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Determination of microbial surface contamination on beef carcasses

Untersuchung der mikrobiellen Oberflächenkontamination von Rinderschlachtkörpern

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Summary

This study determined the microbial surface contamination of a total of 120 half beef carcasses from a high-capacity and a low-capacity beef slaughterhouse in Ankara. Samples were taken by aseptic excision-sampling of the muscle-adipose tissue surface of the flank, brisket and rump areas of each half carcass (0.1–0.2 cm thickness, 10x10 cm^2 per site; 300 cm^2 total). The samples were analyzed for total aerobic plate counts (TAPC), coagulase-positive staphylococci (CPS), Enterobacteriaceae (EB), total coliform counts (TCC), *Escherichia coli* biotype I (EC) and presence of *Salmonella* spp.
Levels of both microbial surface contamination and faecal contamination of the beef carcasses tested were high. Mean log TAPC, EB, TCC, EC and CPS were 4.78, 2.08, 2.03, 1.98 and 3.85 log cfu/cm^2, respectively. EB, TCC, EC and CPS were detected in 81.6 % (98/120), 79.1 % (95/120), 65 % (78/120) and 38.3 % (46/120) of the samples, respectively. *Salmonella* spp. were not detected in any samples.
It is therefore recommended that slaughterhouses introduce internal hygiene measures such as the HACCP system in order to produce beef of good quality and thus protect public health.

Keywords: Beef carcass, microbial contamination, *Escherichia coli* biotype I, *Salmonella* spp.

Zusammenfassung

In der vorliegenden Studie wurde die mikrobielle Oberflächenkontamination von 120 Rinderschlachtkörperhälften in zwei Rinderschlachtbetrieben in Ankara ermittelt, wobei der eine Betrieb hohe und der andere geringe Schlachtzahlen aufwies. Die Proben wurden destruktiv aus dem oberflächlichen Muskel- und Fettgewebe aus dem Bereich der Flanke, der Brust und der Hüfte jeder Schlachkörperhälfte entnommen (0,1–0,2 cm Dicke, 10x10 cm^2 pro Probenahmestellen; 300 cm^2 insgesamt). Die Proben wurden auf die aerobe Gesamtkeimzahl, Koagulase-positive Staphylokokken, Enterobacteriaceae, Coliforme, *Escherichia coli* Biotyp I und *Salmonella* spp. untersucht.
Für die untersuchten Rinderschlachtkörper wurden hohe Keimzahlen sowohl der mikrobiellen Oberflächenkontamination als auch der fäkalen Kontamination ermittelt. Im Durchschnitt ergaben sich Keimzahlen von 4,78, 2,08, 2,03, 1,98 und 3,85 log KbE/cm^2 für die aerobe Gesamtkeimzahl, die Enterobacteriaceae, die Coliformen, *Escherichia coli* Biotyp I bzw. Koagulase-positive Staphylokokken. Enterobacteriaceae, Coliforme, *Escherichia coli* Biotyp I und Koagulase-positive Staphylokokken wurden bei 81,6 % (98/120), 79,1 % (95/120), 65,0 % (78/120) bzw. 38,3 % (46/120) der Proben nachgewiesen. *Salmonella* spp. wurde bei keiner der Proben nachgewiesen.
Demzufolge wird empfohlen, dass die Schlachthöfe betriebseigene Hygienemaßnahmen wie HACCP-Systeme einführen, um Rindfleisch guter Qualität zu produziern und somit die öffentliche Gesundheit zu schützen.

Schlüsselwörter: Rinderschlachtkörper, mikrobielle Kontamination, *Escherichia coli* Biotyp I, *Salmonella* spp.

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Introduction

Beef is an important product among meat species regarding its volume of consumption and production. However, beef and beef products are commonly associated with foodborne infection/poisoning resulting from *Salmonella* spp., *Escherichia (E.) coli, Clostridium (C.) perfringens* and *Staphylococcus aureus. Salmonella* spp., *E. coli* and *C. perfringens* potentially reside on the hide or in the intestinal tract of slaughtered animals (Bean and Griffin, 1990; Vanderlinde et al., 1998; Sumner et al., 2003). The internal surfaces of beef carcasses are generally sterile, and bacterial contamination of carcass surfaces occurs during slaughter and/or dressing procedures (Gracey et al., 1999; Sofos et al., 1999; Duffy et al., 2001; Phillips et al., 2001). The bacteria originate from a variety of sources, such as hides, intestinal contents, contact surfaces and handling by workers (Hansson, 2001). However, if appropriate hygiene measures are taken during the slaughter process, both bacterial surface contamination and faecal contamination of beef carcasses could be minimised (USDA/FSIS, 1996; Gracey et al., 1999).

Duffy et al. (2001) reported that microbiological analyses including total aerobic plate counts, total coliform counts, *E. coli* and *Salmonella* spp. would provide more information about the general level of microbiological contamination of carcasses. However, according to EU Decision 2004/379/EC (Anonymous, 2004), total viable counts and Enterobacteriaceae are used as indicators of hygiene and faecal contamination of carcasses.

This study was undertaken to determine microbial surface contamination on beef carcasses that had been chilled for 30 minutes after slaughter. To evaluate the microbiological quality of beef carcasses, samples were analysed for total aerobic plate counts, coagulase-positive staphylococci, Enterobacteriaceae, total coliform counts, *E. coli* biotype I and presence of *Salmonella* spp. The aim was to provide a basis for improving hygiene measures in slaughterhouses in order to produce beef of good quality and safety and thus to protect public health.

Material and Methods

Study design

This study was undertaken between May and October 2007 at two different-sized beef slaughterhouses (high/low capacity) in Ankara. Each plant was visited six times per month and ten samples were randomly collected during each visit from half beef carcasses that had been chilled for 30 min after slaughter. A total of 120 samples were thus obtained.

The high-capacity slaughterhouse slaughtered between 250 and 300 cattle/day, while the low-capacity slaughterhouse slaughtered 25 to 50 cattle/day. At the high-capacity and the low-capacity slaughterhouse, different workers performed different tasks, and there was a movement of staff between the clean and unclean parts of the slaughter line. Slaughtering techniques (killing, bleeding, skinning, evisceration etc.) were similar in both of the slaughterhouses. In both types of slaughterhouse, carcasses were washed with cold water (+7±1 ºC) before chilling. Total aerobic plate counts were below the level of detection (<1.0x10^1 cfu/ml) in washing water.

Sampling methods

All samples were taken by aseptic excision-sampling of the muscle-adipose tissue surface (0.1–0.2 cm thickness, 10x10 cm = 100 cm^2 per site) of the flank, brisket and rump areas of half beef carcasses as described by USDA/FSIS (1996) and pooling these samples to produce one total 300 cm^2 sample per half carcass. Following collection, all samples were placed in sterile bags and transported to the laboratory in coolers with ice packs for analysis.

Microbiological analysis

The muscle-adipose tissue samples were analysed for total aerobic plate counts (TAPC), coagulase-positive staphylococci (CPS), Enterobacteriaceae (EB), total coliform counts (TCC), *E. coli* biotype I (EC) and presence of *Salmonella* spp. Buffered peptone water (100 ml) was added to each sample before homogenisation in a stomacher (Laboratory Blender 400, Seward, London, UK) for 2–3 min. Tenfold dilutions were then prepared in sterile peptone (0.1 %) water.

Total aerobic plate counts were detected and counted on Plate Count Agar (Oxoid, Basingstoke, Hampshire, UK) incubated at 35 ºC for 48 h. Coagulase-positive staphylococci were detected on Baird-Parker Agar (Oxoid) incubated at 35 ºC for 24–48 h and suspected colonies (grey-black shiny convex colony with narrow white entire margin surrounded by a zone of clearing) were confirmed by the coagulase test on EDTA-coagulase plasma (Remel, Lenexa, KS, USA) within 24 h at 37 ºC (FDA/BAM, 2002). Enterobacteriaceae were detected and counted on Violet Red Bile Glucose Agar (Oxoid) incubated at 30 ºC for 24–48 h. Round, purple-pink, 1–2 mm diameter colonies surrounded by purple haloes were identified as Enterobacteriaceae (Baumgart, 1997). Total coliforms and *E. coli* biotype I were detected and counted on selective *E. coli*/Coliform Chromogenic Agar (Oxoid) incubated at 37 ºC for 18–24 h. Purple and pink colonies were then grown on selective *E. coli*/Coliform Chromogenic Agar according to the manufacturer's instructions. Coliform bacteria were confirmed in Lauryl Sulphate Broth (Oxoid) incubated at 35 ºC for 24–48 h. *E. coli* were confirmed in Brilliant Green Bile Broth (Oxoid) incubated at 44.5 ºC for 24–48 h. Loopfuls from the positive tubes in Brillant Green Bile Broth (growth with gas production) were streaked onto Eosin Methylene Blue Agar (Oxoid), incubated at 35 ºC for 24 h and individual colonies (2–3 mm diameter, exhibiting a greenish metallic sheen by reflected light) were IMViC tested. Colonies exhibiting + + – – results on IMViC tests were identified as *E. coli* biotype I (FDA/BAM, 2002).

Samples were analysed for *Salmonella* spp. following enrichment, isolation and identification procedures as recommended by ISO (Anonymous, 2002). In brief, homogenised samples in buffered peptone water were incubated at 37±1 ºC for 18±2 h to resuscitate damaged cells. One millilitre of resuscitated culture was inoculated into 10 ml of Müller-Kauffmann-Tetrathionate Broth (Oxoid) and incubated at 37±1 ºC for 24±3 h. A subsample of 0.1 ml was inoculated into 10 ml Rappaport-Vassiliadis Broth (Oxoid) and incubated at 41.5±1 ºC for 24±3 h. After enrichment, a loop of each broth culture was inoculated onto both Xylose Lysine Desoxycholate Agar (XLD; Oxoid) and Brilliant Green Agar (Oxoid) and incubated at 37±1 ºC for 24±3 h. Typical *Salmonella* spp. colonies were confirmed using biochemical and serological (Denka Seiken, Tokyo, Japan) tests.

Statistical analysis

All bacterial counts were converted to $\log_{10}$ cfu/cm² values and data were analysed using analysis of variance (GLM) by the SPSS 10.0 statistical package programme (SPSS Inc., Chicago, IL, USA).

Results

As shown in Table 1, the mean log TAPC, EB, TCC, EC and CPS for all samples in the present study were 4.78, 2.08, 2.03, 1.98 and 3.85 log cfu/cm², respectively. *Salmonella* spp. were not detected on any of the beef carcass samples.

As shown in Table 2, EB, TCC, EC, CPS were detected on 81.6 % (98/120), 79.1 % (95/120), 65 % (78/120), 38.3 % (46/120) of the samples, respectively. In the other samples, bacterial counts were below the level of detection (<1.52 log cfu/cm²).

The microbiological results obtained from samples taken from the low-capacity and the high-capacity slaughterhouse are shown in Table 3. Mean counts of TAPC, EB, TCC, EC and CPS were determined as 4.71, 1.98, 1.94, 1.90 and 3.93 log cfu/cm², respectively, in samples from the low-capacity slaughterhouse, while mean counts of TAPC, EB, TCC, EC and CPS of 4.86, 2.18, 2.12, 2.07 and 3.77 log cfu cm^{-2} , respectively, were found in samples from the high-capacity slaughterhouse.

TABLE 3: *Mean number of bacteria (log10 cfu/cm2) on beef carcasses at the low-capacity and the high-capacity slaughterhouse*

Bacteria	Low-capacity slaughterhouse (n=60)			High-capacity slaughterhouse (n=60)		
	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
Total aerobic plate count	4.71 ± 0.13[a]	3.75	5.34	4.86 ± 0.15[a]	3.52	5.62
Enterobacteriaceae	1.98 ± 0.12[b]	1.52	3.78	2.18 ± 0.11[b]	1.52	3.78
Total coliform count	1.94 ± 0.09[c]	1.52	2.98	2.12 ± 0.11[c]	1.52	3.07
E. coli biotype I	1.90 ± 0.08[d]	1.52	2.52	2.07 ± 0.13[d]	1.52	3.20
Coagulase-positive staphylococci	3.93 ± 0.09[e]	3.41	4.30	3.77 ± 0.09[e]	3.14	4.38
Salmonella spp.	not detected			not detected		

[a-e]: Mean logs with the same letter are not significantly different (p>0.05).

Discussion

In the present study, the mean log TAPC were 4.78 cfu/cm². Çalıcıoğlu et al., (2004) reported a mean log TAPC of 4.10 cfu/cm² in samples taken by an aseptic-excision method after chilling for 24 h. Other studies have reported a mean log TAPC of 3.13 (Vanderlinde et al., 1998), 2.59–3.44 (Hansson, 2001), 2.43 (Phillips et al., 2001), 3.88–4.37 (Fries et al., 2002), 1.82 (Sumner et al., 2003) and 1.3 cfu/cm² (Phillips et al., 2006) for beef carcasses, i. e. considerably lower than the values observed in the present study. Slaughterhouse hygiene, slaughter technique and slaughterhouse capacity are important factors in microbial surface contamination of beef carcasses. However, sampling procedures (cotton swabbing, sponging, excising), sampling sites, sampling time and slaughter process stage can also affect the results. For example, the sampling procedure has been reported to have a significant effect on the determination of microbial surface contamination of beef carcasses (Dorsa et al., 1996; Gill et al., 2001; McEvoy et al., 2004).

Murray et al. (2001) reported EB on 21 % of samples taken after chilling, a much lower level than that found in this study. The level of TCC detection (79.1 %) in this study was also higher than that reported by Hansson (2001). *E. coli* biotype I was present in 65% of samples. This is in agreement with results found by Çalıcıoğlu et al. (2004), but it is higher than the levels reported by Hansson (2001), Sumner et al. (2003) and Phillips et al. (2001, 2006). The detection of CPS on 38.3 % of samples in this study was higher than the detection rates of 29 % reported by Vanderlinde et al. (1998), those of 34–42 % determined by Hansson (2001), those of 25.3 % determined by Phillips et al. (2001) and rates of 28.7 % reported by Phillips et al. (2006).

The results in this study are generally in agreement with those reported by Çalıcıoğlu et al. (2004) for slaughterhouses in Elazığ, but are higher than those obtained in other studies undertaken outside Turkey. Differences between the results may be based on differences between countries regarding slaughter hygiene, slaughter techniques and not implementing HACCP processes in slaughterhouses. After bleeding, a plastic bag is not used for sealing off the rectum to avoid faecal contamination during slaughter in Turkey. This results in faecal contamination of carcasses during the evisceration process.

As shown in Table 3, mean numbers of bacteria on beef carcasses at the high-capacity slaughterhouse were higher than at the low-capacity slaughterhouse. However, this difference was not statistically significant (p>0.05). The fact that no significant differences were observed between the slaughterhouses is probably due to the use of the same slaughtering techniques and the movement of staff between the clean and unclean parts of the slaughter line.

The TAPC for 35 % of the samples from the high-capacity slaughterhouse and for 38.3 % of the samples from the low-capacity slaughterhouse was found to be above 5 log cfu/cm², the threshold limit established by EU Direc-

TABLE 1: *Mean numbers (log10 cfu/cm²) of bacteria on all beef carcasses sampled (n=120)*

Bacteria	Mean	Min.	Max.
Total aerobic plate count	4.78	3.52	5.62
Enterobacteriaceae	2.08	1.52	3.78
Total coliform count	2.03	1.52	3.07
E. coli biotype I	1.98	1.52	3.20
Coagulase-positive staphylococci	3.85	3.14	4.38
Salmonella spp.	not detected	–	–

TABLE 2: *Prevalence of Enterobacteriaceae, total coliforms, E. coli biotype I, coagulase-positive staphylococci and Salmonella spp. on beef carcasses*

Bacteria	Number of positive samples/ number analyzed (% positive)
Enterobacteriaceae	98/120 (81.6)
Total coliform count	95/120 (79.1)
E. coli biotype I	78/120 (65.0)
Coagulase-positive staphylococci	46/120 (38.3)
Salmonella spp.	0/120 (0.0)

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tive 2004/379/EC for excision samples. The EB counts in 11.6 % (7/60) of the samples from the low-capacity slaughterhouse and in 15 % (9/60) of the samples from the high-capacity slaughterhouse were also found to be above the threshold limit of 2.5 log cfu/cm^2 established by EU Directive 2004/379/EC for excision samples.

Conclusions

Levels of both microbial surface contamination and faecal contamination of 120 beef carcass samples taken from two Ankara slaughterhouses were found to be high compared with values reported internationally. Therefore, it is recommended that these slaughterhouses introduce hygiene measures with a HACCP system in order to produce beef of good quality and thus protect public health.

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