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The significance of clean and dirty animals for bacterial dynamics along the beef chain

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ABSTRACT

This study investigated the bacterial dynamics along the beef chain for clean and dirty cattle in the slaughter and processing lines, using classic quantitative methods and molecular analyses. In addition, the Norwegian national guidelines for Good Hygiene Practices in Norway were evaluated. In these guidelines, cattle presented for slaughter are categorised according to hide cleanliness, resulting in separate processing lines for meat from very dirty animals and reduced prices to farmers. The study was conducted in two commercial abattoirs in Norway. Two groups were compared; 40 visually clean cattle and 40 visually dirty cattle presented for slaughter, with 20 from each group at each abattoir. The same animals were sampled at five sampling sites: hides, carcass surfaces after dehiding, just before chilling, after chilling, and meat trimmings. Meat trimmings were sampled in only one abattoir. Three hundred and sixty samples were collected by swabbing 100 cm^2 of the brisket area at the first four sampling sites, and sampling 200 g of meat trimmings at the fifth site. The results showed that the hides of dirty cattle had more *Enterobacteriaceae* and higher Aerobic Plate Counts (APC) than visually clean cattle ($P < 0.05$), however there was no significant difference for *Escherichia coli*. For the other sampling sites, there were no differences between the dirty and the clean group. An effect of chilling/drying of the carcass surfaces was demonstrated by the significant reduction in the number of carcasses on which *E. coli* and *Enterobacteriaceae* were detected; from 11% and 39% before chilling to 1% and 16% after chilling, respectively. *Enterobacteriaceae* and *E. coli* were detected in only three and one of the meat trimming samples, respectively. Amplification and sequencing of the 16S rRNA gene from 643 *Enterobacteriaceae* colonies derived from 107 samples demonstrated that *Escherichia/Shigella* were dominant within this family on the hides. However, after dehiding, after grading, and after chilling, the genera *Citrobacter* and *Enterobacter* dominated. The meat trimmings were dominated by the genera *Kluyvera*, *Hafnia*, and unclassified *Enterobacteriaceae*. The relative proportions of *Escherichia/Shigella* were higher for dirty animals than for clean animals, and were higher on hides than from sampling sites further down the chain ($P < 0.05$). The minor differences in contamination on carcass surfaces and meat trimmings between clean and dirty cattle indicate that separate processing lines in Norwegian abattoirs seem to be unnecessary.

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1. Introduction

Faecal contamination of bovine carcass surfaces during slaughter and dressing can include zoonotic agents. In the USA, foods of bovine origin have been linked to about 75% of the Shiga toxin-producing *Escherichia coli* (STEC) foodborne outbreaks (Callaway et al., 2009). Hide-to-carcass contamination is regarded as the major source of contamination, whilst contamination from the alimentary tract to carcass is considered to be easier to control (Buncic et al., 2014; Bosilevac et al., 2005). Hide-to-carcass contamination is a crucial meat safety issue and requires continuous improvement by the meat industry. It is generally accepted that it is impossible to dehide an animal without contaminating the carcass, and it is even more difficult to slaughter and dehide dirty cattle in a strictly hygienic way. However, some studies have shown no association between carcass contamination and the cleanliness of the cattle presented for slaughter (Antic et al., 2010; Van Donkersgoed et al., 1997). Other studies have shown that visually dirty cattle produce carcasses with higher microbial counts than clean

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cattle (Hauge et al., 2012; Serraino et al., 2012). Buncic et al. (2014) observed that results from studies on hide cleanliness and microbiological status of hides and resultant carcasses are inconsistent, and further studies are necessary.

The Codex Code of Hygienic Practice for Meat (2005) states that cattle presented for slaughter must be clean. Several countries have established systems for improving the visual cleanliness of the hides of animals presented at slaughter, these include the UK (Food Standards Agency, 2007), Ireland (Doherty, 1999), Sweden (Swedish Board of Agriculture, 1998), and Finland (Ridell and Korkeala, 1993). The different systems used include hide cleanliness categorisation, measures for cleaning the hides, refusal to slaughter extensively dirty animals, and logistical slaughter (clean animals are slaughtered before dirty animals) (Duffy et al., 2014). The meat industry in Norway introduced national guidelines for Good Hygiene Practices based on hide cleanliness in 2007 (Animalia, 2012). The guidelines include risk categorisation of incoming cattle, since visually dirty cattle are presumed to pose a greater risk to food safety. Accordingly, dirty cattle are sent into a separate processing line where the products are heat-treated and not used for minced meat or cured sausages. The farmers obtain a lower price for very dirty cattle and a smaller deduction for moderately dirty cattle (Hauge et al., 2012). The beef industry in Norway slaughter about 300.000 cattle per year and 4–5% are categorised as dirty (category 1 or 2). The Norwegian herds are relatively small, with an average of 55 cattle per herd.

In order to minimise carcass contamination, the meat industry emphasises staff training to increase the understanding of the importance of good slaughter hygiene. Some abattoirs also invest in decontamination measures, especially in the USA. Several studies have investigated the effects of decontamination interventions applied along the slaughter line, including: hide washing (Arthur et al., 2007), hide clipping before slaughter (Baird et al., 2006), steam and hot water pasteurisation, with or without chemicals, on skinned carcasses (Huffman, 2002), steam vacuuming technique (Phebus et al., 1997), and knife trimming of dirty spots (Castillo et al., 1998). The hygienic status during slaughtering and dressing of cattle has been described in detail (Blagojevic et al., 2012; Bolton et al., 2014; Zweifel et al., 2014), but few studies have focused on the bacterial dynamics along the whole meat chain, including chilling, deboning, and processing. This is a natural consequence of the EU procedure for verifying the hygienic status of beef carcasses, which requires sampling from carcasses before chilling (EC no, 2073/2005). In this study, we addressed this issue by following the same animals along the slaughter- and processing lines, to the end product of meat trimmings. In addition to quantitative analysis, which is the classical method used to assess the hygienic status of carcasses and meat products, 16S rRNA gene sequencing of indicator bacteria (EC no, 2073/2005) was also performed. These analyses describe the diversity of *Enterobacteriaceae* at each sampling site, indicating which bacterial species are introduced, and increase or decrease in relative dominance during the different operations. Molecular assays, such as sequencing of the 16S rRNA gene, have proven useful for the identification and characterisation of bacterial populations from a wide variety of sources (Lehner et al., 2004). A major source of foodborne disease is faecal contamination of beef with *Enterobacteriaceae*, such as *Salmonella*, *E. coli*, and *Yersinia* (Gwida et al., 2014; Wright et al., 2011). Therefore, our study focuses on analyses of the *Enterobacteriaceae* family. The aims of our study were to investigate the bacterial dynamics in beef contamination along the meat chain, comparing visually clean and dirty cattle presented for slaughter, and evaluate the national guidelines for Good Hygiene Practices in Norway (Animalia, 2012).

2. Material and methods

2.1. Carcasses

The study was performed in two commercial abattoirs, one in the southwest region of Norway during January 2013 (abattoir A) and one in the southeast during February 2014 (abattoir B), both during the indoor feeding season. A total of 80 cattle were included in the study, 40 from each abattoir. An expert assessed hide cleanliness, according to the Norwegian guidelines for Good Hygiene Practices (Hauge et al., 2012), directly after stunning and bleeding. Animals with visually clean hides, with only minor quantities of faecal material or mud adhering are categorised as category 0. Moderately dirty animals, with 20–50% of areas on the thighs and/or up to 50% of mid-line cut on the abdomen and brisket covered by dry dirt, are categorised as category 1. Animals with very dirty hides, with more than 50% of the thighs and legs covered in dry dirt and/or more than 50% of mid-line on the abdomen and brisket covered in dry dirt are categorised as category 2. Very dirty cattle with wet dirt are categorised separately; they are processed as though they are category 2 animals, but there are no deductions from the sum paid to the farmers since the soiling might have happened after the animal left the farm. In each of the abattoirs, the cattle were categorised as 20 clean animals and 20 visually dirty animals (mainly as category 2). Animals with moderately dirty hides (category 1), were only included in the study if there were insufficient category 2-animals; three category 1 carcasses were included from abattoir A, and seven category 1 carcasses were included from abattoir B.

2.2. Slaughter lines, chilling, and deboning

The slaughter line speeds in both abattoirs were about 10–15 animals per h during the days of this investigation. The line speed is usually 25–30 carcasses per h, but because sheep and pigs were also being slaughtered at the abattoirs during the time of the study, there were fewer operators on the cattle lines. Dehiding operations in both abattoirs comprised pre-skinning by knife and then use of an upward-puller. No washing of the carcasses was performed at any stage, and knife trimming at the end of the slaughter line was the only decontamination intervention in both abattoirs. Rodding of the oesophagus using clips and bagging of the rectum with a plastic bag were performed before evisceration. The split carcasses entered a chilling tunnel for 2 h at 2–4 °C, and were stored at 4 ± 1 °C. Carcasses in category 2 (from very dirty animals) were stored and deboned separately from the others. The carcasses were deboned and cut into trimmings after two days in the chiller.

2.3. Sampling

In total, 320 swab samples and 40 trimming samples were collected from the same animals (n = 80) at five locations along the value chain. The first sampling location was immediately after stunning and bleeding, where the brisket of the hides was swabbed. The second site was alternately either right or left side of the mid-line immediately after loosening the hide from the brisket area using a knife, and just before mechanical hide pulling. The third site was at the end of the slaughter line, just before chilling, and the fourth site was after chilling. Samples taken from the brisket just after dehiding reflect contamination during dehiding, whilst samples collected at the end of the slaughter line also reflect contamination and hygiene during the steps following dehiding, which are mainly evisceration and knife trimming. In abattoir A, the carcasses were sampled along the slaughter-line over three days, and in abattoir B the carcasses were sampled over two days. At the fifth sampling location, approximately 200 g samples of meat trimmings were collected in plastic bags from each of the 40 carcasses in the deboning department of abattoir A.

At the first four sites along the meat chain, sampling was by swabbing, using cotton-cloths (Mesosoft 10x10 cm, type 157300, Mölnlycke HealthCare, Gothenburg, Sweden) moistened in 10 ml of sterile saline peptone water (pH 7.0 ± 0.2) (Oxoid Ltd., Basingstoke, Hampshire, UK). One cloth was rubbed on a 100 cm^2 area on the brisket surface. The cotton-cloths were placed in individual sterile stomacher bags (BagLight PolySilk, Interscience, St Nom, France). All samples were

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