Food Control 43 (2014) 74–81

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Review

Food Safety Management System validation and verification in meat industry: Carcass sampling methods for microbiological hygiene criteria – A review

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ARTICLE INFO

Article history:
Received 8 November 2013
Received in revised form
17 February 2014
Accepted 25 February 2014
Available online 5 March 2014

Keywords:
HACCP validation and verification
Slaughterhouse hygiene indicators
Carcass non-destructive sampling techniques
'Process-based' microbiological criteria

ABSTRACT

During validation and verification of the system for the proper implementation of HACCP principles, it is essential to rely on microbiological data. Considerable science research has been carried out during the last twenty years on sampling and testing of carcasses for hygiene criteria. This includes the preferable indicator microorganisms to be used, in order to indicate the general hygiene of slaughtering procedures, the evaluation of microbiological data gathered and the sampling methods. Furthermore, European Union (EU) and the United States have adopted the procedures for HACCP validation and verification in their legislation. The aim of this review is to demonstrate the relevant modern trends in this field of food science. In conclusion, microbiological data based on the indicators should be interpreted only to assess general trends in the hygiene process of the operator in order to take corrective action. Microbiological results, obtained only at the end of the slaughtering process, do not provide information on the cause of the problem. Therefore, 'process-based' microbiological criteria which are based on values measured at various stages of the process, including final carcass values, should be used. Finally, in order to implement an adequate monitoring system, non-destructive techniques of carcass sampling could be used instead of excision. The microbial recovery may be lower, but it is proportional to the excision recovery and therefore, non-destructive techniques, like swabbing with sponges, could be a practical sampling method for the estimation of indicators during the slaughtering procedure and hygiene evaluation.

Contents

1. Introduction

HACCP (Hazard Analysis Critical Control Points) implementation in the food industry in general and the influencing factors have been previously reviewed (Milios, Drosinos, & Zoiopoulos, 2012). On the other hand, meat is a highly perishable food and constitutes often the means through which food borne illnesses may spread

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http://dx.doi.org/10.1016/j.foodcont.2014.02.041
0956-7135/

(Adams, 2014; Steele & McMullin, 2007). Therefore, strict application of Good Hygiene Practices (GHP) during slaughtering process is of great importance for Public Health preservation and quality assurance. Furthermore, it is reported that the HACCP systems should take under consideration the distinctiveness of each product, as well as factors related to the production process (Panisselo & Quantick, 2001). For the proper implementation of HACCP principles and, especially, the hazard analysis and the critical control point determination, it is essential to rely on microbiological data gathered from the certain plant during validation of the system (Adams, 2014; Buncic, 2006; Zweifel, Baltzer, & Stephan, 2004).

Moreover, the HACCP system should be verified during implementation, using measurable parameters such as microbiological data. A proper verification procedure should be based on evaluating the microbiological results within the same plant during different periods of time and comparing them with the standards set by legislation. The object of verification is to confirm that the system is working properly and the hazards are effectively kept under control. Microbiological examination, on the other hand, is applied to random samples and does not cover the total surface of the carcass. Therefore, it cannot eliminate the possibility of pathogens' presence. This is the reason for the use of indicator microorganisms, to show the general hygiene and contamination of faecal origin (Brown et al., 2000; Buncic, 2006).

Indicator microorganisms are group of bacteria suggestive of possible prevalence of pathogens. It is important to keep in mind that, there is no proven correlation between indicator organisms and prevalence/levels of pathogens, although there is a possibility that, pathogens might be a positive fraction of indicators. Despite this, it can be accepted that the levels of pathogens are lower than the levels of indicators and also decrease in the population of indicators suggests a similar reduction in the population of pathogens. Furthermore, each indicator is correlated to different pathogens and, therefore, more than one indicator should be used (Brown et al., 2000).

The microbiological parameters that have been used as indicators in slaughterhouses are Total Viable Count (TVC), total coliforms, Enterobacteriaceae, *Escherichia coli*, *Fecal streptococci* and *Aeromonads* (Algino, Badtram, Ingham, & Ingham, 2009; Gill & McGinnis, 1999), while *Listeria* sp., enterococci and bifidobacteria have also been suggested for this purpose (Dencenserie et al., 2008; Gill & Jones, 1995). On the other hand, meat pathogens that have been related in the past with food borne diseases are *Salmonella*, *E. coli* O157:H7, non O157 STEC *E. coli*, *Listeria*, *Campylobacter*, *Clostridium perfrigens* and *Yersinia*. The most important are *E. coli* O157:H7, non O157 STEC *E. coli* and *Salmonella* (Koohmaraie et al., 2005), mainly found in ruminants' meat. Methicillin-resistant *Staphylococcus aureus* in pork meat is also a major global public health concern and could be a safety issue (Lasok & Tenhagen, 2013). *E. coli* O157:H7 is thought to be the most dangerous pathogen for human health, causing hemorrhagic diarrhoea because of the produced toxin (Shiga-toxin) and the damage to the intestine epithelium. It has been proved that *E. coli* O157:H7 can be found in live animals and is related to meat contamination. Non O157 STEC *E. coli* is believed to be the cause for one third of the incidents due to *E. coli* and could be the most significant problem in future. Finally, *Salmonella* can be found in animal faeces and is related to meat contamination, causing 1.4 million incidences per year only in the United States, while *Salmonella* and *Campylobacter* infections are the most commonly reported gastrointestinal diseases across the EU (Bertrand et al., 2010; European Centre for Disease Prevention and Control, 2011).

The current legislation in European Union requires microbiological examinations for certain indicator organisms (hygiene criteria for the slaughtering process), such as TVC, Enterobacteriaceae and *Salmonella* (Anonymous, 2005, 2007). A three-class system, classifying microbiological results from carcasses (for each animal species) into satisfactory, acceptable and unsatisfactory is used to determine the hygiene performance of the operator. As already mentioned there is no proven correlation between indicator organisms and prevalence/levels of pathogens. Therefore, the microbiological data, based on the indicators, should be interpreted only to assess general trends in the hygiene process of the operator, in order to take corrective action such as improvements in slaughter hygiene and review of process controls according to the HACCP system. However, microbiological results described above, used alone, may be insufficient. The results are obtained only after dressing and before chilling, at the end of the slaughtering process and therefore, do not provide information on the cause of the problem (Buncic, 2006).

'Process-based' microbiological criteria correspond to parametres measured at various stages of the process, including final carcass values. Two examples of process-based hygiene performance in slaughterhouses have been proposed. Firstly, the approach of Bolton, Doherty, and Sheridan (2000), which involves microbiological sampling at different stages of the slauhgterline and enables better understanding of the process, as well as the roots of the problem. The second approach is based on evaluation of slaughter hygiene by comparison of bacterial loads on final carcasses with bacterial loads on correlated incoming animals using a numerical parameter (BOIF – Bacterial Output–Input Factor), thus showing the microbiological reduction achieved (Vivas Alegre & Buncic, 2004).

In the present work, we try to review the research made during the last twenty years on sampling and testing techniques of carcasses for establishing hygiene criteria, including preferable indicator microorganisms being used in order to indicate the general hygiene of slaughtering procedures, and on the evaluation of microbiological data gathered, in relation to the sampling methods applied.

2. Sampling and testing of carcasses for hygiene criteria

Sampling and testing of carcasses for hygiene criteria has been used by competent authorities during official inspection of slaughterhouses and also by the companies during HACCP validation and verification (Brown et al., 2000). Stolle (1981) used microbiological testing of bovine carcass surface in order to study the prevalence of *Salmonella* during slaughtering process. By 1975 correlation between salmonellosis incidents in humans and consumption of contaminated food of animal origin had been established. Meat contamination takes place in slaughterhouses during slaughtering process (Arguello, Alvarez-Ordonez, Carvajal, Rubio, & Prieto, 2013). *Salmonella* prevalence in animal faeces, hooves, skin, coat and slaughtering equipment, has been reported (Wesley et al., 2000). From the results of the above study, it was concluded that, in some specific points of slaughtering line, a number of samples were positive for *Salmonella* prevalence. These points were hooves extraction and pelt removal, evisceration, veterinary inspection and carcass trimming. *Salmonella*'s prevalence on carcasses and equipment, at the same points of slaughtering line, proves the cross contamination of carcasses from the animals, through the slaughtering equipment and personnel. It is interesting to point out, that the main aim of the study mentioned above was to determine the prevalence of the pathogen bacteria itself and results were directly related to safety of the meat. But since the HACCP principles introduction, as a means of food hygiene assurance in 1991, the requirement for objective measurement of all microbiological hazards during validation and verification of the system, has been

raised (Gill, 1995). TVC was suggested to be used as an indicator for pathogens contamination in bovine carcasses (Gill, 1995).

Furthermore, Ghafir, China, Dierick, De Zutter, and Daube (2008) showed a significant correlation between *E. coli* counts, Enterobacteriaceae counts and aerobic colony counts for cattle and pig carcasses. From the results of their study it was conducted that *E. coli* counts for pork and beef samples and Enterobacteriaceae counts in pig carcasses were significantly higher in samples contaminated with *Salmonella*. Thus, according to the above authors, *E. coli* may be considered as a good indicator for enteric zoonotic agents such as *Salmonella* for beef and pork samples. In addition, Rudy and Ingham (2009) highlighted the potential use of binary (present or absent) Enterobacteriaceae results for predicting the absence of *Salmonella* on carcasses. Specifically, the absence of Enterobacteriaceae was associated with the absence of Salmonella and, therefore, binary Enterobacteriaceae results might be useful in evaluating beef abattoir hygiene and intervention treatment efficacy.

Jericho, Bradley, Gannon, and Kozub (1992) performed microbiological testing for TVC from bovine carcasses' surface at three stages, in a slaughter and meat cutting plant: i) At the end of the slaughtering process, ii) after chilling and iii) after carcass cutting. At the fist stage, samples were taken either from ten sites of carcasses' surface, or from one site only (thigh). It was concluded from the results of this survey that, in order to evaluate the effectiveness of a Food Safety Management System (FSMS) and obtain comparable and representative results, microbiological data from a standard stage, expanding through a large time period, is required. Therefore, according to this work, sampling of ten different sites of the carcass surface may be less effective than choosing one site, although it can decrease the unevenness of bacterial distribution. Furthermore, from the results of this study the above authors came to the conclusion that, sampling at the end of the process does not determine the contribution of each stage to the problem and therefore cannot detect the cause of the failure.

Another interesting conclusion is that, microbiological results for carcasses immediately after chilling were low, whereas those after cutting, were very high (only 20 min later), as the ratio of psychrotrophic to mesophilic bacteria, is assumed to have been changed by the chilling process. A significant increase in faecal coliform count on meat cuts has been described after the de-boning process of dairy cow carcasses (Charlebois, Trudel, & Messier, 1991). In general, contamination during cutting procedure was considerable. Finally, Jericho et al. (1992) bearing in mind that the specific plant in which the survey was conducted, did not implement a HACCP system and yet, it had acceptable microbiological results, came to the conclusion that prerequisite programmes have to play an important role in food hygiene.

On the contrary, Wilhelm, Rajic, Greig, Waddell, and Harris (2011) assessed the published research investigating the effect of HACCP programmes on microbial prevalence and concentration on food animal carcasses in Australian abattoirs and came to the conclusion that HACCP implementation results to a reduction in microbial prevalence and concentration on beef carcasses. However, the effect of HACCP independent abattoir changes could not be excluded and more primary research and access to relevant data are needed to properly evaluate HACCP programme effectiveness using modelling techniques capable of differentiating the effects of HACCP from other concurrent factors.

Gill and Baker (1998) performed microbiological testing for indicators such as TVC, coliforms and *E. coli* from sheep's carcasses' surface (at forty three different sites of the surface), at four stages of the slaughter line: i) after pelt removal, ii) after evisceration and pluck removal, iii) after dressing operations and iv) after washing. From this survey it was concluded that a large number of aerobic bacteria, such as coliforms, that include *E. coli*, appear mainly at the rumps and the shoulders. The starting source of contamination is animals' skin. During evisceration, pluck removal and trimming, bacteria redistribute from the sites of initial deposition, perhaps due to equipment and personnel. Despite that, the increase of *E. coli* population at the stages of evisceration and dressing operations is not significant. Finally, washing decreases the TVC population of carcass by 0.5 log.

According to Gill and Baker (1998), in general, the hygiene characteristics of the sheep carcass-dressing process are similar to those of the beef dressing process, as they were presented earlier (Gill, Badoni, & Jones, 1996). In both processes, coliforms, largely *E. coli* were deposited on restricted areas of the carcass surface, during skinning, while their numbers were little affected by evisceration, but not affected by trimming. On the contrary, numbers of total aerobic counts were increased by evisceration and numbers of all bacteria were decreased by washing. The major differences between the processes were the narrower range of total counts from sheep than that from beef carcasses and the greater decontamination effect of washing from sheep than from beef carcasses. The difference in the ranges of total counts reflects the fact that large areas of beef carcass surface are not contacted at all by workers or equipment, whereas most of the surface of smaller sheep carcass is handled or contacted by equipment during dressing. The greater effect of washing on sheep carcasses may reflect some physical differences in the states of the contamination on the two types of carcass, such as a larger fraction of the bacteria on the sheep carcass examined being associated with particular matter that did not strongly adhere to the carcass. Finally, the hygiene characteristics of both dressing processes can be objectively assessed from relatively small sets of samples and this technique could, according to the authors, be used for Critical Control Point determination during HACCP validation.

Yang, Badoni, Youssef, and Gill (2012) performed microbiological testing for aerobes, total coliforms and *E. coli* using swab samples obtained from groups of 25 carcasses at various stages of processing at a large beef packing plant. Spraying the uneviscerated carcasses with 5% lactic acid reduced the numbers of aerobes by about 1 log unit. Subsequent carcass-dressing operations i.e. second treatment with 5% lactic acid, pasteurizing, and carcass cooling had no substantial effect upon the number of aerobes on carcasses. The total numbers of coliforms or *E. coli* cells were reduced by the washing of uneviscerated carcasses, but increased after evisceration operations. Numbers were also reduced by spraying with lactic acid and pasteurizing, with no coliforms or *E. coli* being recovered from pasteurized carcass sides. The numbers of aerobes on conveyor belts in the carcass breaking facility were similar to the numbers on cooled carcass, but the numbers of aerobes on cuts and trimmings and the number of coliforms and *E. coli* cells on the products and belts were higher than the numbers on carcasses. The findings indicate that the product can be contaminated with small numbers of *E. coli* during carcass breaking. Gill (2009) had previously suggested the adoption at all packing plants of carcass-dressing procedures and decontaminating treatments to obtain carcasses that meet a very high microbiological standard, as well as the adoption of means for limiting recontamination of product during carcass breaking.

Yalcin, Nizamlioglu, and Gurbuz (2001) performed microbiological testing for faecal coliform counts from beef carcasses surface (rump, brisket and shoulders) at four different stages: i) after dressing, ii) after evisceration, iii) after washing and iv) after chilling. From the results of this study it was concluded that there was a statistically significant increase of fecal coliform counts after dressing, while there were not significant differences in the microorganisms' population at the other stages of the procedure.

Furthermore, processing steps did not increase the faecal coliforms counts on the rump samples, while contamination level of the brisket after washing, was significantly higher than those in the other processing steps. On the contrary, Bacon et al. (2000) had previously reported that coliforms and *E. coli* populations decreased during washing and chilling. According to Yalcin et al. (2001) the inadequate washing was a major contributing factor to the contamination of the brisket site of carcasses and therefore, they suggested that proper washing programme should be implemented for the production of microbiologically clean and safe carcasses.

Reid, Small, Avery, and Buncic (2002) came to the conclusion, that the most contaminated area of beef hide was the brisket, while the least contaminated area was the rump area. During their study, microbiological testing for *E. coli* O157, *Salmonella* spp. and *Campylobacter* spp. was performed before slaughter. Samples were taken from beef hide surface at the areas of rump, flank and brisket. Transfer of the microbial contamination from hide to carcass during de-hiding could take place while making initial cuts through the skin (particularly at brisket area), because of the alternative use of the same hand for handling the hide itself and the carcass surface and during the roll-back of the hide during the process (Hudson, Mead, & Hinton, 1996). In addition, according to Reid et al. (2002), there is little doubt that the increased pathogen contamination on the hides of incoming cattle, results in at least an increased prevalence of pathogens in the abattoir environment (Byrne, Bolton, Sheridan, McDowell, & Blair, 2000) and therefore, further research is necessary in the field of how meat safety can be improved via elimination or reduction of microbial contamination on hides, prior to slaughter.

O'Connor et al. (2012) conducted an interesting systematic review to identify and summarize primary research studies that describe the prevalence of *Salmonella* spp. in pork from slaughter to cooler in the member states of the European Union (EU), Australia, Canada, Hong Kong, Japan, Korea, Mexico, New Zealand, Taiwan, and United States. Relevant studies documented Salmonella spp. prevalence at more than one processing point using the same cohort of pigs or the same production line for the post-cooler component. The carcass sampling points evaluated were stun, bleed, kill, scald, dehair, singe, polish, bung removal, evisceration, split, stamp, final wash, immediately after chill, and 18–48 h after chilling. Their findings suggested that the processing procedures generally resulted in decreased prevalence of *Salmonella* spp. as the carcasses moved towards the cooler.

Yalcin, Nizamlioglu, and Gurbuz (2004) suggested that washing and chilling could be defined as critical control points during slaughtering process. They performed microbiological testing for TVC, total coliforms, and Enterobacteriaceae from sheep carcasses surface (rump, brisket, shoulders and neck) at four different stages: i) after dressing, ii) after evisceration, iii) after washing and iv) after chilling. These results demonstrated, that there was no significant increase of microbial populations during evisceration and that decrease of microbial population during washing was not statistically significant. On the contrary, Ellerbroek, Wegener, and Arndt (1993) as well as Gill and Baker (1998) had previously reported that washing is a significant decontamination stage. Furthermore, Yalcin et al. (2004) came to the conclusion that chilling decreases the microbial populations and, therefore, could be defined as a critical control point. Finally, from the results of the latter study it was concluded that TVC and coliform populations obtained from the neck area varied significantly during the slaughtering procedure and, therefore, neck area should be tested during a hygiene surveillance procedure, such as verification.

Lenahan et al. (2009) monitored the presence of Enterobacteriaceae as indicators of faecal contamination on pig carcasses and examined the potential use of chilling as a critical control point (CCP). Porcine faecal samples and carcass swabs were collected before and after chilling at four Irish pig abattoirs and examined for Enterobacteriaceae and *E. coli* O157:H7. The data showed that overall chilling had the capacity to reduce the numbers of carcasses positive for the presence of Enterobacteriaceae and suggested it could be used as a CCP within a HACCP plan. Lenahan, O'Brien, Kinsella, Sweeney, and Sheridan (2010) came to similar conclusions on the potential influence of chilling monitoring the presence of total viable counts (TVCs), Enterobacteriaceae and *Salmonella* on lamb carcasses.

McEvoy, Sheridan, Blair, and McDowell (2004) performed microbiological testing for TVC, total coliforms, *E. coli* and Enterobacteriaceae from beef carcasses surface (hock, brisket, cranial back, bung, inside round and outside round) at eight different stages: i) after hind leg skinning, ii) hide removal, iii) bung tying, iv) evisceration, v) splitting, vi) washing, vii) chilling and viii) deboning. The aim of this study was to consider microbial contamination at a commercial beef processing facility in relation to EU performance criteria set in Decision 2001/471/EC (Anonymous, 2001) and determine the acceptability of carcass hygiene levels. It should be pointed out that according to the authors' opinion, in order to determine the performance of a HACCP system that has been introduced into a meat plant, it is first necessary to obtain baseline data outlining the initial levels of carcass hygiene. This approach was adopted in the USA by the Food Safety and Inspection Service, which carried out a nationwide baseline data collection programme before the introduction of HACCP (Anonymous, 1994). This study allowed the microbiological criteria for the subsequent HACCP programme to be formulated (Anonymous, 1996). This procedure was not followed by the EU which established performance criteria in the absence of EU wide baseline data. Therefore, the individual plants have to establish their own baseline data and measure their hygiene status against the pre-determined criteria.

Study of McEvoy et al. (2004) indicated that the use of log mean Enterobacteriaceae values will lead to an erroneous assessment of carcass hygiene since the calculation of log means assumes that the Enterobacteriaceae data are log normally distributed (Brown & Baird-Parker, 1982). Furthermore, to be a log normal distribution of values, the bacteria must be detected in more than 80% of samples (Gill & Jones, 2006; Gill, McGinnis, & Bryant, 1998). From the results of that study, it was found that more than 20% of samples resulted to Enterobacteriaceae numbers below the limit of detection. Therefore, McEvoy et al. (2004) suggest that data should be analyzed using the log of the total numbers recovered (log N) than the log mean values. Furthermore, the above authors suggest the use of *E. coli* values instead of mean values of Enterobacteriaceae as a separate attribute in a three-class control chart. Another interesting conclusion of this study was that, while TVCs remain unchanged at the cranial back site during the stages of evisceration and carcass splitting, *E. coli* and Enterobacteriaceae increased by an order of magnitude. The introduction of faecal contamination at these stages may be related to the design of the evisceration table and the contact of the carcass with the splitting stand. Furthermore, data obtained from this study showed heterogeneity of contamination on carcasses at earlier stages of processing, unlike the later stages (before chilling). It is therefore unlikely that microbiological data collected at the stage of chilling will allow corrective actions to be targeted at the specific sites contributing to unacceptable results. Moreover, EU legislation does not measure the effect of chilling as a critical control point. Bacterial numbers were either reduced or remained unchanged as a result of chilling, in this study. The reductions are likely to have occurred as a result of cell death or injury due to low a_w and temperature.

Zweifel et al. (2004) came also to the conclusion that sampling procedure described in EU legislation, i.e. Regulations 2073/2005/EU and 1441/2007/EU (Anonymous 2005, 2007) is unlikely to allow corrective actions to be targeted at the specific sites contributing to unacceptable results. These workers performed microbiological testing for TVC and Enterobacteriaceae from cattle and pig carcasses before chilling at five abattoirs. In addition, Milios, Mataragas, Pantouvakis, Drosinos, and Zoiopoulos (2011) came also to certain conclusions regarding microbiological data obtained during validation and verification of HACCP systems in slaughterhouses. These authors performed microbiological testing for TVC and Enterobacteriaceae from sheep carcasses surface (rump, flank, brisket and shoulder) at four different stages: i) after pelt removal of hind and forelegs, ii) after final pulling or complete pelt removal, iii) after evisceration and iv) before chilling. The aim of this study was to quantify the hygienic status of a lamb slaughterhouse by means of multivariate statistical analysis and demonstrate how this microbiological data could be exploited to improve the lamb slaughter process by constructing control charts. Results showed that pelt removal and evisceration were hygienically uncontrolled. Enterobacteriaceae assessed hygienic characteristics of the freshly produced lamb carcasses better than TVC, with evisceration contributing mostly to the final Enterobacteriaceae counts in the lamb carcasses at the end of the slaughter process. Finally, the above authors reported that, a wealth of information relative to hygienic status of the lamb slaughter process can be obtained by means of multivariate statistical analysis and be used in validation and verification procedures.

It is interesting to note that, according to a technical report of European Food Safety Authority (EFSA, 2010) on the assessment of the comparison of the Australian monitoring programme for carcasses to requirements in Regulation (EC) No 2073/2005 on microbiological criteria on foodstuffs, the EU sampling stage (pre-chilling) reflects the hygiene practices during carcass slaughter and dressing, while the Australian (post-chilling) reflects the level of contamination on the carcass at a later stage in the food chain and can be a poorer reflection of the slaughter line hygiene practices. In addition, Ranta et al. (2010) developed a Bayesian hidden variable model to integrate available limited data of the combined occurrence of three bacterial pathogens, namely *Listeria monocytogenes*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, with causal assumptions along three steps of pork production chain. The model was able to incorporate all data concerning different types of testing at different steps of the chain and provided a tool for quantitative risk assessments and for estimating the causal risk mitigation effects by combining external data with the specific follow-up data. Intervention effects were provided with Bayesian credible intervals indicating the uncertainty due to all information sources included in the model. Combined prevalence in Finnish pork was estimated to be 1–11% and it could be reduced to 0–2% if head was removed intact and rectum sealed off.

Wheatley, Giotis, and McKevitt (2014) examined the effects of eight processing stages (stunning, bleeding, scalding, singeing, polishing, evisceration, final inspection and chilling) on contamination levels of total viable counts (TVC), Enterobacteriaceae and *Salmonella* spp. In total, 95 carcasses from an Irish pork slaughter plant were sampled by swabbing 100 cm^2 of surface at three sites (belly, ham and jowl). There were significant reductions in TVC for all sites after scalding and singeing whilst there was a significant increase in counts after polishing and evisceration compared with preceding stages. Enterobacteriaceae counts indicated hygienic weak points in the examined slaughter plant, leading to faecal (cross)-contamination with elevated counts after stunning, bleeding and evisceration, compared to final counts after chilling. The results showed that contamination can be introduced at various steps in the process and highlighted the importance of monitoring locations other than those required by legislation within the process. Therefore, Wheatley et al. (2014) came to the conclusion that monitoring can be used to establish baseline levels for high-risk stages specific to each plant and to assess the effectiveness of additional interventions.

Finally, Luning et al. (2011) suggested the usefulness of a concurrent diagnosis of Food Safety Management System (FSMS) performance and food safety (FS) output using new tools. Performance is commonly analyzed by checking compliance against preset requirements via audits/inspections, or actual food safety output is analyzed by microbiological testing. *L. monocytogenes*, *Salmonella* spp., *Campylobacter* spp. (food safety indicators), *E. coli*, Enterobacteriaceae (hygiene indicators), and TVC (overall performance) were analyzed at ten critical sampling locations covering both product and environmental samples, using the Microbial Assessment Scheme diagnosis. Riskiness of FSMS context and performance of core FSMS activities were assessed using a diagnostic tool (including 51 indicators and corresponding grids with level descriptions). For the (large) beef meat processor, the FS output diagnosis showed too high TVC, but the high activity scores of their FSMS indicated that this problem could be only solved by supplier measures. The FS output diagnosis of the (small) lamb meat processor showed various contamination problems (but no pathogens) corresponding to various low activity levels in combination with the high-risk context. The combined diagnosis provided clear directions for improvement to move towards more advanced FSMS activity levels or to reduce associated riskiness.

3. Carcass sampling methods

As mentioned in Section 2, carcass sampling and microbiological analysis is widely used for validation and verification purposes. The method used for the recovery of bacterial load is of great importance and should be the most efficacious for recovering the most representative counts of the microbial flora on carcasses, in order to come to solid conclusions on hygiene performance. Microbiologists have been attempting to develop and improve red meat carcass sampling methods since 1930s. Many methods have been developed and evaluated. Non-destructive sampling methods include adhesive contact tape, swabbing, rinsing, direct agar contact, scraping and vacuuming. Various materials for swabbing of carcass surface have been extensively considered (Dorsa, Cutter, & Siragusa, 1996). None of these methods yields 100% recovery of the bacteria present on a carcass surface, when compared to excision (Buncic, 2006; Dorsa et al., 1996; Ingram & Roberts, 1976). In spite of that, non-destructive methods are usually preferred in meat industry, as the excision method damages the carcass surface, affecting its commercial value.

Dorsa et al. (1996) evaluated several non-destructive methods that could be used to sample red meat animal carcasses, against the excision method, such as swabbing with sterile cotton tipped wooden swabs, sterile cheesecloth and sterile sponges. The above authors performed microbiological analyses on samples taken using the four techniques mentioned above, from random beef carcasses and beef tissue inoculated with bovine faeces. From the results of this study, it was concluded that, non-destructive methods can lead to lower bacterial recovery, compared to excision method. In particular, swabbing with cotton tipped swabs does not appear to be a desirable way to sample carcasses, while swabbing with sponges has better results. Moreover, swabbing with sterile cheesecloth led to results that were not significantly lower than excision. Finally, differences were reduced when tissue was highly contaminated. Thereby, Dorsa et al. (1996) came to the conclusion that it is unlikely that excision will ever be a practical

sampling method for a processing plant monitoring programme, while swabbing with sponges is capable of bacteria recovery, proportional to that of excision. Consequently, swabbing with sponges is an adequate method of carcass sampling for rapid process monitoring to detect faecal contamination.

Dorsa, Siragusa, Cutter, Berry, and Koohmaraie (1997) conducted another study regarding aerobic bacteria, *E. coli* O157:H7 and *Salmonella typhimurium* recovery from beef carcass surface tissue, using the sponge swabbing and excision techniques at three points of the processing line: i) pre-wash, ii) post-wash and iii) after chilling. The results of this study proved that aerobic bacteria recovery using the non-destructive technique was lower than that of the excision, but on the other hand, bacterial counts were proportional to the excision counts. Therefore, swabbing with sponges could be a practical sampling method estimating aerobic bacterial populations during the slaughtering procedure and hygiene evaluation. Moreover, Dorsa et al. (1997) concluded that the swabbing technique was very effective for *E. coli* O157:H7 and *S. typhimurium* recovery, even when present at very low levels (1 cfu/100 cm^2). Thus, sponge swabbing can be considered to be an effective way to sample carcasses in a slaughterhouse facility, using standard culture methods or even as a sampling method for rapid immunoassay tests. It is interesting to point out that, according to other researchers, abrasive sampling carcass methods can be as effective for bacterial recovery as destructive methods (Gill & Jones, 2006).

Byrne, Dunne, Lyng, and Bolton (2005) investigated the equivalence of excision and a polyurethane sponge swabbing technique for the recovery of TVC and Enterobacteriaceae, according to 2001/471/EC Decision (Anonymous, 2001), embedded in Regulations 2073/2005/EC and 1441/2007/EC (Anonymous, 2005, 2007). The latter sets microbiological criteria for slaughtering hygiene and permits the use of procedures other than excision if it is demonstrated that these are at least equivalent. Byrne et al. (2005) performed microbiological evaluation of bovine and ovine carcasses by obtaining TVC and Enterobacteriaceae counts using excision and swab sampling method. Samples were taken from four different sites of the carcasses: i) the neck, ii) brisket, iii) flank and iv) rump for cattle and the i) brisket, ii) breast, ii) flank and iv) thorax for sheep. The latter study demonstrated that there were no significant differences between excision and swab TVC and Enterobacteriaceae counts at different sites on bovine and ovine carcasses. This finding provides further evidence that, when factors such as operator-related differences, animal species, sponge materials and sampling locations are controlled, swabbing with a sufficient abrasive material may be as effective as excision for bacterial recovery of carcasses. Furthermore, some researchers (Bolton et al., 2002; Gill & Jones, 2006) have reported that, for some enteric bacteria which have low incidence and uneven distribution on meat carcasses, swabbing with sponges may be more effective than excision, because excision technique samples a smaller area (typically 25 cm^2) than swabbing (typically 100 cm^2).

Lindbland (2007) compared the numbers of total aerobic counts, Enterobacteriaceae, and *E. coli*, recovered by swabbing four carcass sites with gauze (total area 400 cm^2) to those obtained by excision at equivalent sites (total area 20 cm^2). This study suggests that the same process hygiene criteria, as those stipulated for excision, can be used for swabbing with gauze without compromising food safety. For monitoring of low numbers of Enterobacteriaceae and *E. coli*, like those found on swine carcasses at Swedish abattoirs, the results also showed that swabbing of a relatively large area is superior to excision of a smaller area.

Ghafir and Daube (2008) compared the Belgian swabbing sampling method for pig carcasses, covering 600 cm^2 on each half-pig carcass, to the reference destructive method with regard to *E. coli* and aerobic plate counts. They also evaluated *Salmonella* and *Campylobacter* prevalence and their relationship. Recovery was significantly lower for the swabbing method and corresponded to a recovery of 36% for *E. coli* counts and 81% for aerobic plate counts in comparison with the destructive method. On the contrary, there was no significant difference between the swabbing and destructive sampling methods for the prevalence of *Salmonella* or *Campylobacter*. The authors came to the conclusion that the method of swabbing used, is efficient for the sampling of pig carcasses in comparison with the reference destructive method. In addition, Martinez et al. (2009) came to different conclusions during their research. Samples from 240 carcasses were collected from four animal species (porcine, ovine, bovine and equine). Two samples were taken from each carcass, one using the excision method and the other the wet–dry swabbing one. Eight areas from each carcass were sampled. TVC and *E. coli* by excision method revealed statistically significant differences compared to wet–dry swabbing method, while no significant linear relationship was found between carcass surface bacterial counts obtained by wet–dry swabbing method and excision one.

According to a technical report of European Food Safety Authority (EFSA, 2010) and regarding sampling methodology, the EU system requires excision sampling for Aerobic Colony Counts and Enterobacteriaceae, while the Australian system specifies a swabbing approach for Aerobic Colony Counts and *E. coli*. Excision is generally considered having a higher recovery rate of microorganisms. The Australian system is also less sensitive than the EU system as the area sponged is smaller.

Gill and Badoni (2010) investigated the variability of the results when samples are collected by different people using the same procedure. Each carcass, in groups of 25, pig, cattle, or bison carcasses was sampled by five people i.e. two or three people experienced with carcass sampling and two or three without previous experience. Each person sampled a different, randomly selected, site on a dressed carcass side by swabbing an area of approximately 100 cm^2 with a moistened synthetic sponge. The numbers of aerobic bacteria, coliform bacteria, and *E. coli* recovered from each sample were determined. The findings indicated that the numbers of bacteria recovered from carcasses by swabbing with sponges are unlikely to differ substantially as a result of samples being collected by different people using the same procedure.

Ingham, Algino, Ingham, and Rudy (2009) compared the USDA sample preparation procedure with repeatedly squeezing the sponge during a 10 s interval to expel the sample fluid. The USDA sample preparation procedure requires that beef carcass sponge samples are mechanically stomached for 2 min before the sample fluid is squeezed out for analysis. When a large number of sponge samples must be analyzed, the stomaching step can limit throughput. Separate sponge samples were obtained from each half of 100 chilled post intervention beef carcasses from a large-volume abattoir during a 4-month period. All sponge samples were analyzed for *E. coli*, coliforms, Enterobacteriaceae, and *aerobic mesophilic bacteria*. The results suggested that manual squeezing may be a simple and rapid alternative sample preparation method when gram-negative bacteria such as *E. coli*, coliforms, or Enterobacteriaceae are enumerated from beef carcass sponge samples used to monitor operational abattoir hygiene. Finally, it should be noted that, at present, a technical revision of ISO 17604:2003/Amd 1:2009 standard (ISO, 2009) on carcass sampling for microbiological criteria is being prepared in order to accommodate further improvements.

4. Conclusions

For the proper implementation of HACCP principles, it is essential to rely on microbiological data obtained during the

validation of the system. Moreover, the HACCP system should be verified during implementation. The aim of verification is to confirm that the system is working properly and the hazards are effectively kept under control. Indicator microorganisms are used to demonstrate the general hygiene and contamination of faecal origin. TVC, *E. coli* and Enterobacteriaceae counts are suggested to be used as an indicator for pathogens contamination in carcasses. Thus, there is no proven correlation between indicator organisms and prevalence/levels of pathogens. Therefore, the microbiological data based on the indicators should be interpreted only as to assess general trends in the hygiene process of the operator, in order to take corrective action.

However, microbiological results which are obtained only at the end of the slaughtering process, do not provide information on the cause of the problem. Therefore, 'Process-based' microbiological criteria, which are based on characteristics measured at various stages of the process, including final carcass values, should be used. A wealth of information relative to hygienic status of the slaughter process can be obtained by means of multivariate statistical analysis and be used in validation and verification procedures. Furthermore, in order to implement an adequate monitoring system, non-destructive techniques of carcass sampling should be used instead of excision. The microbial recovery may be lower, but it is proportional to the excision recovery and therefore, non-destructive techniques, like swabbing with sponges could be a practical sampling method, for the estimation of indicators during the slaughtering procedure and hygiene evaluation.

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