Food Control 31 (2013) 372–378

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Microbiological contamination of sheep carcases in Finland by excision and swabbing sampling

Satu P. Salmela [a,*,1], Maria Fredriksson-Ahomaa [a], Maija Hatakka [b,2], Mari Nevas [a]

[a] Faculty of Veterinary Medicine, The Department of Food Hygiene and Environmental Health, University of Helsinki, Agnes Sjöbergin katu 2, P.O. Box 66, FI-00014, Finland
[b] Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland

ARTICLE INFO

Article history:
Received 1 May 2012
Received in revised form
7 October 2012
Accepted 13 October 2012

Keywords:
Sheep carcass
Regulation 2073/2005
Excision
Swabbing
Microbiological contamination

ABSTRACT

The aims of this study were to provide data on the microbiological contamination of sheep carcases slaughtered in Finland, and to compare excision and swabbing methods for microbiological sampling of sheep carcases. The results were also compared to the requirements laid down in Regulation (EC) No 2073/2005. A number of 50 sheep carcases were sampled both by excision and by swabbing with gauze at four slaughterhouses. The samples were analysed for total viable counts (TVC's), *Enterobacteriaceae*, and *Escherichia coli*. The same carcases were also sampled for *Salmonella* spp. by swabbing. The mean levels of TVC's and *Enterobacteriaceae*, and the results for *Salmonella* spp. were in line with the requirements laid down in Regulation (EC) No 2073/2005. The mean levels of TVC's for samples by excision and by swabbing were 3.77 log CFU/cm^2 and 3.16 log CFU/cm^2, respectively. *Enterobacteriaceae* were recovered from 72% and 76% of the carcases, and *E. coli* from 48% and 61% by excision and swabbing, respectively. No carcases were found positive for *Salmonella* spp. The mean levels of TVC's, *Enterobacteriaceae* and *E. coli* were significantly higher for the samples by excision than for the samples by swabbing. When the relationship between the sampling methods were analysed, the results for excision samples and swabbing samples were related for the TVC's, and there was a significant correlation between the sampling methods for *Enterobacteriaceae*. The results suggest that swabbing by gauze can be used as an alternative sampling method to excision. In addition, a significant correlation was seen between the results for *Enterobacteriaceae* and *E. coli* for the samples collected by swabbing, suggesting that *E. coli* can be used as an indicator bacterium instead for *Enterobaceteriaceae* for sampling of sheep carcases by swabbing.

1. Introduction

During slaughtering process carcases can be contaminated microbiologically by different routes such as by direct contact with the fleece, equipment and facilities, and the hands of personnel but also by indirect contamination via air (Burfoot et al., 2006; Gill, 1987; Gill & Baker, 1998). The hygienic status of slaughtering process and the microbiological quality of carcases are monitored by microbiological sampling of carcases. In the European Union, this sampling is performed according to Regulation (EC) No 2073/2005 as a part of the slaughterhouses procedures based on HACCP principles and good hygiene practice (Anon, 2005). In the Regulation, the process hygiene criteria indicating the acceptable functioning of the slaughtering process of sheep are set for aerobic colony count (total viable count, TVC), *Enterobacteriaceae* and *Salmonella* spp. The limits for satisfactory, acceptable and unsatisfactory results given for the TVC's and *Enterobacteriaceae* in the Regulation apply only for the samples taken by excision, daily means for unsatisfactory results being >5.0 log CFU/cm^2 TVC's and >2.5 log CFU/cm^2 for *Enterobacteriaceae*. However, food business operators may use other sampling methods, such as swabbing, if they can demonstrate to the competent authority that these procedures provide at least equivalent guarantees of food safety than sampling by excision. For process hygiene criteria even testing against alternative micro-organisms can be allowed.

Previous studies have shown that there are several factors that may influence the number of bacteria recovered from carcases, for example the sampling method, the animal species sampled, the material used for swabbing, sampling site on the carcase, the person who collects the sample, and the micro-organism to be analysed (Byrne, Dunne, Lyng, & Bolton, 2005; Dorsa, Cutter, & Siragusa, 1996; Gill & Baker, 1998; Gill & Jones, 2000; Ingram & Roberts, 1976; Martínez, Celda, Anastasio,

* Corresponding author. Tel.: +46 18 17 55 00.
E-mail address: satu.salmela@helsinki.fi (S.P. Salmela).
[1] Present address: The National Food Agency, P.O. Box 622, SE-751 26 Uppsala, Sweden.
[2] Present address: Dr. Hatakka is presently retired.

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2012.10.017

García, & López-Mendoza, 2010; Pepperell et al., 2005; Prieto, Garcia, Garcia, Otero, & Moreno, 1991; Snijders, Janssens, Gerats, & Cortiaensen, 1984; Zweifel & Stephan, 2003). Also, sampling at different points of time during the slaughtering and chilling process has shown to have an effect on the results (Lenahan, O'Brien, Kinsella, Sweeney, & Sheridan, 2010; McEvoy, Sheridan, Blair, & McDowell, 2004; Prieto et al., 1991). All these factors complicate the comparison of the results from the different studies. In order to establish a universal conversion factor between the results obtained by using the excision and swabbing sampling methods, all the factors listed above should be taken into account (Bolton, 2003). To date, no conversion factor between results obtained by excision and swabbing has been able to set, and the relationship between the results from the sampling by different methods for sheep has been low (Bolton, 2003; Capita, Prieto, & Alonso-Calleja, 2004; Hutchison et al., 2005; Martínez et al., 2009). In many studies, the bacterial counts from sampling by excision have been significantly higher than by swabbing, although swabbing has been shown to give relatively similar results with those collected by excision when swabbing was done more abrasively (Byrne et al., 2005; Dorsa et al., 1996; Gill & Jones, 2000; Martínez et al., 2009, 2010; Pearce & Bolton, 2005; Pepperell et al., 2005).

Mutton production is very low in Finland, and only about 37,000 sheep are being slaughtered annually (Tike, 2011). In 2011, there were 40 slaughterhouses approved for slaughtering sheep in Finland (Evira, 2012). Most of these slaughterhouses are relatively small, and all slaughterhouses slaughter sheep a few days a week or only periodically. Sampling by excision can be seen as a more complex method, and thus many slaughterhouses may wish to choose an alternative sampling method. Also the use of alternative indicator bacteria can be of interest to food business operators, for example *Escherichia coli* being a more specific indicator for faecal contamination than *Enterobacteriaceae* (ICMSF, 1978, pp. 8–11). However, small slaughterhouses may have difficulties to provide sufficient data to support the use of alternative sampling procedures, and would benefit from scientific studies in their planning of risk management measures. The microbiological contamination of sheep carcases in Finland has not been studied before.

The aims of this study were to provide data on the microbiological contamination level of sheep carcases slaughtered in Finland, and to compare excision and swabbing methods in microbiological sampling of sheep carcases. The results were also compared to the requirements laid down in Regulation (EC) No 2073/2005.

2. Materials and methods

2.1. Slaughterhouses

Samples for this study were collected at four different slaughterhouses (A, B, C and D). All the slaughterhouses that participated in the study were small slaughterhouses slaughtering discontinuously, and the speed of the slaughter lines was approximately 15–25 sheep per hour. Fleece removal was initiated manually and completed with vertical hide puller. No tying of oesophagus or bagging of rectum was applied in any of the establishments.

2.2. Collection of samples

Samples were collected by one person on three different occasions at three of the slaughterhouses. At one slaughterhouse only one slaughtering occasion was sampled due to low slaughtering frequency. In total, 50 sheep carcases were sampled.

Samples were collected by both excision and swabbing from each carcase at the end of the slaughter line after dressing but before chilling in accordance with EC Regulation No 2073/2005. Each carcase was sampled at four different sites (breast, brisket, lateral thorax, and flank). At each site, an area of 5 cm^2 was sampled by excision using a sterile template, giving a total sampling area of 20 cm^2 for each carcase. A tissue sample of a thickness of approximately 2 mm was taken out from each site using sterile instruments and gloves, and all the tissue samples from each carcase were pooled for analysis.

Each carcase was also sampled by swabbing method from four sites adjacent to the excision sampling sites. Samples were collected using a sterile template delineating the sampling area of 50 cm^2, the total sampling area being 200 cm^2 for each carcase. One sterile gauze moistened with saline peptone water (distilled water with 0.85% NaCl and 0.10% peptone) was used for swabbing each carcase. Each sampling site was swabbed both vertically and horizontally for at least 20 s, and an unused surface of the same gauze was used for each sampling site. After swabbing the gauze was placed in a sterile container, and 10 ml of saline peptone water added.

In addition, each carcase was sampled for *Salmonella* spp. by swabbing method. Samples were collected from an approximately 5 cm wide area of both inner and outer surface of the carcase stretching from anus to brisket along the ventral cut covering a total sampling area of minimum 1100 cm^2 for each carcase. Two sterile gauzes moistened with 10 ml buffered peptone water (Oxoid, Basingstoke, UK) were used for swabbing each carcase. An unused surface of the gauzes was used for different parts of the sampling site. After swabbing the gauzes were placed in an aseptic Stomacher bag and the bag was sealed for transportation.

Samples were cooled immediately after their collection down to about 4 °C, transported to the laboratory in a cool box containing several ice packs, and stored at 4 ± 1 °C until analysing. Analyses were initiated as soon as possible after arrival to the laboratory, but after storage overnight at the latest.

2.3. Microbiological analyses

Both the samples collected by excision and by swabbing were analysed for total viable counts (TVC's), *Enterobacteriaceae*, and *E. coli*. Before analyses a volume of 100 ml of saline peptone water was added to the excision samples, and 90 ml of saline peptone water to the swab samples. Samples were then homogenized for 2 min, and series of 10-fold dilutions (10^{-6} for TVC's, and 10^{-4} for *Enterobacteriaceae* and *E. coli*) were prepared for analyses.

For TVC's,1 ml of each dilution was pour plated on two parallel plates with plate count agar (Difco, Detroit, USA), and the plates were incubated at 30 °C for 72 h following the procedures in the ISO 4833:2003.

Analyses for *Enterobacteriaceae* were done as described in ISO 21528-2:2004. In short, 1 ml of each dilution was first pour plated with violet red bile glucose agar (Oxoid). After solidification, an additional layer of violet red bile glucose agar was poured on and the plates were incubated at 37 °C for 24 h. Five characteristic colonies per plate were confirmed by oxidase test, and by glucose fermentation test.

E. coli was analysed as described in the Nordic Committee on Food Analysis' Method No 125 (NMKL, 2005). An amount of 1 ml of each dilution was first pour plated with tryptic soy agar (Difco) and preincubated for 1–2 h at room temperature, after which a layer of violet red bile agar (Oxoid) was poured on, and the plates were incubated at 44 °C for 24 h. Five characteristic colonies per plate were confirmed by testing the production of indole and gas in lactose tryptose lauryl sulphate broth (Scharlau, Sentmenat, Spain).

Analyses for *Salmonella* spp. were done according to the Nordic Committee on Food Analysis' Method No 71 (NMKL, 1999). After adding 225 ml buffered peptone water (Oxoid) and massaging the