

Meat Safety

**HYGIENIC ASSESSMENT OF SHEEP CARCASSES AT
SLAUGHTERHOUSES BY DESTRUCTIVE AND NON-DESTRUCTIVE
SAMPLING METHODS**

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Introduction

Regular microbiological examinations of carcasses allow reliable conclusions to be drawn with regard to the long-term hygienic conditions in abattoirs (Mc Evoy et al. 2004, Zweifel et al. 2003, 2005). The EU Commission Decision (2001/471/EC) obliges fresh red meat and poultry meat operators to conduct regular checks on the general hygiene conditions of the production process, including microbiological controls of carcasses, and also makes HACCP methodology principles mandatory. For microbiological monitoring of carcasses Directive 2001/471/EC relies exclusively on total viable counts (TVC) as indicators of hygiene and Enterobacteriaceae as indicators of faecal contamination, and defines microbiological performance criteria for samples obtained by destructive sampling (excision technique). Recent studies suggest that swabbing with abrasive materials and applying the wet-dry double swab technique may be a suitable alternative to excision (Dorsa et al. 1996, Gill et al. 2000). However some authors and Federal authorities have suggested that the microbiological criteria for samples taken by the wet-dry double swab technique and abrasive materials should be set at 20% of the values set for excision samples (Anonymous 2002, Mc Evoy et al. 2004, Zweifel et al. 2005).

Objectives

The aim of the present study was to acquire extensive scientific issues on the hygienic status of sheep slaughtering process. The microbiological contamination of sheep carcasses at two slaughterhouse typologies, EU-approved high capacity slaughterhouses (H.C.S.) and low capacity slaughterhouses (L.C.S, which are allowed to slaughter a maximum of 20 units/week and/or 1,000 units/year), was evaluated by the comparison of the sampling methods recommended by Directive 2001/471/EC (excision and wet-dry swab) with an alternative non-destructive methods (sponge swabbing). Moreover, the practicability and reliability in routine use of these methods was evaluated.

Methodology

267 sheep carcasses were sampled in six slaughterhouses, three EU-approved H.C.S. and three L.C.S. 138 and 129 sheep carcasses were examined respectively at H.C.S. and L.C.S. abattoirs. 187 subjects were Sarda sheep, while 80 were imported from Spain. At each abattoir, sampling was performed weekly within a working day, after slaughtering and before chilling of the carcasses. On each visit, samples were collected involving ten sheep carcasses, randomly chosen. The carcasses were examined at three different sites of the four suggested by the Directive 2001/471/EC: flank (F), brisket (B) and rump (R). On the right side of carcasses, sampling was performed by the destructive method for five sheep (excision, EX) and by the non-destructive method for the remaining five (wet/dry double swab, SW). On the left side of all carcasses, samples were collected by the sponge swabbing method (SP). The sample collection by EX and SW techniques was performed according to the EU Decision criteria. The SP method was performed as following described: at each sampling site (100 cm²), a moistened (7 ml of Buffered Peptone Water, Oxoid-England) sponge (enviro sponge, Tecna, U.S.A.) was rubbed vertically, horizontally and diagonally across the site delineated by a sterile template. Microbiological analyses a) Total viable count (TVC-A.P.H.A., 2001); b) Enterobacteriaceae count (TEC) on Chromocult Coliform Agar (Merck, Germany), incubated at 37 °C for 24-48 h. In the comparison of EX vs SP, a single sponge for all sampling sites was used, while in the comparison of SW vs SP, a sponge for each sampling site was used. Analysis of variance were performed using the GLM procedures. The mean differences were evaluated using the LSD test (Statgraphics Plus, 5.1).

Results & Discussion

The microbiological results, expressed as mean $\pm$ s.d. of log₁₀ cfu/cm² values, were compared to the criteria recommended by Decision 2001/471/EC. a) EX vs SP: the total mean results from 129 sheep carcasses are shown in table 1, while the allocation into the ranges defined by EU Decision is reported in tables 5, 6. Independent from the slaughtering capacity, TVC and TEC mean values were higher ($p < .01$) in the samples collected by the EX than those obtained by the SP. Only TVC mean values were significant higher ($p < .01$) in the L.C.S. than in the H.C.S. (table 2). b) SW vs SP: table 1 shows total mean results from 132 sheep carcasses, while the allocation into the ranges defined by EU Decision is reported in tables 3, 4. Microbiological criteria for samples collected by the SW and SP techniques have been set at 20% of the values set for EX samples (Anonymous, 2002). In the samples obtained by SP, TVC and TEC mean values were higher than those obtained by the SW ($p < .01$). Comparing the results (table 2) in relation to the slaughtering plants capacity, the TVC and TEC mean values were significant higher ($p < .01$) in the L.C.S. than in the H.C.S.. In relation to the sampling site, brisket was the most contaminated: TVC mean values were 2.63 ± 0.64 in the samples obtained by the SP and 1.62 ± 0.75 in those obtained by the SW. Differences between the methods were significant ($p < .01$). In the same sampling site, TEC mean values were 1.23 ± 1.15 and 0.40 ± 1.08 in the samples collected by SP and SW, respectively. As reported by other authors (Vanderlinde et al. 1999), such results are strongly linked to the slaughtering operations. In the flank, the TVC mean values were 2.39 ± 0.76 and 1.60 ± 0.81 in the samples collected by SP and SW respectively, while the TEC mean values were 1.23 ± 1.15 (SP) and 0.40 ± 1.08 (SW). The rump was less contaminated: TVC mean

values were 2.23 ± 0.80 (SP) and 1.48 ± 0.76 (SW), while TEC were 1.10 ± 1.11 (SP) and 0.10 ± 0.92 (SW).

Conclusions

The comparison of a destructive (excision) with a non-destructive method (sponge swabbing) shows significant differences ($p < .01$) between the two techniques. The recovery capacity of the sponge swabbing was lower for all the microbiological considered parameters. Moreover, the use of a single swab for the three sampling sites was unsuitable (dilution effect), particularly for the recovering of Enterobacteriaceae. The excision method was the most reliable and effective in terms of microbial recovering efficacy, but its use is limited because of the destructive effect (Dorsa et al. 1996, Gill et al. 2001, Byrne et al. 2005). Although the lower recovery efficacy, the non-destructive methods are effective and reliable for the hygienic assessment of carcasses at slaughterhouses, and are also suitable for routine use (Reid et al., 2002). With respect to the TVC criteria proposed by Directive 2001/471/EC, most of the carcasses sampled by the three methods were allocated into the acceptable category. Instead over 60 % of samples obtained by the EX were allocated into the unacceptable category for TEC. This percentage decreased until to 17,2% and 39,3% in the samples collected by SP and SW, respectively. The higher prevalence of TVC and TEC were detected in carcasses sampled at L.C.S., independent of the sampling method. The results show that the process management and the slaughterhouse capacity are the main factors affecting the level of sheep carcass contamination (Giuffrida et al. 2002).

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Tables and Figures

Table 1 - Results of the comparison of destructive (EX) and non-destructive (SW and SP) methods for microbiological sampling of sheep carcasses (mean $\pm$ s.d.).

Parameter	EX vs SP		SW vs SP	
	Excision	Sponge swabbing	Dry/wet Swab	Sponge swabbing
Total Viable Count	3.36 $\pm$ 0.76x	2.47 $\pm$ 0.82y	1.57 $\pm$ 0.77y	2.42 $\pm$ 0.75x
<i>Enterobacteriaceae</i>	3.42 $\pm$ 1.40x	1.54 $\pm$ 1.19y	0.24 $\pm$ 1.03y	1.31 $\pm$ 1.15x

(x, y): the mean values in the same line not identified with the same letter are significantly different: $=p < .01$

Table 2 - Results of the comparison of destructive (EX) and non-destructive (SW and SP) methods for microbiological sampling of sheep carcasses (mean $\pm$ s.d.) in relation to the slaughtering plants capacity

Parameter	Sampling Method	Capacity		Sampling Method	Capacity	
		L.C.S.	H.C.S		L.C.S.	H.C.S
Total Viable Count	EX	3.55 $\pm$ 0.68x	319 $\pm$ 0.79y	SP	2.61 $\pm$ 0.82x	2.24 $\pm$ 0.64y
	SP	2.82 $\pm$ 0.82	2.16 $\pm$ 0.68	SW	1.60 $\pm$ 0.79x	1.54 $\pm$ 0.76x
<i>Enterobacteriaceae</i>	EX	3.34 $\pm$ 1.40	3.49 $\pm$ 1.41	SP	1.56 $\pm$ 1.28x	1.07 $\pm$ 0.97y
	SP	1.77 $\pm$ 1.15	1.33 $\pm$ 1.19	SW	0.40 $\pm$ 0.96x	0.09 $\pm$ 1.07y

L.C.S.= low capacity slaughterhouses; H.C.S.= high capacity slaughterhouses; x, y = the mean in the same line not identified with the same letter are significantly different ($p < .01$)

Table 3 – Allocation of the Total Viable Counts results in the categories for process control verification, according to the modified criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 2.8	2.81-4.3	>4.30
Sponge swabbing	L.C.S.	63.1	36.9	0
	H.C.S.	89.6	10.4	0
Dry/wet swab	L.C.S.	100	0.1	0
	H.C.S.	100	0.1	0

L.C.S.= low capacity slaughterhouses; H.C.S.= high capacity slaughterhouses;

Table 4 – Allocation of the *Enterobacteriaceae* Counts results in the categories for process control verification, according to the modified criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		<0.5	0.5-1.8	>1.8
Sponge swabbing	L.C.S.	9.2	53.8	33.8
	H.C.S.	13.4	68.7	14.9
Dry/wet swab	L.C.S.	27.7	40.0	32.3
	H.C.S.	29.9	23.9	46.3

Table 5 – Allocation of the Total Viable Counts results in the categories for process control verification, according to the criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 3.5	3.5 - 5	>5
Excision	L.C.S.	42.6	55.7	1.63
	H.C.S.	58.8	41.1	0

Table 6 – Allocation of the *Enterobacteriaceae* Counts results in the categories for process control verification, according to the criteria recommended by EU Decision 2001/471EC

Method	Capacity	Acceptable range	Marginal range	Unacceptable range
		< 1.5	1.5 - 2.5	> 2.5
Excision	L.C.S.	4.9	29.5	65.6
	H.C.S.	10.3	11.8	77.9