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Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs

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ABSTRACT

Cattle carcasses from two abattoirs were examined at selected stages of slaughter (skinning, evisceration, trimming, washing, blast chilling) for aerobic colony counts (ACC) and *Enterobacteriaceae*. At each stage and abattoir, 50 carcasses were sampled by swabbing at the neck, brisket, flank and rump. After skinning, average ACC on carcasses was 1.5 log CFU cm^{-2} and *Enterobacteriaceae* frequencies at sites were ≤6%. From skinned to washed carcasses, certain abattoir- and site-specific changes occurred. Blasting clearly reduced ACC and *Enterobacteriaceae* results on carcasses from abattoir B, but reductions were limited or lacking in abattoir A. In addition, 100 hides and corresponding chilled carcasses were examined. On hides, average ACC was 5.6 log CFU cm^{-2} and *Enterobacteriaceae* frequencies at sites ranged from 74 to 96%. Average carcass–hide ratios of the two abattoirs were comparable for ACC (0.0182–0.0202%) but differed for *Enterobacteriaceae* counts (abattoir A: 0.4627%; abattoir B: 0.0941%). Such ratios allow comparing process performance between abattoirs in the daily practice.

1. Introduction

To ensure food safety at slaughter, additional measures to the traditional meat inspection procedures are required, in particular because healthy food-producing animals can be carriers of important bacterial pathogens causing human illness (EFSA/ECDC, 2014; Nørrung & Buncic, 2008). Such pathogens might enter the food chain by direct or indirect fecal contamination if good hygiene practices are not warranted. Strict adherence to good practices of slaughter hygiene, along with risk-based preventive measures, is therefore crucial to ensure both public health protection and meat quality. In the European Union (EU), current food hygiene legislation (Reg. (EC) No. 852/2004 and Reg. (EC) No. 853/2004) places the onus for compliance on food business operators. They must apply compulsory self-checking programs following the hazard analysis and critical control point (HACCP) approach.

For assessment of process performance, analysis of the slaughter process is of central importance. To enable risks involved to be estimated and appropriate measures to be taken, slaughter process analysis must also include abattoir-specific microbiological data on carcass contamination during slaughter (Milios, Drosinos, & Zoiopoulos, 2014; Spescha, Stephan, & Zweifel, 2006), especially because carcasses might be contaminated despite the absence of visible contamination (Gill, 2004). For verification of slaughter hygiene conditions in the daily practice, the microbial status of carcasses is often determined by monitoring indicator organisms on carcasses at the end of slaughter (Brown et al., 2000; Ruby, Zhu, & Ingham, 2007; Zweifel, Baltzer, & Stephan, 2005). In the EU, Reg. (EC) No. 2073/2005 and Reg. (EC) No. 1441/2007 set out microbial criteria for carcasses at the end of slaughter. Because of the shortcomings of such end-point criteria, comparison of the microbial contamination on hides and corresponding carcasses has been proposed (Blagojevic, Antic, Ducic, & Buncic, 2011; Vivas Alegre & Buncic, 2004).

The aim of this study was to investigate the effects of certain cattle slaughter process stages on the microbial contamination of carcasses in two large-scale Swiss abattoirs. In addition, the quantitative relationship between the carcass and hide microflora was determined in these abattoirs.

2. Materials and methods

2.1. Abattoirs and slaughter process

This study was based on investigations carried out during seven months (December 2012 to June 2013) in two Swiss abattoirs with annual slaughter capacities of >20 million kg (abattoir A: cattle, sheep, pigs; abattoir B: only cattle). Abattoir A processed up to 60 cattle carcasses per h (on average 85 cattle carcasses per day) and abattoir B up to 75 cattle carcasses per h (on average 450 cattle carcasses per day).

Slaughter operations were performed on slaughter lines featuring separated wet areas and clean areas (Table 1). After being stunned in a stunning box using a captive bolt, animals were shackled by the right rear leg and immediately (within 60 s) exsanguinated. Before skinning, head and hooves were removed. Skinning operations comprised

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Table 1
Operations performed in the cattle slaughter processes.

Location	Process stages in abattoirs A and B
Wet area	Lairage
	Captive bolt stunning; shackling by right rear leg
	Sticking and bleeding [b]
	Removal of head and hooves
	Manual pre-skinning: skin incisions and pre-skinning of rear legs, rump, flank, tail, brisket and forelegs
	Skinning by upward-pulling hide puller [a]
Clean area	Evisceration: brisket sawing, freeing of bung, removal of gut and thoracic viscera [a]
	Carcass splitting with a saw (use of cold water)
	Meat inspection and stamping
	Trimming: trimming of butt, rump and brisket; removal of mesenteric fat, diaphragm remnants and spinal cord [a]
	Carcass weighing and grading
	Final cold water washing [a]
Chiller	Two-stage air chilling process: conventional chilling with preceding blasting [a,b]

[a] Process stages surveyed for bacterial counts in the slaughter process analysis.
[b] Process stages surveyed for bacterial counts in order to determine carcass–hide ratios.

manually performed pre-skinning and mechanized skinning by an upward-pulling hide puller. Before evisceration, carcasses were moved into separated clean areas. Evisceration involved slitting the belly, removal of the gut and removal of thoracic viscera. Carcasses were then split along the midline from back to front with a splitting saw. After trimming, meat inspection, weighing and grading, carcasses were washed with cold potable water to remove visual debris (abattoir A: 12 °C for 16 s; abattoir B: 11 °C for 20 s). Both abattoirs used a two-stage air chilling process. At abattoir A, carcasses were initially blasted with air at 16 m/s and −8.0 °C for about 45 min before entering the chiller (6 m/s at 0–2.0 °C). At abattoir B, air speed and temperature were 11 m/s and 10 °C during blasting (90 min) and 5 m/s and 2.0–4.0 °C in the chiller.

2.2. Sampling

Cattle aged between 3 and 24 months were sampled. The origin of the sampled cattle was distributed throughout Switzerland. Sampling comprised two parts in both abattoirs. First, cattle carcasses were sampled after selected stages of slaughter (skinning, evisceration, trimming, washing, blast chilling). At each stage and abattoir, 50 carcasses were examined (abattoir A: 14 sampling days; abattoir B: 11 sampling days). Second, 100 cattle hides and corresponding carcasses were sampled ($n = 50$ at each abattoir). Samples were collected from hides after sticking (but before skinning) and from carcass in the chiller after blasting. Carcass and hide samples were obtained from the neck, brisket, flank and rump area using the wet-dry double swab technique (Zweifel et al., 2005). At each site, first a moistened swab (0.85% saline solution) and then a dry swab was rubbed across the sampling site (100 cm^2). Samples were transported to the laboratory chilled and microbiological examinations were carried out within 5 h after sampling.

2.3. Aerobic colony counts (ACC) and Enterobacteriaceae

Both swabs of each sampling site were homogenized for 60 s in 20 ml of 0.85% saline solution in a stomacher. Suspensions were plated with a spiral plater (Eddy Jet, IUL SA, Barcelona, Spain) onto plate count agar (Oxoid AG, Pratteln, Switzerland) for ACC and violet red bile glucose agar (VRBG agar; BBL, Cockeysville, MD, USA) for *Enterobacteriaceae*. Agar plates were incubated according to ISO 4833-1:2013 (plate count agar) and ISO 21528-2:2004 (VRBG agar). Counts were calculated as CFU cm^{-2} and the detection limit was 4 CFU cm^{-2} for carcass samples.

2.4. Data analysis

Counts were expressed as log CFU cm^{-2} and compared by reference to mean ($\bar{x}$) values. Evaluation was based on log N (log of summed counts) when the occurrence was too infrequent (<80%) to ensure log normality (McEvoy, Sheridan, Blair, & McDowell, 2004). Values differing by <0.5 ($\bar{x}$) or <1.0 log CFU cm^{-2} (log N) were regarded as similar for practical purposes. For *Enterobacteriaceae*, frequencies were additionally determined. Statistical analysis was performed using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). Analysis of variance and the Bonferroni procedure were used to analyze differences in bacterial counts between process stages, sampling sites and abattoirs. Contingency tables (Chi square test, Fisher exact test) were used to compare *Enterobacteriaceae* frequencies. The level of significance was set at $\alpha = 0.05$. In addition, carcass–hide ratios were calculated (Blagojevic et al., 2011): carcass–hide ratio (%) $= \frac{\sqrt[n]{x1*x2*\ldots*xn}\ (carcass)}{\sqrt[n]{x1*x2*\ldots*xn}\ (hide)} \times 100$, where x is CFU cm^{-2} ($x = 0$ for results below detection limit).

3. Results

3.1. ACC from cattle carcasses during the slaughter process

After skinning, mean log ACC at the different sampling sites ranged from 1.1 to 2.8 log CFU cm^{-2} in abattoir A and 0.9 to 1.7 log CFU cm^{-2} in abattoir B (Fig. 1). At abattoir A, mean log ACC from the neck and rump were similar but differed by about 0.5 and 1.7 log CFU cm^{-2} from the values of the flank ($P < 0.05$) and brisket ($P < 0.05$), respectively. The brisket thereby yielded clearly higher results than the other sites also after evisceration, trimming and washing ($P < 0.05$). At abattoir B, on the other hand, ACC from the rump of skinned carcasses differed clearly from the values of the other sites ($P < 0.05$).

Evisceration (Fig. 1) did not cause significant changes of ACC. Differences to the corresponding mean log ACC after skinning were mainly ≤0.2 log CFU cm^{-2}. After trimming, ACC increases (neck, brisket, rump) and ACC decreases (flank) were observed, but only changes at the neck, flank and rump in abattoir A were >0.5 log CFU cm^{-2} ($P < 0.05$). Amongst sites, significant differences were evident after evisceration (abattoir A: brisket versus other sites, flank versus neck/rump; abattoir B: rump versus other sites) and after trimming (abattoir A: brisket versus other sites, flank versus neck; abattoir B: brisket versus rump). Washing resulted in increases or decreases of mean log ACC (Fig. 1), but only changes at the brisket and rump in abattoir B were significant. In each abattoir, resulting mean log ACC from the neck, flank and rump were comparable (abattoir A: 1.8–2.0 log CFU cm^{-2}; abattoir B: 1.5–1.6 log CFU cm^{-2}). Clearly higher values than for the other sites were found in both abattoirs for the brisket (abattoir A: 2.7 log CFU cm^{-2}, $P < 0.05$; abattoir B: 2.2 log CFU cm^{-2}, $P < 0.05$).

Blasting mainly reduced ACC on carcasses, especially in abattoir B (Fig. 1). At abattoir B, reductions of mean log ACC after blasting ranged from 0.5 to 0.9 log CFU cm^{-2} at the different sites ($P < 0.05$), whereas reductions were lower or lacking in abattoir A (only the increase at the neck was significant in this abattoir). Resulting ACC differed significantly between the two abattoirs and mean log ACC ranged from 1.5 to 2.3 log CFU cm^{-2} in abattoir A (forequarter > flank, $P < 0.05$) and 0.8 to 1.3 log CFU cm^{-2} in abattoir B (brisket > hindquarter, $P < 0.05$).

3.2. Enterobacteriaceae results from cattle carcasses during the slaughter process

Enterobacteriaceae frequencies and log N values are shown in Table 2. Counts of the 93 *Enterobacteriaceae*-positive samples were mainly (82.8%) <1.0 log CFU cm^{-2}. Only two (2.4%) individual samples exceeded 2.0 log CFU cm^{-2}. At sequential stages of slaughter, only few changes of *Enterobacteriaceae* frequencies were significant. Significant differences were evident in abattoir A after blasting at the rump and