.

The influence of plant features on microbial carcass contamination has also been studied by Blagojevic et al. (2011), who investigated two different abattoirs collecting samples at the beginning (before dehiding) and at the end of the slaughterline (before chilling). In this case, the authors reported that the *Enterobacteriaceae* counts did not significantly differ between the two abattoirs.

Also the effect of the plant design was investigated. Prendergast et al. (2004), compared the *Enterobacteriaceae* loads of beef carcasses obtained in two Irish slaughterhouses. The first abattoir was a linear rail plant and had slaughter, dressing and chilling systems on a single floor, while the second abattoir had a serpentine rail on two floors and animals moved from the lairage on a lower level up to a higher level slaughter and dressing line. The authors concluded that the plant design clearly influenced the airborne bacterial numbers, but it did not have any effect on the *Enterobacteriaceae* load of the carcasses, and that the relationship between airborne and carcass contamination is poor.

The effect of plant throughput on indicator bacteria counts was frequently investigated. Two different Australian studies addressed this point. Phillips et al. (2001) carried out a comparison in terms of prevalence of *E. coli* on carcasses among slaughterhouses concentrating on export, slaughterhouses supplying the domestic market and low-throughput slaughterhouses. Although the three types of slaughterhouse differed significantly for standards of construction, government oversight and processing operations, the authors concluded that the incidence of *E. coli* was comparable among the slaughterhouses. Opposite conclusions were drawn by Sumner et al. (2003), who reported significantly lower *E. coli* prevalences on carcasses at low-throughput slaughterhouses (4.7 %) than at high-throughput slaughterhouses (28.4 %). Also Bohaychuk et al. (2011) reported higher mean *E. coli* counts on carcasses from high-throughput plants (-0.23 log cfu/cm^2) than on those from low-throughput plants (-0.54 log cfu/cm^2), but the differences found between the two types of slaughterhouses were not significant. Then, Ozdemir et al. (2010), reported that the mean numbers of bacteria (*E. coli* and *Enterobacteriaceae*) on beef carcasses at high-throughput slaughterhouses were higher (2.07 and 2.18 log cfu/cm^2) than at the low-throughput slaughterhouses (1.90 and 1.98 log cfu/cm^2). Also, in this last case, the difference between the two types of slaughterhouse was not statistically significant, probably because the same slaughtering technique was used in all plants.
Finally, the last retrieved paper reported an opposite finding. Hansson et al. (2001) found a lower prevalence of *E. coli* positive carcasses in high-throughput plants (34 %), than in low-throughput ones (41 %). Also in this last case the difference between the two types of slaughterhouse was not statistically significant.

Authors generally reported lower prevalences/counts in lower throughput plants, but in only one study were the differences between the two types of slaughterhouses significant. Although high-throughput plants applied more modern and specialized techniques than the low-throughput plants, more satisfactory data were generally found at low-throughput plants, which could be due to the lower slaughter rate that may result in better hygiene (Sumner et al., 2003).

Different slaughterhouses could induce different effects on the bacterial counts of carcasses, but the data collected from the retrieved studies did not clarify which one among the different aspects related to different slaughterhouses could have a main effect.

Also the effect of the surveillance system in place in the slaughterhouses was investigated as a factor influencing indicator bacteria counts. For this scope a microbiological baseline was conducted in 16 plants in Australia. *E. coli* was detected in 25 % of carcasses (mean log positives -0.61/cm^2). The survey demonstrated that levels of indicator bacteria on carcasses processed via the co-regulatory system were comparable to those established in abattoirs that operate the traditional system overseen by government inspectors (Bass et al., 2011). Moreover, the application of an online system to monitor faecal contamination caused by dehairing and evisceration operations was also evaluated in order to verify how it could influence carcass hygiene. Tergney and Bolton (2006) demonstrated that the application of this online monitoring system led to a reduction of the faecal contamination rates and consequently it was an effective mean of reducing the enteric counts.

The effect of using physical and chemical decontamination treatments on indicator bacteria counts was another aspect that was commonly investigated in the retrieved studies. Extensive research has been conducted on developing pathogen reduction technologies to improve the microbiological safety of animal carcasses. Among these technologies, steam pasteurisation and steam vacuuming have been recognised as two of the most effective methods for decreasing bacterial populations on animal carcasses.

Treatments with steam or hot water have been tested by different authors. Retzlaff et al. (2005) tested, in a high-throughput beef slaughterhouse, a chamber steam pasteurisation unit and they specifically evaluated the decontamination effect of seven different chamber temperatures (from 71.1 to 87.7 °C)

at the pre-rigor step. When the treatment was conducted at temperatures higher than 85 °C both *E. coli* and *Enterobacteriaceae* were reduced to undetectable levels (<0.4 CFU/cm^2).

Also, Minihan et al. (2003) demonstrated the positive effect of steam pasteurization (90 °C for 10 s of exposure time); the tested treatment significantly reduced the level of both *E. coli* and *Enterobacteriaceae* at the more contaminated sites of the carcasses, but it did not result in complete decontamination of the carcass.

The same conclusions were drawn by Gill and Landers (2003a), who confirmed that among the different decontaminant treatments they investigated, only pasteurisation with steam and hot water was always effective for substantially reducing the bacterial load of beef carcasses.
Pasteurisation equipment, consisting of a fixed cabinet equipped with a carousel that allows each full side of carcass to be treated individually was tested (Corantin et al., 2005). Carcasses were treated under steam pressure at an approximate operating temperature of 74.5 °C for 8 seconds, and the final bacterial loads were significantly lower than those of untreated carcasses. Finally, in the last study (Trivedi et al., 2007), the effectiveness of a steam cleaner suitable for low-to very low-throughput meat-processing plants (economic and easy to operate and install equipment) was verified. Also in this last case, the steam pasteurisation treatment significantly reduced *Enterobacteriaceae* counts at all three anatomical areas sampled on beef carcasses.

The five studies addressing this point showed that steam pasteurisation effectively reduced *E. coli* and *Enterobacteriaceae* counts on beef carcasses. The equipment tested was used both in high and low throughput plants, and all studies reported a clear reduction of both indicator bacteria counts on carcasses due to this decontamination treatment.

Similarly, hot water pasteurisation was demonstrated as an effective way to improve microbiological quality of the beef carcasses, but in this case the improvement of microbiological quality could be negated by a worsening of the organoleptic features.

In a study conducted by Gill and Bryan (2000), beef carcasses were pasteurised with water at 85 °C for different time frames (from 8 to 12 seconds) and all treatments reduced *E. coli* counts, but the reductions increased with longer treatment time and the highest reduction was obtained when the treatment was applied for 11 or 12 seconds. However, the appearance of treated carcasses was judged to be less desirable than that of the untreated ones.

Also Kiermeier et al. (2006), who conducted an investigation to clarify the reasons for higher *E. coli* prevalences reported in some Australian slaughterhouses, concluded that the slaughterhouses using hot water decontamination systems had lowest *E. coli* prevalences and this positive result was also registered in slaughterhouses that had substantial incoming problems related to high level of contaminations of the livestock introduced.

The use of hot water as a pre-evisceration wash in a commercial slaughterhouse was tested also by Bosilevac et al. (2006), and its effect was compared with that of lactic acid washing. The commercial hot water carcass wash cabinet tested, applying 74 °C water for 5.5 s, reduced *Enterobacteriaceae* counts by 2.7 log CFU/100 cm^2 on the pre-evisceration carcasses. Similarly, a lactic acid spray cabinet, applying 2 % lactic acid at approximately 42 °C, reduced *Enterobacteriaceae* counts, but in this case the reduction was more limited (1.0 log CFU/100 cm^2). Moreover, when the two cabinets were used sequentially, a reduction equal to 2.5 log CFU/100 cm^2 was obtained. Consequently, the authors concluded that hot water washing was the more effective procedure for decontamination of beef carcasses before evisceration.

Hence, hot water washing as well as steam pasteurisation appears to be an effective treatment for reducing the bacterial load of beef carcasses at different stages of the slaughterline.

The effect of washing with potable water at ambient temperature was also taken into account by different studies. Mies et al. (2004) and Barboza et al. (2002) concluded that water washing was ineffective in reducing bacterial loads on beef carcasses. The main effect observed after washing with potable water is a redistribution of microbial contamination over the carcass surface, so this would not be an efficacious or desirable way to reduce bacterial loads.

Gill and Landers (2003a) demonstrated that different effects could be accounted for by differences in the washing treatments applied. Moreover, the same authors indicated that washing was ineffective when the initial bacterial load on carcasses was low, but effective when the load was relatively high. A plausible explanation for this finding could be that when counts are high the bacteria are probably associated with particles, which are washed from the carcass by the large volumes of water in washing operation. When numbers are low, the bacteria are probably directly associated with tissues, and so may be refractory to physical removal by washing (Gill and Landers, 2003a).

In the retrieved studies the usage of alternative decontaminant treatments was also investigated and the results obtained were rather controversial.

Mies et al., 2004 evaluated the effectiveness of the following washing systems: single water wash, double water wash, water wash with 0.5 % lactic acid and water wash with 50 ppm of chlorine, to reduce the bacterial contamination on hides of cattle before entering the slaughterhouse. The application of each of the treatment resulted in an increase in the bacterial loads of the carcasses. Also Gill and Landers (2003a), who investigated the antimicrobial effect of different decontaminant treatments applied at four slaughterhouses confirmed that spraying carcasses with 2 % lactic acid is an ineffective treatment in reducing microbial contamination on carcasses. The same conclusions were drawn for peroxyacetic acid spraying. The authors supposed that the apparent failure of these two treatments may be due to the dilution of the applied solution by water present on carcasses from washing or condensation from steam rather than from bacterial resistance.

Barboza et al., (2002) evaluated the effect of organic acids and bacteriocin washes as decontaminants for beef carcasses. Lactic acid and the mixture of lactic acid and nisin produced a reduction of *E. coli* populations on the carcasses, whereas when only nisin was used, no positive effects were reported. The conflicting results reported in the last cited papers could be also due to the step of the slaughterline where the treatments were applied. Mies et al. (2004) described data obtained applying a washing procedure on hides of cattle before entering the slaughterhouse, Barboza et al. (2002) just before carcasses moved into the chilling area.

Moreover, another study (Algino et al., 2007) investigated different physical and chemical decontamination treatments in low-throughput plants, such as dry aging (multiday refrigeration without water), spray-chilling, low pressure and high pressure hot water, 2.5 % acetic acid (commercial vinegar) and a mixture of different acids (citric acid + ascorbic acid + erythorbic acid). All the interventions considered led to a significant decrease in mean levels of *E. coli* and *Enterobacteriaceae* counts on beef carcasses.

Also the application of other chemicals as decontaminants at different steps of the slaughterline could be equally effective as demonstrated by Nou et al. (2003), who tested the chemical dehairing, designed to clean cattle hides before carcass dressing. This treatment led to a significant reduction of approximately 2 logs of *Enterobacteriaceae* counts immediately after hide removal. The process consisting in the removal of hair and extraneous matter from hide with a sodium sulphite solution, the subsequent neutralization with a hydrogen peroxide solution, and a water washing before dehiding.

The effect of chemical decontamination treatments was unclear, since different studies described opposite results related to their effectiveness in reducing bacterial loads on beef carcasses.

The effect of spray-chilling on the bacterial load of beef carcasses was quite controversial. Chilling is considered a crucial phase of the slaughter process, reducing the internal temperature of carcasses and consequently slowing proliferation of most mesophilic bacteria. Air cooling is a process commonly used for this purpose, and it has the positive effect of limiting bacterial growth on carcasses by drying the carcass surface. However, this methodology can provoke water evaporation from carcass surfaces and result in a substantial reduction in carcass weight (and therefore, carcass value). For this reason, in the process termed spray-chilling or spray-cooling, the carcasses are intermittently sprayed with water during the first part of the cooling process in order to limit weight loss.

One study evaluated the effect of a spray-cooling process on *E. coli* counts on carcasses (Gill and Landers, 2003b) in four plants, which applied different decontamination treatments along the slaughterline. This study demonstrated that for two out of the four plants examined, the number of *E. coli* recovered from cooled carcasses were at least 1 log unit higher than the number recovered from dressed carcasses, for one plant the bacterial load remained unchanged and in the last plant, numbers of *E. coli* declined by about 1 log unit during carcass cooling. It is likely that these conflicting results were not due to the spray-cooling step, but they could be partly explained by the effects of the decontamination treatments applied along the line and the bacterial loads of the carcasses just before entering the cooling chamber.

Kinsella et al. (2006) investigated the effect of a chilling system that consisted of a humidification chamber to provide intermittent water spraying of carcasses (spray cycle 2 min on, 1 min off) for 15 h. Carcasses were sampled after dressing and after 24 h in the spray-chilling unit, and the data obtained suggested that this spray-chilling system can limit carcass shrinkage, without significantly altering the surface counts of indicator bacteria.

The two studies addressing the effect of spray-chilling on bacterial loads of carcasses investigated extremely different treatments and, therefore, the results presented cannot be compared. Hence, it was not possible to produce a definitive indication about the effect of spray-chilling on the bacterial load of carcasses.

Only one study considered batch related risk factors on indicator bacteria contamination of beef carcasses. Feed regimen just before moving animals to the slaughterhouse is considered a factor influencing bacterial load of carcasses. Anderson et al. (2005) verified the effect of feed or water administration of an experimental chlorate preparation on *E. coli* on beef carcasses. Data collected confirmed that no main effects of feed and water treatment or any possible interactions were observed on numbers of *E. coli* recovered from hide or carcasses.

In the following tables (Tables 3a-3b-3c), the effect of the risk factors described in the retrieved papers in terms of increase-decrease-no effect of indicator bacteria on beef carcasses are summarized. Those studies which described differences between the variables investigated, but without specifying or investigating which risk factors could influence that variability found, were not included in the list.

Table 3a: Slaughterhouse and farm related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *E. coli* counts on beef carcasses

Effect	Risk factor/Treatment	Comparison factor /control	Reference	N° of studies
Decrease	dry season	wet season	Rigobelo et al. 2008 (BR) Rigobelo et al., 2006 (BR)	2
	low -throughput slaughterhouse	high-throughput	Sumner et al., 2003 (AU)	1
	on line monitoring faecal contamination	control	Tergney and Bolton, 2006 (TR)	1
	decontamination – steam pasteurization temperature > 85°C	temperature 71.1 - 82.2 °C	Retzlaff et al., 2005 (US)	1
	decontamination - steam pasteurization	control - unpasteurized	Minihan et al., 2003 (IE) Corantin et al., 2005 (CA) Gill and Landers, 2003a (CA)	3
	decontamination – hot water	control - untreated	Gill and Bryant, 2000 (CA) Kiermeier et al., 2006 (AU)	2
	decontamination – lactic acid – lactic acid + nisin	control - untreated	Barboza et al., 2002(VE)	1
	decontamination (low-throughput plants) – day aging – acetic acid – mixture of different acids – low and high pressure hot water	control - untreated	Algino et al., 2007 (US)	1
	spray cooling (1 slaughterhouse)	control - before cooling	Gill and Landers, 2003b (CA)	1
Decrease Total				13
Increase	decontamination - single wash/double wash/ water wash with 0.5% lactic acid / water wash with 50 ppm of chlorine	control untreated	Mies et al., 2004 (USA)	1
	spray cooling (2 slaughterhouses)	control - before cooling	Gill and Landers, 2003b (CA)	1
Increase Total				2
No effect	high-throughput slaughterhouse	low-throughput	Phillips et al., 2001 (AU) Bohaychuk et al., 2011 (CA) Ozdemir et al., 2010 (TR) Hansson et al., 2001 (SE)	4
	co-regulatory inspection system	governmental inspection system	Bass et al. 2011 (AU)	1
	washing with water	control - other decontamination treatments	Mies et al., 2004 (US) Barboza et al., 2002 (VE)	2
	decontamination: lactic acid - peroxyacetic acid spraying	control	Gill and Landers, 2003a (CA)	1
	diet - sodium chlorate feed – sodium chlorate water	control	Anderson et al., 2005 (US)	1
	spray cooling (1 slaughterhouse)	[2]control - before cooling	Gill and Landers, 2003b (CA)	1
No effect Total				10

Table 3b: Slaughterhouse related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *Enterobacteriaceae* counts on beef carcasses

Effect	Risk factor/Treatment	Comparison factor /control	Reference	N° of studies
Decrease	dry season	wet season	Ruby et al. 2007 (US)	1
	slaughter characteristics	control	Zweifel et al., 2008 (CH) Collobert et al., 2002 (FR)	2
	high-throughput slaughterhouse	low-throughput	Ozdemir et al., 2010 (TR)	1
	on line monitoring faecal contamination	control	Tergney and Bolton, 2006 (TR)	1
	decontamination – steam pasteurization temperature > 85°C	temperature 71.1 - 82.2 °C	Retzlaff et al., 2005 (US)	1
	decontamination - steam pasteurization	control - unpasteurized	Minihan et al., 2003 (IE) Trivedi et al., 2007 (US)	2
	decontamination - hot water	lactic acid / lactic acid + hot water	Bosilevac et al., 2006 (US)	1
	decontamination (low-throughput plants) – day aging – acetic acid – mixture of different acids – low and high pressure hot water	control - untreated	Algino et al., 2007 (US)	1
	decontamination - chemical dehairing	control - untreated	Nou et al., 2003 (US)	1
Decrease Total				11
No effect	slaughterhouse design: linear rail one floor	serpentine rail and two floors	Prendergast et al., 2004 (IE)	1
	high-throughput slaughterhouse	low-throughput	Ozdemir et al., 2010 (TR)	1
	slaughter characteristics	control	Blagojevic et al., 2011 (RS)	1
	cooling–novel spray-cooling	control	Kinsella et al., 2006 (IE)	1
No effect Total				4

2.3.5. Review question 3

Five papers provided information on the relationship between faecal contamination of beef carcasses and their *E. coli* and/or *Enterobacteriaceae* counts. Two papers provided data on *E. coli*, two papers on *Enterobacteriaceae* and one paper on both the indicator bacteria. All papers considered naturally contaminated carcasses and were conducted in commercial slaughterhouses.

It is generally accepted that the dirtiness of animals at the time of slaughter is directly correlated with the amount of dirt transferred to the carcass, and that procedures avoiding the transfer of dirt from hide to meat can result in improved microbiological quality of the carcasses. Very few studies which investigated these points were retrieved.

In the study conducted by Sarraino et al. (2012), beef carcasses were classified in terms of hide cleanliness at the beginning of the slaughterline and were categorised according to a scale ranging from 1 (very clean) to 5 (very dirty). *Enterobacteriaceae* and *E. coli* counts were estimated for a selection of carcasses from each category and the data collected confirmed that the dirtiest (categories 4 and 5) animals had the highest bacterial loads on carcasses in comparison to the cleanest ones (categories 1, 2 and 3). A direct correlation was observed between visual cleanliness category of hides and the level of microbial contamination of carcasses. Hence, the authors concluded that a pre-slaughter visual evaluation of animals' cleanliness and the consequent application of corrective measures (e.g. rejection of the animals, washing or application of additional procedures to reduce the microbial contamination) could be effective to limit carcass contamination at the end of the slaughterline.

Similar conclusions were drawn by Blagojevic et al. (2012), but in this case the authors demonstrated that the simplification of the categorization scheme, and the classification of the carcasses into only two main categories, including the very dirty animals and the less dirty ones, could be effective in practice to identify the carcasses that should be managed with particular attention since they potentially have the highest microbial loads. Similarly, a simplified classification system, based on three different classes (1: clean, 2: moderately dirty and 3: very dirty) was described in another study conducted in two beef abattoirs in Norway (Hauge et al., 2012). Samples were collected immediately after hide removal, at the beginning and at the end of the slaughterline. After hide removal, animals with visually clean hides produced carcasses with a lower amount of bacteria than dirty ones. However, at the next step of the slaughterline, the dirtiest animals were not associated with significantly higher levels of carcass contamination. This could be due to the fact that the dirtiest carcasses are treated more carefully and such extra care might lead to a clear improvement of the microbiological quality of the carcasses. At the end of the process all carcasses had the same comparable *E. coli* counts regardless of the initial level of contamination.

The same findings were reported by Gill and Landers (2004b), who evaluated the level of contamination of animals carcasses detained for removal of visible contamination. The study was conducted at four slaughterhouses in Canada. A selection of visibly contaminated carcasses were collected on the detaining rail and sampled within the contaminated area, from a site adjacent to the contaminated area and from another non-contaminated area of the carcass, randomly selected. The same sampling scheme was repeated on the carcasses after trimming, and again when the carcasses arrived at chilling after the routine washing and decontamination treatments used in the slaughterhouses had been applied. The data collected confirmed that after completion of the dressing procedures, bacterial loads of sites that had been visibly contaminated, that were adjacent to the trimmed area or that were randomly selected were all similar. Moreover, it emerged that the microbiological quality of the carcasses that were initially detained was generally comparable with, or better than, the other carcasses routinely processed at the same plant.

In the last study dealing with this point, authors (McCleery et al., 2008) evaluated the effect of ante-mortem and post-mortem hide clipping on the microbiological quality of carcasses. In the UK, a clean livestock policy was introduced in 2007 based on the categorization of animals, according to a scale of 1-5, with animals in categories 1 and 2 being the cleanest and consequently considered safe for slaughter without taking further precautions, category 3 animals being rejected at first presentation at ante-mortem and taking additional measures like clipping to facilitate cleaning. Clipping can be conducted both in the lairage or on the slaughterline after stunning and bleeding but before dehiding. Clipping procedures are aimed to remove visible dirt from the hide in order to reduce cross contamination to the carcass during slaughter process. In the study, a selection of carcasses categorised from 1 to 4, according to the level of visual contamination of hides before entering the slaughterhouse, was analysed for *Enterobacteriaceae* counts. Category 1 and 2 animals were slaughtered without further precautions, whereas category 3 and 4 animals were either clipped ante-mortem or post-mortem on the slaughterline. Analysis of data obtained revealed no statistically significant association between the *Enterobacteriaceae* population recovered and animal category or clipping location (ante-mortem or post-mortem). Clipped animals produced carcasses with a comparable microbiological quality to those derived from clean animals.

In conclusion, the studies retrieved confirmed that carcasses derived from clean cattle had lower bacterial load than those derived from visibly dirty animals. However, identification of visibly contaminated cattle and application of effective measures either on animals before entering the slaughterhouse and/or along the slaughterline can lead to lower bacterial contamination or a contamination level of carcasses at the end of the process comparable to that of clean animals. Hence, the studies support the conclusion that the pre-slaughter visual evaluation of the level of hide-carcass

contamination and the application of proper corrective measures along the slaughterline especially for the initially contaminated animals can be an effective approach to reduce carcass contamination at the end of the slaughterline.

Table 21 (Appendix F) summarizes the counts of *E. coli* and *Enterobacteriaceae* on beef carcasses along the slaughterline in relation to the level of visual faecal contamination.

2.4. Pigs

2.4.1. General information about the considered papers

Among the retrieved papers, 31 papers were eligible to provide data on pigs regarding the three review questions. Seventeen papers were conducted in Europe, and the other fourteen outside Europe. All the studies identified described observational studies with the exception of five studies dealing with an experimental design. Eighteen papers provided data on *E. coli*, ten papers on *Enterobacteriaceae* and three papers considered both indicator bacteria.

Fourteen papers provided information on review question 1, fifteen papers addressed review question 2, two papers dealt with both review question 1 and 2, while no papers dealing with the scoring of visual faecal contamination in order to study the effect of this variable on indicator bacteria (review question 3) were retrieved.

The most common sampling method was swabbing (28 out of 31 papers), while skin excision was the sampling method used in three studies. The unit of enumeration most commonly used was log cfu/cm^2. The sampling site on carcasses varied greatly among the retrieved studies as well as the analytical method; in this case the most common was classified as ISO but also Petrifilm, and MPN were used.

More information about the characteristics of the selected papers are available in Table 7 (Appendix C).

2.4.2. General information about the slaughtering process

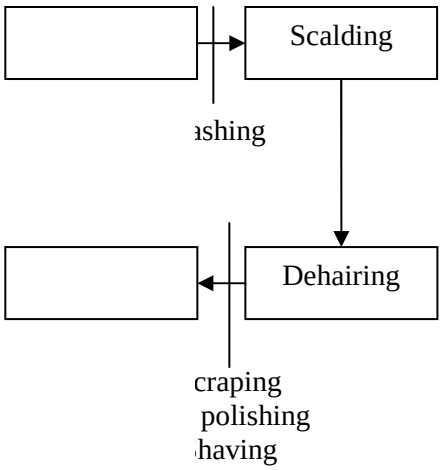

Figure 4: Flow-chart summarizing the activities carried out along the pig slaughterline.

2.4.3. Review question 1

This section is aimed at the evaluation of the effect of the different stages of the slaughterline on the counts of indicator bacteria in swine carcasses. Fourteen papers were retrieved from literature dealing with this issue. In particular, seven papers reported *Escherichia coli* counts, five *Enterobacteriaceae* counts and two counts of both indicator bacteria. In Figure 4, the main slaughtering operations described in the selected studies are presented.

The retrieved papers provided data that were not always comparable. A main challenge in identifying the steps of the slaughterline that lead to a decrease or an increase of indicator bacteria counts on the carcasses is the difficulty of finding studies providing data obtained before and after a single stage. Data have been generally collected at distant sampling points; as a consequence the effective role of a single stage toward a change in bacterial levels is not always evident.

E. coli

Different studies investigating the trend of *E. coli* counts along the slaughterline are described here. Warriner et al. (2002) evaluated the effect of slaughtering on *E. coli* counts by sampling a single carcass every 45-50 minutes. This study evaluated also differences in terms of bacterial loads of carcasses according to the slaughter time in the day. The process was characterised by scraping performed after scalding, and by a double polishing technique performed dry before singeing and wet after it. Moreover, carcasses underwent a washing treatment before chilling. Authors attributed the reduction of bacterial counts to scalding and singeing; however, since the sampling of carcasses after

these specific stages was not possible in the study, these results are presented in tables according to the real sampling points (after scraping and after polishing, respectively). In contrast with the results obtained for *Enterobacteriaceae*, the increase observed during evisceration was not statistically significant for *E. coli*.

Variations of *E. coli* counts were also investigated in an Iberian study. The slaughterline was characterized by a dry and wet scraping technique, as in the previously described paper, and by the anal closure routinely performed before evisceration in order to prevent gut content from leaking. *E. coli* counts decreased during scalding, but increased during evisceration despite anal plugging (Rivas et al., 2000).

Nesbakken et al. (2008) evaluated the effect of blast chilling on different bacterial species. Concerning *E. coli*, a slight reduction was observed after the application of this chilling technique and this reduction was stated as significant.

A Danish study (Wu et al., 2009) investigated the behaviour of *E. coli* by sampling the left hind leg, close to the anus, in a high throughput slaughterhouse (10,000 pigs per day). *E. coli* counts were reduced significantly (by 1-2 log units) at sequential stages of processing and this reduction was attributed by authors to scalding, singeing and chilling. However, sampling points were not located immediately after the stages considered effective. As an example, bacterial loads of samples collected after splitting were compared to ones collected after scalding. In between, several operations had been performed such as singeing, polishing, evisceration and splitting.

Gill et al. (2000) investigated eight slaughterhouses processing 200 to 800 pigs per hour. At one plant only, polished carcasses were pasteurised before dressing commenced. Blast chilling was applied. Sampling was performed by swabbing a random site on carcasses chosen from a pre-designed 83 areas grid. Moreover, *E. coli* counts were estimated by using the hydrophobic grid membrane filter procedure. From each plant, sets of 25 samples were collected at three different sampling points. *E. coli* recovered indicated that substantial numbers of those organisms were added to carcasses during the dressing processes at four of the plants, and that bacterial levels on carcasses were substantially reduced during the chilling without spraying process at two plants.

Tamplin et al. (2001) examined the prevalence and quantity of *E. coli* on swine carcasses at a medium-throughput (7,000 pigs/day) slaughterhouse operating under the hazard analysis and critical control point-based inspection models project (HIMP) program. Carcasses were sampled twice: immediately following exsanguination and after the carcasses were washed, eviscerated and chilled overnight. *E. coli* was found on all carcasses sampled after bleeding and on 30.1 % of the chilled carcasses. Mean numbers recovered on different days varied greatly, in particular after exsanguination (53 to 11,000 cfu/cm^2).

In Canada, a study to evaluate the impact of slaughtering stages on *E. coli* counts on swine carcasses was performed at Lacombe Research Center (LRC) (Jones and Johns, 2012). The objective of the study was to compare the level of F-RNA coliphages with different indicator bacteria. The investigated animals were raised and slaughtered in LRC pilot plant and MPN estimates were provided. Samples were obtained from anal region and from random sites on carcasses. Moreover, samples belonging to a commercial slaughterhouse were also analysed and used to improve the experimental design. In the LRC pilot plant, *E. coli* numbers varied according to the carcass site sampled. However *E. coli* counts in the anal region decreased at each sampling point investigated. Carcasses were sampled before pasteurisation, after pasteurisation, after evisceration and after washing. Random carcass samples showed a decrease in *E. coli* counts only after pasteurisation. The importance of pasteurisation was also confirmed by results obtained in the commercial plant. In this case, carcasses had noticeable decreases of *E. coli* counts after bleeding and after pasteurisation.

Another study was conducted in the LRC and in this case, several operations were separately tested to evaluate which processing resulted in lower *E. coli* counts on carcasses (Bryant et al., 2003). Dehaired carcasses carried relatively large numbers of *E. coli* and singeing did not reduce them. Pasteurisation of carcasses was applied in early stages of the dressing procedure to avoid the degradation of cut muscle surface; however the effectiveness of the treatment was impaired by subsequent recontamination during head removal. The pasteurisation process was then shifted to after head removal, instead of before. However in this case also, the following operations (evisceration, splitting and washing) led to a new increase in bacterial counts. Finally, it was observed that chilled pig carcasses that were pasteurised after head removal and chilled without spraying had acceptable levels of *E. coli*.

Gill and Landers (2004a) analysed results obtained by swabbing routinely processed pig carcasses before and after cooling and demonstrated the cooling process decreased *E. coli* counts.

Namvar and Warriner (2006), in a study aimed at tracing the fate of *E. coli* within a high throughput pig slaughterline, described the changes in counts recovered from the brisket region of swine carcasses. In particular, twelve animals were sampled at 45 minutes intervals during two separate visits after the following operations: bleeding, scalding, scraping, evisceration, splitting, and washing. *E. coli* numbers on carcasses following bleeding, at the beginning of processing, were relatively high, but progressively decreased towards the end of the slaughterline. In particular, *E. coli* counts were significantly reduced by scalding but increased during scraping and were subsequently reduced during the polishing process. *E. coli* was sporadically recovered at low levels from carcasses following evisceration, splitting and the final lactic acid washing. It is interesting to note that a greater number of lactic acid washed carcasses tested positive for *E. coli* compared to those sampled following evisceration or splitting. According to genotype analysis, the authors suggested that the recovered *E. coli* represented a stable endemic population, in particular belonging to the holding area of the abattoir.

Finally, in order to understand which operation would be more effective in reducing *E. coli* counts on pig carcasses, different papers investigating the effects of slaughtering phases were retrieved. Results belonging to the retrieved papers are summarized in Figure 5.

Regarding scalding, all five studies dealing with counts of *Escherichia coli* highlighted a reduction in bacterial numbers (Wu et al., 2009; Namvar and Warriner, 2006; Rivas et al., 2000; Warriner et al., 2002). Namvar and Warriner (2006) in particular described a scalding process of 5 minutes with a water temperature of 65 °C. Warriner et al. (2002) reported a decrease of *E. coli* counts after scraping, but attributed this reduction to the scalding that preceded this step.

Singeing was investigated in one study that observed a decrease in counts (Warriner et al., 2002). In general, the sampling of carcasses after this stage was not performed in the retrieved papers and the considered study attributed to singeing a reduction observed in carcasses after polishing. In contrast, scraping increased bacterial counts according to two out of three studies, both described in one paper (Namvar and Warriner, 2002). According to the same authors, the polishing process was able to reduce *E. coli* counts, but in this case carcasses underwent, after the previous sampling point, additional operations such as polishing, washing and evisceration.

As regards pasteurisation, two papers considering this step recognized it as an effective process to reduce *E. coli* counts (Jones and Johns, 2012; Bryant et al., 2003). The first study, in particular, described a reduction in *E. coli* counts on both sampling sites investigated: a region randomly selected from a grid and the anal region. In this case, carcasses were submitted to a pasteurisation process after polishing. In the second paper, the pasteurisation process was applied after the shaving operation. It should be noted that in this case, the slaughtering process was performed in a pilot plant.

The washing process was investigated in seven studies described in five different papers and it never led to significant results justifying its use as decontamination treatment (Namvar and Warriner, 2006; Warriner et al., 2002; Jones and Johns, 2012; Bryant et al., 2003; Gill et al., 2000b). It is important to underline that in the study performed by Namvar and Warriner (2006), 1.5 % lactic acid was used during this step, but there was no significant effect on *E. coli* counts on carcasses. Moreover a greater number of lactic acid washed carcasses tested positive for *E. coli* compared to those sampled after evisceration and after splitting. According to authors this was probably due to a redistribution of *E. coli* over carcass surface. As previously stated, the described results were not obtained from a punctual evaluation of the slaughtering stage, but were inferred from data belonging from different, not sequential processing stages along the slaughterline.

The final phase of the slaughtering operations is chilling. Five papers dealt with this slaughtering step, but within each study, the study designs were very different from each other. Two studies in particular reported bacterial counts before and after chilling: in both cases the chilling process was able to decrease *E. coli* counts (Nesbakken et al., 2008, Gill and Landers 2004). In one of these cases, the chilling process was characterised by a blast chilling phase (-21.9° for 70 min) (Nesbakken et al., 2008). Two studies were not useful to evaluate this stage because they compared *E. coli* counts obtained after chilling with counts obtained at temporally/physically distant stages, i.e. after bleeding (Tamplin et al., 2001) or after splitting (Wu et al., 2009). The last retrieved paper observed a reduction in *E. coli counts* on carcasses after chilling in only two out of eight plants investigated (Gill et al., 2000).

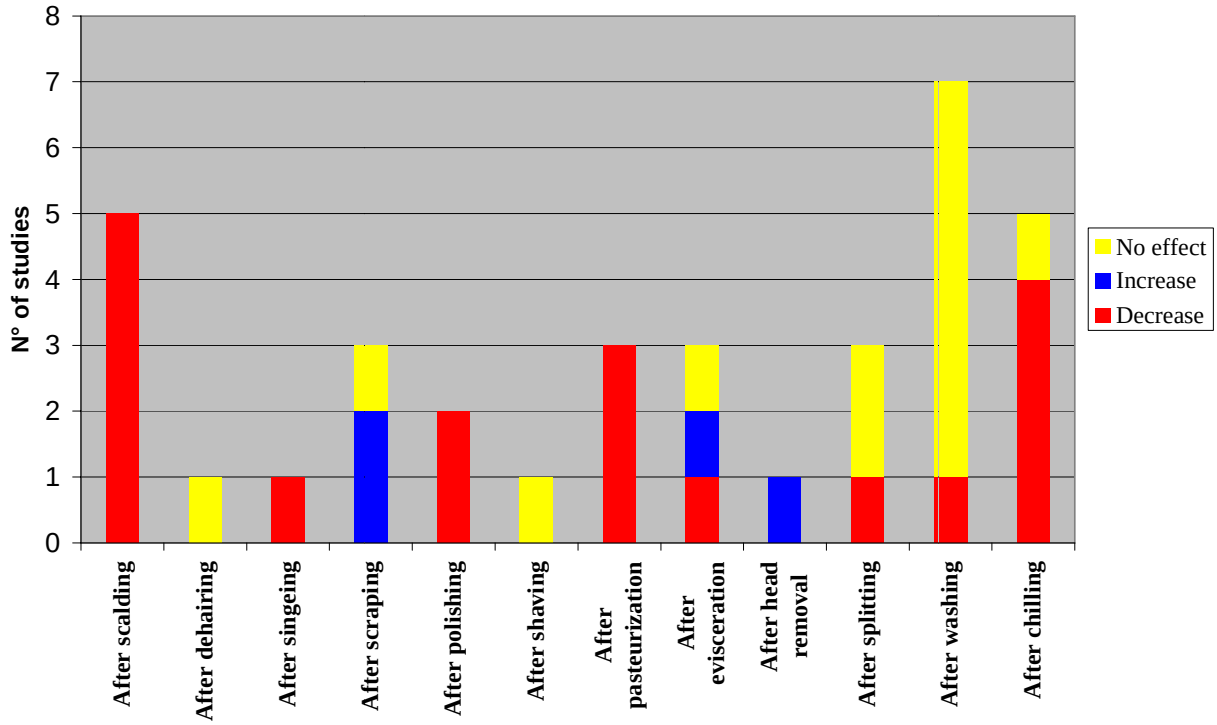

Figure 5: Trends (increase or decrease) of *E. coli* counts at different stages of the slaughterline in pig slaughterhouses**.**

Enterobacteriaceae

One study (Spescha et al. 2006) providing data on *Enterobacteriaceae* along the slaughterline analysed data belonging to different stages in two abattoirs processing 250 and 160 pigs per hour. A characteristic of the first abattoir was the use of blast chilling technique, while in the second abattoir there was a combined dehairing and singeing process. Both plants washed carcasses with potable water before chilling. In both abattoirs, *Enterobacteriaceae* counts decreased after scalding (in all the carcass sites tested) and after chilling and increased after dehairing. Moreover, in the first abattoir, counts decreased after singeing and slightly increased after polishing, while in the second plant they decreased after polishing.

Another study (Duggan et al. 2010) evaluated the relationship between *Enterobacteriaceae* counts and *Salmonella* presence. Results reported in graphics also showed a non statistically significant reduction of *Enterobacteriaceae* counts during chilling.

Lenahan et al. (2006) also evaluated the effect of chilling on *Enterobacteriaceae* counts in carcasses belonging to four different plants. They observed an average reduction even if, at carcass level, this was not always demonstrated since some carcasses showed a slight increase during chilling. The conclusion was that chilling could be an important step to improve the carcass categorisation in relation to Decision 2001/471/EC.

Hurd et al. (2008) evaluated the *Enterobacteriaceae* presence in terms of prevalence in pools of samples and observed an increase in prevalence of *Enterobacteriaceae* on carcasses during and after evisceration. However, it should be noticed that the experimental design of this study was quite complex and had different aims, so these results in terms of counts at different stages are extrapolated from the context. Moreover, sampling sites on carcasses are quite different from usual since swabs were collected from bung and pleura during and after evisceration.

The evisceration stage was evaluated in a study performed in a low-throughput slaughterhouse in Lao PDR processing sixty to eighty pigs per day. In this case the authors observed a significant increase during evisceration (Inthavong et al., 2006), but in general the situation was quite different, in terms of hygienic practices, if compared with other slaughterhouses.

Warriner et al. (2002) evaluated the effect of slaughtering on *Enterobacteriaceae* counts by sampling a single pig carcass every 45-50 minutes in order to also determine differences related to the slaughter time of the day. The process was characterised by scraping after scalding and by double polishing performed dry before singeing and wet after it. Moreover carcasses underwent a washing before chilling. The authors described a reduction in counts after scalding and singeing. On the other hand, an increase in *Enterobacteriaceae* levels was observed after evisceration, which was identified as the key cross-contamination operation.

The microbial contamination of pig carcasses in an Iberian slaughterhouse was the study object of a paper (Rivas et al., 2000) dealing with variation of *Enterobacteriaceae* counts on carcasses during the slaughtering process. The process encompassed a dry and wet scraping technique performed in two stages before and after the singeing phase, respectively, and anal closure before evisceration. *Enterobacteriaceae* counts decreased during scalding and scraping but increased during dehairing and evisceration. According to authors, the unexpected decrease during scraping was not due to this technique but to the singeing process that, as described above, was performed between the two phases of scraping. Interestingly, despite anal plugging, an increase in *Enterobacteriaceae* counts was observed during evisceration.

The effect of different operations on *Enterobacteriaceae* counts are summarized below; results are also shown in Figure 6.

As already observed for *E. coli, Enterobacteriaceae* levels also decreased during scalding in the four studies (Spescha et al., 2006; Rivas et al., 2000; Warriner et al., 2002). This step of pig processing is critical to reduce initial bacterial counts and must be carefully considered in the planning and managing of activity at plant level.

The same studies mentioned before highlighted that the dehairing process was recognised as a source of contamination in two out of three studies. In particular, one study described in the paper by Spescha et al. (2006) determined the joint effects of the singeing and dehairing phases together, and observed an increase in *Enterobacteriaceae* counts. In contrast singeing reduced *Enterobacteriaceae* counts in three different studies (Spescha et al., 2006; Rivas et al., 2000; Warriner et al., 2002).
Evisceration is a key contamination point according to four studies (Rivas et al., 2000; Warriner et al., 2002; Inthavong et al., 2006; Hurd et al., 2008); however Hurd et al. (2008) evaluated the increase in bacterial contamination only in terms of prevalence in the tested pig carcasses.

The last operation that, according to the literature, results in notable variation of carcass contamination is chilling. A decrease in *Enterobacteriaceae* counts was described in three (Lenahan et al., 2009; Spescha et al., 2006) out of four selected studies (Lenahan et al., 2009; Spescha et al., 2006; Duggan et al., 2010). The fourth study (Duggan et al., 2010) describing variation in counts during chilling reported results of *Enterobacteriaceae* counts only in graphics and did not analyse in depth indicator bacteria variations between different stages.

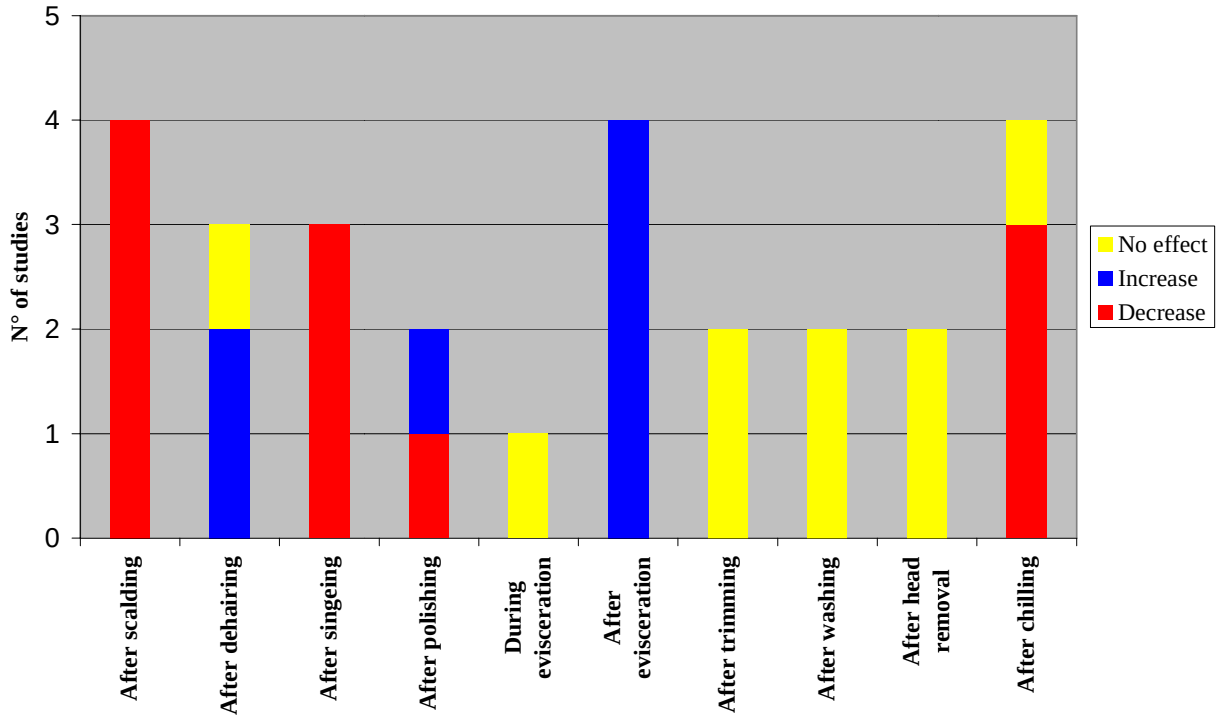

Figure 6: Trends (increase or decrease) of *Enterobacteriaceae* counts at different stages of the slaughterline in pig slaughterhouses

The counts of *E. coli* on small ruminant carcasses described in the selected papers at the different stages of the slaughterline are reported in Table 11 (Appendix D), while the counts of

Enterobacteriaceae are presented in Table 12 (Appendix D). In these tables, the number of samples analysed at each step, the increase or decrease of the counts for each step, the mean and the standard deviation are reported when these data were available.

2.4.4. Review question 2

A total of 18 papers were collected dealing with risk factors potentially influencing bacterial counts on swine carcasses. In particular, 11 papers described studies reporting data on *E. coli*, five papers on *Enterobacteriaceae* and two considered both bacteria.

As already mentioned for review question 1, the comparability of data provided by different studies was hampered by different aspects, such as the sampling method and the unit of enumeration, which were not consistent across studies. Hence, different studies considering the same factor were compared in terms of conclusions drawn and not in terms of counts reported.

Since some papers provided data on both types of indicator bacteria, or considered more than one factor influencing bacterial counts, it was more convenient to consider the individual studies (trials), instead of the papers. In Tables 4a and 4b, the risk factors investigated are summarised for both the indicator bacteria.

E. coli

A total of 30 risk factors or treatments were investigated in relation to *Escherichia coli* counts on carcasses at slaughterhouse in the collected papers.

Only one paper considered the influence of risk factors at farm level. This study in particular considered 12 treatment groups and different conditions regarding the number of daily feeding (2 versus 5), the feed type (pelleted vs mash) and the fasting time before slaughter (4, 14, 24 h) according to a 2×2×3 factorial design. The slaughter process took place in an experimental abattoir. The treatment, according to the statistical model performed, which resulted in the lower *E. coli* counts on the thoracic area was feeding the pigs pelleted feed five times a day followed by a 24 h fast. In contrast, the highest counts were recovered from pigs fed mash, five times a day followed by a 4 h fast. A statistical difference was observed between these two treatments with an a posteriori Tobit analysis. No correlation was found between stomach weight and *E. coli* counts on carcasses and between *E. coli* levels on carcasses and the weight of faeces collected on the truck floor (Saucier et al., 2007).

Attention has been paid also to the management of the animals before slaughtering. Rabaste et al. (2007) evaluated the influence of handling and group dimension on microbial carcass contamination. In particular, pigs were divided in groups of 10 and 30 individuals subjected to rough and gentle handling. The rough treatment consisted in a quickly handling with electric pod; on the contrary, gentle handling was characterised by slow handling managed with the use of a plastic board or whip. Neither handling nor group dimension significantly influenced *E. coli* counts on carcasses. This lack of differences between groups suggested that the stress condition applied had a limited impact on carcass microbial quality.

The effect of the plant throughput on bacterial counts was investigated by Hansson (2000), who compared four low throughput with four high throughput slaughterhouses. The percentage of presumptive *E. coli* (74 %) reported on pig carcasses in high throughput plants (250,000-1,300,000 pigs/year) was higher compared to the percentage (58 %) in low throughput plants (450-800 pigs year). These differences were statistically significant. However, since the study was aimed also at the evaluation of other bacteria, and these did not significantly differ among slaughterhouses, authors

concluded that level of carcass contamination could not be related to the throughput of the slaughterhouse.

In contrast, another study dealing with plant throughput and investigating 50 slaughterhouses in Canada found no relation between abattoir throughput (8000 pigs/year was the cut-off value) and bacterial counts (Bohaychuk et al., 2011). An overall prevalence of 33.7 % *E. coli*-positive swine samples was reported. Most of the carcasses (92.7 %) had levels of *E. coli* lower or equal to 10 cfu/cm^2.

The effect of the surveillance system in place in slaughterhouses was investigated as a potential factor influencing the counts of indicator bacteria. For this scope, a microbiological baseline study was conducted in 7 plants located in New South Wales, Australia. *E. coli* was detected in 63 % of pig carcasses (mean log of positive samples was -0.23 cfu/cm^2). This survey demonstrated that levels of *E. coli* on carcasses processed via the co-regulatory system were comparable to those established in abattoirs that operate the traditional system overseen by government inspectors (Bass et al., 2011).

In another paper, Lindblad et al. (2006) described the results of a microbiological survey performed at Swedish slaughterhouses. Both *E. coli* and *Enterobacteriaceae* were taken into account to assess the baseline value, but only *E. coli* data have been analysed in order to study the effect of the season in which slaughtering was performed. Authors observed that the highest *E. coli* counts on the pig carcasses were obtained in the period June-July; however, the study was conducted during a one year period, so thus the seasonal variation has not been confirmed for data belonging to other years.

Delhalle et al. (2008) studied several managerial factors that could influence microbial status of pig carcasses in the 10 largest Belgian slaughterhouses. In particular, they investigated different plant variables through a questionnaire, data from which were then incorporated into two statistical models (univariate mixed logistic regression and multivariate mixed linear regression) and they then fed the models with microbiological data obtained from a total of 584 carcass samples. Results highlighted in particular that the use of water during lairage cleaning and a high frequency of lairage disinfection resulted in lower *E. coli* counts. In contrast, spraying of live animals when the external temperature was considered hot by operators was correlated with an increase in carcass *E. coli* contamination. The practice of refreshing animals after transport is common. Other possibilities contemplated in the study were spraying 75 % of the time during lairage and spraying automatically in relation to external temperature. According to the authors, however, this conclusion about spraying must be considered carefully. Moreover, the *E. coli* counts on carcasses increased proportionally with the length of processing time between killing and scalding. In contrast, protective factors, which reduced *E. coli* contamination, were a scalding procedure using steam instead of immersion, the disinfection of the splitting machine three times a day and the change of carcass hooks before chilling.

Hamilton et al. (2009) evaluated the effect of decontamination treatments on *E. coli* counts in pig carcasses. The study focused in particular on the effect of two decontamination techniques applied in two different high throughput abattoirs over three days. In each trial, two treatments, one performed with hot water (15 sec with water 81.9-83.5 °C) and one performed with acidified sodium chlorite water (SANOVA solution) were compared with the standard hygiene procedure. Carcasses were sampled using a meat excision technique at belly level. The prevalence of *E. coli* on carcasses in each abattoir was significantly and substantially reduced for both hot water and SANOVA treatment compared to the control. This result was also achieved if one abattoir had higher levels of microbial contamination. Further, the two treatments did not differ significantly. It should be noticed that although SANOVA treatment is not approved according to EU legislation, no restriction exists regarding the use of hot water, and thus, this could be an effective efficient alternative operation to reduce *E. coli* levels on slaughtered pig carcasses.

Different treatment conditions occurring during slaughter were investigated by several papers. One study reported data aimed at evaluating the effect of different risk factors toward the variations in *E. coli* counts on pig carcasses. Samples were collected before washing and after chilling in 10 low-throughput slaughterhouses in Wisconsin (Algino et al., 2009). Between the selected sampling points, there was a reduction in *E. coli* levels on both skinned and unskinned carcasses. As regards the washing treatment, it was found to be always effective in reducing *E. coli* counts, regardless of the water temperature applied. In fact, in this study, washing was performed with water temperatures ranging from <12.8 °C to >32.2 °C in different trials. Also both the tested chiller hold times (1 or 2 days) effectively reduced *E. coli* levels on the carcasses.

In contrast, another study investigating the effect of pig carcass washing failed to demonstrate any effective lowering of *E. coli* levels. More specifically, the authors divided carcasses in three groups: normally processed (anal plugging was routinely applied before evisceration), but no Good Manufacturing Practices (GMP) applied at this step to avoid intestinal ruptures; unwashed GMP carcasses (which were processed with particular attention to the Good Manufacturing Practices during evisceration to avoid intestinal ruptures) and washed GMP carcasses (25 sec of high pressure cold water at the end of the slaughterline). Results highlighted the effectiveness of implementing GMP during evisceration in order to reduce of *E. coli* counts. In contrast, no effect was associated with the use of carcass washing (Rivas et al., 2000).

Since tissues exposed during the sticking of pigs are likely to be contaminated with bacteria during slaughtering, it is required to trim the tissue around wound before evisceration. Gill and Badoni (2001) investigated the effect of this practice as well as the effect of pasteurisation by analysing these tissues before and after their application. The authors concluded that while the pasteurisation of carcasses can reduce the numbers of *E. coli* on the cut tissue of sticking wounds, the trimming is probably ineffective for that purpose probably because of contamination during the routine operation.

The technique of trimming contaminated sites on pig carcasses detained because of visible contamination was investigated. One paper in particular described the effect of trimming the contaminated site on the microbial level of carcasses by sampling on both the specific contaminated sites and a random site on the carcasses (Gill and Landers, 2004a). Moreover, the effect of chilling was investigated through the previously mentioned sampling scheme. Visibly contaminated sites harboured high level of bacteria and were considered potential sources of bacterial spreading during processing. According to the authors, the trimming technique, as well as the cooling process, were found to be effective in decreasing *E. coli* counts on contaminated carcass sites as well as on randomly selected ones.

Conter et al. (2006) investigated the effect of pasteurisation treatment in an Italian slaughterhouse in which a prototype steam decontamination unit was installed before evisceration during the pig slaughter process. This treatment provided a significant reduction of *E. coli* counts on pig carcasses.

Table 4a: Slaughterhouse and farm related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *E. coli* counts on swine carcasses

Effect	Risk factor/Treatment	Compared factor/ Control	Reference	N° of studies
Decrease	Acidified sodium chlorite washing	Standard hygiene procedure	Hamilton et al. 2009 (AU)	1
	Chilling	No chilling	Gill and Landers 2004 (CA)	1
		Prewashed carcass	Algino et al. 2009 (US)	1
	Chilling contaminated site	No chilling	Gill and Landers 2004 (CA)	1
	Cleaning and disinfection three times a day	Others regimen	Delhalle et al., 2008 (BE)	1
	Frequency of lairage disinfection	Other frequencies	Delhalle et al., 2008 (BE)	1
	Hot water carcass washing (83.5°C)	Standard hygiene procedure	Hamilton et al. 2009 (AU)	1
	Lairage cleaning with water	Other cleaning solutions	Delhalle et al., 2008 (BE)	1
	Low throughput slaughterhouses	High throughput slaughterhouses	Hansson 2000 (SE)	1
	Meal frequency 5, Feed type (pelleted), fasting time (24 h)	Meal frequency (2; 5), Feed type (pelleted; mash), fasting time (4; 14; 24 h)	Saucier et al. 2007 (CA)*	1
	New hooks for carcasses before chilling	Same hooks	Delhalle et al., 2008 (BE)	1
	Pasteurized sticking wounds	Not pasteurized sticking wounds	Gill and Badoni 2001 (CA)	1
	Scalding with steam	Immersion scalding	Delhalle et al., 2008 (BE)	1
	Steam treatment (before pasteurization)	No steam treatment	Conter et al., 2006 (IT)	1
	Trimming of the contaminated site	No trimming	Gill and Landers 2004 (CA)	1
	Unwashed+GMP	Unwashed	Rivas et al. 2000 (ES)	1
	Washing	Prewashed carcass	Algino et al. 2009 (US)	1
Decrease Total				17
Increase	June-July	Other months	Lindblad et al. 2007 (SE)	1
	Skinned	Unskinned	Algino et al. 2009 (US)	1
	Spraying when ext temp is considered hot	Spraying with others rules	Delhalle et al., 2008 (BE)	1
	Time between killing and scalding	Continuous variable	Delhalle et al., 2008 (BE)	1
Increase Total				4
No effect	Co-regulatory system	Governmental inspection system	Bass et al. 2011 (AU)	1
	Gentle handling	Rough handling	Rabaste et al. 2006 (CA)**	1
	Low throughput slaughterhouses	High throughput slaughterhouses	Bohaychuk et al. 2011 (CA)	1
	Small group (10 animals)	Large group (30 animals)	Rabaste et al. 2006 (CA)**	1
	Sticking wounds trimming	Not trimmed sticking wounds	Gill and Badoni 2001 (CA)	1
	Washed+GMP	Unwashed+GMP	Rivas et al. 2000 (ES)	1
	Washing T	Prewashed carcass	Algino et al. 2009 (US)	1
No effect Total				7
Total				28

* Risk factor referring to farm management; ** Risk factor referring to pre slaughter operations

Enterobacteriaceae

A total of 19 papers were retrieved dealing with risk factors or treatments in relation to *Enterobacteriaceae* counts on pig carcasses at slaughter.

Zweifel et al. (2007) evaluated the microbiological contamination of pig carcasses in different low-throughput Swiss abattoirs with a median annual slaughter of 600 animals. In total, 3,000 samples were collected from 750 carcasses. Authors noticed that *Enterobacteriaceae* accounted only for a small subset of microorganisms. Because log normality was not ensured, log N values were used for comparison (log of the total number recovered per square centimetre). Results highlighted an important difference between carcass contamination within the selected abattoirs, with the percentage of positive sites on carcasses ranging from 9 % to 22 %. Thus *Enterobacteriaceae* was considered a useful indicator of abattoir-specific hygienic weak points in low throughput plants.

The influence of plant features on microbial carcass contamination was also studied by Blagojevic et al. (2011), who investigated two different abattoirs (A and B), through the collection of 100 samples from randomly selected pig carcasses. In particular, the ratio between carcass (before chilling) and skin microflora was calculated in order to assess the process with respect to its ability to reduce the transfer of incoming microbial loads onto dressed carcasses. In both slaughterhouses, *Enterobacteriaceae* load was significantly reduced during processing. However, the authors observed that, despite the EU process hygiene criteria (PHC, based on daily mean log value for carcasses) not discriminating between the processes in the two abattoirs, the process in plant B was clearly more hygienic according to the comparison of individual carcasses. Consequently a PHC based on the ratio evaluated in the study was suggested.

Both papers described the impact of slaughterhouse characteristics on the processing hygiene, while other studies tried to demonstrate which specific stages and factors exerted deleterious effects on microbial contamination.

The use of anal plugging prior to scalding and dehairing was investigated in a EU licensed low-throughput abattoir (Purnell et al., 2010). These carcasses were produced for the pork rather than the bacon market and were consequently not singed after dehairing. A significant increase in *Enterobacteriaceae* counts around the anal areas of unplugged carcasses after scalding and dehairing was observed, while slight non significant decreases were observed on plugged carcasses. According to the authors, the results, together with visual observation during the work, clearly showed that faecal leakage can occur during these processing stages and that methods avoiding it could be useful to reduce pig carcass contamination.

Different risk factors were investigated in another study in which the authors considered the effect of skinning, washing temperature and chilling duration in 10 very low-throughput abattoirs in Wisconsin (Algino et al., 2009). In each slaughterhouse, carcass halves were sampled before washing and the other halves of the corresponding carcasses were sampled after chilling. *Enterobacteriaceae* counts decreased in both skinned and unskinned carcasses between pre-washing and chilling; however, leaving the carcasses unskinned during chilling was associated with lower mean levels of *Enterobacteriaceae*. Washing was effective in reducing *Enterobacteriaceae* levels on carcasses when water temperature ranged from <12.8° to 32.2 °C, but not when temperature was >32.2 °C. Authors supposed that the highest water temperature resulted in the least chilling and contraction of the skin and underlying tissue thus enhancing bacterial attachment. Also, chilling for both the tested chiller holding times (1 or 2 days) was effective in significantly reducing *Enterobacteriaceae* levels on the swine carcasses.

Trivedi et al. (2006) evaluated the effectiveness of a commercially-available domestic steam cleaner for reducing naturally occurring bacterial populations on freshly slaughtered pig carcasses in four low or very low throughput abattoirs. A 60 second steam treatment was applied after the final wash and before any organic acid treatment on three anatomical sites on the right half of the carcasses. The effect on *Enterobacteriaceae* level was evaluated immediately after the treatment and 24-h later after cold storage. The steam treatment significantly reduced microbial contamination at all anatomical locations with no differences in term of mean *Enterobacteriaceae* population immediately after and 24 h after the application of steam. The authors thus suggested the use of household domestic steam cleaning systems as a critical control measure in low and very low throughput meat processing plants.

The effect of GMP and washing during processing was investigated by Rivas et al. (2000). These authors divided carcasses in three groups: normally processed (anal plugging was routinely applied before evisceration, but GMP was not implemented during evisceration); unwashed GMP carcasses (which were processed with particular attention to the Good Manufacturing Practices during evisceration) and washed GMP carcasses (25 sec of high pressure cold water at the end of the slaughterline). Interestingly, results showed the effectiveness of implementing GMP during evisceration thus demonstrating that anal plugging could be more efficient against *Enterobacteriaceae* levels when GMP are implemented. In contrast no significant effect was observed on carcass *Enterobacteriaceae* levels after the addition of the washing treatment during processing (Rivas et al., 2000).

Finally, Tomovic et al. (2011) evaluated the effect of rapid chilling of swine carcasses on bacterial populations. Two regimens of rapid chilling were applied. Treatment 1 consisted of 3h at -31 °C and then 2-4 °C until 8 h post-mortem, while the first phase of the second treatment was equivalent, but then carcasses were left at 2-4 °C for 24 h. Interestingly, results highlighted that treatment 2 decreased *Enterobacteriaceae* counts in a highly significant way; in contrast treatment 1 did not significantly decrease them. Authors concluded that rapid chilling can improve the microbiological safety of pork if a rapid chilling treatment is implemented together with a 24 h duration of the entire chilling operation.

Tables 17 and 18 (Appendix E) report *E. coli* and *Enterobacteriaceae* counts from the studies dealing with the effect of the slaughterhouse and farm related features (general and at different stages of the slaughtering line) on the indicator bacteria on pig carcasses.

Table 4b: Slaughterhouse related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *Enterobacteriaceae* counts on swine carcasses

Effect	Treatment/Risk Factor	Compared factor/Control	Reference	N° of studies
Decrease	Anal plugging	No anal plugging	Purnell et al. 2010 (UK)	1
	GMP applied during evisceration to reduce ruptures	No such GMP applied	Rivas et al. 2000 (ES)	1
	Chilling time	Pre-chilling	Algino et al. 2009 (US)	2
	Chilling time (3h -31°C + 24 h 2-4 °C)	Pre chilling	Tomovic et al., 2011 (RS)	1
	Steam decontamination	No steam treatment	Trivedi et al. 2006 (US)	1
	Washing T° (< 32.2 °C)	Pre-washing	Algino et al. 2009 (US)	4
Decrease Total				11
Effect	Plant features	Another plant	Blagojevic et al. 2011 (SRB)	1
	Plant features	Different plants	Zweifel et al. 2008 (CH)	1
Effect Total				2
No effect	Chilling time (3 h - 31 °C+ 8 h 2-4 °C)	Pre-chilling	Tomovic et al., 2011 (RS)	1
	Washing	Unwashing	Rivas et al. 2000 (ES)	1
No effect Total				2
Total				15

2.5. Small ruminants

2.5.1. General information about the considered papers

Among the retrieved papers, 21 papers were eligible in order to provide data on small ruminants regarding the three review questions.

Half of the papers (11) were conducted in Europe, while the remainder were from Australia (4), USA (2), Canada (1), Brazil (1), Morocco (1) and India (1). All papers described observational studies with the exception of one paper; ten papers provided data on *E. coli*, eight papers on *Enterobacteriaceae* and three papers considered both indicator bacteria.

Four papers provided information on review question 1, thirteen papers addressed review question 2, one paper dealt with review question 3, while three papers answered all three review questions.

Twenty out of twenty-one papers described studies that analysed pooled samples; in most of the papers, samples were collected from two to five regions of the carcass.

The most common sampling method was swabbing (16 out of 21 papers), followed by excision and the unit of enumeration mostly used was log cfu/cm^2 (15 out of 21 papers).

Alternative plate count was the analytical method most frequently used (10 out of 21 papers) followed by Petrifilm (seven papers).

Concerning the sampled area, it should be pointed out that a wide variability among studies exists; the surface of the sampled area ranged from 5 to 100 cm^2.

Table 8 (Appendix C) reports general information concerning the selected 21 papers and the review questions (1, 2, 3) for which each paper provided pertinent data.

2.5.2. General information about the slaughtering process

The six papers providing information on the presence and counts of *E. coli* and *Enterobacteriaceae* on carcasses along the slaughtering process seldom reported information about the features of the different steps of the slaughterline. The slaughter procedure for small ruminants is similar to that of cattle (see Figure 2, paragraph 2.2.2), and it commonly starts with mechanical stunning (penetrative and non-penetrative) with a captive bolt pistol. Electrical stunning is increasingly an option. This is followed by bleeding in a hanging position.

An important step takes place after bleeding: the dressing (or skinning or flaying) operation. Different types of dressing can be used according to the features of the abattoir: in conventional dressing, sheep carcasses are shackled by first one then the other hind leg while the unshackled hind leg and rump are skinned. Then the carcasses are suspended from a gambrel by both rear legs for skinning of the forequarters and evisceration. In low-throughput slaughterhouses, the carcass is lowered onto a cradle for pelt removal; head and feet are removed and dressing initiated. Then the carcass is raised again and flaying completed. An alternative type of process, referred to as inverted dressing, involves the carcasses being skinned while suspended by the forelegs, with suspension by the rear legs being adopted only after skinning is completed. This second type of dressing is usually considered an improvement compared to the previous one, due to the fact that it reduces the labour required for skinning and reduces the contamination of the carcasses.

After dressing, evisceration takes place: offals are separated, inspected and cleaned. Condemned material is separated and disposed of in a sanitary manner. Finally, the carcasses are submitted to chilling, standing in a tunnel for 2 h at 2-4 °C and then stored at 4±1 °C (conventional chilling). Before the chilling step, carcasses can be split and cut.

In some slaughterhouses, washing is an additional step before chilling: the primary object of carcass washing is to remove visible soiling and bloodstains and to improve carcass appearance after chilling. Soiled carcasses should be sprayed/washed with potable water immediately after dressing before the soiling material dries, thus minimizing the time for bacterial growth.

Along the slaughterline, the unclean operations, therefore, include stunning, bleeding and dehiding, while the clean operations are evisceration, carcass splitting and carcass dressing.

2.5.3. Review question 1

Among the six papers, three papers provided data on *E. coli* counts and three papers on *Enterobacteriaceae*. Concerning both the indicator bacteria, five out of six papers performed studies in which samples were collected at more than one step along the slaughterline, while one out of six papers reported data on carcasses sampled immediately before and after one step of the slaughterline (i.e. chilling).

The retrieved papers provided data that were not always comparable due to several reasons: the slaughter processes described are rather heterogeneous, the sampled area and the analytical methods used vary considerably. For these reasons, it is not appropriate to provide a direct comparison among the different studies, as it would not be meaningful. Rather, it seems more informative to analyse the trend of the indicator bacteria contamination at the different stages of the slaughterline within each of the studies, according to the conclusions drawn by the authors. Figures 7 and 8 show respectively the effect in terms of decrease, increase or no effect of the different stages of the slaughterline on *E. coli* and *Enterobacteriaceae* counts on small ruminant carcasses, as reported in the eligible studies.

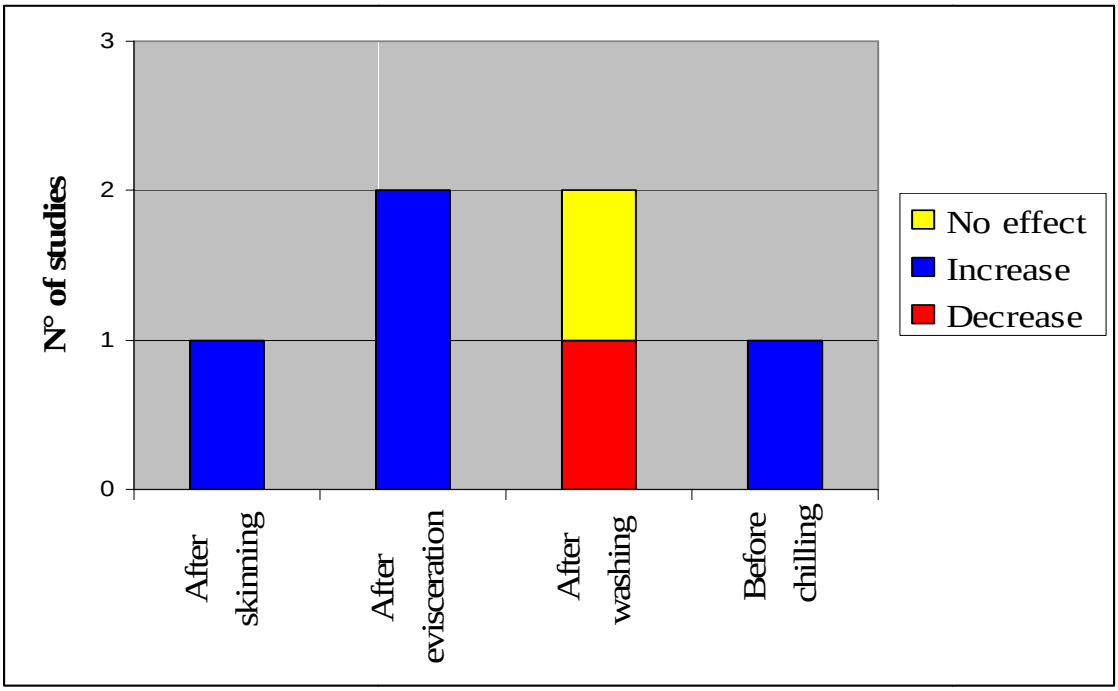

Figure 7: Trends (increase and decrease) of *E. coli* counts at different stages of the slaughterline in small ruminant slaughterhouses

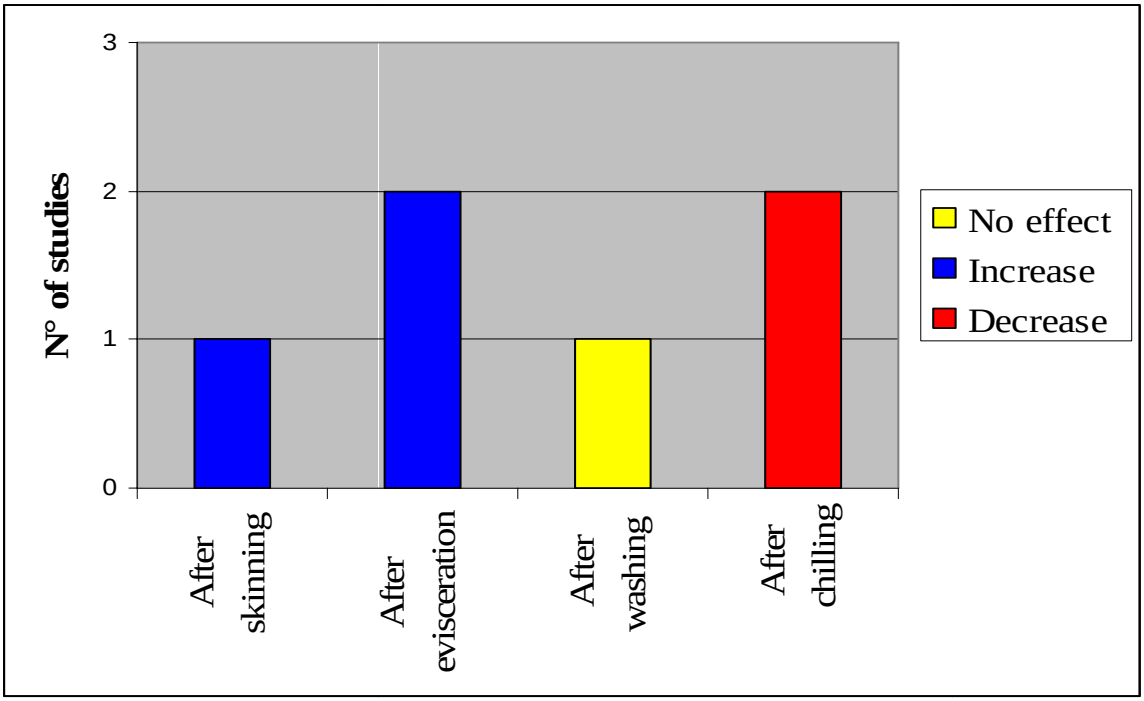

Figure 8: Trends (increase and decrease) of *Enterobactreiaceae* counts at different stages of the slaughterline in small ruminant slaughterhouses

As far as *E. coli* counts are concerned, one study performed by Hauge et al. (2011b), collected swab samples from the brisket area (100 cm^2) of 35 lambs at two different points of the slaughterline: at the

beginning (after fleece removal) and at the end (before chilling). The two sampling points were chosen in order to investigate the microbial transfer from fleece to the carcass surface during skinning. *E. coli* counts expressed as log cfu/100 cm^2, had the following values: 1.78 and 2.71 at the beginning and at the end of the slaughter process, respectively. The authors concluded that there is an increasing level of contamination of carcasses along the slaughtering process and that the counts at the end of the slaughterline, just before chilling were significantly ($p<0.05$) higher than at skinning.

The microbial load of sheep/goat carcasses along the slaughterline was investigated by another study (Bhandare et al., 2007), in which swab samples were taken at three different points along the slaughterline: after flaying, after washing and after evisceration. Samples were collected from different sites of the carcass (neck, shoulder, flank, rib, brisket, rump) and analysed in pools. *E. coli* prevalence and mean counts were reported at the three different sampling points: 11 % and 3.5 log cfu/cm^2 after flaying; 21 % and 3.9 log cfu/cm^2 after evisceration; 8.3 % and 3.1 log cfu/cm^2 after washing.

Finally, differences in the levels of indicator bacteria can be found not only along the steps of the slaughterline but also at different sampling regions of the carcass. Martineli et al. (2011) collected swab samples from an area of 20 cm^2 of 30 carcasses from the forequarter and hindquarter surface after skinning, evisceration and washing processes. The highest value of *E. coli* counts was found on forequarter (0.31 log cfu/cm^2) and the lowest on hindquarter (0.03 log cfu/cm^2) after skinning. A gradual reduction (not significantly different) of these counts was observed in the forequarter (from 0.31 after skinning to 0.07 log cfu/cm^2 after washing).

Concerning *Enterobacteriaceae* counts, two out of three papers concluded that the chilling step represented an important point in reducing the load of these indicator bacteria on carcasses. In fact, Yalcin et al. (2003) collected samples (by the excision technique - 10 cm^2 of surface area) from four areas of the same carcass at four different slaughterline stages: after dressing, after evisceration, after washing and after chilling. Altogether, 176 samples were analysed in pools in order to determine *Enterobacteriaceae* counts, leading to the following mean values (expressed as log cfu/cm^2): 0.38 (after dressing); 0.75 (after evisceration); 0.58 (after washing) and 0.11 after chilling. The authors stated that even if not significant ($p>0.05$), evisceration brought an increase, while chilling brought a reduction of *Enterobacteriaceae* counts. However, the study concluded that chilling represents one of the most important steps in improving the hygienic quality of carcasses.

The same conclusion about the chilling step was drawn by another study (Lenahan et al., 2010) in which swab samples were taken from lamb carcasses before and after chilling in five different abattoirs in Ireland. In this case also, samples were collected from several areas of the carcass (flank, lateral thorax, breast, brisket) and analysed in pools. Chilling reduced *Enterobacteriaceae* counts on 51 % of carcasses tested, counts remained unchanged on 23 % of carcasses, while they increased on 26 %. This may be due to re-growth or contamination just before or during the chilling process.

A third paper by Milios et al. (2011), investigated the hygienic status of a lamb slaughterhouse along the process. Swab samples from different sites of the carcass were collected during pelt removal (A- After pelt removal of hind and forelegs/ before pulling; B- After pulling/before evisceration) and the evisceration steps (C- After evisceration/before pluck removal; D- After pluck removal/before chilling). The study showed that *Enterobacteriaceae* counts progressively increased in each subsequent step after the first sampling point and evisceration contributed mostly to the final count. The recorded counts (log cfu/cm^2) were the following: sampling point A-0.76; sampling point B-2.27; sampling point C-2.68; sampling point D-2.90. In particular, the stage after evisceration/before pluck removal was the determinant stage for the prediction of the carcasses contamination rate at the end of the lamb slaughterline.

According to the retrieved papers, an increasing level of contamination along the slaughterline was recorded by three out of six papers; in particular, the skinning and the evisceration steps frequently caused increases of *E. coli* and *Enterobacteriaceae*. Furthermore, it seems clear that the different steps of the slaughterline can have an influence on counts, but on the other hand, the levels of indicator bacteria can change according to the sampled region. In fact, one paper reported that the forequarters had a higher level of *E. coli* compared to the hindquarters.

Along the slaughterline, the most effective point in order to reduce the microbial contamination was the chilling step: two papers concluded that this phase seems to be the most effective point in order to reduce the counts, thus being a control point along the slaughterline.

Finally, concerning the washing step, the results are not clear or unanimous; according to two studies, washing had no effect in reducing the counts, while in another study it reduced *E. coli* counts before the chilling step.

The counts of *E. coli* on small ruminant carcasses described in the selected papers at the different stages of the slaughterline are reported in Table 13 (Appendix D), while the counts of *Enterobacteriaceae* are presented in Table 14 (Appendix D). In these tables, the number of samples analysed at each step, the increase or decrease of the counts for each step, the mean and the standard deviation are reported when these data were available.

2.5.4. Review question 2

The aim of this section is to describe results of the papers investigating the effects of farm management and slaughter characteristics, as well as other factors, on *E. coli* and *Enterobacteriaceae* loads on small ruminant carcasses.

The great variability among the selected papers is due to the multitude of aspects considered in the different studies and to the complexity of the slaughterline. Moreover, other variables like the indicator organism considered, the sampling and analytical methods used, the setting where the studies were carried out and the specific steps of the slaughterline investigated, render the available data barely comparable.

According to the defined search process and the established eligibility criteria, a total of 16 papers dealing with risk factors were obtained. Since some papers provided data on both types of indicator bacteria, or considered more than one factor influencing bacterial counts, it was more convenient to consider the individual studies (trials), instead of the papers.

Altogether, 19 studies related to factors which could potentially influence indicator bacteria counts on small ruminant carcasses were described in the retrieved papers.

Studies were divided according to the indicator bacteria considered and the factor investigated, resulting in a total of 11 studies dealing with *E. coli*. Of these, one study focused on batch information, while ten studies examined slaughtering techniques or annual season. Concerning *Enterobacteriaceae* counts and risk factors, eight studies investigated the slaughtering techniques.

As already mentioned for review question 1, the comparability of data provided by different studies was hampered by several aspects, such as the sampling method and the unit of enumeration, which were not consistent across studies. Hence, different studies considering the same factor were compared in terms of conclusions drawn and not in terms of counts reported.

Among the retrieved papers, only one study dealt with factors related to the batch characteristics. Hauge et al. (2011b) investigated *E. coli* counts on carcasses associated with different shearing regimes as: unshorn lambs; lambs shorn in the abattoir immediately before slaughter; lambs shorn on farm three days before slaughter; lambs shorn on farm seven days before slaughter. Analysis of the samples collected from the brisket area at two points of the slaughterline (after removal of the fleece and before chilling) led to the following conclusions:

- at the skinning point, *E. coli* counts were higher on the carcasses shorn immediately before slaughter compared with those on farm;

- lower levels of *E. coli* were detected on shorn lambs compared to unshorn lambs at the skinning; at this sampling point, shearing was effective in reducing *E. coli* loads on carcasses;

- increasing contamination of carcasses was recorded along the slaughterline; thus, any reduction of *E. coli* on shorn lambs at the skinning point was negated further along the line, so that *E. coli* numbers on shorn lambs were not significantly different from unshorn lambs at the end of the slaughterline.

Consequently, the application of a shearing regime can be unsuccessful if other control measures, like attention to slaughter hygiene during the process (especially during evisceration), are not properly implemented and monitored.

Among factors related to the slaughterhouse, three factors, the effect of the season, the inspection of lymph nodes for caseous lymphadenitis and the plant features were investigated by the retrieved papers.

As far as the season was concerned, Duffy et al. (2001*)* did not find any differences ($p>0.05$) between *E. coli* counts on carcasses in the spring versus the fall or winter seasons. In the above mentioned study swab samples were collected from six different lamb slaughterhouses at 24 hours after chilling; three of the six plants applied organic acid rinse to carcasses before chilling. In another study (Cohen et al., 2006), raw meat samples were collected from several slaughterhouses in Morocco in two different sampling periods: a hot season (April to September) and a cold season (November to March). The authors concluded that season had no effect on the recorded *E. coli* counts.

Concerning the microbial contamination due to the handling of lymph nodes during the inspection of adult sheep carcasses for caseous lymphadenitis (CLA), Jordan et al. (2012) collected swab samples from two regions (rump and scapular region) of carcasses before and after inspection. Data analysis showed that the contribution of inspection to higher counts of both indicator bacteria (*E. coli* and *Enterobacteriaceae*) was statistically significant ($p<0.001$). In fact, the routine inspection of carcasses for CLA had a detrimental impact on carcass hygiene; curtailed inspection procedures coupled with an improvement in live animal management and the use of data regarding the occurrence of lesions at slaughter could improve the hygienic status of carcasses.

Three studies investigated the effect of slaughterhouse throughput on *E. coli* counts on small ruminant carcasses. In an Australian study (Phillips et al., 2001), swab samples of sheep carcasses after chilling were collected from differing throughput slaughterhouses: domestic slaughterhouses, export slaughterhouses and Low-Throughput Slaughterhouses (LTSs). The samples were analysed for several microorganisms including generic *E. coli* counts, which were found to be less prevalent on sheep carcasses from LTSs (21.4 %) compared with domestic (32.7 %) and export (35.2 %) slaughterhouses. A similar conclusion was drawn by Sumner et al. (2003), who analysed samples collected from carcasses after chilling from medium and low-throughput slaughterhouses. *E. coli* was detected less frequently on carcasses from LTSs (18.5 %) compared with medium throughput (61.5 %) abattoirs.

However, within LTSs there was a considerable in-plant disparity between the hygienic status of carcasses. Finally, another Australian study (Bass et al., 2011) conducted a microbiological survey in order to examine carcasses from slaughterhouses that processed a broad range of slaughter volumes, thus differing in terms of complexity of construction and processing. The results showed a wide range of prevalences (from 8.7 % to 89.5 %) of *E. coli* among the different slaughterhouses: consequently, taken alone, the "snapshot" of the slaughterhouse may be misleading. Those authors concluded that integration of each plant's microbiological data with information involving livestock handling and process elements should be combined in order to be effective.

Among the retrieved studies, one study (Feizullah and Daskalov, 2010) combined together the slaughterhouse throughput (low or high throughput) with the season. Samples were collected from lamb carcasses after washing/before chilling. Concerning *E. coli* counts, the authors found those carcasses from the low-throughput slaughterhouse during the spring had significantly ($P<0.001$) lower *E. coli* counts compared to those collected from the low-throughput slaughterhouse in the winter and high-throughput slaughterhouse in the spring. With regards to *Enterobacteriaceae* counts, differences between the two slaughterhouses were significant ($P<0.001$) for the autumn, winter and spring season. In fact significant variations were reported between the spring and the winter seasons for the low-throughput slaughterhouse, with lower counts found on carcasses in spring (1.30 log cfu/cm^2 in spring versus 3.18 log cfu/cm^2 in winter); between the winter and the autumn seasons for the high-throughput slaughterhouse, lower counts were found in winter (1.27 cm^2 in winter versus 6.05 log cfu/ cm^2 in autumn).

Eight papers studied the influence of specific practices applied at one or more stages of the slaughterline on indicator bacteria counts on carcasses.

The results reported in different studies are presented following the normal order of the slaughter processing operations. In particular, the effect of the following practices on the levels of indicator bacteria will be discussed: the application of a pre-slaughter washing treatment; the dressing procedure; skin-on versus conventionally dressed carcasses; pasteurisation treatment and the chilling treatment.

Concerning the application of a pre-slaughter washing treatment, Kannan et al. (2007*)* investigated the effect of this treatment on goat carcass bacterial counts, as a strategy to reduce the faecal contamination on skin/hides of animals. The comparison between the control group (no washing) with the treated group (1 min spray washing with potable water) led to the conclusion that *E. coli* counts did not significantly decrease during spray washing.

The type of dressing was investigated as a potential risk factor influencing indicator bacteria loads on carcasses in two studies. Gill et al. (2000a) concluded that the substitution of inverted for conventional dressing might reduce of 1.5 log units *E. coli* counts on sheep carcasses. Another study (White et al., 2002) comparing different types of pelt removal methods (Cradle dressing; Hybrid method and Frame method) found that the Hybrid and the Frame systems, considered as inverted dressing, produced significantly lower *Enterobacteriaceae* counts ($p<0.01$) compared to the conventional Cradle dressing. Thus, the greatest reduction in microbiological contamination was achieved using inverted dressing as it minimized hand contact during pelt removal.

The microbiological status of skin-on sheep carcasses was studied and discussed in relation to conventionally dressed carcasses by Fisher et al. (2007*)*. Current EU legislation prohibits the production of ruminant carcasses with the skin left on and flaying during the dressing procedure is a statutory requirements. However, demand for these skin-on, singed products (associated to specific organoleptic qualities) by several ethnic groups resident in the United Kingdom is evident and may well occur in other European countries. Skin-on carcasses had lower *Enterobacteriaceae* counts

($p<0.001$) before chilling than conventionally dressed carcasses. However, this difference was eliminated by the application of a chilling step that reduced the counts mainly on conventionally dressed carcasses. Moreover, the application of a toasting step (an additional exposure to gas flame) on skin-on carcasses after evisceration significantly reduced *Enterobacteriaceae* counts before chilling, thus toasting is a recommended step for these kind of products.

Hauge et al. (2011a) evaluated the microbiological effects of hot water pasteurisation of lamb carcasses. The application of a pasteurising treatment (82 °C± for 8 s) on carcasses after dressing/before chilling led to a 99.5 % reduction of *E. coli* counts, corresponding to a mean reduction of 1.85 log CFU per carcass ($P<0.001$). Also *Enterobacteriaceae* counts were significantly reduced by 2.37 log CFU per carcass after pasteurisation. The reduction of *Enterobacteriaceae* counts after the application of steam was also reported by Milios et al. (2011). The use of steam application (8-10 passes of steam spraying pistol on each side of the carcass) after pluck removal/before chilling reduced *Enterobacteriaceae* counts (almost 1 log CFU/cm^2) without adverse effects on the organoleptic characteristics of lamb carcasses.

Finally, the chilling step represented an important point along the slaughtering process in order to control the growth of bacteria and to ensure food safety. Several methods and time-temperature combinations can be used to cool the carcass. The vascular perfusion chilling (VPC) is a method investigated by Brown et al. (2009), in which very fine ice particles in a solution of sodium chloride and water circulated through the vascular system, offering significant reductions in cooling time. The system involves attaching a specially designed catheter to the carotid artery in the neck of the animal, a chilled isotonic solution of sugars and salts is then pumped through the arterial/venous system for approximately 3 min thereby removing as much residual blood as possible from within the carcass. The study concluded that VPC was capable of a rapid initial reduction of carcass temperature in comparison with air chilling; however uptake of perfusate into the carcass occurred, reducing the cooling period. Concerning *Enterobacteriaceae* counts on the carcasses belonging to the control group and the treatment group (VPC), no conclusions can be drawn due to the fact that numbers of *Enterobacteriaceae* on the pooled surface samples and in the deep tissue samples taken from the loin before chilling and at 24 h were below the detection limits for all but two samples. Comparison between three different chilling treatments on lamb carcasses was studied by Rubio et al. (2012). The treatments differed in the following time-temperature combinations: Conventional treatment - carcasses at 2 °C for 24h; Ultra-rapid treatment - carcasses at 20 °C for 3.5h then 2 °C until 24 h; Slow treatment - carcasses at 12 °C for 7h then 2 °C until 24 h. Carcasses of ultra-rapid treatment had the lowest *Enterobacteriaceae* counts, however, the carcasses subjected to this treatment were susceptible to cold shortening and consequently to a loss of meat quality.

According to the retrieved papers several conclusions can be drawn on the effect of different types of risk factors on indicator bacteria counts. Annual season had no significant effect on the counts: in fact, two papers investigating this factor concluded that there was no difference between bacterial loads on carcasses during the warm season compared to the cold season. However, one paper investigating the slaughterhouse throughput coupled with the season, concluded that season has an influence on indicator bacteria counts, with the lowest counts being detected in spring for low-throughput slaughterhouses and in winter for high-throughput slaughterhouses.

A process that can lead to an increasing of the counts of indicator bacteria is the handling of lymph nodes during the inspection of carcasses for caseous lymphadenitis; thus, reduced inspection procedures coupled with the use of data regarding the management of animals could reduce indicator bacteria loads on carcasses.

Another factor that can have an impact on counts is the throughput of the slaughterhouse. Three papers concluded that low-throughput slaughterhouses produce carcasses with lower prevalences and counts of indicator bacteria compared to high-throughput slaughterhouses.

The application of treatments along the slaughterline and the effect of the slaughtering technique on indicator bacteria levels on carcasses were investigated by eight papers. Washing animals before slaughter does not significantly reduce the microbial load. On the other hand, hot water pasteurisation of carcasses after dressing is effective; several authors concluded that this treatment leads to a significant reduction of the prevalence and counts of *E. coli* and *Enterobacteriaceae* on carcasses. Another step that can have an effect in reducing carcass microbial load is the chilling phase. The use of experimental chilling treatments can be effective in order to quickly reduce carcass temperature and bacterial load; however a loss of meat quality is recorded.

Finally, the dressing technique has an effect on indicator bacteria counts on carcasses, leading to an increase or reduction. In fact, inverted dressing, which minimizes contact between hands and carcass during pelt removal, is considered by several authors as the technique to be adopted in order to limit carcass contamination.

Tables 5a and 5b summarize the effect (in terms of decrease, increase and no effect) of risk factors related to the slaughterhouse on *E. coli* and *Enterobacteriaceae* counts.

Finally, Tables 19 and 20 (Appendix E) report *E. coli* and *Enterobacteriaceae* counts from the studies dealing with the effect of the slaughterhouse features (general and at different stages of the slaughterline) on the counts of indicator bacteria.

Table 5a: Slaughterhouse related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *E. coli* counts on small ruminant carcasses

Effect	Risk factor/Treatment	Compared factor/Control	Reference	N° of studies
Decrease	low throughput-spring	low throughput-winter; high throughput-spring	Feizullah and Daskalov (2010) (BG)	1
	Low Throughput Slaughterhouses (LTSs)	Domestic-export slaughterhouses	Phillips et al. (2001) (AU)	1
	LTSs	Medium throughput slaughterhouses	Sumner et al. (2003) (AU)	1
	low throughput–sampling point	high throughput-sampling point	Feizullah and Daskalov (2010) (BG)	1
	Inverted dressing	Conventional dressing	Gill et al. (2000) (CA)	1
	Hot water pasteurization	Untreated	Hauge et al. (2011a) (NO)	1
Decrease Total				**6**
Increase	Pre-Inspection	Post-inspection	Jordan et al. (2012) (AU)	1
Increase Total				**1**
No effect	Spring	Fall or winter	Duffy et al. (2001) (US)	1
	Hot season	Cold season	Cohen et al. (2006) (MA)	1
	Slaughterhouse throughput	broad range of volume	Bass et al. (2011) (AU)	1
	Pre-slaughter washing	no washing	Kannan et al. (2007) (US)	1
No effect Total				**4**

Table 5b: Slaughterhouse related risk factors investigated in the retrieved studies leading to decrease/increase/or with no effect on *Enterobacteriaceae* counts on small ruminant carcasses

Effect	Risk factor/Treatment	Compared factor/Control	Reference (study)	N° of studies
Decrease	Inverted dressing	Conventional dressing	White et al. (2002) (GB)	1
	Skin on carcasses	conventionally dressed	Fisher et al. (2007) (GB)	1
	Ultra rapid chilling	Conventional and slow chilling	Rubio et al. (2012) (ES)	1
	Hot water pasteurization	Untreated	Hauge et al. (2011a) (NO)	1
	Steam application	Untreated	Milios et al. (2011) (GR)	1
	low throughput-spring	high throughput-winter	Feizullah and Daskalov (2010) (BG)	1
Decrease Total				**6**
Increase	low throughput-winter	high throughput-autumn	Feizullah and Daskalov (2010) (BG)	1
	Pre-Inspection	Post-inspection	Jordan et al. (2012) (AU)	1
Increase total				**2**
No effect	Vascular perfusion chilling	conventional chilling	Brown et al. (2009) (GB)	1
No effect Total				**1**

2.5.5. Review question 3

Three papers investigated the relationship between faecal contamination of carcasses and their *E. coli* and/or *Enterobacteriaceae* counts: all the studies were conducted in Europe. In one paper (Hauge et al., 2011b), visual faecal contamination related to *E. coli* counts was studied, in another (Byrne et al., 2006), visual faecal contamination in relation to *Enterobacteriaceae* counts was studied, while in the last one (Whyte et al., 2002), the focus was more on the development of a method to assess gross visible contamination on carcasses according to different types of pelt removal techniques.

Concerning the sampling points, two out of three papers collected samples at one point along the slaughterline, mainly after pelt removal, while regarding the sampled areas, two out of three papers took samples at several sites of the carcass. The sampling method used was swabbing and the analytical method of enumerating the bacteria (*E. coli* and/or *Enterobacteriaceae*) was the alternative plate count. Counts were expressed as log cfu/cm^2 or log cfu/total sampled area.

Hauge et al. (2011b) investigated *E. coli* carcass contamination associated with fleece cleanliness. In total, 140 lambs of 5 months of age, grazed in the hills for 3-4 months and finished on grass on home pasture were slaughtered in a commercial slaughterhouse.

The animals were divided into four groups according to the shearing regimes: 35 lambs shorn in the abattoir immediately before slaughter (day 0); 35 lambs shorn on-farm three days before slaughter (3 days); 35 lambs shorn on-farm seven days before slaughter (7 days); 35 lambs not shorn before slaughter (unshorn).

After stunning and bleeding (before fleece removal), all the lambs were assessed and scored on visual cleanliness of the fleece (0– 3 scale) by a skilled observer. The score '0' represented a visually clean fleece (minor faecal material or mud in the fleece); a score of '1' represented small spots of dirt under the belly, legs, and tail; a score of '2' represented a generally dirty fleece; and a score of '3' represented a very dirty fleece (faecal material or mud under the belly, legs, and tail).

Immediately after the removal of the fleece, swab samples were collected from the brisket area (100 cm^2) for analysis of *E. coli* (pour plate according to NMKL method No. 125 - Nordic Committee on

Food Analysis, 2005). Mean cleanliness scores for the four shearing groups were: 0.66 (0 days), 0.60 (3 days), 0.63 (7 day), and 2.49 (unshorn); thus the unshorn lambs were dirtier and had a higher score value than shorn lambs ($p<0.05$). Mean *E. coli* values at skinning were of 1.65, 1.88, 2.16, and 2.49 log cfu/100 cm^2 for carcasses with cleanliness score '0', '1', '2' and '3'; however these values were not significantly different.

This study demonstrated that, on average, visually clean animals tended to produce less microbially-contaminated carcasses than dirty animals. While efforts to improve hygiene during slaughter and skinning are undeniably important, significant improvements in the hygiene of ovine carcasses can be made by controlling cleanliness of the live animals.

Byrne et al. (2006) studied the risk factors associated with the transfer of bacterial contamination from the fleece to the ovine carcass, with the aim to provide a scientific basis for the development of a "clean sheep policy". In this study, sheep in lairage were visually inspected by the veterinary inspector at the slaughterhouse and graded (based on the visual inspection of the fleece) into five categories: (A) clean and dry; (B) clean and wet; (C) dirty and dry; (D) dirty and wet and (E) with visible faecal dags.

Microbiological evaluation of the carcasses was conducted using the swab sampling method. *Enterobacteriaceae* counts were obtained from 40 animals per category at four separate sites (brisket, shoulder, flank and rump) immediately after pelt removal.

Enterobacteriaceae were detected in 37.6 % of samples tested; the mean values in the five categories were: 2.7, 2.9, 4.4, 3.9 and 4.4 log cfu/ 4.000 cm^2. Contamination levels were similar over the four sampled sites (brisket, shoulder, flank and rump). In this study, the *Enterobacteriaceae* counts recovered from dirty sheep were higher than the counts found on the clean sheep regardless of dryness or wetness. Moreover, the parameter "fleece dryness/wetness" did not affect the *Enterobacteriaceae* count on the carcass when the fleece was clean, while this parameter did influence *Enterobacteriaceae* count when the fleece is dirty, resulting in overall higher counts. *Enterobacteriaceae* counts suggested that dirt was a contributing risk factor regardless of wetness or dryness of the animal. The clean sheep policy should, therefore, differentiate between clean and dirty sheep and require additional hygiene measures for the latter.

Whyte et al. (2002) investigated methods of reducing lamb carcass contamination in low-throughput abattoirs (no more than 20 livestock units/week) where cradle dressing was employed. In the study, a modified cradle design and several improved pelt removal methods were developed and tested. Moreover, a method of scoring gross visible contamination on the depelted carcass was developed in order to measure gross visible contamination when comparing different methods of pelt removal.

Gross, visible contamination (straw, wool, dirt and faecal material) was quantified from the same regions of the carcass using a method based on the use of sheets of colourless, clear adhesive plastic film (90 by 130 mm – 3M Pat-it). The adhesive sheets were applied to the carcass surface and then fixed to a light blue cartridge paper background. Quantification of the degree of gross visible contamination was based on the quantity of material adhering to the adhesive film. This was graded between 0 (no visible contamination) and 10 (maximum visible contamination).

Concerning the modified pelt removal procedure, measurements of gross visible contamination (mainly hair) at the shoulder and abdomen regions showed that a modified pelt removal procedure was significantly better than the conventional method ($p<0.01$ and $p<0.05$ respectively). In relation to the inverted dressing procedures (Cradle, Hybrid and Frame methods), the Cradle method of pelt removal produced the most visibly contaminated carcasses for all carcass sites apart from the shoulder, for which a similar contamination score to that of the Hybrid method was recorded.

Correlation between the microbiological contamination of the carcass and the level of gross contamination was not possible because minor changes in TVC (Total Viable Counts) and *Enterobacteriaceae* (less than 1 log cfu/cm^2) were accompanied by large changes in gross visible contamination scores.

In conclusion, the degree of gross visible contamination at the carcass sampling sites closely reflected the contact that was likely to occur between the carcass and the hands of the slaughterman or the fleece. Significant improvement in gross visible contamination was achieved by adoption of inverted dressing, as this minimizes hand contact with the carcass during pelt removal.

Despite the fact that the number of retrieved studies providing data for review question 3 was quite limited, the available data suggested that:

1) the distinction between clean and dirty carcasses could be an important starting point in order to improve the hygiene of ovine carcasses;

2) additional hygiene measures should be applied for high-risk (dirty) animals (i.e.: slaughtering at the end of the day; reduced line speed; thorough cleaning of operator hands, arms and aprons; the use of inverted dressing; greater spacing between carcasses);

3) modifications to pelt removal methods which reduce contact between the carcass and the hands of the slaughterman or the fleece can significantly improve gross visible contamination.

Table 22 (Appendix F) summarizes the counts of *E. coli* and *Enterobacteriaceae* on small ruminant carcasses along the slaughterline in relation to the level of visual faecal contamination.

CONCLUSIONS AND REMARKS

- A total of 87 papers were used to collect data for the three review questions. Forty-two papers provided pertinent data about beef carcasses, 31 papers about swine carcasses and 21 papers about small ruminants.

- A high level of variability among the different studies, due to different aspects and to the complexity of the slaughterlines, was evidenced. Some variables, like the sampling and analytical methods used, the area of carcass sampled, the unit of enumeration used, the specific step of the slaughterline investigated and the decontamination treatments applied along the slaughterline, render the available data barely comparable and could lead to conflicting conclusions among studies describing counts at the same stage of the slaughterline or investigating the same risk factor.

- Further studies are needed to state precisely the effect of slaughterline step on *E. coli* and *Enterobacteriaceae* counts on carcasses regarding in particular poorly investigated steps as also those with contrasting results. Studies should be performed by sampling carcasses before and after specific steps in order to avoid confounding.

- Studies dealing with risk factor should be addressed in particular by posing particular attention to the choice of control groups in order to better appreciate results.

- In general a better agreement should be obtained within the scientific community in particular regarding the presentation of results. A standardized unit of enumeration could help in reaching this objective. Confidence interval should be preferred to facilitate comparison among studies and research synthesis.

REVIEW QUESTION 1. Presence of the indicator organisms *E. coli* and *Enterobacteriaceae* and their counts on carcasses during different stages in the slaughterline

- The main challenge in identifying the steps of the slaughterline that led to a decrease or an increase of indicator bacteria counts on pig and ruminant carcasses was the paucity of studies that provided data obtained before and after a single stage. Data have generally been collected at distant sampling points and in between, different treatments have usually been used. As a consequence, the effective role of the single stages in a change in counts was not always evident.

Beef carcasses

- Evisceration and trimming led to an increase of *E. coli* counts up to 1 log (cfu/cm^2) in one study, whereas in other studies (5), changes of *E. coli* and *Enterobacteriaceae* counts correlated to these slaughter phases were trivial (within 0.5 log).

- Washing after evisceration was effective to reduce the *E. coli* load of beef carcasses in one study (reduction up to about 1 log), whereas some other studies demonstrated that washing with potable water had no effect on *E. coli* and *Enterobacteriaceae* counts (variation within 0.5 log compared to the previous step of the slaughterline).

- The application of different sequential decontamination treatments, such as hot water, pasteurisation and washing with acids after evisceration always led to a drop (higher than 1 log) of both *E. coli* and *Enterobacteriaceae* counts on beef carcasses.

- Chilling does not effectively reduce bacterial load on beef carcasses. Different studies reported conflicting data in terms of reduction/increase of *E. coli* and *Enterobacteriaceae* loads due to chilling treatment of beef carcasses at the end of the slaughterline.

Pigs

- Scalding effectively reduces both *E. coli* and *Enterobacteriaceae* counts on pig carcasses. For both indicator bacteria observed reductions were in some cases higher than 3 log (cfu/cm^2) and were described in all sampling regions tested. Therefore, scalding should be carefully considered in the implementation of GMP and HACCP within pig slaughterhouses.

- Pasteurisation effectively reduces *E. coli* counts on pig carcasses up to 1.86 log (cfu/100 cm^2) under experimental slaughter conditions. However, more studies are needed in commercial slaughterhouses.

- Carcass washing does not effectively reduce microbial contamination on pig carcasses.

- Chilling is effective in reducing *E. coli* and *Enterobacteriaceae* counts on pig carcasses, and consequently, it should be carefully implemented in the context of slaughter operations. Achieved reductions varied greatly according to sampling site on carcasses.

Small ruminants

- Skinning and evisceration are the two main steps along the slaughterline where increases (from 0.5-1.0 to 2.0 log cfu/cm^2) of *E. coli* and *Enterobacteriaceae* on carcasses occur, as reported by the four available papers.

- Carcass washing does not effectively reduce microbial contamination on small ruminant carcasses.

- Chilling is effective in reducing *Enterobacteriaceae* counts (two papers) of small ruminant carcasses, as it reduced microbial counts by more than 0.5 log cfu/cm^2.

REVIEW QUESTION 2. Information that could explain the variability of the *E. coli* and *Enterobacteriaceae* counts along the slaughterline

Beef carcasses

- Pre-slaughter diet on farm (batch-related risk factor) does not affect microbial contamination of beef carcasses. However, this was addressed in only one retrieved study.

- Annual season has a direct impact on indicator bacteria prevalence and counts on beef carcasses; the lowest levels of carcass contamination were obtained during coldest months and dry season.

- Slaughterhouse characteristics have an influence on carcass contamination levels but it was difficult to assess which factors had the greatest impact on the counts. The slaughterhouse throughput was not found to be clearly correlated to the counts of *E. coli* or *Enterobacteriaceae* on beef carcasses. Although in only one study differences between indicator bacteria counts obtained in low and high throughput slaughterhouses were significant, lower prevalences/counts were generally reported in lower throughput plants.

- Steam pasteurisation and hot water washing are effective ways to improve the microbiological quality of beef carcasses, as reported for review question 1. The different equipment-procedures tested were used both in high and low throughput slaughterhouses, and all studies reported a clear reduction for both *E. coli* or *Enterobacteriaceae* counts on beef carcasses up to undetectable levels.

- The effect of washing carcasses with potable water at ambient temperature is unclear, as mentioned for review question 1. Different effects could be accounted for by differences in the washing treatments applied as well as differences in the initial bacterial load of carcasses, since washing seems to be ineffective when the initial bacterial load on carcasses is low, but effective when the load is relatively high.

- The effect of chemical decontamination treatments (e.g. washing-spraying with lactic acid, chlorine, peroxyacetic acid, nisin) is unclear, since different studies described opposite results related to their effectiveness in reducing bacterial loads on beef carcasses. These conflicting results could be due to the different chemicals tested, as well as the procedures followed and the steps of the slaughterline where the treatments were applied.

Pigs

- A high number of different risk factors were investigated in the retrieved papers but comparisons are not possible since few studies considered the same factor and also in this case study design and operational environments were uncomparable.

- Hot water (>80 °C) based treatments, such as pasteurization, are effective in reducing both *E. coli* counts (three studies) and *Enterobacteriaceae* counts (one study) on whole carcasses and on specific sites. These results agree with papers described in review question 1.

- Anal plugging was found effective in reducing carcass contamination during evisceration in one study. However other authors suggested the importance of GMP during evisceration and plugging to maximize the prevention of contamination.

Small ruminants

- Carcasses of shorn lambs had significantly lower (1.0 log cfu/cm^2) counts of *E. coli* at the beginning of the slaughterline compared to those of unshorn lambs (in the only retrieved study of this batch-related risk factor). However, this advantage is lost during the slaughtering process if other control measures are not applied.

- Annual season has no effect on microbial contamination of small ruminant carcasses. However, the association of the annual season with slaughterhouse throughput (one study) led to the conclusion that indicator bacteria counts are lower during spring for low-throughput slaughterhouses and during winter for high-throughput slaughterhouses.

- The effect of the slaughterhouse throughput on the prevalence of indicator bacteria on carcasses was unclear. Some studies concluded that carcasses from low-throughput slaughterhouses had lower prevalence of *E. coli* than those from high-throughput plants while another study observed that the prevalence of *E. coli* differed greatly among the investigated plants.

- Hot water pasteurisation of carcasses after skinning is effective, as it significantly reduces the prevalence and level (from 1.0 to 2.0 log cfu/cm^2) of indicator bacteria on small ruminant carcasses.

- Modified dressing procedures, which reduce contact between hands and pelts, are advisable, as they reduce the indicator bacterial load by 1.5 log unit on carcasses compared to conventional dressing.

REVIEW QUESTION 3. The potential relationship between the counts of *E. coli* and *Enterobacteriaceae* and visual faecal contamination on ruminant carcasses along the slaughterline

Beef carcasses

- Clean cattle produce carcasses with lower bacterial loads (about 0.5-1 log) than do dirty carcasses.

- Pre-slaughter visual classification of the level of animal dirtiness and the application of proper corrective measures was effective in reducing microbial contamination of carcasses, as demonstrated by the retrieved studies addressing this issue.

- Application of effective measures either on dirty animals before entering the slaughterhouse and/or on dirty carcasses along the slaughterline can lead to contamination level of carcasses comparable to or lower that of clean animals at the end of the slaughterline.

Pig carcasses

- The literature research did not provide any papers dealing with the possible relationship between visual faecal contamination on pig carcasses and counts of indicator bacteria.

Small ruminants

- Visually clean small ruminants inspected at the lairage or before fleece removal, are less contaminated than dirty animals. Thus, an effort should be made in order to classify clean and dirty animals and apply additional hygienic measures for the latter group.

- Modified dressing methods which reduce contact between hands and fleece with the carcass, can improve gross visible contamination of small ruminant carcasses. However, due to the fact that methods to assess gross visible contamination on carcasses differ among studies, it's not possible to compare data provided by the available studies.

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APPENDICES

Appendix A. Relevance Screening

The checklists used for the assessment of the relevance of the retrieved papers are reported below. Neutral means that the answer was informative only and did not lead to paper exclusion.

First level assessment – step 1

Questions	Answers	Inclusion/Exclusion
Language of the paper	English	Inclusion
	French	Inclusion
	Italian	Inclusion
	Spanish	Inclusion
	German	Exclusion
	Other	Exclusion
Is the paper a primary research paper?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper consider main livestock species?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper provides data on presence/counts of (non pathogenic) *Escherichia coli* or *Enterobacteriaceae*?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Is the main aim of the paper the investigation of antimicrobial resistance and does the paper provide no pertinent data for the scope of the review?	Yes	Exclusion
	No	Inclusion
	Unknown	Inclusion

First level assessment – step 2

Questions	Answers	Inclusion/Exclusion
Which animal species are considered in the paper ?	Ruminants	Neutral
	Swine	Neutral
	Poultry	Neutral
	Horses	Neutral
	Others	Neutral

Does the paper provide data on more than one stage of the slaughterline or data on risk factors influencing the loads of indicator bacteria on carcasses?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper provide data on carcasses?	Yes	Inclusion
	No	Exclusion
	Unknown	Inclusion
Does the paper consider a real setting?	Yes	Neutral
	No	Neutral
	Unknown	Neutral

Second level assessment

Questions	Answers	Inclusion/Exclusion
Where was the paper conducted?	Europe	Neutral
		Neutral
	Central and south America	Neutral
	Africa	Neutral
	Asia	Neutral
	Oceania	Neutral
Does the paper provide original data?	Yes	Inclusion
	No	Exclusion
Which species is/are considered?	Cattle	Inclusion
	Sheep	Inclusion
	Goat	Inclusion
	Swine	Inclusion
	Horse	Inclusion
	Other	Exclusion
Does the study provide data on carcasses?	Yes	Inclusion
	No	Exclusion
Which indicator organism/s is/are considered in the paper?	*Escherichia coli*	Inclusion
	Enterobacteriaceae	Inclusion
	None of them	Exclusion
	Real setting	Inclusion

Which kind of scenario is considered?	Pilot slaughterhouse	Inclusion
	Artificial contamination with *E. coli* and/or *Enterobacteriaceae* strains	Exclusion
Which kind of sample is collected?	Swab	Neutral
	Skin Excision	Neutral
	Meat excision	Neutral
	Other	Neutral
Which is the study aim?	Counts at different stages	Inclusion
	Factors influencing the counts	Inclusion
	Relation between visual faecal contamination and counts of indicator bacteria	Inclusion
	None	Exclusion
If a factor influencing the counts was considered, is that factor related to:	Batch characteristics	Neutral
	Slaughterhouse features	Neutral
	Sampling method	Neutral

Appendix B. Data Collection

The forms used for the collection of data from the selected papers are reported below.

General information

Questions	Answers
Reference ID	
Type of reference	Paper
	Other
Start year of the study	(YYYY)
End year of the study	(YYYY)
Country where the study was conducted	
Type of study	Observational
	Experimental
Study aim	Counts at different stages
	Factors influencing the counts
	Relationship between visual faecal contamination and counts
Species considered in the study	Cattle
	Swine
	Sheep
	Goat
	Horses
Age (months)	
Number of plants involved	1
	2
	3
	More
Plant 1 - Number of animal slaughtered/hour	
Plant 1 - Number of animals slaughtered/day	
Plant 1 - Number of animal slaughtered/year	
Plant 2 - Number of animal slaughtered/hour	

Plant 2 - Number of animals slaughtered/day	
Plant 2 - Number of animal slaughtered/year	
Plant 3 - Number of animal slaughtered/hour	
Plant 3 - Number of animals slaughtered/day	
Plant 3 - Number of animal slaughtered/year	
Does the paper describe the use of any decontaminant treatments?	Yes
	No
	NR
Sampling method	Swab
	Skin excision
	Meat excision
If a swab method was used, specify:	Sponge
	Dry and wet
	Gauze
Sampling area on the carcasses:	Delimited
	Undelimited
	Not specified
Type of sample	Single
	Pool
Sampled area on the carcass	Neck
	Brisket
	Flank
	Rump
	Jowl
	Belly
	Back
	Ham
	Other
Dimension of the sampled area (cm^2)	Single sample area (cm^2)
Sample size (number)	
	E. coli

Indicator bacteria considered	*Enterobacteriaceae*
	Both
Analytical method	ISO
	Alternative plate count
	Petrifilm
	MPN
	Other
Significance level (alfa) e.g. 0.05	
Power (1-beta) e.g. 0.80	
Effect size (delta)	

Data collection

Questions	Answers
Reference ID	
Study aim	Counts at different stages
	Factors influencing the counts
	Relationship between visual faecal contamination and counts
Species considered	Bovine
	Swine
	Goat
	Sheep
Risk factor considered	
Classification of faecal contamination (how carcasses were classified)	
When was faecal contamination evaluated?	Ante-mortem
	Post-mortem
Indicator bacteria considered	*Enterobacteriaceae*
	E. coli
Unit of enumeration	CFU/g
	CFU/cm^2
	logCFU/g

	logCFU/cm^2
	Other
Does the paper describe how negative results have been considered?	Yes
	No
	No negative results
Experimental group n°	
Slaughtering stage	
Slaughtering stage features	
Sampling region	
Counts	N
	Mean
	SD
	Min
	Max
	5 percentile
	95 percentile
	Prevalence (%)
	other
Sampling region	
Counts	N
	Mean
	SD
	Min
	Max
	5 percentile
	95 percentile
	Prevalence (%)
	other
Sampling region	
Counts	N
	Mean
	SD
	Min
	Max

	5 percentile
	95 percentile
	Prevalence (%)
	other
Sampling region	
Counts	N
	Mean
	SD
	Min
	Max
	5 percentile
	95 percentile
	Prevalence (%)
	other
Stage/s where counts decrease	
Stage/s where counts increase	
Effect of the RF on carcass counts	Increase
	Decrease
	None
Effect of faecal contamination on carcass counts	Increase
	Decrease
	None
Final considerations (comments)	

Appendix C. General characteristics of the selected papers

Table 6: General characteristics of the 41 papers providing data on beef carcasses. (E: experimental; O: observational; EC: *Escherichia coli*; EB*: Enterobacteriaceae*)

Reference (country)	Type of study	Sample characteristics		Indicator bacteria	Method characteristics		Review question
		Type of sample	Region sampled		Analytical method	Unit of enumeration	
Blagojevic et al., 2012 (RS)	O	Swab (sponge)	brisket, flank, rump, neck	EB	Petrifilm	logCFU/cm^2	3
Yang et al., 2012 (CA)	O	Swab (sponge)	randomly selected	EC	Membrane filter	log CFU/100 cm^2	1
Nero 2012 (BR)	O	Swab	not specified	EC - EB	Petrifilm	logCFU/ cm^2	1
Serraino et al., 2012 (IT)	O	Swab (sponge)	brisket, groin, hock	EC - EB	ISO	logCFU/ cm^2	3
Hauge et al., 2012 (NO)	O	Swab (sponge)	brisket, belly	EC	NMKL	log CFU/100 cm^2	3
Bass et al., 2011 (AU)	O	Swab (sponge)	brisket, flank, rump, jowl, midloin	EC	Petrifilm	logCFU/ cm^2	2
Blagojevic et al., 2011 (RS)	O	Swab (sponge)	brisket, flank, rump, perianal region, neck	EB	Petrifilm	CFU/ cm^2	2
Bohaychuk et al., 2011 (CA)	O	Swab (sponge)	not specified	EC	Alternative plate count	CFU/ cm^2	2
Ozdemir et al., 2010 (TR)	O	Skin excision	brisket, flank, rump	EC - EB	ISO	logCFU/ cm^2	2
McCleery et al., 2008 (GB)	O	Meat excision	brisket, flank, rump, neck	EB	ISO	logCFU/ cm^2	3
Rigobelo et al., 2008 (BR)	O	Swab (sponge)	rump	EC	Qualitative analysis	presence/absence	1-2
Zweifel et al., 2008 (CH)	O	Skin excision	brisket, flank, rump, neck	EB	Alternative plate count	logCFU/ cm^2	2
Algino et al., 2007 (US)	E	Swab (sponge)	brisket, flank, rump	EC - EB	Alternative plate count	logCFU/ cm^2	2
Ruby et al., 2007 (US)	O	Swab (sponge)	inside - outside area + navel-plate-brisket-shank area	EB	Petrifilm	log CFU/100 cm^2	1-2
Trivedi et al., 2007 (US)	E	Swab (sponge)	rump, midline, neck	EB	Alternative plate count	logCFU/ cm^2	2
Tergney and Bolton, 2006 (TR)	O	Swab (sponge)	brisket, flank, rump, anus, hock	EC - EB	ISO - Alternative plate count	NR	2
Bosilevac et al., 2006 (US)	E	Swab (sponge)	brisket, foreshank, top round surface, anus hock	EB	Petrifilm - Bactometer	log CFU/100 cm^2	2
Kiermeier et al., 2006 (AU)	O	Swab	not specified	EC	Qualitative analysis	presence/absence	2
Kinsella et al., 2006 (IE)	O	Meat excision	brisket, flank, rump, neck	EB	ISO	logCFU/ cm^2	2
Rigobelo et al., 2006 (BR)	O	Swab (sponge)	near the anus	EC	Qualitative analysis	presence/absence	2
Anderson et al., 2005 (US)	E	Swab (sponge)	between the bung and the hock	EC	Alternative plate count	log CFU/swab	2

Table 6 (Continued): General characteristics of the 41 papers providing data on beef carcasses. (E: experimental; O: observational; EC: *Escherichia coli*; EB*: Enterobacteriaceae*; NR: not reported)

Reference (country)	Type of study	Sample characteristics		Method characteristics			Review question
		Type of sample	Region sampled	Indicator bacteria	Analytical method	Unit of enumeration	
Corantin et al., 2005 (CA)	O	Swab (gauze)	not specified	EC	Petrifilm	logCFU/cm^2	2
Retzlaff et al., 2005 (US)	E	Meat excision	ventral midline area	EC - EB	Petrifilm	logCFU/cm^2	2
Arthur et al., 2004 (US)	O	Swab (sponge)	inside - outside area + plate-brisket-shank area	EB	Petrifilm - Bactometer	logCFU/100 cm^2	1
Gill and Landers, 2004b (CA)	O	Swab (sponge)	randomly selected + contaminated area + adjacent to the contaminated area	EC	Membrane filter	CFU/100 cm^2	3
Madden et al., 2004 (IE)	O	Swab (sponge)	brisket	EB	ISO	logCFU/cm^2	1
McEvoy et al., 2004 (GB)	O	Swab (dry and wet)	brisket, hock, cranial back, bung	EC - EB	Alternative plate count	logCFU/cm^2	1
Mies et al., 2004 (US)	E	Swab (sponge)	brisket, belly, round	EC	Petrifilm	logCFU/cm^2	2
Prendergast et al., 2004 (IE)	O	Swab (dry and wet)	brisket	EB	ISO	logCFU/cm^2	2
Gill et al., 2003 (CA)	O	Swab (gauze)	randomly selected	EC	Membrane filter	cfu/100cm^2	1
Gill and Landers, 2003a (CA)	O	Swab (gauze)	randomly selected	EC	Membrane filter	log CFU/100cm^2	2
Gill and Landers, 2003b (CA)	O	Swab (gauze)	randomly selected	EC	Membrane filter	log CFU/100cm^2	2
Minihan et al., 2003 (IE)	O	Swab (sponge)	rump, midline, neck	EC - EB	ISO	log CFU/1000 cm^2	2
Nou et al., 2003 (US)	O	Swab (sponge)	anal-hock area	EB	Petrifilm	log CFU/100 cm^2	2
Sumner et al., 2003 (AU)	O	Swab (sponge)	Brisket, flunk, rump	EC	Petrifilm	Log CFU/cm^2	
Barboza et al., 2002 (VE)	O-E	Swab (sponge)	brisket, renal site, neck	EC	Petrifilm	logCFU/cm^2	1-2
Collobert et al., 2002 (FR)	O	Skin excision	not specified	EB	Alternative plate count	logCFU/cm^2	2
Hansson, 2001 (SE)	O	Swab (sponge)	loin, sternum	EC	Alternative plate count	CFU/cm^2	2
Phillips et al., 2001 (AU)	O	Swab (sponge)	brisket, flank, rump	EC	Petrifilm	presence/absence	2
Gill and Bryant, 2000 (CA)	E	Swab (gauze)	randomly selected	EC	Membrane filter	log of the total number recovered from 2500 cm^2	2
Bacon et al., 2000 (US)	O	Swab (sponge)	brisket, flank, rump	EC	Petrifilm	log CFU/100 cm^2	1

Table 7: General characteristics of the 31 papers providing data on swine carcasses. (E: experimental; O: observational; EC: *Escherichia coli*; EB*: Enterobacteriaceae;* HGMFT: Hydrophobic Grid Membrane Filtration technique)

Reference (country)	Type of study	Sample characteristics		Indicator bacteria	Method characteristics		Review question
		Type of sample	Region sampled		Analytical method	Unit of enumeration	
Jones and Johns, 2012 (CA)	O -E	Swab (gauze)	random, anal	EC	HGMFT	logCFU/100 cm^2 (MPN)	1
Bass et al., 2011 (AU)	O	Swab (sponge)	brisket, rump, ventral jowl (if possible)	EC	Petrifilm	logCFU/cm^2	2
Bohaychuk et al., 2011 (CA)	O	Swab (sponge)	adjacent areas same carcass side	EC	ISO	CFU/cm^2	2
Blagojevic et al., 2011 (RS)	O	Swab (sponge)	jowl, belly, ham, perianal	EB	Petrifilm	CFU/cm^2	2
Tomovic et al., 2011 (RS)	E	Swab (sponge)	jowl, belly, back, ham	EB	ISO	logCFU/cm^2	2
Duggan et al., 2010 (IE)	O	Swab (sponge)	jowl, belly, back, ham	EB	ISO	logCFU/g	1
Hamilton et al., 2010 (AU)	E	Meat excision	Belly strip	EC	Petrifilm	logCFU/g	2
Purnell et al., 2010 (UK)	E	Swab (sponge)	anus	EB	ISO	logCFU/ cm^2	2
Wu et al., 2009 (DK)	O	Swab (sponge)	left leg close to the anus	EC	Petrifilm	logCFU/100 cm^2	1
Algino et al., 2009 (US)	O-E	Swab (sponge)	jowl, belly, ham	EC - EB	Petrifilm	logCFU/ cm^2	2
Lenahan et al., 2009 (IE)	O	Swab (sponge)	whole side	EB	ISO	logCFU/ cm^2	1
Hurd et al., 2008 (US)	O	Swab (sponge)	bung, skin, pleura	EB	ISO	Prevalence	1
Delhalle et al., 2008 (BE)	O	Swab	ham, pelvis, forelimb, sternum	EC	ISO	logCFU/ cm^2	2
Zweifel et al., 2008 (CH)	O	Skin excision	neck, belly, back, ham	EB	ISO	logCFU/ cm^2	2
Nesbakken et al., 2008 (NO)	O	Swab	ham	EC	ISO	logCFU/ cm^2	1
Lindblad et al., 2007 (S)	O	Swab (sponge)	neck, belly, back, ham	EC	ISO	logCFU/ cm^2	2
Rabaste et al., 2007 (CA)	O	Swab (sponge)	brisket, internal rib cage, front feet top	EC	HGMFT	logCFU/983 cm^2 (MPN)	2
Saucier et al., 2007 (CA)	E	Swab (gauze)	flank, thoracic inside	EC	HGMFT	logMPN	2
Trivedi et al., 2007 (US)	E	Swab (sponge)	jowl, belly, ham	EB	ISO	logCFU/ cm^2	2
Conter et al., 2006 (I)	E	Skin excision	jowl, belly, back, ham	EC	ISO	logCFU/ cm^2	2
Inthavong et al., 2006 (LA)	O	Swab	jowl, belly, ham	EB	ISO	logCFU/cm^2	1

Table 7 (Continued): General characteristics of the 31 papers providing data on swine carcasses. (E: experimental; O: observational; EC: *Escherichia coli*; EB*: Enterobacteriaceae;* HGMFT: Hydrophobic Grid Membrane Filtration technique)

Reference (country)	Type of study	Sample characteristics		Indicator bacteria	Method characteristics		Review question
		Type of sample	Region sampled		Analytical method	Unit of enumeration	
Spescha et al., 2006 (CH)	O	Swab (dry and wet)	neck, belly, back, ham	EB	ISO	logCFU/ cm^2	1
Namvar and Warriner et al., 2006 (CA)	O	Swab (sponge)	brisket	EC	Petrifilm	logCFU/100cm^2	1
Gill and Landers, 2004a (CA)	O	Swab (gauze)	contaminated + randomly selected	EC	HGMFT	logTotal (MPN)	1 -2
Warriner et al., 2002 (UK)	O	Swab (sponge)	brisket	EC-EB	ISO	CFU/100 cm^2	1
Tamplin et al., 2001 (US)	O	Swab (sponge)	neck, belly, ham	EC	Petrifilm	CFU/ cm^2	1
Hansson, 2001 (S)	O	Swab (sponge)	loin, sternum	EC	ISO	CFU/ cm^2	2
Gill and Badoni, 2001 (CA)	O	Swab (sponge)	sticking wound	EC	HGMFT	MPN	2
Gill et al., 2000b (CA)	O	Swab (sponge)	random	EC	HGMFT	MPN	1
Rivas et al., 2000 (ES)	O-E	Swab	neck, abdomen	EC - EB	ISO	logCFU/ cm^2	1-2
Bryant et al., 2003 (CA)	E	Swab	back, jowl, ham, belly	EC	HGMFT	MPN/100 cm^2	1

Table 8: General characteristics of the 21 papers providing data on small ruminant carcasses. (E: experimental; O: observational; EC: *Escherichia coli*; EB*: Enterobacteriaceae*)

Reference (country)	Type of study	Sample characteristics		Indicator bacteria	Method characteristics		Review question
		Type of sample	Region sampled		Analytical method	Unit of enumeration	
Rubio et al., 2013 (ES)	O	Skin excision	brisket, rump	EB	Petrifilm	log CFU/cm^2	2
Jordan et al., 2012 (AU)	O	Swab (sponge)	rump and scapular region	EC-EB	Petrifilm	CFU/cm^2	2
Bass et al., 2011 (AU)	O	Swab (sponge)	brisket, flank, midloin	EC	Petrifilm	log CFU/cm^2	2
Hauge et al., 2011a (NO)	O	Swab (sponge)	outside (mid-line of the abdomen, under the forelegs, around rectum and hind legs) and inside the carcass	EC - EB	Alternative plate count	log CFU/carcass	2
Hauge et al., 2011b (NO)	O	Swab (sponge)	Brisket	EC	Alternative plate count	log CFU/100 cm^2	1-2-3
Martineli et al., 2011 (BR)	O	Swab	forequarter - hindquarter leg	EC	MPN	log CFU/cm^2	1
Milios et al., 2011 (GR)	O	Swab (sponge)	brisket, flank, rump, shoulder	EB	Alternative plate count	log CFU/cm^2	1-2
Feizullah and Daskalov, 2010 (BG)	O	Swab (sponge)	leg, chest-outer and inner surface, neck	EC - EB	ISO	log CFU/cm^2	2
Lenahan et al., 2010 (IE)	O	Swab (dry and wet)	brisket, flank, lateral thorax, breast	EB	ISO	log CFU/cm^2	1
Brown et al., 2009 (GB)	E	Meat excision	lateral thorax, flank, brisket, breast	EB	Alternative plate count	log CFU/cm^2	2
Bhandare et al., 2007 (IN)	O	Swab	neck, brisket, flank, rump, shoulder	EC	Alternative plate count	log CFU/cm^2	1
Byrne et al., 2007 (IE)	O	Swab (sponge)	brisket, flank, rump, shoulder	EB	Alternative plate count	log CFU/4000cm^2	3
Fisher et al., 2007 (GB)	O	Skin excision	rump, belly, flank, brisket, shoulder, neck	EB	Alternative plate count	log CFU/cm^2	2
Kannan et al., 2007 (US)	O	Swab (sponge)	brisket, flank, leg	EC	Petrifilm	log CFU/cm^2	2
Cohen et al., 2006 (MA)	O	Meat excision	not specified	EC	Alternative plate count	log CFU/g	2
Yalcin et al., 2004 (TR)	O	Skin excision	neck, brisket, leg, shoulder	EB	Alternative plate count	log CFU/cm^2	1
Sumner et al., 2003 (AU)	O	Swab (sponge)	brisket, flank, rump	EC	Petrifilm	log CFU/cm^2	2
Whyte et al., 2002 (GB)	O	Swab (dry and wet)	shoulder, abdomen and lateral surface of the rear leg	EB	Alternative plate count	log CFU/cm^2	2-3
Phillips et al., 2001 (AU)	O	Swab (sponge)	brisket, flank, rump	EC	Petrifilm	log CFU/cm^2	2
Duffy et al., 2001 (US)	O	Swab (sponge)	flank, breast, leg	EC	Petrifilm	log CFU/cm^2	2
Gill et al., 2000a (CA)	O	Swab (sponge)	two sites for each carcass selected from a grid of 43 areas on one side of the carcass surface	EC	Membrane filter	CFU/100 cm^2	2

Appendix D. Counts at different stages along the slaughterline

Table 9: *Beef* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase; ND: not detected)

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
Gill et al., 2003 (CA)	Killing Hide removal		1) after hide removal	randomly selected	425	52	3.16	log of the total number recovered from 25 samples (cfu/100 cm^2)		
		washing carcasses and spray with lactic acid (2%)	2) after spraying with lactic acid			76	3.41			
	Evisceration									
	Trimming	vacuum/hot water clean								
		washing	3) before washing			68	3.43			
			4) after washing			64	2.57		X	
		pasteurization and spray with 2% lactic acid	5) after pasteurization and spraying with lactic acid			4	0.3		X	
	Cooling		6) after chilling			32	2.18			X
Yang et al., 2012 (CA)	Killing Hide removal		1) after hide removal*	randomly selected	200	12	1.62	log of the total number recovered from 25 samples (cfu/100 cm^2)		
		washing	2) after washing			8	0.30		X	
		spraying with lactic acid (5%)	3) after spraying with lactic acid			0	ND			
	Evisceration		4) after evisceration			0	ND			
	Trimming		5) after trimming			20	1.43			X
		washing	6) after washing			8	1.00			
		spraying with lactic acid (5%)	7) after spraying with lactic acid			0	ND		X	
		pasteurization (steam at > 90°C)	8) after pasteurization			0	ND		X	
	Cooling									

* hide-on carcasses were washed with 1.5% of sodium hydroxide at 55°C and then washed with chlorine water
ND: none detected

Table 9 (Continued): *Beef* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase)

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
McEvoy et al., 2004 (GB)	Killing			hock	1728			log N: log of the total number of recovered/cm^2		
	Hide removal		1) after hide removal			41.7	2.91			
	Evisceration		2) after evisceration			25	2.74			
	Trimming	splitting	3) after splitting							
		washing with warm water (35-40°C)	4) after washing			16.7	2.02		X	
	Cooling	24 h	5) after chilling			5.9	1.3		X	
	Killing			brisket						
	Hide removal		1) after hide removal			47.3	2.98			
	Evisceration		2) after evisceration			30.6	2.79			
	Trimming	splitting	3) after splitting							
		washing with warm water (35-40°C)	4) after washing			22.3	2.47		X	
	Cooling	24 h	5) after chilling			6.1	1.18		X	
	Killing			cranial back						
	Hide removal		1) after hide removal			0	0			
	Evisceration		2) after evisceration			5.6	1			X
	Trimming	splitting	3) after splitting							
		washing with warm water (35-40°C)	4) after washing			11.2	2.13			X
	Cooling	24 h	5) after chilling			6.1	1.4		X	
	Killing			bung						
	Hide removal		1) after hide removal			41.2	2.41			
	Evisceration		2) after evisceration			33.4	2.67			
	Trimming	splitting	3) after splitting							
		washing with warm water (35-40°C)	4) after washing			26.6	2		X	
	Cooling	24 h	5) after chilling			5.9	1.95			

Table 9 (Continued): *Beef* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase; NR: not reported) * min - max 8 plants sampled; ** min - max considering the three sampling area

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
Bacon et al., 2000 (US)	Killing Hide removal		1) after hide removal	flank, brisket, rump	1280		2.6 - 5.3*	least squares means for the log10 values (log CFU/100cm2)	X	
	Evisceration Trimming	steam vacuum of spot contamination (plants 1,2,3,4,5,6,7,8) washing (water 29 - 38°C) (plants 1,2,3,4,5) lactic (1,6 - 2,6%) or acetic acid rinsing (plants 1,2,3,4)								
		thermal pasteurization (plants 1,2,3,4,5,6,7,8) washing (16 to 32°C) (plants 1,2,3,4,5,6,7,8) lactic (1,6 - 2,6%) or acetic acid rinsing (plants 1,2,3,4,6,7,8)	2) after final washing				1.0 - 3.0*		X	
	Cooling	24 h (plant 5,6,7,8) 36 h (plants 1,2,3,4)	3) after chilling				0.9*		X	
Barboza et al., 2002 (VE)	Killing Hide removal Evisceration		1) after hide removal				0.3 - 0.5**			
	Trimming		2) after splitting	neck, brisket, renal site	192		1.3 - 1.7**	log CFU/cm^2		X
	Cooling		3) after washing				1.2 - 1.4**			
Nero et al., 2012 (BR)	Killing Hide removal Evisceration Trimming		1) after bleeding 2) after hide removal 3) after evisceration	NR	65		NR	NR	X	
	Cooling		4) after last washing							
Rigobelo et al., 2008 (BR)	Killing Hide removal Evisceration		1) after hide removal	rump near the anus	216	58	NR	NR		
	Trimming		2) after splitting and trimming			38			X	
	Cooling		3) after last washing in the cooler			32			X	

Table 10: *Beef* - Counts of *Enterobacteriaceae* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase)

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
Arthur et al., 2004 (US)	Killing									
		high-pressure water rinses and steam vacuum	1) before hide removal				6.2 (on the hide)			
	Hide removal									
		steam vacuum	2) after hide removal				1.4		X	
	Evisceration Trimming	wash cabinet - cold water + lactic acid (2-3%)		inside and outside area + navel-plate-brisket-foreshank area	288			log cfu/100 cm^2		
		wash cabinet (water 90°C) wash cabinet (peroxyacetic acid) steam pasteurization cabinet	3) after evisceration				1.7			
	Cooling		4) after decontamination treatments				0.2		X	
			5) after spray chilling (29 h)				0.4			
Ruby et al., 2007 (US)	Killing									
			1) before hide removal				4.9			
	Hide removal	trim and steam vacuum								
			2) after hide removal				1.04		X	
	Evisceration Trimming	spraying with lactic acid (5%)								
		steam vacuum	3) after evisceration	inside and outside + navel-plate-brisket-shank area	18989		0.8	log CFU/100 cm^2		
		spray with ambient temp. Water hot water lactic acid (5%)								
			4) after decontamination treatments				- 0.38		X	
	Cooling									
			5) after chilling (36-48h)				0.2			X

Table 10 (Continued): *Beef* - Counts of *Enterobacteriaceae* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase)

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
McEvoy et al., 2004 (GB)	Killing				1728			log N: log of the total number of recovered/cm2		
	Hide removal		1) after hide removal	hock		69.4	3.44			
	Evisceration		2) after evisceration			58.3	2.75		X	
	Trimming	splitting	3) after splitting							
		washing with warm water (35-40°C)	4) after washing			44.5	3.03			
	Cooling	24 h	5) after chilling			15.2	1.48		X	
	Killing									
	Hide removal		1) after hide removal	brisket		75	2.84			
	Evisceration		2) after evisceration			63.9	2.82			
	Trimming	splitting	3) after splitting			63.9	2.71			
		washing with warm water (35-40°C)	4) after washing			63.9	2.45			
	Cooling	24 h	5) after chilling			33.4	1.74		X	
	Killing									
	Hide removal		1) after hide removal	cranial back		13.9	1.84			
	Evisceration		2) after evisceration			16.7	1.97			
	Trimming	splitting	3) after splitting			38.9	3.42			X
		washing with warm water (35-40°C)	4) after washing			66.7	2.64		X	
	Cooling	24 h	5) after chilling			33.4	3.03			
	Killing									
	Hide removal		1) after hide removal	bung		66.7	2.93			
	Evisceration		2) after evisceration			80.6	3.35			
	Trimming	splitting	3) after splitting			72.3	3.51			
		washing with warm water (35-40°C)	4) after washing			55.9	3.13			
	Cooling	24 h	5) after chilling			21.2	2.42		X	

Table 10 (Continued): *Beef* - Counts of *Enterobacteriaceae* reported at different stages of the slaughterline. (N: number of samples; D: decrease; I: increase; NR: not reported)

Reference	Stages of the slaughterline	Additional operations - decontamination treatments	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	D	I
Nero et al., 2012 (BR)	Killing		1) after bleeding	not reported	65		NR	NR		
	Hide removal		2) after hide removal				NR		X	
	Evisceration		3) after evisceration				NR			
	Trimming						NR			
			4) after last washing				NR			
	Cooling						NR			
Madden et al., 2004 (IE)	Killing	high-pressure washing with hot water		brisket	100			log cfu/cm2		
	Hide removal		1) after hide removal				0.7			
	Evisceration									
	Trimming									
			2) after splitting				0.63			
			3) after washing				1.02			
	Cooling									

Table 11: *Swine* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; M: mean; SD: standard deviation; D: decrease; I: increase; NR: not reported) * reduction assessed according to the t-paired test

Reference	Operations	Sampling points	Sampled region	N	%	Counts (M and SD)	Unit of enumeration	D	I
Jones and Johns 2012 (CA)	10 sec 86°	Before pasteurization	Anal	25	100	3.80 (0.93)	Log CFU/100 cm^2		
		After pasteurization	Anal	25	100	2.42 (0.94)		X	
		After evisceration	Anal	25	84	1.32 (1.40)		X	
		After washing	Anal	25	64	-0.09 (1.27)		X	
	10 sec 86°	Before pasteurization	Random	25	92	1.79 (1.13)	Log CFU/100 cm^2		
		After pasteurization	Random	25	64	-0.07 (1.11)		X	
		After evisceration	Random	25	56	-0.33 (1.17)			
		After washing	Random	25	44	-0.67 (0.97)			
Wu et al. 2009 (DK)	(singeing, polishing, evisceration)	After stunning	Left leg	105		5.07 (4.95-5.18)	Geometric mean log CFU/100 cm^2 (CI)		-
		After scalding	Left leg			4.14 (3.92-4.36)		X	
		After splitting	Left leg			2.03 (1.89-2.18)		X	
		After cooling	Left leg			NR		X	
Nesbakken et al. 2008 (NO)		Before chilling	Ham	60		NR	Log CFU/cm^2		
	Air Blast. -21.9° 1h	After chilling	Ham	60		NR		X*	
Namvar and Warriner 2006 (CA)	CO_2 (tunnel)	After bleeding	Brisket	12		4.84 +/- 0.85	Log CFU/100 cm^2		
	65° for 5 min	After scalding	Brisket	12		<1,17		X	
		After scraping	Brisket	12		4.01+/-1.23			X
	Dry polishing and wash	After evisceration	Brisket	12		<1,17		X	
		After splitting	Brisket	12		<1,17			
	Lactic acid 1,5%	After washing	Brisket	12		<1,17			
	CO_2 (tunnel)	After bleeding	Brisket	12		4.48+/- 0.65			
	65° for 5 min	After scalding	Brisket	12		<1.17		X	
		After scraping	Brisket	12		3.51 +/- 0.88			X
	Dry polishing and wash	After evisceration	Brisket	12		<1.17		X	
		After splitting	Brisket	12		<1.17			
	Lactic acid 1,5%	After washing	Brisket	12		<1.17			
Gill and Landers 2004a (CA)		Before chilling	Random	25	64	1.64	log cfu of the total number for 2500 cm^2		
		After chilling	Random	25	64	1.11		X	
Warriner et al. 2002 (UK)	Scalding Dry pol./singeing/wet pol.	After bleeding	Brisket	10	90	3.86×10^4	CFU/100 cm^2		
		After scraping	Brisket	10	100	6.39×10^3		X	
		After polishing	Brisket	10	100	5.18×10^2		X	
		After washing	Brisket	10	100	5.44×10^2			
Tamplin et al. 2001 (US)		After bleeding	Neck, belly, ham	100	100	1700	CFU/ cm^2		
		After chilling	Neck, belly, ham	122	30.1	1.1			
Gill et al. 2000b (CA)	After evisceration	After polishing	Random	200		NR	MPN CFU 100 cm^2		
		After washing	Random	200		NR			
		After chilling	Random	200		NR			

Table 11 (Continued): *Swine* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; M: mean; SD: standard deviation; D: decrease; I: increase; NR: not reported)

Reference	Operations	Sampling points	Sampled region	N	%	Counts (M and SD)	Unit of enumeration	D	I
Rivas et al. 2000 (ES)		After bleeding	Neck, abdomen	216		3.36 (0.45)	logCFU/ cm^2		
		After scalding	Neck, abdomen			0.10 (0.16)		X	
		After dehairing	Neck, abdomen			0.45 (0.42)			
		After scraping	Neck, abdomen			0.05 (0.12)			
		After evisceration	Neck, abdomen			1.06 (0.98)			X
		End	Neck, abdomen			1.16 (0.97)			
Bryant et al. 2003 (CA)		After dehairing	Random	25	92	1.25(1.02)	MPN logCFU/ cm^2 (SD)		
		After shaving	Random	25	100	1.29 (0.81)			
		Before head removal	Random	25	4	NR			
		After head removal	Random	25	56	NR			X
		after pasteurization	Random	25	12	NR		X	
		After washing	Random	25	16	NR			

Table 12: *Swine* - Counts of *Enterobacteriaceae* reported at different stages of the slaughterline. (N: number of samples; M: mean; SD: standard deviation; D: decrease; I: increase; NR: not reported)

Reference	Sampling points	Sampled region	N	%	Counts (M and SD)	Unit of enumeration	D	I
Duggan et al. 2010 (IE)	Before chilling	Ham, back, belly, jowl	30		NR	Log CFU/cm^2		
	After chilling	Ham, back, belly, jowl			NR			
Lenahan et al., 2009 (IE)	Before chilling	Whole side	480		Not aggregated	Log CFU/ cm^2		
	After chilling	Whole side	480		Not aggregated		X	
Hurd et al. 2008 (US)	Before scalding	Skin	7	82.1				
	During evisceration	Bung	7 pool of 5	96.4				
	After evisceration	Pleura	7 pool of 5	92.9			X	
Inthavong et al. 2006 (LA)	Before evisceration	Ham, back, belly, jowl	62		2.81 (23-3.1)	Log CFU/ cm^2		
	After evisceration	Ham, back, belly, jowl			2.98 (2.1-3.3)			X
Spescha et al. 2006 (CH)	After bleeding	Neck, belly, back, ham	100	100	4.57; 4.51; 4.57; 4.65	Log CFU/ cm^2 logN(Neck, Belly,Back, Ham)		
	After scalding	Neck, belly, back, ham	100	0-6	1.38; 1.94; 1.,20; NR		X	
	After dehairing	Neck, belly, back, ham	100	82-98	3.95; 3.84; 4.47; 4.56			X
	After singeing	Neck, belly, back, ham	100	12-42	2.51; 1.86; 3.05; 2.62		X	
	After polishing	Neck, belly, back, ham	100	7-27	2.60; 2.02; 1.51; 2.20			X
	After trimming	Neck, belly, back, ham	100	13-49	2.96; 2.28; 2.51, 1.,94			
	After washing	Neck, belly, back, ham	100	18-36	2.68; 2.47; 2.18; 2.08			
	After head removal	Neck, belly, back, ham	100	19-36	2.74; 2.16; 3.29; 2.23			
	After chilling	Neck, belly, back, ham	100	0-14	1.98; 0.60; 0.09; NR		X	
	After bleeding	Neck, belly, back, ham	100	99-100	6.10; 6.05; 6.08; 6.11			
	After scalding	Neck, belly, back, ham	100	2-22	3.26; 1.68; 1.64; 1.64		X	
	After dehairing and singeing	Neck, belly, back, ham	100	56-86	6.09; 5.20; 5.55; 5.78			X
	After polishing	Neck, belly, back, ham	100	76-87	4.09; 4.00; 3.68; 3.56		X	
	After trimming	Neck, belly, back, ham	100	83-92	4.65; 4.59; 4.27; 4.40			X
	After washing	Neck, belly, back, ham	100	69-85	4.35; 3.,86; 3.89; 3.87			
	After chilling	Neck, belly, back, ham	100	17-43	3.62; 2.68; 3.00; 2.,84		X	
Warriner et al. 2002 (UK)	After bleeding	Brisket	10	90	1.05 x 10^6	CFU/100 cm^2		
	After scraping	Brisket	10	100	1.32 x 10^4		X	
	After polishing	Brisket	10	100	1.,84 x 10^3		X	
	After evisceration and washing	Brisket	10	100	4.29 x 10^3			X
Rivas et al. 2000 (ES)	After bleeding	Neck, abdomen	-		3.54 (0.20)	Log CFU/ cm^2		
	After scalding	Neck, abdomen	-		0.12 (0.29)		X	
	After dehairing	Neck, abdomen	-		0.84 (0.40)			X
	After singeing and scraping	Neck, abdomen	-		0.23 (0.19)		X	
	After evisceration	Neck, abdomen	-		1.18 (0.84)			X
	End	Neck, abdomen	-		1.39 (0.98)			

Table 13: *Small ruminants* - Counts of *E. coli* reported at different stages of the slaughterline. (N: number of samples; M: mean; SD: standard deviation; NR: not reported)

Reference (country)	Stages of the slaughterline	Operations	Sampling points	Sampled region	N	%	Counts (M and SD)	Unit of enumeration	Increase	Decrease
Bhandare et al., 2007 (IN)	NR	NR	After flying	Pool (neck, shoulder, flank, brisket, rump)	32	11.1	3.55 (0.08)	log CFU/cm^2	-	-
			After washing		32	8.3	3.11 (0.05)		-	-
			After evisceration	Pool (brisket, rib and flank)	32	20.8	3.95 (0.06)		X	-
Hauge et al., 2011b (NO)	Skinning	Fleece removal - manual and mechanical (inverted dressing)	After removal of fleece (start of slaughter)	Brisket	35	NR	2.79	log CFU/100 cm^2	-	-
	Cooling	After trimming, grading and steam vacuum treatment	Before chilling (End of slaughter)		35		2.99		X	-
Martineli et al., 2011 (BR)	Pelt removal	NR	After skinning	Forequarter legs	30	NR	0.31 (0.84)	log CFU/cm^2	X	-
				Hindquarter legs	30		0.03 (0.16)		-	-
	Evisceration		After evisceration	Forequarter legs	30		0.08 (0.21)		-	-
				Hindquarter legs	30		0.10 (0.25)		X	-
	Washing		After washing	Forequarter legs	30		0.07 (0.00)		-	X
				Hindquarter legs	30		0.00 (0.00)		-	X

Table 14: *Small ruminants* - Counts of *Enterobacteriaceae* reported at different stages of the slaughterline. (N: number of samples; M: mean; SD: standard deviation; NR: not reported)

Reference (country)	Stages of the slaughterline	Operations	Sampling points	Sampled region	N	%	Counts (M and SD)	Unit of enumeration	Increase	Decrease
Yalcin et al., 2004 (TR)	Pelt removal	NR	After dressing	Pool (Leg; shoulder, brisket, neck)	44	NR	0.38 (0.16)	log CFU/cm^2	-	-
	Evisceration		After evisceration		44		0.75 (0.21)		X	-
	Washing		After washing		44		0.58 (0.21)		-	-
	Cooling		After chilling (24 h)		44		0.11 (0.08)		-	X
Lenahan et al., 2010 (IE)	5 Abattoirs	NR	Before chilling	Pool (flank, lateral thorax, breast, brisket)	400	NR	-0.35-1.16	log CFU/cm^2	-	-
			After chilling		400		-0.32-0.43		-	X
Milios et al., 2011 (GR)	Pelt removal	NR	A- After pelt removal of hind and forelegs/ before final pulling	Pool (rump, flank, brisket, shoulder)	60	NR	0.76 (0.80)	log CFU/cm^2	-	-
			B-After pulling/before evisceration		60		2.27 (0.53)		X	-
	Evisceration		C-After evisceration/before pluck removal		60		2.68 (0.62)		X	-
			D-After pluck removal/before chilling		60		2.90 (0.55)		X	-

Appendix E. Risk factors: detailed results

Table 15: *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; NR: not reported; SD: standard deviation)

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Rigobelo et al., 2006 (BR)	sampling	season	rain season	pre-evisceration - post evisceration - post processing	rump, near the anus	80	30 - 70 - 27.5	-	-	yes
			dry season				22.5 - 55 - 17.5	-	-	
Rigobelo et al., 2008 (BR)			rain season	pre-evisceration - post evisceration - post processing	rump, near the anus	216	44	-	-	yes
			dry season				20	-	-	
Phillips et al., 2001 (AU)	slaughterhouse	plant throughput	slaughterhoused for the export	after chilling (12 h)	rump, flank, brisket	1275	11.3	-	-	no
			slaughterhouses for the domestic market				8.8	-	-	
			low throughput plants				7.9	-	-	
Sumner et al., 2003 (AU)			slaughterhouse	after chilling (8-48 h)	flank, brisket	159	28.4	-	-	yes
			low throughput plants				4.7	-	-	
Bohaychuk et al., 2011 (CA)			low throughput plants	during chilling	NR	1036		-0.54 (-0.68/-0.40)	log CFU/cm^2	no
			high throughput plants					-0.23 (-0.43/-0.02)		
Hansson, 2001 (SE)			high throughput plants	at the end of the slaughterline	loin, sternum	200	34	-	-	no
			low throughput plants				41	-	-	
Ozdemir et al., 2010 (TR)			high throughput	NR	rump, flank, brisket	120	-	2.07 (0.13)	log CFU/cm^2	no
			low throughput				-	1.90 (0.08)		
Bass et al., 2011 (AU)		type of surveillance applied in the plant	co-regulatory system	after chilling (at least 4 hours)	flank, brisket	NR	-	reported as aggregated data	-	no
			traditional system				-		-	
Tergney and Bolton, 2006 (TR)		application of an online monitoring system to monitor faecal contamination	application of the monitoring - yes	final inspection stand (before trimming)	anus, rump, brisket, flank, hock	180	-	no detailed data	log CFU/cm^2	yes
							-			

Table 15 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; SD: standard deviation)* log of the total number recovered from 2500 cm^2

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Retzlaff et al., 2005 (US)	decontamination	steam pasteurization /temperature	71.1 °C pre-treatment	pre rigor - before/after treatment	ventral midline area	280	5	Min: 0.4 Max: 0.8	log CFU/cm^2	no
			71.1 °C post-treatment				20	Min: 0.4 Max: 5.0		
			73.9 °C pre-treatment				15	Min: 0.4 Max: 1.7		no
			73.9 °C post-treatment				5	Min: 0.4 Max: 0.8		
			76.7 °C pre-treatment				5	Min: 0.4 Max: 0.8		no
			76.7 °C post-treatment				0	<0.4		
			79.4 °C pre-treatment				20	Min: 0.4 Max: 6.6		no
			79.4 °C post-treatment				10	Min: 0.4 Max: 4.1		
			82.2 °C pre-treatment				10	Min: 0.4 Max: 1.7		marginally effective
			82.2 °C post-treatment				0	<0.4		
			85.0 °C pre-treatment				30	Min: 0.4 Max: 2.5		yes
			85.0 °C post-treatment				0	<0.4		
			87.8 °C pre-treatment				5	Min: 0.4 Max: 0.8		yes
			87.8 °C post-treatment				0	<0.4		
Minihan et al., 2003 (IE)		steam pasteurization	untreated neck	pre chilling	neck, midline, rump	30	100	0.84 (0.45)	log CFU/1000 cm^2	yes
			pasteurized neck				97	0.79 (0.37)		
			untreated midline				93	0.76 (0.39)		
			pasteurized midline				77	0.60 (0.44)		
			untreated rump				90	0.98 (0.77)		
			treated rump				67	0.47 (0.34)		
Corantin et al., 2005 (CA)			before treatment	Not reported	randomly selected sites	1003	14.2	0.06 (0.19)	log CFU/cm^2	yes
			after treatment				1.8	0.01 (0.05)		
Gill and Bryant, 2000 (CA)		hot water pasteurization / temperature	8 sec - before treatment	pre chilling	randomly selected sites	250	96	2.95	CFU/100 cm^2*	yes
			8 sec- after treatment				64	1.81		
			9 sec - before treatment				84	2.91		
			9 sec- after treatment				52	1.66		
			10 sec - before treatment				80	3.08		
			10 sec- after treatment				12	0.95		
			11 sec - before treatment				76	3.88		
			11 sec- after treatment				32	1.08		
			12 sec - before treatment				76	3.58		
			12 sec- after treatment				12	0.85		

Table 15 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; SD: standard deviation)

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Mies et al., 2004 (US)	decontamination	different treatments	single water wash double water wash water wash with 0,5% lactic acid water wash with 50 ppm chlorine	pre slaughter - before/after treatment	brisket - belly - inside round	120	-	-	log CFU/cm2	yes
Barboza et al., 2002 (VE)		different treatments	lactic acid (before treatment) lactic acid (after treatment) nisin (before treatment) nisin (after treatment) lactic acid + nisin (before treatment) lactic acid + nisin (after treatment)	after washing - before/after treatment	neck	192	-	1.1 0.5 1.0 1.0 1.0 <0.2	log CFU/cm^2	yes no yes
Algino et al., 2007 (US)		different treatments	dry-aging 4 days before treatment dry-aging 4 days after treatment dry-aging 6 days before treatment dry-aging 6 days after treatment dry-aging 7 days before treatment dry-aging 4 days after treatment acetic acid spray before treatment acetic acid spray after treatment mixture of different acids before treatment mixture of different acids after treatment low pressure hot water before treatment low pressure hot water after treatment high pressure hot water before treatment high pressure hot water after treatment	prechilling before/after treatment	flank, brisket, rump	265	9 0 61 3 21 11 47 13 24 7 24 3 18 3	0.64 -1.38 -0.76 -1.35 0.10 -0.66 -0.34 -1.05 -0.33 -1.23 0.13 -1.24 -0.13 -1.29	log CFU/cm^2	yes

Table 15 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts. (N: number of samples; SD: standard deviation)

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/RF effect
Gill and Landers, 2003a (CA)	decontamination treatments	comparison of 4 plants	plant A after skinning	-	randomly selected sites	500	60	2.97	CFU/100 cm^2*	Yes
			plant A after washing and spraying with lactic acid				56	2.76		
			plant A before washing				52	2.63		
			plant A after washing				52	2.51		
			plant A after spraying with peroxyacetic acid then steam pasteurization				0	None detected		
			plant B after skinning				20	2.20		
			plant B after washing and spraying with lactic acid				52	2.04		
			plant B before washing				92	3.56		
			plant B after washing				92	3.19		
			plant B after steam pasteurization and spraying with lactic acid				0	None detected		
			plant C after skinning				48	3.99		
			plant C after washing and spraying with lactic acid				60	2.74		
			plant C before washing				60	2.78		
			plant C after washing				32	2.45		
			plant C after steam pasteurization and spraying with lactic acid				0	None detected		
			plant D after skinning				64	4.01		
			plant D before washing				68	4.16		
			plant D after washing				60	3.16		
			plant C after spraying with lactic acid				32	2.93		

* $\log_{10}$ of the total number of *E. coli* recovered from 25 samples

Table 15 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; NR: not reported; SD: standard deviation) * $\log_{10}$ of the total number of *E. coli* recovered from 25 samples

Reference (country)	Treatment/Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Gill and Landers, 2003b (CA)	spray chilling	comparison of 4 plants	plant A before cooling	cooling - before/after treatment	randomly selected sites	200	24	1.08	CFU/100 cm^2*	controversial effect
			plant A after cooling				4	0.0		
			plant B before cooling				0	none detected		
			plant B after cooling				4	0.0		
			plant C before cooling				12	0.48		
			plant C after cooling				16	1.40		
			plant D before cooling				44	1.59		
			plant D after cooling				68	2.98		
Anderson et al., 2005 (US)	diet	different feed-water treatments	administration of sodium chlorate - water	NR	bung and hock	64	-	reported as aggregated data s	-	no
			administration of sodium chlorate - water						-	
			no treatment						-	

Table 16: *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *Enterobacteriaceae* counts (N: number of samples; NR: not reported; SD: standard deviation) § Data reported for different stages of the slaughterline; ^ counts obtained on brisket samples; * prevalence obtained on the entire carcass

Reference (country)	Treatment/Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Ruby et al., 2007 (US)	sampling	season	colder months warmers months	different stages of the slaughterline	inside outside round + navel-plate-brisker-shank area	18989	- -	Aggregated data 0.97	logCFU/100cm^2	yes yes
		sampling time /year	2005 2006				-	1.04		
Ruby et al., 2007 (US)		location	East Midwest Southwest				-	Aggregated data§		yes
Prendergast et al., 2004 (IE)		plant design	A: linear rail - one floor B: serpentine rail - two floors	end of the slaughterline, after washing	brisket	NR	- -	-0,61 -0,57	logCFU/cm^2	no
Ozdemir et al., 2010 (TR)		plant throughput	high throughput low throughput	NR	rump, flank, brisket	120		2.18 (0.11) 1.98 (0.12)		no
Collobert et al., 2002 (FR)		plant	A B C D	end of the slaughterline	NR	233	- - - -	0.70 1.30-1.43 0.60-0.66 1.18-1.65	log/CFU/cm^2	yes
Blagojevich et al., 2011 (RS)	slaughterhouse	plant	A hide B hide A dressed carcass B dressed carcass	before dehiding before chilling	rump, flank, brisket, neck	100		1.97 x 10^2 2.92 x 10^2 1.06 x 10^1 0.59 x 10^1	mean CFU/cm^2	no
Zweifel et al., 2008 (CH)		plant	A B C D E F G H I J K L M P Q	NR	neck, brisket, flank, rump	50 50 50 50 50 50 50 50 50 50 50 50 50 50 50	30* 20* 55* 33.3* 23.3* 33.3* 8.3* 6.7* 16.7* 3.3* 16.7* 0.0* 11.7* 8.3* 36.7*	not detected Max: 2.20^ Max: 2.30^ Max: 1.60^ Max: 1.90^ Max: 1.60^ Max: 1.6^ not detected not detected not detected Max: 1.9^ not detected Max: 1.6^ not detected Max: 4.24^	log CFU/cm^2	some differences were detected among plants

Table 16 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *Enterobacteriaceae* counts (N: number of samples; SD: standard deviation)

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/ RF effect
Retzlaff et al., 2005 (US)	decontamination	steam pasteurization / temperature	71.1 °C pre-treatment	pre rigor - before/after treatment	ventral midline area	280	20	Min: 0.4 Max: 9.1	log CFU/cm^2	no
			71.1 °C post-treatment				25	Min: 0.4 Max: 24.8		
			73.9 °C pre-treatment				25	Min: 0.4 Max: 1.7		no
			73.9 °C post-treatment				5	Min: 0.4 Max: 1.7		
			76.7 °C pre-treatment				25	Min: 0.4 Max: 3.3		no
			76.7 °C post-treatment				15	Min: 0.4 Max: 17.3		
			79.4 °C pre-treatment				30	Min: 0.4 Max: 9.9		no
			79.4 °C post-treatment				10	Min: 0.4 Max: 4.1		
			82.2 °C pre-treatment				5	Min: 0.4 Max: 17.3		marginally effective
			82.2 °C post-treatment				5	Min: 0.4 Max: 1.7		
			85.0 °C pre-treatment				45	Min: 0.4 Max: 9.9		yes
			85.0 °C post-treatment				0	<0.4		
			87.8 °C pre-treatment				15	Min: 0.4 Max: 5.0		yes
			87.8 °C post-treatment				0	<0.4		
Minihan et al., 2003 (IE)		steam pasteurization	untreated neck	prechilling	neck, midline, rump	30	90	1.71 (1.06)	log CFU/1000 cm^2	yes
			pasteurized neck				57	0.85 (0.95)		
			untreated midline				97	1.92 (0.73)		
			pasteurized midline				70	0.96 (0.84)		
			untreated rump				97	2.25 (1.20)		
			treated rump				87	1.46 (0.90)		
Trivedi et al., 2007 (US)		steam pasteurization	before treatment	before/after treatment	neck, midline, rump	72	-	1.36	log/CFU/ cm^2	yes
			immediately after treatment				-	0.52		
			24 h after treatment				-	0.50		
Nou et al., 2003 (US)		chemical dehairing	treated carcasses	pre evisceration before/after treatment	anal-hock area	480	-	1.4 (0.7)	log CFU/ 100 cm^2	yes
			untreated carcasses				-	3.2 (1.0)		

Table 16 (Continued): *Beef* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *Enterobacteriaceae* counts (N: number of samples; SD: standard deviation) #logN: log of the total number recovered/cm^2 - mean of the four sites sampled

Reference (country)	Treatment/ Risk factor	Specifications	Experimental group	Sampling points	Sampled region	N	%	Counts (SD)	Unit of enumeration	Treatment/RF effect
Bosilevac et al., 2006 (US)		Comparison of different treatments	lactic acid before treatment	pre evisceration before/after treatment	brisket, foreshank, anus-hock, top round	768	-	4.0	log CFU/100 cm^2	yes
			lactic acid after treatment				-	3.0		
			hot water before treatment				-	4.4		
			hot water after treatment				-	1.7		
			lactic acid + hot water before treatment				-	4.7		
			lactic acid + hot water before treatment				-	2.2		
Algino et al., 2007 (US)	decontamination	different treatments	dry-aging 4 days before treatment	prechilling before/after treatment	flank, brisket, rump	265	9	0.96	log CFU/cm^2	yes
			dry-aging 4 days after treatment				3	-1.21		
			dry-aging 6 days before treatment				84	-0.08		
			dry-aging 6 days after treatment				33	-0.87		
			dry-aging 7 days before treatment				26	0.78		
			dry-aging 4 days after treatment				16	0.37		
			acetic acid spray before treatment				58	0.15		
			acetic acid spray after treatment				30	-0.84		
			mixture of different acids before treatment				28	0.04		
			mixture of different acids after treatment				22	-0.57		
			low pressure hot water before treatment				27	0.57		
			low pressure hot water after treatment				12	-0.86		
			high pressure hot water before treatment				19	0.22		
			high pressure hot water after treatment				15	-0.66		
Kinsella et al., 2006 (IE)	chilling	spray chilling	spray chilling before treatment	before/after chilling	neck, flank, brisket, rump	30	-	2.30#	log CFU/cm^2	no
			spray chilling after treatment				-	2.25#		
			conventional chilling before treatment				-	2.55#		
			conventional chilling after treatment				-	2.39#		

Table 17: *Swine* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; NR: not reported)

Reference	Treatment/Risk Factor	Experimental group	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	RF effect
Bohaychuk et al. 2011 (CA)	Plant throughput (cut-off 8000)	Low throughput	During chilling	NR	1069	33.7%	0.01(-0.15-0.16)	LogCFU/cm^2	
		High throughput	During chilling	NR			0.23(0.06-0.40)		
Hamilton et al. 2010 (AU)	Hot water-acidified sodium chlorite Abattoir A and B	A-Control		Belly	42	92.9%	0.89 (0.11)	LogCFU/g	
		B-Hot water(83.5°)		Belly	41	9.8%	-0.83 (0.21)		X
		C-Acidic treatment		Belly	40	12.5%	-0.75 (0.19)		X
		A-Control		Belly	150	69.3%	0.45 (0.08)		
		B-Hot water(81.9°)		Belly	150	22%	-0.65 (0.11)		X
		C-Acidic treatment		Belly	100	30%	0.60 (0.,13)		X
Algino et al. 2009 (US)	Skinning	Unskinned	Before washing	Bell, ham, jowl	121		-0.3	logCFU/ cm^2	X
			After chilling	Bell, ham, jowl			-1. 2		
		Skinned	Before washing	Bell, ham, jowl	60		0.5		
			After chilling	Bell, ham, jowl			-0.61		
	Washing T°	Washing T <12.8°	Before washing	Bell, ham, jowl	26		1.01		
			After chilling	Bell, ham, jowl			-0.71		
		Washing T 12.8°-21.1°	Before washing	Bell, ham, jowl	97		-0.43		
			After chilling	Bell, ham, jowl			-1.24		
		Washing T 21.1-32.2	Before washing	Bell, ham, jowl	42		0.09		
			After chilling	Bell, ham, jowl			-0.91		
		Washing T >32.2	Before washing	Bell, ham, jowl	16		0.34		
			After chilling	Bell, ham, jowl			-0.31		
	Chilling time	1 day chilling	Before washing	Bell, ham, jowl	112		-0.29		X
			After chilling	Bell, ham, jowl			-1		
		2 days chilling	Before washing	Bell, ham, jowl	69		-0.04		
			After chilling	Bell, ham, jowl			-1.01		
Delhalle et al., 2008 (BE)	Mixed models considering different RF	Spraying if ext temp hot	During chilling	Ham, back, forelimb, sternum	584		0.59 (0.22)	model parameter logCFU/ cm^2	
		Time between killing and scalding	During chilling	Ham, back, forelimb, sternum			0.23 (0.1)		
		Scalding with steam	During chilling	Ham, back, forelimb, sternum			-0.65 (0.29)		X
		Washing and disinfection splitting machine three times a day	During chilling	Ham, back, forelimb, sternum			-0.89 (0.37)		X
		Lairage cleaning with water	During chilling	Ham, back, forelimb, sternum			-0.56 (0.08)		X
		Frequency of lairage disinfection	During chilling	Ham, back, forelimb, sternum			-0.76 (0.15)		X
		New hooks for carcasses before chilling	During chilling	Ham, back, forelimb, sternum			-0.69 (0.08)		X
Lindblad et al. 2007 (S)	Season		Before chilling	Ham,back,Belly,Neck	541	57%	0.05 (0.58)	logCFU/ cm^2	X
Gill and Landers 2004a (CA)	Detained carcass, contamination	Visibly contaminated carcasses	Before trimming	Contaminated site	25	60%	4.78	log of the total number for 2500 cm^2	
			After trimming	Contaminated site	25	80%	2.92		X
			After chilling	Contaminated site	25	32%	1.7		X
			Before trimming	Random	25	72%	2.37		
			After trimming	Random	25	76%	2.24		
			After chilling	Random	25	32%	1.69		X

Table 17 (Continued): *Swine* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *E. coli* counts (N: number of samples; NR: not reported)

Reference	Treatment/Risk Factor	Experimental group	Sampling points	Sampled region	N	%	Counts	Unit of enumeration	RF effect
Hansson 2001 (S)	Plant throughput	Low throughput (450-800 pigs/year)	Before chilling	Loin, sternum	100	58%		logCFU/ cm^2	X
		High throughput >250,000	Before chilling	Loin, sternum	100	74%			
Gill and Badoni 2001 (CA)	Pasteurization, sticking wound trimming		Before pasteurization	Sticking wound	25	80%	2.09	logCFU/100 cm^2	X
			After pasteurization	Sticking wound	25	28%	0.85		
			After trimming	Sticking wound	25	40%	1.56		
Rivas et al. 2000 (ES)	Washing and GMP (anal plugging before evisceration)	Unwashed-non GMP	Before chilling	Neck, abdomen	36		1.20 (0.72)	logCFU/ cm^2	X
		Unwashed, GMP	Before chilling	Neck, abdomen	54		0.24 (0.43)		
		Washed and GMP	Before chilling	Neck, abdomen	35		1.13 (0.34)		
Bass et al. 2011 (AU)	Plants features	B	During chilling	Rump, brisket, jowl	19	42%	-0.6	logCFU/ cm^2	
		D	During chilling	Rump, brisket, jowl	5	40%	-0.6		
		L	During chilling	Rump, brisket, jowl	17	29%	0.06		
		M	During chilling	Rump, brisket, jowl	8	12.5%	-0.45		
		N	During chilling	Rump, brisket, jowl	5	60%	-0.27		
		O	During chilling	Rump, brisket, jowl	20	95%	-0.17		
		P	During chilling	Rump, brisket, jowl	3	10%	-0.55		
Saucier et al. 2007 (CA)	Meal frequency (2; 5), Feed type (pelleted; mash), fasting time (4; 14; 24 h)	P-2-4h	After evisceration	Thoracic area, flanks	8	100%	1.31 (0.43)	CFU/926 cm^2 from MPN estimate	X
		P-2-4h	After evisceration	Thoracic area, flanks	8	37.5%	1.77 (0.14)		
		P-2-14h	After evisceration	Thoracic area, flanks	8	25%	1.89 (0.54)		
		P-2-24h	After evisceration	Thoracic area, flanks	8	25%	1.99 (0.55)		
		P-5-4h	After evisceration	Thoracic area, flanks	8	37.5%	1.79 (0.25)		
		P-5- 14h	After evisceration	Thoracic area, flanks	8	25%	1.8 (0.30)		
		P-5- 24h	After evisceration	Thoracic area, flanks	8	87.5%	2.07 (0.49)		
		M-2-4h	After evisceration	Thoracic area, flanks	8	37.5%	1.81 (0.22)		
		M-2-14h	After evisceration	Thoracic area, flanks	8	50%	2.18 (0.60)		
		M-2-24h	After evisceration	Thoracic area, flanks	8	25%	1.83 (0.25)		
		M-5-4h	After evisceration	Thoracic area, flanks	8	12.5%	1.8 (0.28)		
		M-5-14h	After evisceration	Thoracic area, flanks	8	62.5%	1.92 (0.34)		
		M-5-24h	After evisceration	Thoracic area, flanks	8	25%	2.1 (1.12)		
Rabaste et al. 2006 (CA)	Handling	Rough	NR	Int Rib cage, brisket, feet	10	52%	1.45 (0.69)	CFU/983 cm^2 from MPN estimate	
		Gentle	NR	Int Rib cage, brisket, feet	10	68%	1.49 (0.57)		
	group size	10	NR	Int Rib cage, brisket, feet	10	65%	1.48 (0.58)		
		30	NR	Int Rib cage, brisket, feet	10	55%	1.46 (0.67)		
Conter et al. 2006 (I)	Steam treatment (after evisceration)	Steam treatment	After evisceration	Jowl, belly, back, ham	54		NR	logCFU/ cm^2	
		Control	After evisceration	Jowl, belly, back, ham	32		NR		

Table 18: *Swine* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *Enterobacteriaceae* counts (N: number of samples; D: decrease; I: increase)

Reference	Treatment/ Risk Factor	Experimental group	Sampling points	Sampled area on the carcass	N	%	Counts	Unit of enumeration	RF Effect
Purnell et al. 2010 (UK)	Anal plugging	Plugged	Before scalding	Anal region	34		2.18 (1.28)	logCFU/cm^2	
		Plugged	After scalding and dehairing	Anal region	34		1.91 (0.95)		
		Unplugged	Before scalding	Anal region	34		1.86 (1.01)		
		Unplugged	After scalding and dehairing	Anal region	34		3.01 (1.08)		X
Algino et al. 2009 (US)	Skinning	Unskinned	Before washing	Bell, ham, jowl	121		0.57	logCFU/cm^2	
			After chilling	Bell, ham, jowl			-0.86		X
		Skinned	Before washing	Bell, ham, jowl	60		1.28		
			After chilling	Bell, ham, jowl			0.41		X
	Washing T°	Washing T <12.8°	Before washing	Bell, ham, jowl	26		2.05		
			After chilling	Bell, ham, jowl			0.52		X
		Washing T 12.8°-21.1°	Before washing	Bell, ham, jowl	97		0.31		
			After chilling	Bell, ham, jowl			-1		X
		Washing T 21.1-32.2	Before washing	Bell, ham, jowl	42		1.06		
			After chilling	Bell, ham, jowl			-0.14		X
		Washing T >32.2	Before washing	Bell, ham, jowl	16		1.09		
			After chilling	Bell, ham, jowl			0.61		
	Chilling time	1 day chilling	Before washing	Bell, ham, jowl	112		0.64		
			After chilling	Bell, ham, jowl			-0.43		X
		2 days chilling	Before washing	Bell, ham, jowl	69		1.08		
			After chilling	Bell, ham, jowl			-0.45		X
Zweifel et al. 2008 (CH)	Plant features	A	Before/during chilling	Neck, belly, back, ham	50	22%	2.30;2.92;2.38;1.90	logCFU/cm^2 (log Total Number recovered)	
		B	Before/during chilling	Neck, belly, back, ham	50	22%	2.60;2.45;2.80;2.45		
		C	Before/during chilling	Neck, belly, back, ham	50	32%	2.20;NR;3.3.1;3.56		
		D	Before/during chilling	Neck, belly, back, ham	50	44%	3.84;2.56;3.44;3.17		
		E	Before/during chilling	Neck, belly, back, ham	50	8%	3.17;2.38;1.90;2.08		
		F	Before/during chilling	Neck, belly, back, ham	50	14%	2.08;2.45;2.20;1.60		
		G	Before/during chilling	Neck, belly, back, ham	50	56%	3.15;2.51;3.08;3.30		
		H	Before/during chilling	Neck, belly, back, ham	50	14%	2.51;ND;ND;1.60		
		I	Before/during chilling	Neck, belly, back, ham	50	14%	1.90;1.60;1.90;2.64		
		J	Before/during chilling	Neck, belly, back, ham	50	26%	1.90;2.20;3.09;2.51		
		K	Before/during chilling	Neck, belly, back, ham	50	10%	2.38;1.60;2.45;1.60		
		L	Before/during chilling	Neck, belly, back, ham	50	22%	2.72;2.45;3.29;2.20		
		M	Before/during chilling	Neck, belly, back, ham	50	16%	2.30;2.38;2.60;2.08		
		N	Before/during chilling	Neck, belly, back, ham	50	56%	2.72;2.98;3.45;3.20		
		O	Before/during chilling	Neck, belly, back, ham	50	2%	1.60; ND; ND; ND		

Table 18 (Continued): *Swine* - Counts at different stages of the slaughterline reported in papers describing risk factors influencing *Enterobacteriaceae* counts (N: number of samples; D: decrease; I: increase)

Reference	Treatment/ Risk Factor	Experimental group	Sampling points	Sampled area on the carcass	N	%	Counts	Unit of enumeration	RF effect
Trivedi et al. 2006 (US)	Steam decontamination 60 sec 82-85° on 3 anatomical areas	25 % of carcasses acid treated	Before steam treatment	Ham	72		134	logCFU/cm^2	X
				Belly	72		219		
				Jowl	72		212		
				All	72	61,6%	188		
		25 % of carcasses acid treated	After steam treatment	Ham	72		008		
				Belly	72		028		
				Jowl	72		026		
				All	72	33,8%	021		
		75% of carcasses acid treated	24h after steam treatment	Ham	72		032		
				Belly	72		053		
				Jowl	72		046		
				All	72	25,5%	044		
Rivas et al. 2000 (ES)	Washing and GMP (anal plugging before evisceration)	Unwashed-non GMP	Before chilling	Neck, abdomen	36		121 (074)	logCFU/cm^2	X
		Unwashed, GMP	Before chilling	Neck, abdomen	54		025 (045)		
		Washed and GMP	Before chilling	Neck, abdomen	35		014 (032)		
Tomovic et al. 2011 (RS)	Chilling length (3 h at -31.1° followed by 2-4° chill room (8 or 24h)	8 h	Before chilling	Ham, back, belly, jowl	24	70%	053	logCFU/cm^2	
			After chilling	Ham, back, belly, jowl		54%	039		
		24 h	Before chilling	Ham, back, belly, jowl	24	58%	04		
			After chilling	Ham, back, belly, jowl		25%	009		X
Blagojevic et al. 2011 (RS)	Plant features	A	After stunning	Ham, belly, jowl	50		778x10^3(162x10^5-537x10)	CFU/cm^2 geometric mean	
			Before chilling	Ham, belly, jowl	50		894x10(141x10^3-063x10)		X
		B	After stunning	Ham, belly, jowl	50		419x10^3(204x10^5-468x10)		
			Before chilling	Ham, belly, jowl	50		097x10(955x10^2-009x10)		X

Table 19: *Small ruminants* - Counts at different stages of the slaughterline reported in papers describing factors related to the slaughterhouse influencing *E. coli* counts (N: number of samples; NR: not reported; M: mean; SD: standard deviation)

Reference (country)	Treatment/Risk Factor	Experimental group	Sampling point/s	Sampled region/s	N	%	Counts (M and SD)	Unit of enumeration	Treatment /RF effect
Gill et al., 2000a (CA)	Dressing procedure	Conventional set 1 (carcasses suspended by the rear legs)	After the final wash in the dressing procedure	Pool (Two sites for each carcass selected from a grid that specifies 43 areas on one side of the carcass surface)	25	15/25	total number of bacteria recovered: 4.40	CFU/100 cm^2	-
		Conventional set 2			25	17/25	total number of bacteria recovered: 4.11		-
		Inverted dressing 1 (carcasses suspended by the foreleg)			25	11/25	total number of bacteria recovered: 2.58		Yes
		Inverted 2			25	15/25	total number of bacteria recovered: 2.60		
Duffy et al., 2001 (US)	Season	Spring	post 24h carcass chilling	Pool (flank, breast, leg)	1259	NR	0.63	log CFU/cm^2	No
		Fall or winter			1261		0.76		
Phillips et al., 2001 (AU)	Plant throughput	All the slaughterhouses	After chilling	Pool (rump, flank and brisket)	921	29.2	0.17 (0.60)	log CFU/cm^2	-
		Export slaughterhouses			270	35.2	NR		-
		Domestic slaughterhouses			306	32.7	NR		-
		Low throughput plants			345	21.4	NR		Yes
Sumner et al., 2003 (AU)	Plant throughput	Medium throughput plants (4)	After chilling (8-48 h)	Pool (rump, flank and brisket)	148	61.5	0.39	log cfu/cm^2	No
		Low throughput plants (11)			216	18.5	-0.01		
Cohen et al., 2006 (MA)	Seasonal effect	Hot season (April to September)	NR	Raw meat	26	44.2 (on the total samples)	1.2 (0.6)	log CFU/g	No
		Cold season (November to March)			26		1.3 (0.6)		
Kannan et al., 2007 (US)	Pre-slaughter spray washing	Control (no wash)	After skinning/before evisceration	Pool (flank, brisket, leg)	10	NR	2.1	log CFU/cm^2	No
		Washed (1 min spray washed - potable water)			10		2.1		
Feizullah and Daskalov, 2010 (BG)	Capacity and Season	Low throughput (15-20 animals per day)/Spring	After washing/Before chilling	Pool (leg, chest -outer and inner surface, neck)	15	NR	1.00	log CFU/cm^2	Marginally effective
		Low throughput/Winter			15		1.65		
		High throughput (100-200 animals per day)/Spring			15		2.00		
	Sampling point	High throughput		Leg (lateral)	12		2.00		Yes
		Low throughput			12		0.7		

Table 19 (Continued): *Small ruminants* - Counts at different stages of the slaughterline reported in papers describing factors related to the slaughterhouse and to the batch (one study) influencing *E. coli* counts (N: number of samples; NR: not reported; M: mean; SD: standard deviation).

Reference (country)	Treatment/Risk Factor	Experimental group	Sampling point/s	Sampled region/s	N	%	Counts (M and SD)	Unit of enumeration	Treatment /RF effect
Bass et al., 2011 (AU)	Plant throughput	Plant A (goat)	After chilling	midloin, flank, brisket	323	29	-0.38	log CFU/cm^2	some differences were detected among plants -
		Plant C (sheep)				68.9	-0.28		
		Plant D				89.5	0.41		
		Plant E				26.8	-0.16		
		Plant F				41.4	0.08		
		Plant G				87.5	-0.22		
		Plant H				40	-0.20		
		Plant I				57.9	-0.33		
		Plant J				60	-0.10		
		Plant K				24.1	-0.38		
		Plant L				54.5	-0.05		
		Plant M				42.1	-0.35		
		Plant N				70	0.12		
		Plant O				8.7	0.40		
		Plant P				86.2	0.02		
Hauge et al., 2011a (NO)	Pasteurisation (82°C+-1 for 8 s): after dressing and grading/ before chilling	Control	After evisceration- dressing/before chilling	Outside (mid-line of the abdomen, under the forelegs, around rectum and hind legs) and inside the carcass	90	66 (59/90)	2.39	log CFU/carcass (4500 cm^2)	-
			24 h after chilling		90	43 (39/90)	0.98		Yes
		Pasteurisation	After evisceration-dressing/before chilling		90	26 (23/90)	0.54		Yes
			24 h after chilling		90	21 (19/90)	0.37		Yes
Hauge et al., 2011b (NO)	Shearing regime	Unshorn	After removal of fleece (start of slaughter)	Brisket	35	80	2.79	log CFU/100 cm^2	-
			Before chilling (End of slaughter)		35		2.99		-
		Shorn 0 days abattoir	After removal of fleece (start of slaughter)		35		1.78		No
			Before chilling (End of slaughter)		35		2.71		
		Shorn 3 days on farm	After removal of fleece (start of slaughter)		35	62	1.49		
			Before chilling (End of slaughter)		35		2.68		
		Shorn 7 days on farm	After removal of fleece (start of slaughter)		35		1.73		
			Before chilling (End of slaughter)		35		2.69		
Jordan et al., 2012 (AU)	Inspection of lymph nodes	Pre-inspection	NR	Scapula	148	35	7	CFU/cm^2	Yes
				Rump	148	84	42		
		Post-inspection	NR	Scapula	148	67	22		
				Rump	148	93	52		

Table 20: *Small ruminants* - Counts at different stages of the slaughterline reported in papers describing factors related to the slaughterhouse influencing *Enterobacteriaceae* counts (N: number of samples; NR: not reported; M: mean; SD: standard deviation)

Reference (country)	Treatment/Risk Factor	Experimental group	Sampling point/s	Sampled region/s	N	%	Counts (M and SD)	Unit of enumeration	Treatment/ RF Effect
Whyte et al., 2002 (GB)	Dressing procedure	Cradle dressing (the carcass was hung by the rear legs before the pelt was pulled off manually)	After skin removal	Pool (Shoulder, abdomen and lateral surface of the rear leg)	48	NR	0.47	log CFU/cm^2	Yes
		Hybrid method (use of the cradle to support the lamb while the pelt was released from the forelegs and brisket; then the carcass is lifted in a vertical position (inverted))			48		0.14		
		Frame method (carcass carcass manipulation at an optimum working position and encourage pelt to hang down and away from the carcass during the pelt removal - inverted dressing)			48		0.03		
Fisher et al., 2007 (GB)	Skin on vs conventionally dressed	No toast (singe, wash and eviscerate)	Before evisceration	Pool (rump, belly, flank, brisket, shoulder, neck)	60	24/60	0.964	log CFU/cm^2	Yes
		Toast	After evisceration		60	3/60	0.433		
		Toast before splitting	Before splitting		60	10/60	0.569		No
		Toast after splitting	After splitting		60	7/60	0.509		
		Toast before inspection	Toasted and then inspected		60	6/60	0.424		No
		Toast after inspection	Inspected and then toasted		60	4/60	0.429		
		Skin on	After singeing/pre chilling		60	2/60	0.420		Yes
		Conventional (skin removed)	After dressing/pre-chilling		60	26/60	0.955		
Brown et al., 2009 (GB)	Vascular perfusion chilling (very fine ice particles in a solution of sodium chloride and water)	Control	After dressing/Before chilling	Pooled surface samples (lateral thorax, flank, brisket, breast)	10	1/10	1.4	log CFU/cm^2	No
		Treatment (4 mins perfusion)	At 24 h after held in the chill-room		10	1/10	2.0		
Feizullah and Daskalov, 2010 (BG)	Slaughterhouse throughput and Season	Low throughput/ Spring	After washing/Before chilling	Pool (leg, chest -outer and inner surface, neck)	15	NR	1.30	log CFU/cm^2	Yes
		Low throughput/ Winter			15		3.18		
		High throughput/ Winter			15		1.27		Yes
		High throughput/ Autumn			15		6.05		

Table 20 (Continued): *Small ruminants* - Counts at different stages of the slaughterline reported in papers describing factors related to the slaughterhouse influencing *Enterobacteriaceae* counts (N: number of samples; NR: not reported; M: mean; SD: standard deviation)

Reference (country)	Treatment/Risk Factor	Experimental group	Sampling point/s	Sampled region/s	N	%	Counts (M and SD)	Unit of enumeration	Treatment/ RF Effectiveness
Hauge et al., 2011a (NO)	Pasteurisation (82°C+-1 for 8 s): after dressing and grading/ before chilling	Control	After evisceration-dressing/before chilling	Outside (mid-line of the abdomen, under the forelegs, around rectum and hind legs) and inside the carcass	90	100	3.78	log CFU/carcass (4500 cm^2)	Yes
			24 h after chilling		90	NR	1.94		
		Pasteurisation	After evisceration-dressing/before chilling		90	66	1.41		
			24 h after chilling		90	NR	0.49		
Milios et al., 2011 (GR)	Steam application (8-10 passes of steam spraying pistol at each side of the carcass)	Before Steam Application	After pluck removal/Before chilling	Pool (rump, flank, brisket, shoulder)	60	NR	3.74 (0.51)	log CFU/cm^2	Yes
		After Steam Application			60		2.67 (0.60)		
Jordan et al., 2012 (AU)	Inspection of lymph nodes	Pre-inspection	NR	Scapula	148	46	11	CFU/cm^2	Yes
				Rump	148	87	66		
		Post-inspection	NR	Scapula	148	77	26		
				Rump	148	96	57		
Rubio et al., 2013 (ES)	Chilling	CT: Conventional treatment (2°C for 24h)	Before post -treatment (after dressing)	Rump and brisket	20	NR	3.24 (0.16)	log CFU/cm^2	-
			Post-mortem treatment		20		3.31 (0.16)		-
		UT: Ultra rapid treatment (-20°C for 3.5h then 2°C until 24 h)	Before post-mortem treatment (after dressing)		20		3.04 (0.17)		Yes
			Post-mortem treatment		20		2.43 (0.17)		
		ST: Slow treatment (12 °C for 7h then 2°C until 24h)	Before post-mortem treatment (after dressing)		20		3.44 (0.17)		-
			Post-mortem treatment		20		3.22 (0.17)		-

Appendix F. Faecal contamination

Table 21: *Beef* - Relationship between visual faecal contamination and *E. coli* (EC), *Enterobacteriaceae* (EB) counts along the slaughterline. (N: number of samples; NR: not reported; O: observational; SD: standard deviation) * min - max values obtained for the 4 sampled slaughterhouses; C0: category 0 - C1: category 1 - C2: category 2 - C3: category 3 - C4: category 4 - C5: category 5

Indicator bacteria	Reference (country)	Study	Plants	Decontaminants	Sampled area on the carcass	Classification of faecal contamination	N	Unit of enumeration	Sampling point	Experimental group	Mean (SD)
EB	McCleery et al., 2008 (GB)	O	1	NR	neck, brisket, flank, rump	1-2 clean animals - 3-4 animals classed as dirty and requiring special provisions to be taken before being slaughtered (clipped ante-mortem or online) - 5 animal rejected	362	Log CFU/cm^2	NR		No punctual data were available
EB	Blagojevic et al., 2012 (RS)	O	2	No	neck, brisket, flank, rump	1: clean and dry; 2: slightly dirty; 3: dirty; 4: very dirty	100	Log CFU/cm^2	before chilling	C1 C2 C3 C4	0.81 (0.74) 0.78 (0.63) 0.83 (0.68) 1.49 (0.60)
EB	Serraino et al., 2012 (IT)	O	1	No	brisket, groin, flank, hock	1 (clean-dry) to 5 (filthy-wet)	75	Log CFU/cm^2	before chilling	C1 C2 C3 C4 C5	0.3 (0.01) 0.3 (0.01) 1.8 (2.2) 1.0 (1) 1.4 (0.9)
EC							75			C1 C2 C3 C4 C5	0.3 (0.01) 0.3 (0.01) 1.1 (1.1) 0.9 (1.1) 1.1 (0.8)
EC	Gill and Landers, 2004b (CA)	O	4	plant A: washing with 200 ppm peroxyacetic acid; plant B: steam + 2% lactic acid; plant C: hot water + 2% lactic acid; plant D: 2% lactic acid	contaminated area + adjacent to the contaminated area + randomly selected areas	NR	75	log/ 2500 cm^2	CA - before trimming CA - after trimming CA - after dressing ACA -before trimming ACA after trimming ACA after dressing RS - before trimming RS - after trimming RS - after dressing	plant A, B, C, D	2.67* - 6.32* 1.54 * - 5* none detected* - 2.8* 1.4* - 3-94* 1.23* - 3.63* none detected* - 3.09* 1.65* - 4.46* 1* - 2.54* none detected* - 1.91*
EC	Hauge et al., 2012 (NO)	O	2	No	brisket, belly	0:clean; 1: moderately dirty; 2: very dirty	324	Log CFU/100 cm^2	after dehiding end of slaughterline after dehiding end of slaughterline after dehiding end of slaughterline	C0 C0 C1 C1 C2 C2	0.97 0.28 1.38 0.33 1.67 0.51

Table 22: *Small ruminants* - Relationship between visual faecal contamination and *E. coli* (EC), *Enterobacteriaceae* (EB) counts along the slaughterline (N: number of samples; M: mean; O: observational)

Indicator bacteria	Reference (country)	Study	Plant	Decontaminants	Sampled region	Classification of faecal contamination	N	Unit of enumeration	Sampling point	Experimental group	M	
EC	Hauge et al., 2011b (NO)	O	1	No	brisket	0: visually clean fleece; 1: small spots of dirt; 2: generally dirty fleece; 3: very dirty fleece	35	log CFU/100 cm^2	At skinning	C0	1.65	
							35			C1	1.88	
							35			C2	2.16	
							35			C3	2.49	
EB	Byrne et al., 2007 (IE)	O	1	No	brisket, flank, rump, shoulder	(A) clean and dry; (B) clean and wet; (C) dirty and dry; (D) dirty and wet; (E) with visible faecal dags	40	log CFU/4000cm^2	After skinning	A	2.7	
							40			B	2.9	
							40			C	4.4	
							40			D	3.9	
							40			E	4.4	
EB	Whyte et al., 2002 (GB)	O	1	No	Shoulder	Score between 0 (no visible contamination) and 10 (maximum visible contamination)	9	log CFU/cm^2	After skinning	Cradle dressing	0.47	4.3*
					Flank		9					2.7*
					Hindquarters		9					5.6*
					Lower foreleg		9					9.0*
					Shoulder		9			Hybrid dressing	0.14	3.4*
					Flank		9					1.4*
					Hindquarters		9					0.8*
					Lower foreleg		9					6.3*
					Shoulder		9			Frame dressing	0.03	1.7*
					Flank		9					1.6*
					Hindquarters		9					0.7*
					Lower foreleg		9					4.4*

* mean gross contamination score

ABBREVIATIONS

ACC: Aerobic colony count

AT: Austria

AU: Australia

BR: Brazil

BG: Bulgaria

CA: Canada

CZ: Czech Republic

ES: Spain

GB: United Kingdom

IE: Ireland

IN: India

LA: Lao People's Democratic Republic

IT: Italy

MA: Morocco

NO: Norway

PHC: Process Hygiene Criteria

PHI: Process Hygiene Indicators

RS: Serbia

SE: Sweden

TR: Turkey

TW: Taiwan

US: United States

VE: Venezuela

ZA: South Africa