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Original article

Assessment of the microbiological conditions of red-meat carcasses from bacterial counts recovered by sampling via excision or swabbing with cotton wool

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Summary 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 (EX) and the other the wet–dry swabbing method (SW). Eight areas from each carcass were sampled. Most of the samples obtained by SW revealed total aerobic viable counts (TVC) levels of between 3.1 and 4.0 log CFU cm^{-2}, while most of the values corresponding to excision were located between 4.1 and 5.0 log CFU cm^{-2}. Moreover, *Enterobacteriaceae* (EC) counts were only detected above 3.0 log CFU cm^{-2} in 0.85% of the carcasses when the samples were collected by swabbing, while the excision method revealed that 13.75% of the carcasses presented EC greater than 3.0 log CFU cm^{-2}. TVC and EC by EX revealed statistically significant differences compared to SW, while no significant linear relationship was found between carcass surface bacterial counts obtained by SW and EX.

Keywords Carcasses sampling, *Enterobacteriaceae*, excision, Regulation (EC) 2073/2005, swabbing, total aerobic viable counts.

Introduction

Regulation (EC) 852/2004 and Regulation (EC) 2073/2005 (European Commission, 2005) of the European Union mean that every red-meat slaughterhouse has to operate according to Hazard Analysis and Critical Control Point (HACCP) principles. Microbiological sampling and testing of carcasses has been introduced to verify that procedures based on HACCP function correctly in slaughterhouses. The microbiological analysis of carcass surfaces has become an important information source in developing and implementing HACCP systems for red-meat slaughtering and dressing operations (Capita *et al*., 2004).

According to Regulation (EC) 2073/2005 (European Commission, 2005), amended by Regulation (EC) 1441/2007, microbiological criteria for evaluating carcasses of cattle, pigs, sheep, goats and horses include aerobic colony counts and *Enterobacteriaceae* counts on carcasses after dressing but before chilling. The excision sampling method is exclusively recommended to asses the hygienic quality of carcasses. Swabbing is also permitted but only if a correlation has previously been found between the excision and swabbing techniques. Swabbing is acceptable only when substantial fractions of bacteria, present on sampled areas are recovered (Dorsa *et al*., 1996; Palumbo *et al*., 1999) and when correlation with excision is high (Hutchison *et al*., 2005). It is necessary to know the percentage of bacteria recovered by different sampling methods to compare the microbiological data obtained through different techniques (Capita *et al*., 2004). As yet no universal quantitative conversion factor between excision and swabbing results has been established (Bolton, 2003; Capita *et al*., 2004).

It is generally accepted that the excision method recovers significantly higher numbers of bacteria from meat surfaces compared with swabbing (Dorsa *et al*., 1996; Gill & Jones, 2000; Gill *et al*., 2001). Therefore, excision is the most effective bacterial carcass sampling method (Dorsa *et al*., 1996; Sharpe *et al*., 1996; Untermann *et al*.,1997; Palumbo *et al*., 1999); and it represents the reference method against which other sampling

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methods must be evaluated (Pepperell *et al.*, 2005). By contrast, swabbing obtains high variability in recoveries, ranging from between 0.01% and 100% (Eisel *et al.*, 1997; Gill & Jones, 2000; Capita *et al.*, 2004; Pepperell *et al.*, 2005). Nevertheless, swabbing is now the most commonly used carcass sampling method in the context of mandatory HACCP systems in red-meat abattoirs within the EU (European Union) (Pepperell *et al.*, 2005).

Several studies related to carcass surface microbiology use non-destructive methods (Marjatta & Korkeala, 1997; Untermann *et al.*, 1997; Hansson, 2001; Bryant *et al.*, 2003; McEvoy *et al.*, 2004) and swabbing methods are commonly used in practice without previous assessment of the relationship between results from both excision and swabbing methods (Capita *et al.*, 2004). Moreover, there is limited published information about the microbiological quality of horse meat (Gill, 2005) and no works have been found evaluating the effectiveness of the wet–dry swabbing method in the recovery of total aerobic plate counts and *Enterobacteriaceae* counts in equine carcasses.

This study aims to compare swabbing and excision methods for the recovery of total aerobic viable counts (TVC) and *Enterobacteriaceae* counts (EC) from bovine, equine, ovine and porcine carcasses surface; and to analyze the effect of microbial load and animal species on the percentage of TVC recovered by the wet–dry cotton swabbing method.

Materials and methods

Animal species examined

A total of 240 carcasses from porcine, ovine, bovine and equine species (60 of each) were sampled at six slaughterhouses (equine carcasses were only sampled at three slaughterhouses) in the East of Spain. Each slaughterhouse had three different slaughter lines (bovine-equine, ovine and porcine) and two visits were made (four for equine). All samples were taken under the normal work conditions in each slaughterhouse. The sampling areas were the rump (perianal area), flank, brisket and neck for bovine, equine, and ovine carcasses; and the ham (perianal area), loin, brisket and jowl on porcine carcasses.

Sampling methods

Two samples were taken from every carcass, one by excision and another by wet–dry cotton swabbing. The total number of samples analyzed was 480 (240 by excision and 240 by swabbing).

The carcasses were selected from the slaughter chain by systematic random sampling. First a constant (k) was calculated dividing the total number of animals to be slaughtered that day (N) by the wanted sample size ($n = 5$). Then, out of every k carcasses, one was chosen for sampling.

Eight areas were sampled from each carcass (four areas on the left half-carcass and four areas on the right half-carcass), four areas by excision (EX) and the other four by the wet–dry cotton swabbing method (SW). In this way, the sampling areas were alternated in every carcass.

Samples were collected by the same person at the end of the process, before the carcasses were taken to the refrigerating room. Excision-based sampling (EX) involved removing a 5 cm^2 sliver of tissue, with a maximum depth of 3 mm, from the four zones on each carcass sampled, making a total of 20 cm^2 per carcass. A flame-sterilized stainless-steel squared tube impregnated with colouring (Allura red, E-129) was used to mark the area. Each tissue sample was immediately intersected and withdrawn with a sterile scalpel and pincers. The four tissue samples from each carcass were placed inside a sterile plastic container, previously filled with 10 mL of peptone water (0.1% peptone, 0.85% NaCl).

Two sterile cotton swabs (a wet one and a dry one) were used for wet–dry swab sampling (SW). Samples were collected within an area of 100 cm^2 (50 cm^2 on ovine carcasses) from the four previously listed sites. These sampling areas were delineated with an alcohol-sanitized disposable plastic template. Firstly, for each sampling area, a swab was moistened in peptone water (0.1% peptone, 0.85% NaCl) for at least 5 s, and then rubbed firmly across the carcass surface with fifteen strokes in vertical, horizontal and diagonal directions, for at least 30 s. Immediately after rubbing with a wet swab, the procedure was repeated within the same template with a dry swab. The total surface sampled in each carcass was 400 cm^2 (200 cm^2 on ovine carcasses). Finally, in the slaughterhouse, the ends of the four pairs of wet and dry swabs were combined into a single sample in a sterile container holding 10 ml of peptone water.

Immediately after sample collection using both methods, and after placing them inside their corresponding containers, both were shaken vigorously for 5 s, to help the microorganisms move from the tissue and cotton fibres to the water solution. Later on, the containers were placed in a refrigerator at 4 °C. Samples were transported to the laboratory in a cold-box containing ice packs within 1 h of collection.

Microbiological analysis

Total aerobic viable counts and EC were determined by standard plate-count methods, according to the criteria specified by ISO 4833:2003 and ISO 21528-2:2004, respectively. All the analyses were made in the same laboratory. The material collected by excision (10 mL of peptone water with excised slivers of tissue) was placed

inside a stomacher bag with 90 mL of peptone water and homogenised for 2 min in a stomacher (model Lab-Blender 400, Seward Medical, London, UK). The content taken by swabbing (10 mL of peptone water plus the ends of the eight swabs) was placed in a plastic container with 90 mL of peptone water. The containers with the swabs were vigorously vortexed for 2 min so that all the microorganisms inside the cotton wool were successfully transferred to the water solution.

Each sample homogenate (excision or swab) was then diluted decimally in peptone water, and 1 ml aliquots were added to suitable Petri dishes, reaching 10^{-6} dilution (TVC) and 10^{-4} dilution (EC).

Samples were analysed within 24 h of collection. The culture media used were PCA (Plate Count Agar; Oxoid CM325; Unipath, Basingstoke, UK) for TVC and VRBG (Violet Red Brilliant Green Agar; Oxoid CM485; Unipath, Basingstoke, UK) for EC. PCA plates were incubated at 37 °C for 48 h before colonies were counted. *Enterobacteriaceae* were incubated at 37 °C for 24 h. *Enterobacteriaceae* presence was confirmed by oxidase testing and ability to metabolize glucose.

Data analysis

All bacterial counts were expressed as CFU cm^{-2} of carcass surface and they were transformed into log values (log CFU cm^{-2}). A log value of −0.5 CFU cm^{-2} was assumed, for those samples in which *Enterobacteriaceae* were not detected (Gill & Jones, 2000; Gill *et al*., 2001).

The recovery percentage by swabbing was calculated dividing counts obtained by SW by counts found by EX, then multiplying the result by 100. The statistical package SAS® (Statistical Analysis System, SAS Institute, Cary, NC, USA) was used for analysis of variance (ANOVA) of bacterial count following the general linear model procedure. The data were analysed using a one-way ANOVA.

To statistically analyse the effect of microbial load and carcass species on the percentage of TVC recovered by the wet–dry cotton swabbing method (SW), the carcasses were classified into three levels: TVC low (<4.0), medium (4.00–4.50) and high (>4.50).

The model used was: $Y_{ij} = \mu + S_i + \mathrm{TVC}_j + S_i * \mathrm{TVC}_j + \epsilon_{ij.}$

Where, Y_{ij} = percentage of TVC recovered by the wet–dry cotton swabbing method; μ = general average; S_i = the animal carcass species (four levels). TVC_j = level of TVC (log CFU cm^{-2}) obtained by excision (three levels). ϵ_{ij} = residual error of the model.

Significant differences were found with a significance level of 95% ($P < 0.05$). We also used linear regression (procedure REG of SAS) to quantitatively measure the strength of the relationship between excision and swabbing sampling. The correlation between TVC and EC was calculated using Pearson correlation coefficient.

Results and discussion

Carcass distribution according to microbial load, animal species and sampling method are shown in Table 1 (TVC) and Table 2 (EC). TVC were detected in every carcass by both excision and swabbing. However EC were only detected in 72.5% and in 50% of the samples taken by excision and swabbing respectively. Most of the samples obtained by swabbing (48.33%) revealed TVC levels of between 3.1 and 4.0 log CFU cm^{-2}, while most of the values found by excision (50.85%) were located between 4.1 and 5.0 log CFU cm^{-2}. It is important to highlight that TVC exceeded 4.1 log CFU cm^{-2} in only 5.85% of the samples when they were collected by swabbing (in no sample was TVC greater than 5.0 log CFU cm^{-2}). Moreover, EC counts were only detected above 3.0 log CFU cm^{-2} in 0.85% of the carcasses when the samples were collected by swabbing, while the excision method revealed that 13.75% of the carcasses presented EC greater than 3.0 log CFU cm^{-2}.

Table 1 Distribution of carcasses (%) according to total aerobic viable counts (log CFU cm^{-2}), animal species and sampling method

		Range log CFU cm^{-2}					
Specie	**Sampling**	**ND**	**1.1–2.0**	**2.1–3.0**	**3.1–4.0**	**4.1–5.0**	**5.1–6.0**
Bovine	Excision	0	0	13.3	35.0	45.0	6.7
Bovine	Swabbing	0	21.7	58.3	20.0	0	0
Equine	Excision	0	0	0	8.3	60.0	31.7
Equine	Swabbing	0	3.3	25.0	60.0	11.7	0
Ovine	Excision	0	0	1.7	38.3	45.0	15.0
Ovine	Swabbing	0	10.0	38.3	46.7	5.0	0
Porcine	Excision	0	0	0	38.3	53.4	8.3
Porcine	Swabbing	0	1.7	25.0	66.6	6.7	0
Average	Excision	0	0	3.8	30.0	50.9	15.4
Average	Swabbing	0	9.2	36.7	48.3	5.9	0

ND, not detected.

Table 2 Distribution of carcasses (%) according to *Enterobacteriaceae* counts (log CFU cm^{-2}), animal species and sampling method

		Range log CFU cm^{-2}					
Specie	**Sampling**	**ND**	**0.1–1.0**	**1.1–2.0**	**2.1–3.0**	**3.1–4.0**	**4.1–5.0**
Bovine	Excision	31.7	0	26.6	31.7	10.0	0
Bovine	Swabbing	75.0	18.3	6.7	0	0	0
Equine	Excision	30.0	0	10.0	46.7	11.6	1.7
Equine	Swabbing	45.0	31.6	15.0	6.7	1.7	0
Ovine	Excision	30.0	0	26.7	33.3	10.0	0
Ovine	Swabbing	53.3	23.3	16.7	5.0	1.7	0
Porcine	Excision	18.3	0	26.7	33.3	20.0	1.7
Porcine	Swabbing	26.7	21.7	43.4	8.2	0	0
Average	Excision	27.5	0	22.5	36.3	12.9	0.9
Average	Swabbing	50.0	23.7	20.5	5.0	0.9	0

ND, not detected.

Moreover, the Pearson correlation coefficient between TVC and EC was very low (0.29 and 0.48 for EX and SW respectively), thus, we cannot establish a relationship between both counts.

The SW method recovered few TVC compared to the results obtained by EX (Table 3). Besides, significant differences ($P < 0.05$) in TVC were detected among animal species and microbial-load levels. The recovery percentages with SW compared with EX from porcine, ovine, bovine and equine carcasses were 17.43%, 14.69%, 9.24% and 7.02% respectively. When TVC was higher than 4.50 log CFU cm^{-2} the recovery percentage by SW was only 4.99% and when TVC was between 4.00 and 4.50 log CFU cm^{-2} the percentage was 13.50%.

In all the studied species, statistically significant differences ($P < 0.001$) were found for TVC between samples obtained by excision and samples obtained by swabbing (Table 4). Likewise, EC was higher ($P < 0.001$) in the samples obtained by excision than in those obtained by swabbing. These results do not agree with those by Hutchison *et al.* (2005), who reported that both methods revealed similar prevalence of *Enterobacteriaceae* in ovine and porcine carcasses, although they did observe higher prevalence and counts with the excision method in bovine carcasses.

Thus, when presenting results of *Enterobacteriaceae* counts it is important to know the approach used when *Enterobacteriaceae* are not detected by the analytical technique. Nevertheless, Regulation (EC) 2073/2005 (European Commission, 2005) does not indicate what should be done in these cases. There are different proposals (Rivas *et al.*, 2000; Duffy *et al.*, 2001; McEvoy *et al.*, 2004; Hutchison *et al.*, 2005), although the most widespread appears to be to assign the value −0.5 log CFU cm^{-2} to the samples when *Enterobacteriaceae* are not detected (Gill & Jones, 2000; Bolton, 2003; Bryant *et al.*, 2003; Pearce & Bolton, 2005). It is worth pointing out that the percentage of carcasses in which *Enterobacteriaceae* is detected can vary greatly among the different species and slaughterhouses. Usually, there is high degree of variation between the counts on the near-adjacent surface of a carcass (Hutchison *et al.*, 2005). For this reason, we have taken two samples from each carcass, one by excision and the other by swabbing in near-adjacent areas. Furthermore, a number of factors can also influence the percentage of bacteria recovered, such as the pressure exerted on the carcass with the swab, the duration of cleaning with the swab and the differences between people who collect the samples (Berry *et al.*, 1978; Snijders *et al.*, 1984). For this reason all the samples in this study (EX and SW) were collected by the same person. Therefore, it is often difficult to compare the results of different studies assessing the microbial load of the carcass surface, particularly because of the differences between the sample collection methods used, sampling areas, microbiological techniques, detection limits of the techniques, data analysis procedure, countries and different periods of time (Brown *et al.*, 2000; Capita *et al.*, 2004).

Table 4 Statistics for total aerobic viable counts (TVC) and *Enterobacteriaceae* counts (EC) in different species using two sampling methods

		Sampling method					
		Excision			Swabbing		
Animal specie	Microbial group	Mean	SD	Median*	Mean	SD	Median*
Bovine	TVC	4.05a	0.77	4.11	2.49b	0.66	2.54
Bovine	EC	1.50a	1.47	2.44	−0.16b	0.65	0.69
Equine	TVC	4.74a	0.53	4.72	3.31b	0.59	3.31
Equine	EC	1.61a	1.49	2.35	0.39b	1.00	0.87
Ovine	TVC	4.29a	0.66	4.18	3.03b	0.69	3.11
Ovine	EC	1.49a	1.41	2.18	0.32b	1.02	1.08
Porcine	TVC	4.28a	0.56	4.15	3.27b	0.49	3.29
Porcine	EC	2.00a	1.34	2.54	0.85b	0.98	1.09
Average	TVC	4.34a	0.68	4.36	3.03b	0.69	3.11
Average	EC	1.65a	1.43	2.30	0.35b	0.99	1.05

Averages in the same row with different letter present statistically significant differences ($P < 0.001$).

*In the case of EC, the median has been calculated only considering the carcasses where EC has been detected.

Table 3 Total aerobic viable counts recoveries (%) from carcasses by swabbing method (SW) compared with those by the excision method (EX)

Microbial load by EX[1]	Bovine			Equine			Ovine			Porcine			Total		
	n	*X*	%[2]	*n*	*X*	%[2]	*N*	*X*	%[2]	*n*	*X*	%[2]	*n*	*X*	%[2]
<4.00	29	3.38	16.65ay	5	3.81	12.01ay	22	3.61	22.70ayz	19	3.71	25.92az	75	3.56	20.46a
4.00–4.50	10	4.27	7.02by	18	4.30	10.77ayz	15	4.19	18.90az	21	4.20	17.45bz	64	4.24	13.50b
>4.50	21	4.87	2.44cz	37	5.07	4.53bz	23	5.01	4.28bz	20	4.92	9.34cz	101	4.98	4.99cz
Total	60	4.05	9.24x	60	4.74	7.02xy	60	4.29	14.69yz	60	4.28	17.43z	240	4.34	12.09

[1]log CFU cm^{-2}.

[2]Percentage of recovery by SW compared with EX.

n, number of carcasses; *X*, means of log_{10} counts.

Values in the same column (a, b, c) and values in the same row (x, y, z) with different letters present statistically significant differences ($P < 0.05$).

Thus, the percentage of carcasses with positive isolations of *Enterobacteriaceae* can oscillate between 24.0% and 86.0% in porcine (Gill & Jones, 2000; Hansson, 2001; Hutchison *et al*., 2005; Pearce & Bolton, 2005), between 23.4% and 89.0% in bovine (Madden *et al*., 2004; McEvoy *et al*., 2004; Hutchison *et al*., 2005; Pearce & Bolton, 2005) and between 15.2% and 79.3% in ovine species (Vanderlinde *et al*.,1999; Zweifel & Stephan, 2003; Hutchison *et al*., 2005; Pearce & Bolton, 2005).

The linear regression to quantitatively measure the strength of the relationships between excision and swabbing methods was calculated. Because the coefficient values R^2 were very low in all species for both TVC ($R^2 < 0.45$) and EC ($R^2 < 0.20$), we conclude that the bacterial numbers from samples collected by swabbing are not related to those on the same carcass sampled by excision. Similar findings and conclusions have been reported previously in bovine, ovine and porcine livestock (Hutchison *et al*., 2005) comparing counts from near-consecutive carcasses.

However, when the TVC was lower than 4.00 log CFU cm^{-2} the percentage increased to 20.46%. Thus, we can conclude that the percentage of microorganisms recovered by swabbing diminishes as TVC obtained by excision increases or when the microbial load is high. For each of the four species examined, the percentage of TVC recovered was significantly smaller ($P < 0.05$) when TVC was higher than 4.50 log CFU cm^{-2}. At this level of TVC, no significant differences were detected among species. This may be because of the fact that cotton is not a very abrasive material and cannot capture the bacteria which are firmly stuck to the carcass surface (Capita *et al*., 2004). Also, because of the small size of the cotton swabs, they might be saturated quickly during sampling. Our results agree with Cenci-Goga *et al*. (2007), who observed, in an *in vitro* study with bovine skin cuts, inoculated with various suspensions of marker microorganisms (*Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus*), that the percentage of microorganisms recovered by the wet–dry cotton swabbing method decreased as microbial load increased.

One of the acknowledged problems of swabbing methods is the high variability of recoveries achieved. Many factors influence the recovery percentage: nature of the swab material (Dorsa *et al*., 1996; Gill & Jones, 2000; Reid *et al*., 2002; Pearce & Bolton, 2005; Pepperell *et al*., 2005), animal species (Gill & Jones, 2000; Pepperell *et al*., 2005), whether samples were collected immediately after processing or after a period of cold storage (Yu *et al*., 2001; McEvoy *et al*., 2004), number of swabs used to take the sample (Capita *et al*., 2004), microbial group (Prieto *et al*., 1991; Pepperell *et al*., 2005), nature of the tissue, humidity and texture of the surface where the sample is collected (Gill & Jones, 2000; Gill *et al*., 2001). Consequently, it is difficult to compare the results obtained by swabbing methods between different studies. A universal quantitative conversion factor between excision and swabbing results has not yet been established (Bolton, 2003; Capita *et al*., 2004). Recent studies show that through sample collection with polyurethane sponges (Byrne *et al*., 2005; Pearce & Bolton, 2005) or cellulose acetate sponges (Gill & Jones, 2000) similar counts are obtained to those collected by the excision method. Nevertheless, these works present low TVC counts (generally lower than 3.5 log CFU cm^{-2} by excision). Therefore, it may be necessary to study the effectiveness of these sponges when the counts are higher (>4 log CFU cm^{-2}) as in this case these studies reveal greater differences between the two methods.

The combination of factors influencing the percentage of microorganisms recovered by swabbing can make it difficult to interpret the results and verify production-process hygiene based on the HACCP system. Therefore, large-scale evaluation of the swabbing method is needed to determine whether it is suitable to verify HACCP efficacy (Hutchison *et al*., 2005).

The order in which the animal was slaughtered did not influence TVC (Table 5) in any of the studied species. Therefore the choice of a random sampling method, as indicated by the Regulation (EC) 2073/2005 (European Commission, 2005), would be sufficient to indicate the level of hygiene in the production process. It should be mentioned that in the slaughterhouses under study, the average number of animals slaughtered daily was small: forty bovine, fifteen equine, 270 porcine and 160 ovine. Therefore, if a greater number of animals are slaughtered, the time-course of the microbial counts should be studied throughout the whole working day, as the environmental conditions in slaughter chains (temperature, relative humidity, air currents) could modify and influence the microbial counts, favouring carcass contamination and the multiplication of the microorganisms they hold (Latre *et al*., 1997; Marjatta & Korkeala, 1997; Burfoot *et al*., 2006). The scarce difference observed in TVC on different porcine carcasses (0.10 log CFU cm^{-2}) could be because during the

Table 5 Total aerobic viable counts recoveries (log CFU cm^{-2} ± SD) obtained by the excision method according to slaughter order and animal species

	Slaughter order				
	q	***q* + *k***	***q* + 2*k***	***q* + 3*k***	***q* + 4*k***
Bovine	4.22 ± 0.72	3.83 ± 0.83	4.30 ± 0.84	4.00 ± 0.84	3.90 ± 0.60
Equine	4.87 ± 0.51	4.76 ± 0.44	4.75 ± 0.48	4.67 ± 0.45	4.63 ± 0.76
Ovine	4.49 ± 0.67	4.08 ± 0.58	4.45 ± 0.78	4.20 ± 0.57	4.25 ± 0.72
Porcine	4.32 ± 0.62	4.32 ± 0.64	4.25 ± 0.49	4.32 ± 0.53	4.22 ± 0.58

q, first carcasses selected at random; *k*, total number of animals to sacrifice this day/wanted sample size ($n = 5$).

scalding and dehairing phases cross-contamination takes place among carcasses. Microbial contamination of the carcasses also increases during dehairing (Rivas *et al.*, 2000) and there is a redistribution of the bacterial load inside the carcasses.

The excision method, commonly known as the 'destructive method', probably gets this second name from certain works (Fliss *et al.*, 1991; Palumbo *et al.*, 1999; Werlein, 2001; Ransom *et al.*, 2002) where a large amount of tissue is taken from carcasses. However, the reduced sample size taken for this work (four slivers of tissue samples of 5 cm^2 of surface with a maximum depth of 3 mm) did not reduce carcass quality because in no case was the muscular part damaged during sample collection. The time spent in taking the sample by excision was approximately half the time spent by the wet–dry cotton swabbing method (3 min vs. 6 min).

This study reveals that a drawback of the swabbing method is the low and very variable percentage of microorganisms recovered, as compared with the excision method. This prevents us from establishing a quantitative conversion factor between the counts obtained by the two methods. For these reasons we do not recommend the wet–dry cotton swabbing method to be used to assess production-process hygiene in slaughterhouses.

In conclusion, the swabbing method recovered very few TVC on carcasses compared with the excision method; moreover it depended on the microbial load on carcasses and the animal species (the percentage of TVC recovered by swabbing diminished as TVC obtained by excision increased). Consequently, the microbial load should be considered when evaluating microorganism recovery efficacy of the non-destructive methods under study.

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References

European Commission. (2005). Regulation (EC) no. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, **338**, 1–26.

Berry, B.W., Joseph, A.L. & Ai-Ti, C. (1978). Bacterial sampling techniques on beef, pork and lamb adipose tissue. *Applied and Environmental Microbiology*, **35**, 978–979.

Bolton, D.J. (2003). The EC Decision of 8th June 2001 (EC/471/2001): excision versus swabbing. *Food Control*, **14**, 207–209.

Brown, M.H., Gill, C.O., Hollingsworth, J.I. *et al.* (2000). The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology*, **62**, 7–16.

Bryant, J., Brereton, D.A. & Gill, C.O. (2003). Implementation of a validated HACCP system for the control of microbiological contamination of pig carcasses at a small abattoir. *Canadian Veterinary Journal*, **44**, 51–55.

Burfoot, D., Whyte, R., Tinker, D. *et al.* (2006). Importance of airborne contamination during dressing of beef and lamb carcasses. *Journal of Food Protection*, **69**, 2828–2836.

Byrne, B., Dunne, G., Lyng, J. & Bolton, D.J. (2005). Microbiological carcass sampling methods to achieve compliance with 2001/471/EC and new hygiene regulations. *Research in Microbiology*, **156**, 104–106.

Capita, R., Prieto, M. & Alonso-Calleja, C. (2004). Sampling methods for microbiological analysis of red meat and poultry carcasses. *Journal of Food Protection*, **67**, 1303–1308.

Cenci-Goga, B.T., Miraglia, D., Ranucci, D. *et al.* (2007). An in vitro system for the comparison of excision and wet–dry swabbing for microbiological sampling of beef carcasses. *Journal of Food Protection*, **70**, 930–936.

Dorsa, W.J., Cutter, C.N. & Siragusa, G.R. (1996). Evaluation of six sampling methods for recovery of bacteria from beef carcass surfaces. *Letters in Applied Microbiology*, **22**, 39–41.

Duffy, E.A., Belk, K.E., Sofos, J.N. *et al.* (2001). Microbial contamination occurring on lamb carcasses processed in the United States. *Journal of Food Protection*, **64**, 503–508.

Eisel, W.G., Linton, R.H. & Muriana, P.M. (1997). A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. *Food Microbiology*, **14**, 273–282.

Fliss, I., Simard, R.E. & Ettriki, A. (1991). Comparison of three sampling techniques for microbiological analysis of meat surfaces. *Journal of Food Protection*, **56**, 249–251.

Gill, C.O. (2005). Safety and storage stability of horse meat for human consumption. *Meat Science*, **71**, 506–513.

Gill, C.O. & Jones, T. (2000). Microbiological sampling of carcasses by excision or swabbing. *Journal of Food Protection*, **62**, 167–173.

Gill, C.O., Badoni, M. & McGinnis, J.C. (2001). Microbial sampling of meat cuts and manufacturing beef by excision and swabbing. *Journal of Food Protection*, **64**, 325–334.

Hansson, I.B. (2001). Microbiological meat quality in high and low capacity slaughterhouses in Sweden. *Journal of Food Protection*, **64**, 820–825.

Hutchison, M.L., Walters, L.D., Avery, S.M. *et al.* (2005). Comparison of wet–dry swabbing and excision sampling methods for microbiological testing of bovine, porcine, and ovine carcasses at red meat slaughterhouses. *Journal of Food Protection*, **68**, 2155–2162.

Latre, M.V., Vela, A.I., Lara, C., Cubero, G. & Rodríguez, A.A. (1997). Puntos críticos a considerar como fuente de contaminación bacteriológica de canales en mataderos. *Alimentaria*, **282**, 33–36.

Madden, R.H., Murray, K.A. & Gilmour, A. (2004). Determination of the principal points of product contamination during beef carcass dressing processes in Northern Ireland. *Journal of Food Protection*, **67**, 1494–1496.

Marjatta, T.R. & Korkeala, H.J. (1997). Airborne bacteria and carcass contamination in slaughterhouses. *Journal of Food Protection*, **60**, 38–42.

McEvoy, J.M., Sheridan, J.J., Blair, I.S. & Dowell, D.A. (2004). Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. *International Journal of Food Microbiology*, **92**, 217–225.

Palumbo, S.A., Klein, P., Capra, J., Eblen, S. & Millar, A.J. (1999). Comparison of excision and swabbing sampling methods to determine the microbiological quality of swine carcass surfaces. *Food Microbiology*, **16**, 459–464.

Pearce, R.A. & Bolton, D.J. (2005). Excision vs sponge swabbing – a comparison of methods for the microbiological sampling of beef, pork and lamb carcasses. *Journal of Applied Microbiology*, **98**, 896–900.

Pepperell, R., Reid, C.A., Nicolau, S. *et al.* (2005). Experimental comparison of excision and swabbing microbiological sampling methods for carcasses. *Journal of Food Protection*, **68**, 2163–2168.

Prieto, M., García, M.L., García, M.R., Otero, A., López, A.T.M. & Moreno, B. (1991). Distribution and evolution of bacteria on lamb carcasses during aerobic storage. *Journal of Food Protection*, **54**, 945–949.

Ransom, J.R., Belk, K.E., Bacon, R.T., Sofos, J.N., Scanga, J.A. & Smith, G.C. (2002). Comparison of sampling methods for microbiological testing of beef animal rectal/colonal faeces, hides, and carcasses. *Journal of Food Protection*, **65**, 621–626.

Reid, C.A., Avery, S.M., Hutchison, M.L. & Buncic, S. (2002). Evaluation of sampling methods to assess the microbiological status of cattle hides. *Food Control*, **13**, 405–410.

Rivas, T., Vizcaíno, J.A. & Herrera, F.J. (2000). Microbial contamination of carcasses and equipment from an Iberian pig slaughterhouse. *Journal of Food Protection*, **63**, 1670–1675.

Sharpe, A.N., Isigidi, C., Kingombe, B. *et al.* (1996). Efficient non destructive sampler for carcasses and other surfaces. *Journal of Food Protection*, **59**, 757–783.

Snijders, J.M.A., Janssen, M.H.W., Gerats, G.E. & Corstiaensen, G.P. (1984). A comparative study of sampling techniques for monitoring carcass contamination. *International Journal of Food Microbiology*, **1**, 229–236.

Untermann, F., Stephan, R., Dura, U., Hofer, M. & Heimann, P. (1997). Reliability and practicability of bacteriological monitoring of beef carcass contamination and their rating within a hygiene quality control programme of abattoirs. *International Journal of Food Microbiology*, **34**, 67–77.

Vanderlinde, P.B., Shay, B. & Murray, J. (1999). Microbiological status of Australian sheep meat. *Journal of Food Protection*, **62**, 380–385.

Werlein, H.D. (2001). Comparison of destructively and rinsing gained samples to determine TVC of pig carcasses by bioluminescence. *Meat Science*, **59**, 165–168.

Yu, S.L., Cooke, P.H. & Tu, S.I. (2001). Effects of chilling on sampling of bacteria attached to swine carcasses. *Letters in Applied Microbiology*, **32**, 205–210.

Zweifel, C. & Stephan, R. (2003). Microbiological monitoring of sheep carcass contamination in three Swiss abattoirs. *Journal of Food Protection*, **66**, 946–952.