International Journal of Food Microbiology 146 (2011) 170–175

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Evaluation of control over the microbiological contamination of carcasses in a lamb carcass dressing process operated with or without pasteurizing treatment

K. Milios [a], M. Mataragas [b,*], A. Pantouvakis [c], E.H. Drosinos [b], P.E. Zoiopoulos [d]

[a] Veterinary Service, Prefectural Administration of Aitoloakarnania, 47 Iroon Politechniou, GR-302 00 Mesologgi, Greece
[b] Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Food Quality Control and Hygiene, Iera Odos 75, GR-11855 Athens, Greece
[c] University of Piraeus, 80 Karaoli & Dimitriou, GR-185 34 Piraeus, Greece
[d] Laboratory of Animal Science, School of Management of Natural Resources and Enterprises, University of Western Greece, 2 G. Seferi, GR-301 00 Agrinio, Greece

ARTICLE INFO

Article history:
Received 5 July 2010
Received in revised form 7 February 2011
Accepted 18 February 2011

Keywords:
Hygiene
Lamb
Multivariate analysis
Quality
Slaughterhouse
Steam application

ABSTRACT

The aim of this study was to quantify the hygienic status of a lamb slaughterhouse by means of multivariate statistical analysis, to demonstrate how the microbiological data could be exploited to improve the lamb slaughter process by constructing control charts and to evaluate the potential effect of an intervention step such as steam application on the microbiological quality of lamb carcasses. Results showed that pelt removal and evisceration were hygienically uncontrolled. TVC and *Enterobacteriaceae* progressively increased from the stage 'after pelt removal of hind and forelegs/before final pulling' to the stage 'after evisceration/before pluck removal' thus indicating possible deposition of microorganisms during these operations. It seems that the processing stages of freshly produced carcasses were better distinguished by *Enterobacteriaceae*, with evisceration contributing mostly to the final *Enterobacteriaceae* counts. Application of steam during the lamb slaughter process reduced microbial counts without adverse effects on the organoleptic characteristics of the carcasses. Moreover, the construction of control charts showed that decontamination with steam contributed to the maintenance of an in control process compared to that before the application of steam, suggesting the potential use of steam as an intervention step during the lamb slaughter process.

1. Introduction

The lamb slaughter process includes operations with or without intervention that affect microbial contamination originated from fleece and visceral contents (Bolton et al., 2001). Prerequisite programmes and the Hazard Analysis Critical Control Point System (HACCP) are applied to intervene towards controlling carcass contamination. Pelt removal of hind and forelegs, final pulling or complete pelt removal, evisceration, pluck removal, steam application or hot water washing, chilling, chilled and frozen storage, and metal detection are potential critical points for the microbial contamination of lamb carcasses during slaughter process.

The effectiveness of steam application for decontaminating carcasses is well-known for pork (Gill and Jones, 2006) and beef (Bolton et al., 2001; Gill and Bryant, 1997; Nutsch et al., 1997; Nutsch et al., 1998) but such data are limited for lamb. It should be noted that application of steam or hot water washing are not performed in Greek lamb slaughterhouses due to concerns for organoleptic characteristics of the carcasses, i.e. lean and fat appearance, color, odor or overall acceptability of the carcasses.

Microbiological records obtained by the HACCP system are hardly exploited further. Control charts are infrequently used in the food industry to supervise the microbiological quality of the produced foods. Statistical Process Control or Statistical Quality Control (SPC or SQC) should be introduced in a food safety management system to evaluate processes being in or out of control (Augustin and Minvielle, 2008).

Microbial counts as hygiene or quality indicators are used in meat slaughterhouses for evaluating the effective application of the HACCP system. Commission Regulations (EC) No. 2073/2005 and 1441/2007 report that Total Viable Counts (TVCs) and *Enterobacteriaceae* should be used as hygiene indicators of freshly produced lamb carcasses. The above legislation requires the microbiological testing of the carcasses prior to chilling and the use of control charts, e.g. the cumulative count control charts for *Salmonella* presence on carcasses to monitor the slaughterhouses hygiene (Anonymous, 2005; 2007).

Multivariate statistics may serve as a tool to determine or even to reassess reported critical control points, to determine which microbiological parameters should be analyzed in order to evaluate product quality, to update an existing food safety system and finally to identify operations, which have an impact on the microbiological quality of the carcasses. Therefore, the objective of the present study was to evaluate, by means of statistical tools, the control over carcass microbiological contamination in a lamb dressing process, operating with or without a pasteurizing treatment.

* Corresponding author. Tel.: +30 210 529 4683, +30 210 529 4704; fax: +30 210 529 4683.
E-mail address: mmat@aua.gr (M. Mataragas).

0168-1605/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2011.02.023

2. Materials and methods

2.1. Experimental design and sample collection

2.1.1. Data set 1

The study was carried out at an EU-approved medium-scale slaughterhouse with 3 process lines (cattle, sheep and pigs) at the district of Aitolia in Western Greece. The facility has a total annual capacity of 800,000 kg of meat. The sheep slaughter line is basically used for the slaughter of lambs (up to a maximum carcass weight of 15 kg). The slaughterhouse was visited 20 times over a five-month period (i.e. one day per week for 20 weeks). Swabs from three different carcasses were sampled at each visit corresponding to the beginning, middle and end of the lamb slaughterhouse operation. Carcass sampling was performed using the non-destructive method, i.e. swabbing with sponges (Anonymous, 2001; 2005). The same carcass and the same sites, namely rump, flank, brisket and shoulder, were sampled at four different processing steps i.e. after pelt removal of hind and forelegs/before final pulling (stage A), after final pulling or complete pelt removal/before evisceration (stage B), after evisceration/before pluck removal (stage C) and after pluck removal/before chilling (stage D). This amounts to 20 visits × 3 carcasses × 4 processing steps = 240 swabs in total. A pooled sample consisted of eight sponges corresponding to a sampling carcass area of 800 cm^2, i.e. 4 sampling sites × 100 cm^2 per sampling site × 2 sides of carcass. Sponging at each carcass site consisted of 10 vertical passes (up and down being considered as one pass) and 10 horizontal passes (side-to-side being considered as one pass) with a pressure equivalent to those would be used to remove dried blood from the carcass (Lenahan et al., 2010). After expelling the excess air, the stomacher bags with sponges were folded down. The samples were transferred with isothermal iceboxes to the laboratory, stored at 0–4 °C and analyzed within 24 h.

2.1.2. Data set 2

Steam was applied on lamb carcasses as an intervention step. It was performed after pluck removal and immediately before chilling. Steam application consisted of 8–10 vertical passes of the steam spraying pistol (Crown, CS 160 H, Athens, Greece) pointing at each side of the carcass (up and down being considered as one pass). The critical limits applied are those used for beef and pork decontamination, i.e. atmospheric temperature inside steam chamber *ca.* 90 °C and duration of steam application *ca.* 8–10 s, since available data for lamb carcasses were limited (Bolton et al., 2001; Gill and Bryant, 1997; Nutsch et al., 1997; Nutsch et al., 1998). Samples were collected before and after steam application by the method used for Data set 1 and handled as mentioned above.

2.2. Microbiological analysis

Samples were analyzed for Total Viable Counts (TVCs) and *Enterobacteriaceae*. An aliquot of 100 ml of sterile 0.1% (w/v) saline peptone water (0.1% peptone and 0.85% NaCl) was added into a stomacher bag containing a pooled sample and homogenized in a stomacher (Lab Blender, Seward, London, UK) at low speed and room temperature for 2 min. Serial decimal dilutions in Ringer solution were prepared and 0.1 or 1 ml samples of appropriate dilutions were spread or poured on agar plates, respectively. TVCs were determined on Plate Count Agar (PCA, Merck, Darmstadt, Germany), incubated at 30 °C for 72 h and *Enterobacteriaceae* in Violet Red Bile Dextrose Agar (VRBDA, Merck, Darmstadt, Germany), overlaid with 5 ml of the same medium and incubated at 37 °C for 24 h.

2.3. Preliminary evaluation of slaughterhouse hygiene

Slaughterhouse hygiene was preliminarily evaluated by means of a scoring system, namely the Hygiene Assessment System (HAS) (Anonymous, 1999; Pawsey, 2002; Pinillos and Jukes, 2008). The HAS system is divided into 5 sections: A) ante-mortem procedures, B) slaughter and dressing procedures, C) personnel and practices, D) maintenance and hygiene of premises and E) general conditions and management. The HAS score was: 0, high risk – seriously defective practices; 30, defective practices; 60, normally satisfactory/occasionally defective practices; and 100, minimum risk – always well done practices (Pawsey, 2002).

2.4. Statistical analysis

2.4.1. Lamb slaughter process including only non intervention (pelt removal and evisceration) slaughter control points

All bacterial counts were transformed to logarithmic values ($\log_{10}$ cfu/cm^2). Values for the mean $\log_{10}$ (X_{mean}) and the standard deviation (*SD*) of the log values were calculated for each data set on the assumption of a log Normal distribution of the counts (Gill et al., 1998; Gill and McGinnis, 1999). Compliance of the log-transformed values with a Normal distribution as well as the skewness and kurtosis of the fitted Normal distribution were checked with the Anderson–Darling test. A value for the $\log_{10}$ of the data set arithmetic mean (log*A*) was also calculated with the formula (Kilsby and Pugh, 1981):

$$logA = X_{mean} + \left[ln(10) \times \left(SD^2 / 2 \right) \right] \quad (1)$$

Descriptive analysis of data sets was done to provide a global view of their distribution and to test their normality. Correlation between TVC and *Enterobacteriaceae* was tested by means of Pearson correlation (r).

Multivariate analysis of variance (MANOVA) and discriminant function analysis (DFA) were performed to investigate how the different processing steps affect the contamination and the effectiveness of monitoring with TVC and *Enterobacteriaceae*. Significant differences between the sampling points may indicate potential critical points along the lamb slaughter process (Gonzalez-Miret et al., 2006).

Multilevel modeling and multiple regression analysis were carried out with the *Enterobacteriaceae* data to study the rate of the variable change and to predict microbial count changes (Lekroengsin et al., 2007):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_\nu X_\nu + \ldots + \varepsilon \quad (2)$$

where Y is the *Enterobacteriaceae* counts in stage D (dependent variable), X is the *Enterobacteriaceae* counts in the different processing stages A to C during the three production times (independent variable), β_0 is the regression coefficient, β_ν is the regression coefficient of any independent variable X_ν, ν is the number of independent variables and ε is the residual error.

2.4.2. Lamb slaughter process including non intervention (pelt removal and evisceration) and intervention (steam application) slaughter control points

In addition to the above descriptive analysis, TVC and *Enterobacteriaceae* were compared by means of dependent *t*-test since the same carcasses were sampled before and after steam application. Control charts were also constructed to detect any increase in the counts of the *Enterobacteriaceae* and to evaluate the lamb slaughterhouse hygiene (Anonymous, 2006a, 2006b; Montgomery, 2000). These included individual (X_i) control chart accompanied by a moving range (*MR*) control chart and cumulative sum (*CUSUM*) control chart accompanied by *CUSUM* signal chart (Beauregard et al., 1992; Jarvis, 2008). *CUSUM* chart was used because it is more sensitive to changes in the production process. The data set 1 was used to construct the parameters of the control charts (central value and critical limits). Then, the control charts were reset and the new data obtained from

the intervention experiments (data set 2) were plotted to detect changes in the performance of the lamb slaughterhouse hygiene. Distribution of the microbial counts was determined using the @Risk v4.5 (Palisade Corp., New York, USA) while the statistical analysis was performed using the SPSS v15.0 (SPSS, Inc., Chicago, Ill., USA). All the calculations were carried out in the Microsoft Excel 2007 (Microsoft, Redmond, WA, USA).

2.5. Organoleptic evaluation

The organoleptic characteristics of the carcasses treated with steam were assessed by five experienced people in the field of lamb meat market, through a questionnaire. Each question was a statement about lean and fat appearance, color, odor as well as overall acceptability of the carcasses after 1, 2 and 3 days of the application of steam. A five-point Likert scale, ranging from 1 – being the highest quality score as "no effect of steam on organoleptic characteristics of carcasses" – to 5 – being the lowest quality score as "maximum (negative) effect of steam on the organoleptic characteristics of carcasses" was followed.

3. Results and discussion

3.1. Preliminary evaluation of slaughterhouse hygiene

The level of the hygienic conditions in the abattoir was preliminarily evaluated with the HAS. It is based on the assessment of the risk associated with the activities involved during lamb slaughter process. The HAS score was 45.7 indicating usually defective/occasionally satisfactory practices. The majority of the problems was identified in the section C (sub-score 0.9) and E (sub-score 3.5), especially in management with a very low score. Section A (sub-score 5.6), B (sub-score 24.4) and D (sub-score 11.25) were marked relatively high. Certain problems occurred in section B, due to the fact that specific activities such as washing of carcasses or existence of intervention steps, i.e. steam application or hot water washing, were given zero mark. Such operations are not applied in Greek lamb slaughterhouses because it is believed that the application of water causes adverse effects on the appearance of carcasses, making them undesirable to consumers.

3.2. Lamb slaughter process including only non intervention (pelt removal and evisceration) slaughter control points

The distribution of the microbial counts did not significantly deviate from normality (P-value>0.05, Table 1) for all processing stages. The X_{mean} for TVC and *Enterobacteriaceae* progressively increased in each subsequent processing step after the first sampling point (stage A). Differences were observed in the *SD* between the processing steps for both TVC and *Enterobacteriaceae*. Therefore, for each microbiological parameter, the X_{mean} values between stages cannot be directly compared and so the log*A* values should be used instead, which include the variance of the microbiological counts (Kilsby and Pugh, 1981).

In general, there was a moderate correlation between TVC and *Enterobacteriaceae* counts. The r values were 0.40, 0.64, 0.57 and 0.53 (P-value <0.001) for the stage A, B, C and D, respectively. Although this moderate correlation could be indicative of inadequate handling of carcasses, the evaluation of the process should be based on enumeration of *Enterobacteriaceae* or on both *Enterobacteriaceae* and TVC, rather than on enumeration of TVC only. Otherwise erroneous conclusions on the hygienic performance of the various operations could be drawn. The absence of strong correlation between TVC and *Enterobacteriaceae* (>0.7–0.8), which characterize the microbiological quality of carcasses and the lamb slaughter process hygiene, suggests that these microbiological parameters provide different information. Indeed, *Enterobacteriaceae* are widely used as a hygiene indicator microorganism, whereas TVC provide mainly information relative to shelf life. Gill et al. (1996) found a weak correlation (0.21) between a subgroup of *Enterobacteriaceae* (*Escherichia coli*/coliforms) and aerobic counts during the assessment of a beef carcass dressing process. Spoilage microorganisms such as *Pseudomonas* spp., and acinetobacteria/moraxellae were present as major fractions of most microflora. *Enterobacteriaceae* together with lactobacilli and *Brochothrix thermosphacta* were the minority. Moreover, *Enterobacteriaceae* as well as *E. coli*/coliforms are accepted indicators for fecal contamination and hence for the potential presence of enteric pathogens (International Commission on Microbiological Specifications for Foods, ICMSF, 1988).

Table 1
Statistics for TVC and *Enterobacteriaceae* counts obtained from 60 randomly sampled carcasses during the lamb slaughter process including only non intervention (pelt removal and evisceration) slaughter control points.

Microbiological parameter	Processing step[a]	Distribution[b] and arithmetic mean ($\log_{10}$ cfu/cm^2)			
		X_{mean}	*SD*	95% confidence interval	log*A*
TVC	A	3.05	0.58	1.92 to 4.19	3.44
	B	3.75	0.71	2.36 to 5.14	4.33
	C	4.23	0.63	3.00 to 5.46	4.68
	D	4.37	0.55	3.30 to 5.44	4.71
Enterobacteriaceae	A	0.76	0.80	0.10 to 2.33	1.49
	B	2.27	0.53	1.23 to 3.30	2.59
	C	2.68	0.62	1.47 to 3.89	3.12
	D	2.90	0.55	1.82 to 3.97	3.24

[a] A, after pelt removal of hind and forelegs/before final pulling; B, after final pulling or complete pelt removal/before evisceration; C, after evisceration/before pluck removal; and D, after pluck removal/before chilling.
[b] Normal distribution of microbial counts at all stages (P-value>0.05).

The results from MANOVA showed that the counts between the various processing steps differed significantly (P-value<0.001). Furthermore, ANOVAs for dependent variables also showed significant changes between the different sampling points (P-value<0.001). Pairwise comparisons between processing stages revealed significant differences in TVC (P-value<0.001) and *Enterobacteriaceae* (P-value<0.001). Their counts progressively increased from the stage A to the stage C but no change was observed from the stage C to the stage D (P-value = 0.99 and 0.36 for TVC and *Enterobacteriaceae*, respectively). The pelt removal and evisceration processes indicated possible contamination, since increase in microbiological counts on carcasses were observed. The occurrence of *Enterobacteriaceae* counts (≥2.0–3.0 $\log_{10}$ cfu/cm^2) suggests that the pelt removal and evisceration operations were hygienically uncontrolled. TVC counts fell within the marginal limits in both operations. It is evident that if the latter were taken into account to assess the hygienic status, this would result in erroneous conclusions (Table 1). The hygiene performance criteria used were based on the Commission Regulation (EC) No. 2073/2005 and its amendment (EC) No. 1441/2007 (Anonymous, 2005; 2007). The recommended method is that of the collection of samples by excision whereas, in the case of swabbing, the criteria are different. It is assumed that the swab samples amount up to the 20% or less of the counts obtained by excision (Anonymous, 2001). Therefore, the swab criteria for TVC on lamb were ($\log_{10}$ cfu/cm^2): acceptable<2.8, marginal 2.8–4.3 and unacceptable>4.3, whereas for *Enterobacteriaceae,* were: acceptable<0.8, marginal 0.8–1.8 and unacceptable>1.8 (Lenahan et al., 2010). These findings further support the previous observations made for TVC and *Enterobacteriaceae* regarding their use for the hygienic characterization of lamb carcasses. Finally, the significant increase, observed after pelt removal and evisceration, could suggest that these steps constitute critical points.

DFA identified the first two variates as significant (P-value for variate 1<0.001 and for variate 2 = 0.008) to discriminate the groups, i.e. processing stages. However, the first variate accounted for the majority of variance (97.7%), whereas the second variate accounted

for only 2.3%. Group differences shown by MANOVA can be explained in terms of one underlying dimension. The results also showed that *Enterobacteriaceae* had a greater contribution to the variate 1 than the TVC, but the opposite was true for the variate 2. Hence, the following conclusions can be derived from the MANOVA and DFA: (i) the processing steps during lamb slaughter process, especially the pelt removal and evisceration, are likely to affect the contamination of the carcasses (deposition of bacteria), (ii) the extent of contamination can be measured by both TVC and *Enterobacteriaceae* (two significant variates) and (iii) the group separation can be best explained in terms of one underlying dimension, which in this context was likely to be *Enterobacteriaceae* (greater contribution to the first variate than TVC). *Enterobacteriaceae* counts could be used to discriminate between all groups, while TVC could not. Therefore, *Enterobacteriaceae* better differentiated the processing stages of freshly produced carcasses.

Enterobacteriaceae data were analyzed to describe the changes of their counts over time (multilevel growth models) and to predict their numbers at the end of slaughter process on lamb carcasses, i.e. stage D (multiple regression). In this context, time could be considered the different stages sampled. *Enterobacteriaceae* counts progressively increased from the baseline hygienic status at stage A (or time 0) onwards. The results showed that the trend in the data is best described by a second-order polynomial (P-value<0.001). This reflects the initial high increase in *Enterobacteriaceae* counts from stage A to stage B but a lower increase in *Enterobacteriaceae* counts at the subsequent stages. A large variability was observed in the *Enterobacteriaceae* counts at stages across carcasses as reflected by the significant variance in intercepts [$\mathrm{var}(u_{oj}) = 0.47$, P-value = 0.001]. Significant changes in *Enterobacteriaceae* counts over time, reflected by the significant variance in slopes [$\mathrm{var}(u_{1j}) = 0.03$, P-value = 0.01], was also observed. The covariance between the slopes and intercepts, $\mathrm{cov}(u_{oj}, u_{1j}) = -0.78$, P-value<0.01, suggests that as intercepts increased, the slope decreased. This means that carcasses with good hygienic status at the beginning (stage A) of slaughter process (low intercepts) were highly affected by slaughter operations (steep positive slope) resulting in low hygienic status at the end of the process (stage D). Carcasses with low hygienic status at stage A (high intercepts), were little affected by slaughter operations (flatter slope). This information was not obvious from the previous analysis.

The multiple regression model was developed to quantify the changes in the microbiological counts in the four different sampling points and also to evaluate the effect of the different production times, i.e. beginning, middle and end of the lamb slaughterhouse operation, on carcasses contamination levels. The results showed that the *Enterobacteriaceae* counts in stage C (after evisceration/before pluck removal), irrespective of the production time (P-value = 0.52), was the determinant stage (P-value<0.001) for the prediction of the carcasses contamination rate at the end of the lamb slaughter process. The inclusion of the stage A and B did not make a significant contribution to predicting the outcome (P-value = 0.20). The regression coefficient (β) for the stage C was 0.68 (P-value<0.001) yielding the following equation:

$$Ec_D = 1.90 + 0.68 \times Ec_C \quad (3)$$

where Ec_D and Ec_C are the *Enterobacteriaceae* counts in lamb carcasses at stage D and C, respectively.

The above regression coefficient for the stage C indicates the individual contribution of the predictor to the model or the relationship between the outcome and the predictor. However, the standardized form of the regression coefficient provides a better insight into the importance of the predictor in the model. The standardized β (0.76) indicates the number of *SD*s that the outcome will change as a result of one *SD* change in the predictor. The value indicates that as the *Enterobacteriaceae* counts increase by one *SD* (0.62) in stage C, *Enterobacteriaceae* counts increase by 0.76 *SD*s in stage D. The *SD* in stage D is 0.55 and so this constitutes a change of 0.42 $\log_{10}$ cfu/cm^2 in the *Enterobacteriaceae* counts (0.76×0.55). Therefore, it could be concluded that in the present work *Enterobacteriaceae* counts in lamb carcasses at stage C were a significant predictor of *Enterobacteriaceae* counts in lamb carcasses at the end of lamb slaughter process.

3.3. Lamb slaughter process including non intervention (pelt removal and evisceration) and intervention (steam application) slaughter control points

Carcasses decontamination was performed with steam at stage D to investigate its effect on the lamb carcasses hygienic and organoleptic characteristics. Steam is not applied in Greek lamb slaughterhouses for carcass appearance reasons, as it has already been mentioned. However, no adverse effects on the above characteristics were observed as evaluated by the five member panel after 1, 2 and 3 days of the steam application and all questions were marked by score 1.

In Table 2 the results of the distribution of the microorganisms before and after carcasses decontamination with steam are presented. The distribution of the microbiological counts before and after steam application did not significantly deviate from normality (P-value>0.05). The X_{mean} for TVC and *Enterobacteriaceae* counts decreased after the application of steam. However, differences in the *SD* were observed and the log*A* values before and after steam application were calculated with the Eq. 1. After steam application almost 1 $\log_{10}$ cfu/cm^2 reduction was observed in *Enterobacteriaceae* counts (P-value<0.001), whereas the effect was smaller on TVC (reduction by 0.7 $\log_{10}$ cfu/cm^2) (P-value<0.001). James et al. (2000) also applied steam to lamb carcasses for 8 s with an atmospheric steam vessel and they obtained 1 $\log_{10}$ cfu/cm^2 reduction in total bacterial counts, without adversely affecting the lean appearance, colour, odour or the overall acceptability of the lamb carcasses.

Few works have been conducted with reference to the application of Statistical Process Control (SPC) to microbiological data (Augustin and Minvielle, 2008; Gill and Jones, 2006; Hayes et al., 1997; Murphy et al., 2005; Srikaeo and Hourigan, 2002). Hence, in order to evaluate the lamb slaughterhouse hygiene and to detect increase in the microbial counts (out of control process), the data from *Enterobacteriaceae* counts, before and after intervention, were used to develop control charts. To achieve this, the data collected immediately before chilling were analyzed further. Compliance with Commission Decision (EC) 471/2001 and its amendment (EC) 379/2004 requires the microbiological testing of carcasses prior to chilling (Anonymous, 2001, 2004). Control charts such as the individual (X_i) accompanied by a moving range (*MR*) (classical Shewhart control charts) and the cumulative sum (*CUSUM*) were constructed. Although many microbiological data are collected from survey studies and samples examination, no consideration is made regarding their further application. When microbiological counts with levels above the

Table 2
Statistics for TVC and *Enterobacteriaceae* counts obtained from 60 randomly sampled carcasses during the lamb slaughter process including non intervention (pelt removal and evisceration) and intervention (steam application) slaughter control points.

Microbiological parameter	Processing step[a]	Distribution[b] and arithmetic mean ($\log_{10}$ cfu/cm^2)				
		X_{mean}	*SD*	95% confidence interval	log*A*	Δlog*A*[c]
TVC	BSA	5.89	0.31	5.27 to 6.51	6.00	−0.72
	ASA	5.07	0.43	4.23 to 5.90	5.28	
Enterobacteriaceae	BSA	3.74	0.51	2.73 to 4.74	4.04	−0.95
	ASA	2.67	0.60	1.49 to 3.85	3.09	

[a] BSA, before steam application; and ASA, after steam application. Steam was applied after pluck removal and immediately before chilling.
[b] Normal distribution of microbial counts at all stages (P-value>0.05).
[c] Difference in log*A* before and after steam application.

detection limit that follow a normal distribution are available, their levels ($\log_{10}$ cfu/cm^2) can be used to construct classical Shewhart control charts.

The X_i and *MR* control charts were chosen because their construction is simple and are commonly used to monitor a process based on the microorganism levels (Anonymous, 2006a; 2006b). The first chart merely involves the plotting of the individual results over time while the second involves the difference between the present sample result X_i and the previous sample result X_{i-1} ($X_i - X_{i-1}$, $i = 2, 3,\ldots, n$). To develop the aforementioned control charts, baseline data should be collected. The data set 1 was used to create the baseline control charts and to calculate the central value and critical limits. After calculating the central value and the critical limits, horizontal lines were placed on the control charts and the baseline data were plotted. As it can be seen from Fig. 1, the baseline data produced an in control process (samples 1 to 20). The control charts were reset and the new collected data set 2 was plotted (samples 21 to 30). Both charts, X_i and *MR* were then viewed for out of control sequences, i.e. any point exceeding a control limit and/or eight consecutive points on the same side of the average. In most cases, the microbiological data require the presence only of the upper critical limit (Anonymous, 2006a; 2006b).

Fig. 1a demonstrates a process with a positive shift in *Enterobacteriaceae* counts before steam application (X_i control chart). The process showed a positive shift after the sample number 22 as identified by the eight consecutive points above average (sample numbers 23 to 30) and confirmed by the out of control points, marked with circle, exceeding the *UCL* on sample number 23, 24, 27 and 30. Although the process average shifted up, there is no indication that the variation increased (Fig. 1b). After steam application, the control charts indicated an in control process (Fig. 1c and d). Classical Shewhart control charts are useful when large process shifts occur (≥ 2 *SD*). However, when small shifts occur (0.5 to 1.5 *SD*), X_i and *MR* control charts are not so sensitive to detect changes. Thus, a *CUSUM* chart was constructed to confirm that the process after steam application was actually in control. *CUSUM* control charts are constructed differently and are able to detect small changes in the process. The *CUSUM* chart (Fig. 2a) identified a major adverse trend, before steam application from sample 22–23, indicating that the process was uncontrolled. No adverse trend in the data was observed after steam application. However, in order the *CUSUM* control chart to be more informative, a *CUSUM* signal chart should be constructed (Fig. 2b and c). The cumulative upper signal values display a continuous rise (positive shift) from sample number 22 to sample number 30, with sample numbers 25 to 30 all exceeding the *USAL* before steam application (Fig. 2b). On the other hand no changes in the process were observed after steam application, (Fig. 2c).

4. Conclusions

The hygienic status of the lamb slaughterhouse, as assessed by HAS, was relatively low. The major problems were related to personnel and practices as well as management. The relatively low hygiene standards of the abattoir were confirmed by the increase of *Enterobacteriaceae* counts (hygiene indicator microorganism) during the lamb slaughter process and the out of control process before steam application. On the other hand, the application of steam contributed to the maintenance of an in control process. The present work showed the potential use of steam as an intervention step during lamb slaughter process, achieving reduction of the

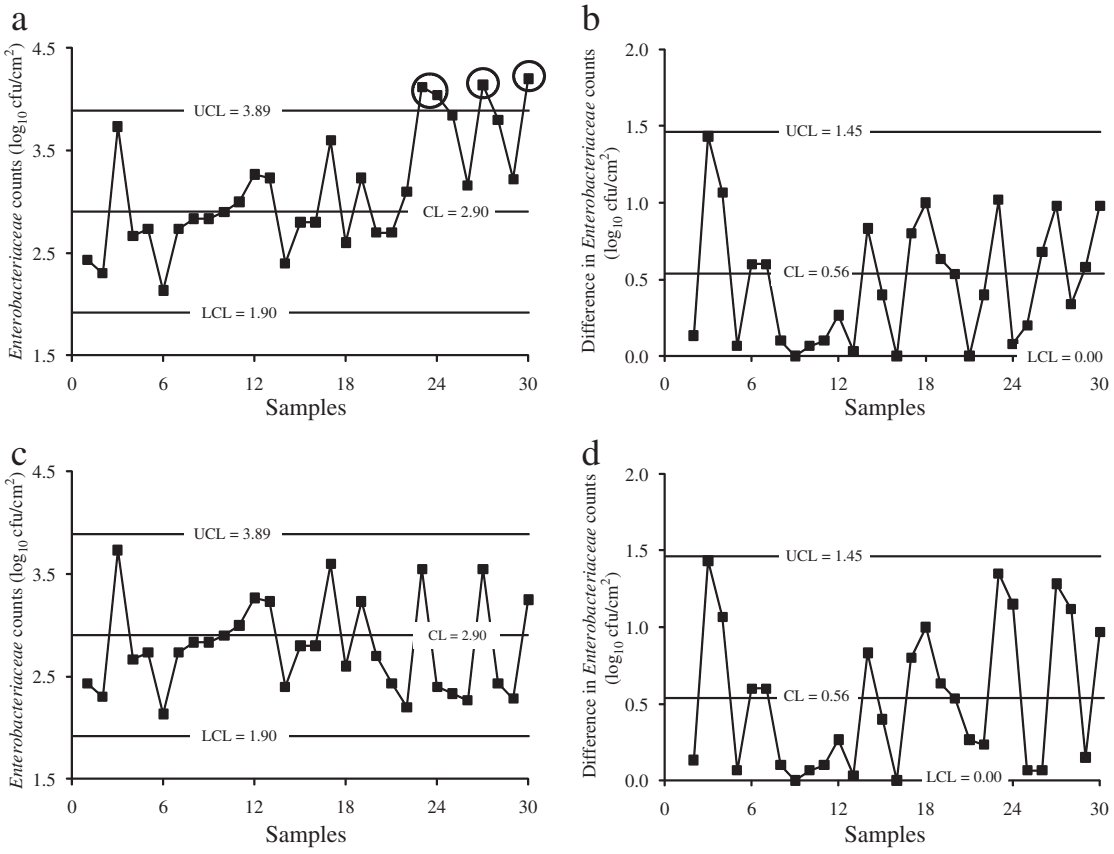

Fig. 1. Baseline data (samples 1 to 20; data set 1) plotted on X_i (control chart for individual units monitoring the central tendency of the process, i.e. the mean) (a and c) and *MR* (moving range control chart estimating the variability of the process) (b and d) control charts before (a and b) and after (c and d) steam application with central lines and critical limits derived from the baseline data. New data (samples 21 to 30; data set 2) were plotted to identify potential out of control process. Circles indicate out of control points exceeding the *UCL*.

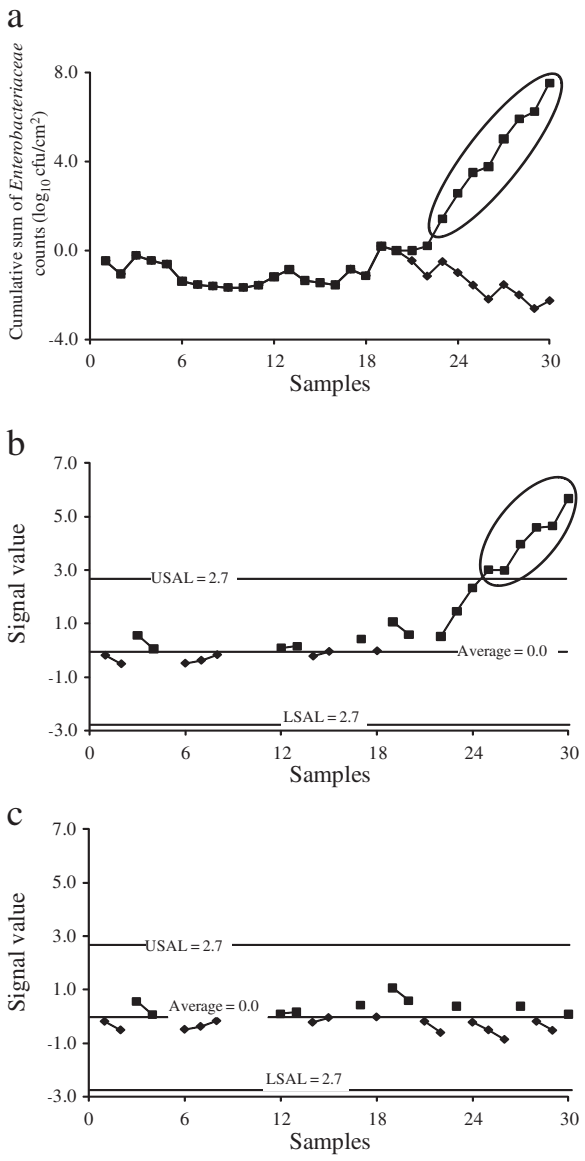

Fig. 2. *CUSUM* control chart of the *Enterobacteriaceae* counts (a) and *CUSUM* signal chart of the *Enterobacteriaceae* data before (b) and after steam application (c) from carcass sampling during lamb slaughter process. Circles indicate adverse trends (a) and out of control points exceeding the *USAL* (b and c). ■) before steam application values (a) and cumulative upper signal values (b and c) and ♦) after steam application values (a) and cumulative lower signal values (b and c).

microbiological counts without any adverse effects on the organoleptic characteristics of the carcasses.

Acknowledgment

The authors would like to express their gratitude to the slaughterhouse enterprise for providing permission to conduct this study.

References

Anonymous, 1999. Red meat slaughterhouse: assessment score sheet MH9 (Rev. 10/99). Produced by Meat Hygiene Service (MHS). Ministry of Agriculture, Fisheries and Food (MAFF), UK.

Anonymous, 2001. Commission Decision of 8 June 2001 on laying down rules for the regular checks on the general hygiene carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat (2001/471/EC). Official Journal of the European Communities L165, 48–53 21/06/2001.

Anonymous, 2004. Commission Decision (EC) No. 379/2004 of 26 April 2004 on amending Decision 2001/471/EC as regards bacteriological tests in certain meat establishments (2004/379/EC). Official Journal of the European Union L144, 1–4 30/04/2004.

Anonymous, 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L338, 1–26 22/12/2005.

Anonymous, 2006a. Final report and executive summaries from the AOAC International Presidential Task Force on Best Practices in Microbiological Methodology: Part II F Statistical Process Control. USFDA. http://www.cfsan.fda.gov/~acrobat/bpmm-f.pdf.

Anonymous, 2006b. Final report and executive summaries from the AOAC International Presidential Task Force on Best Practices in Microbiological Methodology: Part II F Statistical Process Control – 1. Appendices for Statistical Process Control. USFDA. http://www.cfsan.fda.gov/~acrobat/bpmm-f1.pdf.

Anonymous, 2007. Commission Regulation (EC) No. 1441/2007 of 5 December 2007 an amendment of the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Official Journal of the European Union L322, 12–29 7/12/2007.

Augustin, J.-C., Minvielle, B., 2008. Design of control charts to monitor the microbiological contamination of pork meat cuts. Food Control 19, 82–97.

Beauregard, M.R., Mikulak, R.J., Olson, B.A., 1992. A practical guide to statistical quality improvement. Van Nostrand Reinhold, New York, USA.

Bolton, D.J., Doherty, A.M., Sheridan, J.J., 2001. Beef HACCP: intervention and non-intervention systems. International Journal of Food Microbiology 66, 119–129.

Gill, C.O., Bryant, J., 1997. Decontamination of carcasses by vacuum-hot water cleaning and steam pasteurizing during routine operations at a beef packing plant. Meat Science 47, 267–276.

Gill, C.O., Jones, T.H., 2006. Setting control limits for *Escherichia coli* counts in samples collected routinely from pig or beef carcasses. Journal of Food Protection 69, 2837–2842.

Gill, C.O., McGinnis, J.C., 1999. Improvement of the hygienic performance of the hindquarters skinning operations at a beef packing plant. International Journal of Food Microbiology 51, 123–132.

Gill, C.O., McGinnis, J.C., Badoni, M., 1996. Use of total or *Escherichia coli* counts to assess the hygienic characteristics of a beef carcass dressing process. International Journal of Food Microbiology 31, 181–196.

Gill, C.O., McGinnis, J.C., Bryant, J., 1998. Microbial contamination of meat during the skinning of beef carcass hindquarters at three slaughtering plants. International Journal of Food Microbiology 42, 175–184.

Gonzalez-Miret, M.L., Escudero-Gilete, M.L., Heredia, F.J., 2006. The establishment of critical control points at the washing and air chilling stages in poultry meat production using multivariate statistics. Food Control 17, 935–941.

Hayes, G.D., Scallan, A.J., Wong, J.H.F., 1997. Applying statistical process control to monitor and evaluate the hazard analysis critical control point hygiene data. Food Control 8, 173–176.

International Commission on Microbiological Specifications for Foods (ICMSF), 1988. Indicator microorganisms, 2nd ed. Microorganisms in Foods, Vol. 2. University of Toronto Press, Toronto, Canada, pp. 3–14.

James, C., Thornton, J.A., Ketteringham, L., James, S.J., 2000. Effect of steam condensation, hot water or chlorinated hot water immersion on bacterial numbers and quality of lamb carcasses. Journal of Food Engineering 43, 219–225.

Jarvis, B., 2008. Statistical process control using microbiological data, Statistical Aspects of the Microbiological Examination of Foods, 2nd ed. Academic Press, London, UK, pp. 225–258.

Kilsby, D.C., Pugh, M.E., 1981. The relevance of the distribution of microorganisms within batches of food to the control of microbiological hazards from foods. The Journal of Applied Bacteriology 51, 345–354.

Lekroengsin, S., Keeratipibul, S., Trakoonlerswilai, K., 2007. Contamination profile of *Listeria* spp. in three types of ready-to-eat chicken meat products. Journal of Food Protection 70, 85–89.

Lenahan, M., O'Brien, S.B., Kinsella, K., Sweeney, T., Sheridan, J.J., 2010. Assessment of lamb carcass hygiene before and after chilling at five Irish abattoirs. Food Control 21, 313–318.

Montgomery, D.C., 2000. Introduction to Statistical Quality Control, 4th ed. John Wiley & Sons, New York, USA.

Murphy, R.Y., Osaili, T., Beard, B.L., Marcy, J.A., Duncan, L.K., 2005. Application of statistical process control, sampling, and validation for producing *Listeria monocytogenes*-free chicken leg quarters processed in steam followed by impingement cooking. Food Microbiology 22, 47–52.

Nutsch, A.L., Phebus, R.K., Riemann, M.J., Schafer, D.E., Boyer, J.E., Wilson, R.C., Leising, J.D., Kastner, C.L., 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. Journal of Food Protection 60, 485–492.

Nutsch, A.L., Randall, K., Phebus, R.K., Riemann, M.J., Kotrola, J.S., Wilson, R.C., Boyer, J.E., Brown, T.L., 1998. Steam pasteurization of commercially slaughtered beef carcasses: evaluation of bacterial populations at five anatomical locations. Journal of Food Protection 61, 571–577.

Pawsey, R.K., 2002. Hygiene improvement at source. Cases Studies in Food Microbiology for Food Safety and Quality. Royal Society of Chemistry, Cambridge, UK, pp. 410–428.

Pinillos, R.G., Jukes, D.J., 2008. Hygiene assessment system (HAS) scores – an analysis of the available data from English slaughterhouses. Food Control 19, 806–816.

Srikaeo, K., Hourigan, J.A., 2002. The use of statistical process control (SPC) to enhance the validation of critical control points (CCPs) in shell egg washing. Food Control 13, 263–273.