DETERMINATION OF MICROBIAL CONTAMINATION, PH AND TEMPERATURE CHANGES IN SHEEP AND CATTLE CARCASSES DURING THE SLAUGHTER AND PRE-COOLING PROCESSES IN KONYA, TURKEY

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ABSTRACT

This study was conducted to determine microbial contamination, pH and internal temperature changes in sheep and cattle carcasses during slaughter and chilling stages. Samples were analysed for the presence of *Salmonella* spp. and Enterobacteriaceae and aerobic colony counts (ACC) were performed. Air sampling was also performed in slaughtering areas and chilling rooms.

Mean values of ACCs were between 2.57±0.61 and 4.71±0.24 log CFU/cm^2 and between 3.51±0.48 and 5.19±0.28 log CFU/cm^2, whereas Enterobacteriaceae counts were between 0.89±0.46 and 2.61±0.10 log CFU/cm^2 and between 0.55±0.37 and 3.63±0.39 log CFU/cm^2 in cattle and sheep carcasses, respectively. Enterobacteriaceae contamination in the shoulder region of cattle carcasses after washing, Enterobacteriaceae contamination in all regions in sheep carcasses after chilling and ACC in the shoulder region of sheep carcasses after chilling all exceeded the limits of EC regulation (EC No 2073/2005).

Keywords: air sampling, Enterobacteriaceae, *Salmonella* spp., slaughtering stages, ACC

1. INTRODUCTION

It is desirable to keep the initial microorganism load in meat as low as possible and to observe hygiene rules during slaughtering. Therefore, it is very crucial that slaughtered animals are cut according to hygiene rules in slaughterhouses. Carcasses naturally have a low level of microbial flora and can be regarded to be sterile immediately after slaughtering. Microbial contamination can occur in slaughtered animals in most of the stages throughout the slaughtering process. The slaughtering steps are basically followed by bleeding, dressing, evisceration, washing and finally storage. The hygienic bleeding and dressing system described by FAO is to allow the animal to move up from the back leg to an upright position to allow the bleeding to continue until the blood flow reaches negligible level. Besides, inadequate hygiene conditions along the dressing cause the bacteria to spread from the carcass to the knives and to the hands of the operators. Contamination of carcasses may also occur through direct contact with equipment or hands of the personnel or may also occur indirectly through microorganisms in the air of the slaughterhouse following slaughter (UNTERMAN *et al*., 1997, GILL and BAKER, 1998, BURFOOT *et al*., 2006).

The European Union legislation has declared that the Enterobacteriaceae content and Aerobic colony count (ACC) should be used as hygiene criteria throughout the slaughtering process and that measures should be taken if the values increase above the criteria for slaughtered animals during the slaughtering process (Barco *et al*., 2015). The legislation required monitoring of the above bacterial groups as process hygiene criteria for cattle, sheep and other slaughtered animals and were declared to be Hazard Analysis and Critical Control Point (HACCP) indicators for an acceptable food processing system (EC No 2073/2005; Barco *et al*., 2015). According to the legislation, the ACC and Enterobacteriaceae limits for carcasses of cattle and sheep were declared as minimum (m) and maximum (M) values were 3.5 log CFU/cm^2 - 5.0 log CFU/cm^2 and 1.5 log CFU/cm^2 - 2.5 log CFU/cm^2, respectively.

The European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ) has also introduced the current requirement that the interior temperature of the carcass should not higher than 7 °C immediately after the post-mortem examination, before transporting. A panel of researchers has stated that temperature-time profiles can be applied to obtain similar or reduced levels of carcass contamination and that contamination levels at this temperature range are typically related to the initial level of contamination.

In most studies on carcass surface microbiology, non-invasive methods are used, such as the swab method (MCEVOYA, 2004). The swab method is the preferred method for carcass sampling according to HACCP requirements for European Union slaughterhouses (Pepperell *et al*., 2005). There are a number of published studies in which swab sampling methods have been utilised (ANDERSON *et al*., 2005; BLAGOJEVIC, 2012; BARCO *et al*., 2015; PETRUZZELLI *et al*., 2016; ALONSO-CALLEJA *et al*., 2017).

Carcass samples used in this study were slaughtered using procedures based on 'Good Hygiene Practices' and 'HACCP' principles, related to European Union Regulation 852/2004. The main purpose of the present study was to determine whether the Turkish Food Codex Hygiene Criteria and Commission Regulation (EC) No 2073/2005, are met in cattle and sheep slaughterhouses in the Konya province, which is the biggest producer of red meat in Turkey. In addition, we aimed to detect the airborne contamination in slaughtering area and cold storage rooms, to identify the sources of contamination during slaughtering and to detect the incidence (presence or absence) of *Salmonella* spp. contamination in sheep and cattle carcasses.

2. MATERIALS AND METHODS

2.1. Sample collection

In this study, changes in microbial flora, pH and temperature in cattle and sheep carcasses during different slaughter stages were investigated in three different large-scale slaughterhouses (with a daily cutting capacity of at least 40 cattle, according to the classification of Turkish slaughterhouses) between December 2013 and April 2016 in Konya, Turkey. Swab samples moistened with sterile buffered peptone water (BPW) were collected using the swab technique consisting of 5 vertical and horizontal passes described by USDA with slight modification. We swabbed an area of 10 × 10 cm^2 from five randomly chosen regions of the carcasses, including two shoulders, two rumps and briskets, after three different cutting stages (dressing, evisceration and washing) and after storage of the same carcass in chilling rooms for 24 h. A total of 480 samples from sheep (n = 240) and cattle (n = 240) were collected from the carcasses. Samples were cold chain transported to the laboratory, and microbiological analyses were performed within 3 h of sampling. Samples were cold chain transported to the laboratory

2.2. Microbiological analysis

ACC were performed as follows: 1 ml from a 1:10 diluted swab sample was poured onto plate count agar (PCA, Merck 105463) plates. Incubation was performed under aerobic conditions at 37°C for 24 h. The total number of Enterobacteriaceae was determined according to the International Organization for Standardization (ISO) 21528–2:2004. The procedure was as follows: 1 ml of serial dilutions in Buffered Peptone Water (BPW, Merck, Germany), were poured onto violet red bile glucose agar (VRBG, Merck, Germany) and incubated at 37°C for 24 h. Typical colonies grown on plates were quantified after incubation. Isolation and identification of *Salmonella* spp. was performed using the method recommended by ISO 6579:2002 + A1:2007 with slight modifications. Accordingly, swab samples in BPW were incubated overnight at 37°C for pre-enrichment. For selective enrichment, 0.1 ml of the pre-enriched culture was added to 10 ml of modified Rappaport-Vassiliadis broth (MRVB, Merck, Germany) and incubated at 41.5°C for 24-48 h. Subsequently, 0.1 ml from the enriched culture was streaked onto Xylose Lactose Tergitol 4 (XLT4, Merck, Germany) agar supplemented with XLT4 Selective Supplement (Merck, Germany). These plates were incubated at 37°C for 24 h. DNA isolation was performed from five selected black or black-centred colonies on each plate which were considered to be 'presumptive *Salmonella* colonies' grown on XLT4 agar.

2.3. Conventional m-PCR for detecting *Salmonella* spp.

DNA isolation from suspicious *Salmonella* colonies was performed using the boiling method. Following optimisation of PCR conditions, conventional multiplex-PCR (m-PCR) was performed. Gene primers used for *Salmonella* spp. are shown on Table 1.
The m-PCR master mix comprised 1 U *Taq* Polymerase and *Taq* buffer (5 mM KCl and 0 mM Tris-HCl), 1.5 mM $MgCl_2$, 0.025 mM of each primer, 0.9 μM Inv-A primers and 0.4 μM IE1 and Flic-C primers in a 20-μl reaction volume. The m-PCR protocol comprised an initial denaturation step for 5 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 58°C and 30 s at 72°C, with a final extension step of 7 min at 72°C (PAIAO *et al*., 2013).

Table 1. The primer pairs used in this study.

	Primers	Product length	Reference
Salmonella spp. (Inv-A)	F:GTGAAATTATCGCCACGTTCGGGCAA R:TCATCGCACCGTCAAAGGAACC	284 bp	Rahn *et al.*, 1992
S. enteritidis (IE-1)	F:AGTGCCATACTT TTAATGAC R:ACTATGTCGATACGGTGGG	316 bp	Wang and Yeh, 2002
S. typhimurium (Flic-C)	F:CCCGCTTACAGGTGGACTAC R:AGCGGGTTTTCGGTGGTTGT	432 bp	Paiao *et al.*, 2013

2.4. Air sampling

Air sampling was employed to determine the number of ACC and fungal counts in slaughter operation and cold storage rooms during processing. PCA (Merck, Germmany) was used for ACC and potato dextrose agar (Merck, Germany) supplemented with 10% tartaric acid solution was used for fungal counts in the air sampler (Air Ideal 3P, Biomerieux, France). The air sampler device was placed 1-1,5 m above the floor along the slaughter line and chilling rooms. In the sampling areas, 190 l of air was vacuumed by placing the Petri dishes on the vacuum surface of the device. The plates were incubated before determining microbial counts. The samples were taken six independent times on different sampling days.

2.5. Determination of pH and temperature

pH and temperature values of the carcass were measured during different sampling stages using a portable pH and temperature probe (Testo 205, Germany). Changes in pH and temperature of sheep and cattle carcasses were also determined during the following slaughtering steps: dressing, evisceration, washing and chilling.

2.6. Statistical analysis

Data obtained from the study was analysed using SPSS software package 21.00. Data was subjected to variance analysis (one-way ANOVA) and two sample t-tests in accordance with the experimental design. Significant differences ($p < 0.05$) were identified using multicomparisons of the means, with Duncan's test, within the variance analysis. Means and standard errors of the means were reported.

3. RESULTS AND DISCUSSION

In our study, ACC in cattle carcasses were observed between 2.57±0.61 and 4.71±0.24 log CFU/cm^2 and Enterobacteriaceae counts were observed between 0.89±0.46 and 2.61±0.10 log CFU/cm^2. Further, contamination with Enterobacteriaceae in the shoulder region after washing was found to exceed the limits of EC regulation for cattle carcasses. Our highest ACC in cattle carcass were observed in shoulders and rumps after dressing and in briskets after washing (Fig. 1). Contamination levels were not found to be statistically different ($p > 0.05$) for ACC in shoulders, rumps and brisket regions at different stages of cattle slaughtering.

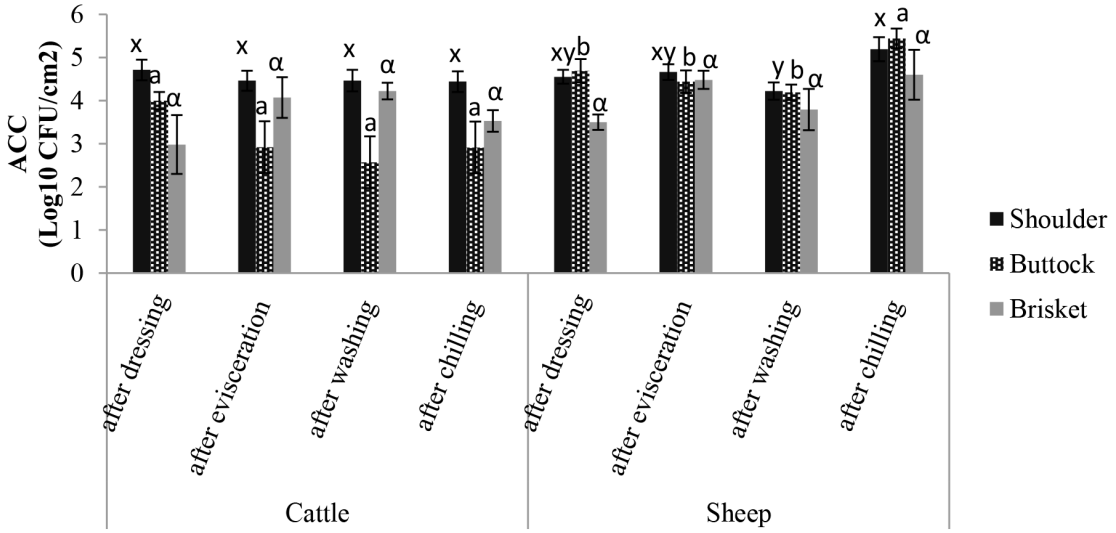

Figure 1. ACC at different slaughtering stages of cattle and sheep carcasses ($log_{10}CFU/cm^2$) Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) indicate significant difference ($p<0.05$).

Although there were no statistically significant differences in the total number of Enterobacteriaceae in cattle carcasses ($p > 0.05$) in shoulders, rumps and brisket regions at all stages of slaughtering ($p > 0.05$; Fig. 2), we observed that Enterobacteriaceae contamination was the highest after the washing stage. This can be explained by partial contamination originating from faeces or internal organs that are spread to other regions during washing. In sheep carcass samples, the highest level of Enterobacteriaceae contamination was observed at the chilling stage, and this was statistically significant ($p < 0.05$; Fig 2). In sheep carcasses, mean values of ACC were between 3.51±0.48 and 5.19±0.28 log CFU/cm^2, whereas Enterobacteriaceae counts were between 0.55±0.37 and 3.63±0.39 log CFU/cm^2. Further, ACC in the shoulder region and Enterobacteriaceae contamination in all regions after the chilling stage exceeded the limits of EC regulation. The highest ACC were found in all sampled parts after the chilling stage (Fig 1) and this was statistically significant ($p < 0.05$). In a similar study, ZWEIFEL *et al*. (2014) obtained similar results in cattle carcasses and they determined that chilling was the most important stage for preventing contamination.

Cattle and sheep carcasses were compared in terms of slaughtering steps and contamination levels in different sampling regions. Statistical graphs and a comparison table of ACC and Enterobacteriaceae count from both sheep and cattle carcasses at different slaughtering stages are given below ($p < 0.05$, Table 2). ACC were higher in the shoulder region of cattle carcasses than in that of sheep carcasses ($p < 0.05$, Table 2). Similar differences were observed in ACC in rump area after washing and that in brisket after the dressing process in the two carcasses ($p < 0.05$).

Further, ACC in the rump area of sheep carcasses had higher contamination than that in the rump area of cattle carcasses during the dressing stage. After evisceration, contamination in the brisket of sheep carcasses was higher than that in the brisket of cattle carcasses ($p < 0.05$, Fig. 3.).

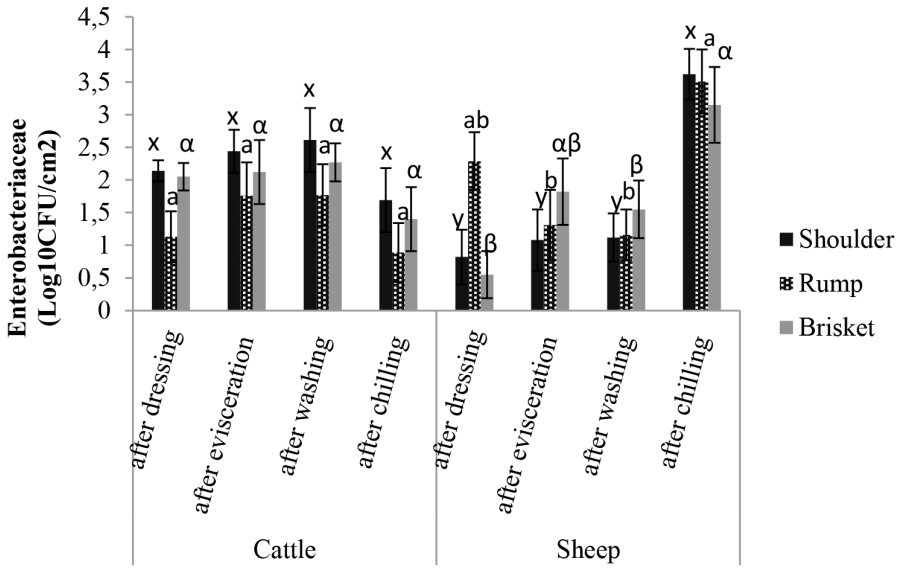

Figure 2. *Enterobacteriaceae* counts at different slaughtering stages of cattle and sheep carcasses ($log_{10}CFU/cm^2$). Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

Table 2. Comparison the ACC and *Enterobacteriaceae* contamination levels of cattle and sheep carcasses.

	Processing steps		Shoulder x± Sx	Rump x± Sx	Brisket x± Sx	P
ACC (Log CFU/cm^2)	after dressing	cattle	4,71±0,24^a	2,99±0,69^b	4,01±0,21^{ab}	*
		sheep	4,55±0,17	4,70±0,28	3,51±0,48	
	after evisceration	cattle	4,46±0,23	4,08±0,47	2,93±0,68b	
		sheep	4,66±0,18	4,45±0,26	4,48±0,21	
	after washing	cattle	4,46±0,26^a	4,22±0,20^a	2,57±0,61^b	**
		sheep	4,22±0,21	4,19±0,19	3,79±0,48	
	after chilling	cattle	4,44±0,24	3,54±0,26	2,92±0,68	
		sheep	5,19±0,28	5,44±0,24	4,60±0,59	
Enterobacteriaceae (Log CFU/cm^2)	after dressing	cattle	2,14±0,16^a	2,06±0,22^a	1,14±0,40^b	*
		sheep	0,83±0,42^b	2,30±0,45^a	0,55±0,37^b	*
	after evisceration	cattle	2,45±0,33	2,13±0,50	1,77±0,52	
		sheep	1,09±0,47	1,31±0,54	1,82±0,51	
	after washing	cattle	2,61±0,10^a	2,28±0,30^a	1,77±0,48^b	*
		sheep	1,12±0,38	1,16±0,39	1,55±0,44	
	after chilling	cattle	1,69±0,50	1,41±0,50	0,89±0,46	
		sheep	3,63±0,39	3,52±0,49	3,15±0,59	

x,y: values within a row with different letters are significantly different ($p<0.05$), x: mean, Sx: standard error of mean *: $p<0.05$;**: $p<0.01$.

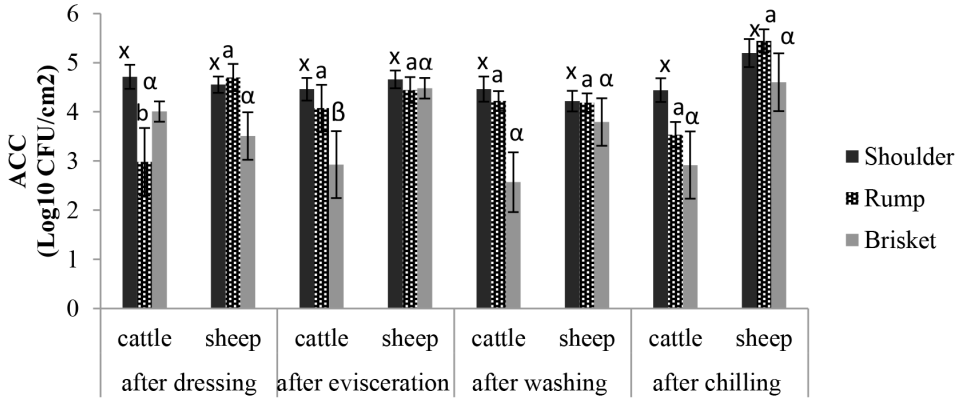

Figure 3. Comparison the ACC Levels of Cattle and Sheep Carcasses. Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

The shoulder region of cattle carcasses after dressing and in the shoulder and rump regions after washing had higher Enterobacteriaceae contamination values than those of sheep carcasses ($p < 0.05$, Fig 4.), this implied that the washing process in cattle was inadequate.

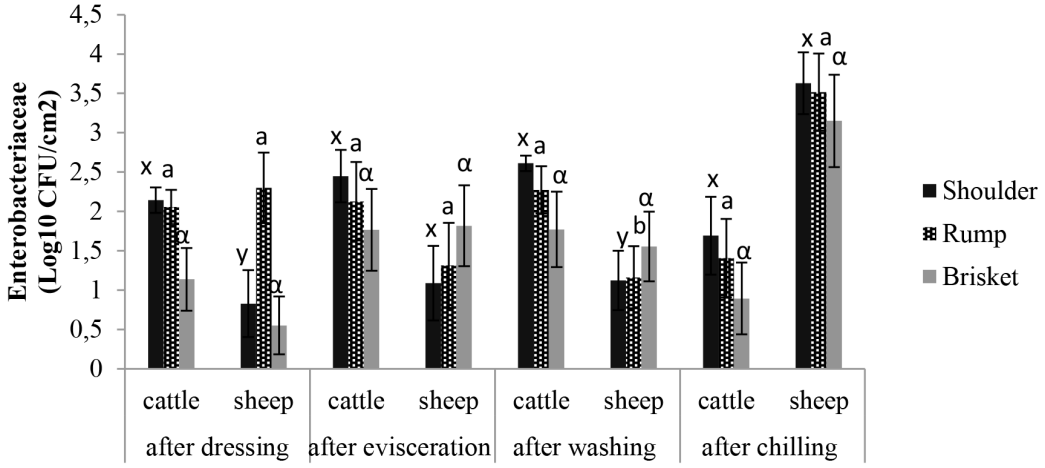

Figure 4. Comparison *Enterobacteriaceae* spp. Contamination Levels of Cattle and Sheep Carcasses.Data are presented as mean±standart error. Data with different superscript (x,y; a,b; α,β) above each bar indicate significant difference ($p<0.05$).

Other previous studies (BARBOZA DE MARTINEZ *et al*., 2002; MIES *et al*., 2004) have supported these findings and stated that the washing process during slaughtering may be ineffective in decreasing the rate of bacterial contamination. Therefore, many investigators suggest that washing should be done with effective disinfectants and that more strict preventive measures should be taken. A similar study (BARBOZA de MARTINEZ *et al*., 2002) investigated the effect of nisin and lactic acid against bacterial loads, including ACC, total coliforms and *E. coli* in cattle carcasses. Researchers stated that washing alone did not reduce the bacterial load and that spraying with a mixture of lactic acid and nisin provided the highest reduction in all bacterial groups tested. In another study (MIES *et al*., 2004), researchers assessed the effect of washing cattle carcasses with or without

disinfectant solutions prior to slaughtering on aerobic plate, coliform, *E. coli* and *Salmonella* counts. The greatest reductions were observed in mean logs of bacterial counts in groups sprayed with 4%-6% ethanol and lactic acid.

Our microbial counts are comparable with those obtained in other surveys at the national and international level using similar techniques (swabbing or sponging) (GILL and BAKER, 1998; DUFFY *et al*., 2001; YALÇıN *et al*., 2004; PEARCE *et al*., 2005; SALMELA *et al*., 2013; PETRUZELLI *et al*., 2016). GILL and BAKER (1998) examined aerobic counts and coliform and *E. coli* contamination rates on randomly selected sheep carcass surfaces using the swab method in Canada. The highest *E. coli* load was detected on shoulder and rump regions after the dressing stage and that the ACC load was the highest after the evisceration stage. Duffy *et al*. in 2001 investigated lamb carcasses in the United States using sponge sampling to detect the presence of *Salmonella* spp and *E. coli* and determine ACC and total coliform loads after the chilling process. *Salmonella* spp. was detected in 1.5% samples, whereas ACC, total coliform and *E. coli* counts were observed at 4.42, 1.18 and 0.70 log CFU/ cm^2, respectively. A similar study in Turkey by YALÇıN *et al*. (2004) stated that ACC in sheep carcasses were 2.96, 3.10, 2.81 and 1.69 log CFU/ cm^2 after dressing, evisceration, washing and chilling steps, respectively. Moreover, in Ireland PEARCE *et al*. (2005) reported that ACC in sheep carcasses, as tested by the excision method, in the thorax, shoulder-neck, chest-brisket, and flank areas were 3.4, 3.6, 3.5 and 2.4 log CFU/cm^2, respectively, whereas measurements using the polyurethane sponge method indicated 2.9, 2.8, 3.3 and 2.5 log CFU/cm^2 and that using the cellulose acetate sponge method indicated 2.7, 2.5, 2.7 and 2.3 log CFU/cm^2 in the same stages, respectively. SALMELA *et al*. (2013) also assayed ACC of carcasses sampled by excision and swab methods in Finland; results were 3.77 and 3.16 log CFU/cm^2, respectively. Researchers reported that ACC and Enterobacteriaceae and *E. coli* counts were higher in the excision method than the swab method. The authors reported that Enterobacteriaceae was detected using the excision and swab methods in 72% and 76% carcasses, respectively, whereas *E. coli* was detected in 48% and 61% carcasses, respectively. Similar to our findings, PETRUZZELLI *et al*. (2016) investigated ACC and Enterobacteriaceae contaminations in ovine, bovine and swine carcasses in Italy using the sponge method following EC regulations. They found that contamination levels in bovine, ovine and swine carcasses, when measuring ACC were 1.96, 2.27 and 2.27 log cfu/cm^2, respectively, whereas Enterobacteriaceae counts were 0.01, 0.27 and 0.20, respectively. They also stated that cattle carcasses had significantly lower levels of ACC and Enterobacteriaceae counts than swine and ovine carcasses. ALONSO-CALLEJA *et al*. (2017) also studied lamb carcasses in two slaughterhouses in Spain. They stated that total viable counts (TVC, 2.74 log CFU/cm^2) were higher than Enterobacteriaceae (2.21 log CFU/cm^2) counts and that there was a high correlation between them. They also stated that 0% and 30.8% of the samples in abattoir A and 10% and 40% of the samples in abattoir B exceeded EC regulations for TVC and *Enterobacteriaceae* counts, respectively.

An overall evaluation demonstrated that as the samples progressed through the steps of the slaughtering process, an increase in the total number of microorganisms in the shoulders, rump and brisket regions was observed after dressing in both cattle and sheep carcasses. In this context, GILL *et al*. (2003) argued that despite the use of decontamination methods, such as pasteurisation, hot-water washing or lactic acid spraying, after the evisceration step, the increase in bacterial counts after chilling of carcasses was related to the proliferation of initial microorganisms rather than subsequent contaminations. However, it has been determined that ACCs, as one of the determinative criteria of slaughtering hygiene, are also in conformity with the Turkish Food Codex Regulations on Microbiological Criteria of Meat and Meat Products and Commission, except in the shoulder region of sheep carcasses after the chilling stage. In addition, Enterobacteriaceae

limits were exceeded in shoulder, brisket and rump regions of sheep carcasses after the chilling process and in the shoulder region of cattle carcasses after washing. Thus, it is very important to take necessary precautions to prevent contamination, particularly during the process of washing and evisceration during slaughtering. Likewise, it is thought that the chilling process should be performed at maximum performance and speed and that carcass decontamination methods should be applied, if necessary, to minimise the growth of pathogenic and spoilage microorganisms.

The present study aimed to determine the presence of *Salmonella* spp. in sheep and cattle slaughtering process. However, no *Salmonella* spp. were detected in the 480 samples analysed, which is highly satisfactory in terms of slaughtering hygiene and public health. SALMELA *et al*. (2013) did not detect any *Salmonella* spp. by swab and excision methods in carcasses in any of the samples, similar to that observed our present study. Nevertheless, MADDEN *et al*. (2001) found that three of the 200 samples from cattle carcasses were contaminated with *Salmonella* spp. CHAVEZ *et al*. (2015) used the sponge sampling technique to collect samples (n = 142) after the washing step and before the chilling step and determined that 18% cattle carcasses were contaminated with *Salmonella* spp. at the three inspected abattoirs. Similarly, HALD *et al.* (2003), collected pig carcass samples from 12 slaughterhouses in five countries of Europe. The researchers stated no *Salmonella* was found in one country while 5.3 % of 3485 samples found to be positive in the other four countries. In a recent study in Ethiopia, MULUNEH and KIBRET (2015) found 7.6 % of the beef carcasses collected from an abattoir positive for *Salmonella* spp.

We observed that as the sheep and cattle slaughtering process progressed, the temperature progressively decreased in all carcass regions and that this was statistically significant ($p < 0.05$; Table 3). The temperature observed after storage did not reach the desired low temperature (4°C-6°C), indicating that cooling is insufficient. This demonstrates the necessity for systematically controlling the size of carcasses, internal temperature, air flow and humidity of the chilling rooms. It was observed that inner temperatures of sheep carcasses after chilling storage were lower than cattle. Nevertheless, it has been observed that this decrease in temperature does not provide any advantage in reducing the microbial load. As a matter of fact, according to the EFSA panel on BIOHAZ (2014a,b), it has been declared that it is important to go to the transport stage while the chilling process is performed on the carcasses so that the number of spoilage bacteria cannot reach an unsatisfactory level.

Table 3. pH and temperature (°C) values of the shoulder and rump regions at different slaughtering stages.

		Sheep		Cattle	
		Shoulder	Rump	Shoulder	Rump
	Processing Steps	x±Sx	x±Sx	x±Sx	x±Sx
pH	after dressing	6.40±0.09^{a}	6.35±0.05^{a}	6,40^{a}±0,09	6,35^{a}±0,05
	after evisceration	6.48±0.12^{a}	6.14±0.05^{ab}	6,48^{a}±0,12	6,14^{ab}±0,05
	after washing	6.33±0.23^{a}	6.15±0.01^{ab}	6,33^{a}±0,23	6,15^{ab}±0,01
	after chilling	5.61±0.28^{b}	5.28±0.04^{c}	5,61^{b}±0,28	5,28^{c}±0,04
	P*	***	***	***	***
°C	after dressing	38.66±0.22^{a}	38.48±0.38^{a}	32,77^{ab}±1,54	34,47^{b}±1,93
	after evisceration	38.12±0.26^{a}	37.46±0.47^{a}	33,85^{a}±2,82	37,50^{a}±0,35
	after washing	36.27±0.68^{b}	37.22±0.92^{a}	28,23^{b}±3,95	37,05^{ab}±0,85
	after chilling	7.72±0.77^{c}	8.13±0.85^{b}	11,43^{c}±1,18	13,36^{c}±1,24
	P*	***	***	***	***

Air sampling results for ACC and fungal counts were found to be lower in the chilling rooms than in the slaughtering area (Table 4; $p < 0.05$). These values indicated that the hygiene in chilling rooms is satisfactory. This can explain why microbial counts were reduced in the chilling rooms compared with those in the slaughtering area and increasing general hygienic conditions is critical for reduction of carcass microbial growth. In a similar study, PRENDERGAST *et al.* (2004) also investigated the relation between airborne bacterial counts and carcass contamination in two abattoirs in Ireland. The ACC were found to be between 1.79-3.49 $\log_{10}$CFU/m^3 at different stages of slaughtering. They also stated the clean areas of slaughtering process had a lower microbial load than the dirty areas. Researchers found the correlations between carcass contamination and aerial load was low. Similarly, BURFOOT *et al*. (2006) noted that airborne contamination of cattle and sheep carcasses during the evisceration phase is less of a concern than other contamination sources.

Table 4. AC, Yeast and Molds Counts in the Slaughtering Room and Cold Storage Room ($\log_{10}$cfu/m^3).

Areas	ACC (x±Sx)	Yeast and Molds (x±Sx)
Slaughtering room	2.54±0.01^a	1.23±0.12
Cold storage room	1.98±0.03^b	1.04±0.01
P	***	

a, b, c: The differences between different letter values in the same column are significant ($p < 0.05$). N: Number of samples. x: Mean value. Sx: Standard error of mean, *: p<0,05;**: p<0,01;***: p<0,001.

4. CONCLUSION

In the present study, Enterobacteriaceae limits were exceeded in shoulder, brisket and rump regions of sheep carcasses after the chilling process and in the shoulder region of cattle carcasses after washing. Further, ACC in the shoulder region and Enterobacteriaceae contamination in all regions after the chilling stage exceeded the limits of EC regulation. The highest ACC were found in all sampled parts after the chilling stage. This is thought to be due to the provision of a suitable environment as a result of poorly cooled chilled rooms to gradually increase microbial load, which is relatively low in cutting stages. Furthermore, it is satisfactory that *Salmonella* spp. is not detected in the present study, but it should be considered that this result may be due to possible insufficiency of the swabbing method compared with the excision.

In conclusion, the microbial quality of meat for consumption is closely related to public health. Thus, it is critical to understand specific microbial risks of each work step, from slaughtering to chilling of carcasses. Microbiological analyzes which are carried out only at the end of the process, may not provide realistic information on the main causes of the microbial contamination. Therefore, the microbiological criteria which are 'Process-based' related to measurements including different methods at various stages of the process should be preferred and more detailed risk assessments should be undertaken to assess and develop preventive measures that can reduce sources of contamination during the most critical stages of slaughtering.

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Paper Received April 12, 2018 Accepted June 14, 2018