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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

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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).

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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