MICROBIOLOGICAL ASPECTS OF OVINE PELT REMOVAL ASSISTED BY AIR INFLATION. Severini M., Ranucci D., Cenci Goga B.T., Miraglia D.

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#### Introduction

Sheep and lambs are hand-depelted in the majority of European slaughterhouses and assisting manual pelt removal by air inflation is a common practice in Italy as well as in several other European countries. The use of this method in the European Union is permitted by the Directive 95/23/EC which amended Directive 64/433/EEC on animals of live weight lower than 15 kg on the condition that all hygienic specifications are respected and the safe implementation of the inflation method is guaranteed by the veterinary service officers (Severini, 1996). The main practical advantages of this method are: a) less labour- and time-consuming pelt removal; b) a uniform and smooth carcass surface; c) reduction of skin damage (Severini et al., 1994).

The better quality of carcasses has been documented in our previous studies (Trevisani et al., 1996). Due to the fact that the main concern was the surface microbial contamination, in these studies the microbial load of the carcass surface was also investigated by swab samples. The results showed no significant differences between lambs depelted with and without air inflation (Cenci Goga et al., 1996; Trevisani et al., 1996). However, the risk that air infiltrating subcutaneous tissues could spread bacteria under the surface was highlighted. This contamination can not be detected by examining swab samples, as we did in our experiment. The need of further investigation aimed at assessing the possible presence of bacteria in the air infiltrating subcutaneous tissues was stressed. In this occasion, the preliminary results of a recent experiment carried out to further evaluate the microbial risk of using air inflation for ovine pelt removal are presented.

#### Materials and methods

#### Trial methodology

Nine randomly selected carcasses of lambs slaughtered in a commercial abattoir were examined in order to evaluate the average surface microbial contamination in that specific environment. As usual, the carcasses were hind-leg suspended and compressed air was injected at the level of the left fore-leg immediately after bleeding when the head was still on. Single wet swab samples  $(100 \text{ cm}^2)$  were collected after evisceration at three sites: shoulder, outside hind leg, and lumbar region. Subsequently, six lambs slaughtered in the above-mentioned abattoir were examined in order to evaluate possible microbial contamination specifically due to air infiltration into subcutaneous tissues. The shoulder is one of the sites where this can commonly occur. Therefore, double swab samples  $(20 \text{ cm}^2)$  and excision samples with a surface area of  $20 \text{ cm}^2$  and a depth of about 5mm were taken from adjacent areas of the shoulder site immediately after pelt removal before evisceration started (left shoulder), and then after a 24-hour-chilling period (right shoulder). The samples taken from four carcasses depelted with air inflation were compared with those from two carcasses skinned without air inflation.

### Microbiological analyses

Each sample was placed in 10 ml of Maximum Recovery Diluent (Oxoid CM733B) and stored in ice-cooled insulated containers. The samples were then transported to the laboratory and analyzed within four hours. The samples were mechanically shaken for 2 minutes and 10-fold dilutions prepared in Maximum Recovery Diluent pre-cooled to 1°C. Agar Plate Counts (APC) were obtained on Plate Count Agar (Oxoid CM325), incubated at 30°C for 72 hours and at 20°C for 7 days. Duplicate counts were made on quarter sections of the agar surface inoculated with 0.01 ml of appropriate dilutions of the sample. The number of coliform organisms (VRB) was counted using Violet Red Bile Agar (Oxoid CM107B), incubated at 30°C for 24 hours. The number of total Enterobacteriaceae (VRBG) was counted using Violet Red Bile Glucose Agar (Oxoid CM485B), incubated at 30°C for 24 hours and at 20°C for 7 days. This medium was inoculated with 0.1 ml of the diluted samples because the counts were expected to be lower than the APC. The numbers of presumed Staphylococcus aureus and Staphylococcus spp. were enumerated using Baird Parker Agar Base (OxoidCM275B) with added Egg Yolk Tellurite Emulsion (Oxoid SR054C), incubated at 37°C for 48 hours. This medium was also inoculated with either 0,01 or 0.1 ml of the diluted samples, since the counts were expected to be lower than the APC. The minimum levels of detection for APC and Enterobacteriaceae or presumed S. aureus were 10 and 1 cfu/cm<sup>2</sup>, respectively. The weighted mean counts were calculated and the values transformed to log10 cfu/cm2. The APC at 30°C and the numbers of Enterobacteriaceae and S. aureus were transformed to log10 number of colony forming units per gram (log10 cfu/g). The lower limit of detection was 10 cfu/g (log<sub>10</sub> = 1.0). For statistical analysis, samples yielding no growth, i.e. below the limit of detection, were scored as 1 cfu/g to allow log10 transformation. Anaerobic bacteria (RCM) were counted using Reinforced Clostridial Medium (Oxoid CM149B).

#### **Results and discussion**

The results of the first trial are reported in Table 1. The mean values of APC are very similar among the three sampled sites and show an average acceptable microbiological status (Hadley et al., 1997) even if differences do exist among the carcasses for each sample site. The highest value is  $4.90 \log_{10} \text{ cfu/cm}^2$ , recorded at the shoulder site. The average number of total coliform organisms is also acceptable (Fliss et al., 1991) and only one carcass showed a relatively high value at one sample site. The average number of *Staphylococcus* spp. is acceptable in all the sampled sites, but relevant differences were observed among the carcasses. Colonies with the characteristics of *Staphylococcus aureus* were never detected.

The results of the second trial are reported in Tables 2 and 3. In general, the average microbial contamination detected by the swab test on the carcass surface immediately after depelting of the inflated and non inflated lambs is very similar and the mean values are slightly lower than those recorded in the carcasses from the first trial.

Table 1. Samples taken after the evisceration from inflated lambs (Trial 1). Values expressed as Log cfu/ cm<sup>2</sup>

	Shoulder		Lum	bar region	Outside hind leg		
	mean	st. dev	mean	st. dev	mean	st. dev	
APC 30°C	3.10	1.20	3.43	0.53	3.05	0.53	
VRB 30°C	0.12	0.63	1.20	0.88	0.62	0.45	
BP	1.36	1.09	2.42	0.96	2.20	0.79	

#### Table 2. Samples taken after depelting, before evisceration (Trial 2). Values expressed as Log cfu/ cm<sup>2</sup>

	Non inflated carcasses				Inflated carcasses			
	Swab		Exc	ision	Swab Exci		ision	
	mean	st. dev	mean	st.dev	mean	st.dev	mean	st.dev
APC 30°C	2.24	0.83	2.37	0.38	2.28	0.55	2.12	0.78
APC 20°C	2.24	0.58	1.94	1.19	2.36	0.68	2.14	0.81
VRBG 30°C	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.55
VRBG 20°C	0.00	0.00	0.00	0.00	0.47	0.58	0.40	0.79
BP	1.83	0.92	1.24	1.75	1.86	0.49	1.76	0.49

Table 3. Samples taken after 24-hour-chilling period (Trial 2).

 values ex	pressed as Log cfu/cm <sup>-</sup>	
	Non inflated carcasses	S

	Non inflated carcasses					Inflated carcasses			
	Swab		Exc	ision	Swab Excis		sion		
	mean	st. dev	mean	st.dev	mean	st.dev	mean	st.dev	
APC 20°C	2.17	0.74	2.90	0.40	2.86	0.63	3.38	0.36	
VRBG 20°C	0.92	1.30	1.32	0.03	1.45	0.98	1.65	1.10	

These data suggest that evisceration contributes to enhancing the surface microbial contamination, even if slightly. The situation does not change after the 24-hour-chilling period when the number of psychrotrophic bacteria remains stable (Table 3). The comparison between swab samples and excision samples shows that the values are quite similar. At each sampling time the microbial load detected in the excision samples was not significantly higher than in the swab samples, thus proving that the number of bacteria which were present in the subcutaneous tissues was absolutely irrelevant. Furthermore, anaerobic bacteria were never detected in any of the examined carcasses. The slight increase in total psychrotrophic bacteria recorded in the excision samples of both inflated and non inflated carcasses after the chilling period might be attributed to the operations which took place after skinning (such as evisceration and pre-chilling carcass handling) and could have affected the surface microbial load before refrigeration. Even more, the observed values were lower than the acceptable level.

## Conclusions

2

The preliminary data of our investigation aimed at evaluating the microbial risk in subcutaneous tissues of lamb carcasses hand-depelted by means of air inflation show that no significant bacterial contamination is detectable in the air infiltrated tissues and confirm that the overall hygienic risk related to the air inflating method to assist ovine pelt removal is very low on the condition that well-defined specifications on the operating methodology are given and implemented.

# References

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