

ASSESSMENT OF MICROBIOLOGICAL LOAD OF SMALL RUMINANT CARCASSES, LIVERS, SOME LYMPH NODES, TOOLS AND KNIFE SAMPLES IN SLAUGHTERHOUSE

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Abstract: The aim of this study was to determine the microbiological loads of small animal carcasses, carcass lymph nodes, whole liver surface, liver lymph nodes and some tools contacting with carcass and offal. Total 630 samples taken from small animal carcasses, livers, hepatic lymph nodes, subiliac and prescapular lymph nodes, staff knives and slaughterhouse tools samples (stainless steel table, plastic crates, offal carts) were investigated for mesophilic aerobic bacteria, *Enterobacteriaceae*, *Escherichia coli* counts and *Salmonella* spp. The mean total aerobic mesophilic bacteria (TAMB), *Enterobacteriaceae* and *E. coli* numbers of the carcasses were 3.6, 0.6, and 0.1 log₁₀ CFU/cm², respectively, and the most contaminated region among the carcass sampling points was flank. The mean TAMB, *Enterobacteriaceae* and *E. coli* counts of the liver surfaces were 6.0, 3.7, 2.9 log₁₀ CFU/liver, respectively. The average TAMB, *Enterobacteriaceae* and *E. coli* numbers of the knives were found as 6.3, 2.9 and 2.1 log₁₀ CFU/blade, and the average TAMB, *Enterobacteriaceae* and *E. coli* counts of the slaughterhouse surfaces were 5.1, 1.6, 0.5 log₁₀ CFU/cm². *Salmonella* spp. was detected in 4% of the liver samples and 10% of the knives samples. Consequently, the presence of *Salmonella* on the surface of livers and blades, and high number of *E. coli* on the livers, blades and tools show that a public health risk may arise at any time, and staff should pay extra attention to the “Good Hygiene Practices” and Food Safety Management Systems (such as HACCP) applied in slaughterhouses.

Key words: carcass; liver; lymph node; microbiological quality; *Enterobacteriaceae*; *Escherichia coli*; *Salmonella* spp.

Introduction

Red meat is one of the important animal protein sources in human diet, and the meat under the skin of a healthy animal is considered sterile. However, carcass contamination is inevitable during the slaughtering process such as skinning and evisceration (1). Most of the microbial contamination on the carcass surface comes from different sources such as hide, intestinal contents, slaughterhouse equipment/tools and workers during the slaughtering.

Salmonella is one of the pathogenic bacteria causing foodborne diseases (2). It is known that the animal skin, gastrointestinal tract and feces are the primary sources of *Salmonella* contamination for the carcass surface during the slaughter process (3). Besides, the studies conducted on carcass lymph nodes have shown that those nodes may harbor pathogenic microorganisms such as *Salmonella* spp. (4). It is known that completely removing lymph nodes from the carcass is impossible. The lymph nodes remaining in carcass meat become a part of the meat product after the mincing process of meat (5). Edible offal that is derived from carcass may be exposed to cross contamination with pathogen microorganisms in case of poor hygiene

in slaughterhouse (6). Most of the studies related to the *Salmonella* prevalence in lymph nodes, edible offal, carcass and on slaughterhouse tools have been conducted in beef carcasses and beef slaughterhouses (3-5, 7, 8), however, the studies conducted in small animal carcasses are very limited (9-11). Hence, this study was conducted to investigate (i) the microbiological condition of small animal carcasses and liver surfaces in slaughterhouse, (ii) *Salmonella* prevalence of carcass lymph nodes and hepatic lymph nodes in small animals, and (iii) the bacterial load of staff blade and some slaughterhouse tools contacting with offal.

Materials and methods

The study was conducted in a slaughterhouse that has beef and sheep/goat slaughter-lines with a line speed of approximately 150 small animals per hour, and there was no automatic hide puller for small animal carcasses. Samples of the study were composed of total 630 samples taken from small animal carcasses (200 samples taken from 50 carcasses; four sampling sites including rump, flank, brisket and neck regions were sampled for each carcass), livers (50), hepatic lymph nodes (taken from 100 livers), subiliac (100 pairs, one pair per carcass) and prescapular lymph nodes (100 pairs, one pair per carcass), staff knives (blade) (40) and some tools (stainless steel tables (15), plastic crates (15), offal carts (10)) used in slaughterhouse. The animals sampled were not separated as goat and sheep; they all were defined as small animal because the slaughterhouse was cutting them as mixed, and the staff was using the same blade, same plastic crates, offal carts and tables for both goat and sheep. The lymph nodes samples were collected as a pair per carcass. The samples were collected between February and May 2018, and the slaughterhouse was visited once a week during this period.

Sampling procedures

Carcass samples were taken from four sampling sites including rump, flank, brisket and neck regions indicated in the ISO 17604 (12) at the stage after washing but before chilling. Briefly, samples were taken from carcass regions by swabbing an area of 100 cm² using sterilized stainless steel template (10 cm×10 cm) and sterile sponge (World

bioproducts, EZ-Reach™, US/Canada) which was premoistened with 25 ml sterile buffered peptone water (BPW) (Biokar, Beauvais/France). The entire liver surface was sampled by the sterile sponge premoistened with 25 ml sterile BPW. Samples from tools (stainless steel table, plastic crates, offal carts) were collected by swabbing an area of 100 cm² using the sterilized template (10 cm×10 cm) and the premoistened sterile sponge. Staff knives were sampled by swabbing the both surfaces of the blade with the premoistened sterile sponge. Hepatic, subiliac and prescapular lymph nodes were taken using sterile scalpel. All visible hepatic lymph nodes on the liver surface were collected.

Microbiological analysis

All the samples were transported to the laboratory in a thermo cool box containing pre-frozen ice bags within 2-3 h. The sponge samples taken from carcasses, livers, blades and tools were homogenized in a stomacher (Bag mixer 400, Interscience, France) for 2 min. Analysis of aerobic plate counts, *E. coli* and *Enterobacteriaceae* in all the samples were conducted according to the methods described in ISO 4833, ISO 16649-2 and ISO 21528-2, respectively (13-15). Briefly, Plate Count Agar (PCA), Tryptone Bile X-glucuronide (TBX) agar and Violet Red Bile Glucose (VRBG) Agar (Biokar, Beauvais/France) were used for the detection of aerobic plate count (APC), *E. coli* and *Enterobacteriaceae* counts. TBX and VRBG plates were incubated at 37°C for 24 h, and PCA plates were incubated at 30°C for 72 h. The adipose tissues surrounding the each lymph nodes were removed as much as possible, and each lymph nodes were dipped into 70% alcohol for 5 min in order to disinfect the outside of the node. After this procedure, the lymph nodes were kept in open air to remove the residual alcohol for 5 min. And then, hepatic, subiliac and prescapular lymph nodes were separately placed into sterile stomacher bags, and they were crushed with rubber mallet from outside of the stomacher bag. After this, 100 ml sterile BPW were added into stomacher bags and homogenized in a stomacher (BagMixer 400, Interscience, France) for 1 min, and the homogenized samples were incubated at 37°C for 24 h for *Salmonella* pre-enrichment procedure. Analysis of *Salmonella* spp., in all samples were conducted according to the methods described in ISO 6579 (16). Briefly; after pre-enrichment

procedure, 0.1 ml of the sample was added to 10 ml Rappaport-Vassiliadis broth (RVS; Oxoid, Hampshire/England) and 10 ml Muller-Kauffmann tetrathionate novobiocin broth (MKTTn; Oxoid, Hampshire/England). RVS and MKTTn were incubated at 41.5°C and 37°C for 24 h, respectively. RVS and MKTTn enrichments cultures were streaked to Xylose-Lysine-Deoxycholate agar (XLD; Lab M, Lancashire/United Kingdom) and Xylose-Lysine-Tergitol agar (XLT4; Lab M, Lancashire/United Kingdom), and the plates were incubated at 37°C for 24-48 h. At the end of the incubation, five suspected colonies with black center were transferred to tubes containing Triple Sugar Iron Agar and Lysine Iron Agar (Merck, Darmstadt/Germany) and incubated at 37°C for 24 h. After incubation, presumptive positive *Salmonella* colonies were confirmed with *Salmonella* latex test (Oxoid, Hampshire/United Kingdom) and Microgen GN-ID A (Microgen, Camberley/United Kingdom).

Statistical analysis

Statistical analysis were made using SPSS version 22 (IBM SPSS, IBM Corporation, USA). The microbiological data were converted to Log₁₀ CFU. One way analysis of variance (ANOVA) was used to compare samples taken from different regions (rump, flank, brisket and neck) of the carcasses. Statistical significance level was accepted as P<0.05.

Results and discussion

In Turkey, decontamination of carcasses with any chemicals is not allowed, but washing with water. Carcass samples were taken after carcass washing stage before chilling. In the present study, the highest APC count in the carcass regions was found in the flank by average APC number of 3.6 log₁₀ CFU/cm² (Table 1), and significant difference was observed between the flank and neck regions (P<0.05). Similarly, the highest *Enterobacteriaceae* and *E. coli* numbers were found in the flank while the lowest numbers were in the rump and neck regions (P<0.05). Since the microbiological results were expressed as log₁₀ CFU/cm² in the study, statistical analysis for *E. coli* numbers of the rump, brisket and neck regions was not performed (because some samples had *E. coli* number of <25 CFU/100 cm² and negative logarithmic values).

Gürbüz et al. (17) reported that the highest level of *Enterobacteriaceae* and APC were in rump and brisket regions after washing of sheep carcasses, respectively. However, unlike our study, they had chosen the three sampling points (rump, shoulder and brisket), not including flank region. In our study, the possible reason of the high microbial contamination of the flank compared to the other regions may be due to the dirty hands and blades of the staff. Staff hands and blades were frequently touching the flank region during the evisceration process. The average APC, *Enterobacteriaceae* and *E. coli* numbers of 50 carcasses were 3.6, 0.6 and 0.1 log₁₀ CFU/cm², respectively. When the values obtained for *E. coli* in rump, brisket and neck regions of the carcasses were converted from CFU/100 cm² to CFU/cm², negative log values were obtained. Negative log value was used to imply that there was less than 1 bacterium in per cm² (Table 1). In Europe, the studies conducted in Italy, Spain, Finland and Poland (18-21) reported that APC and *Enterobacteriaceae* counts on sheep carcasses were between 2.27-3.88 log₁₀ and between 0.27-1.03 log₁₀ CFU/cm², respectively. Small differences among the bacterial load of carcasses should be taken as normal. It should be kept in mind that some factors such as slaughter practices, the number of animals cutting per hours, sampling time (season), sampling methods (sponge, excision or swabbing) and hygienic conditions and procedures in slaughterhouses can affect the bacterial load on carcasses.

Salmonella spp. was not detected in 50 carcass samples collected in this study (Table 1). Similar to our result, some researchers reported no *Salmonella* spp. in sheep carcasses by excision and swabbing methods (17, 20-22). On the other hand, some researchers found that *Salmonella* spp. prevalence in small animal carcasses was between 0.62% and 14.1% (23-27). It is difficult to make comparison among the studies in point of the *Salmonella* prevalence. Since, some factors such as number of animals that harbor *Salmonella* spp. on their hide and in feces due to husbandry practices, sample numbers, sampling time (season) and frequency, sampling methods (sponge, excision or swabbing) and hygienic procedures in slaughterhouses should be taken into consideration. It should be noted that only 400 cm² surface area was sampled for each carcass in this study, hence, actual *Salmonella* prevalence may be higher than 0%.

Table 1: The mean Aerobic Plate Count (APC), *Enterobacteriaceae*, *Escherichia coli* counts and *Salmonella* spp. prevalence of the small animal carcasses and carcass regions (\log_{10} CFU/cm²±SD) (n=50)

	Carcass (general)	Carcass regions			
		Rump	Flank	Brisket	Neck
APC	3.6±0.8	3.1±1.2 ^{AB}	3.6±0.9 ^B	3.2±0.9 ^{AB}	3.0±1.1 ^A
<i>Enterobacteriaceae</i>	0.6±0.8	0.3±0.9 ^A	0.7±0.9 ^B	0.4±0.9 ^{AB}	0.1±0.8 ^A
<i>Escherichia coli</i>	0.1±0.9	-0.4±0.8	0.04±0.9	-0.2±0.9	-0.4±0.8
<i>Salmonella</i> spp. ^a	0/50	0/50	0/50	0/50	0/50

^{AB}: The values with different superscript within the same row are significantly different (P<0.05)

^a: *Salmonella* positive sample/Total samples

Table 2: The average Aerobic Plate Count (APC), *Enterobacteriaceae* and *Escherichia coli* on the blade, liver and some slaughterhouse tools (plastic crates (15), stainless steel tables (15) and offal carts (10)) samples

	Blade ^a (n=40)	Liver ^b (n=50)	Slaughterhouse tools ^c (n=40)
APC	6.3±1.0	6.0±0.7	5.1±0.9
<i>Enterobacteriaceae</i>	2.9±1.3	3.7±0.9	1.6±0.8
<i>Escherichia coli</i>	2.1±1.1	2.9±0.9	0.5±0.9

^a: Log₁₀ CFU/blade

^b: Log₁₀ CFU/liver

^c: Log₁₀ CFU/cm²

Table 3: *Salmonella* spp. prevalence on the knife blade, liver, some slaughterhouse tools [plastic crates (15), stainless steel tables (15) and offal carts (10)] and in lymph nodes.

Samples	Positive samples/Total samples
Knife	4/40
Liver	2/50
Slaughterhouse surfaces	0/40
Portal (hepatic) lymph nodes	0/100
Prescapular lymph nodes	0/100
Subiliac lymph nodes	0/100

Knife blades and other tools (plastic crates, offal carts, working tables) used by the staff at the slaughterhouse can be one of the contamination sources of the carcass (1, 28). In the present study, the average numbers of APC, *Enterobacteriaceae* and *E. coli* on the blades were detected as 6.3, 2.9 and 2.1 log₁₀ CFU/blade, respectively (Table 2). *Salmonella* spp. was detected in 4 (10%) out of 40 blades (Table 3). Bakhtiary et al. (29) reported that *Salmonella enterica* was found on knives in a sheep and beef slaughterhouse in Iran. In the samples taken from the stainless steel table, plastic crates and offal carts, the mean numbers of APC, *Enterobacteriaceae* and *E. coli* were 5.1, 1.6 and 0.5 log₁₀ CFU/cm², respectively (Table 2). *Salmonella* spp. was not found on the surface

samples taken from stainless steel table, plastic crates and offal carts. To our knowledge, there are limited studies indicating bacterial load of staff knives in slaughterhouse. Hence, some references used in this study are old. Bell (30) detected APC number of 3.61 log₁₀ CFU/cm² on the blade used for hide skinning. In another study, APC number on the blade was detected as 5.04 log₁₀ CFU/cm² (31). Bell and Hathaway (31) and Bell (30) noted the bacterial load of the blade as per cm². If it is assumed that the entire surface area of a blade is more than 10 cm², then it can be said that the results of the studies are similar to each other. When the results obtained from tool and blades were evaluated, it was seen that the tool and blade samples had a high amount of microorganisms,

and they could pose an important role in the cross contamination of carcass and offal. In addition, the presence of *Salmonella* spp. in 10% of the blades showed that hygienic condition of blades should be taken care. The Regulation (EC) 853/2004 reported that “they (slaughterhouses) must have facilities for disinfecting tools with hot water supplied at not less than 82°C, or an alternative system having an equivalent effect” (32). The slaughterhouse where this study was conducted has equipment for disinfecting blades with hot water at 82 °C, however, it was observed that the equipment were not used by the staff. The results show that it is extremely important that staffs are to be informed and educated about the hygiene rules and public health. Moreover, staffs who do not comply with the hygiene rules should be warned during the slaughter process.

Liver surfaces can be contaminated with microorganisms during the production stage, and it can pose a risk for the public health. Woldemariam et al. (10) examined 107 small animals (sheep and goat) liver in Ethiopia and found that 5 (4.7%) of the livers were *Salmonella* spp. positive. In the present study, the average APC, *Enterobacteriaceae* and *E. coli* numbers of the 50 small animal livers collected from slaughterhouse were 6.0, 3.7 and 2.9 log₁₀ CFU/liver, respectively (Table 2). *E. coli* was detected in 90% of the livers collected (detection limit was ≥ 1.4 log₁₀ CFU/liver). *Salmonella* spp. was detected in 2 (4%) livers surface, while it was not found in any of the hepatic lymph nodes collected from 50 livers, (Table 3). This result indicates that *Salmonella* that were detected on the surface of the livers most likely originates from the hands or knife blades of the staff or the surfaces contacting with the livers such as offal carts, plastic crates. The presence of high amount of microorganisms on the surfaces of tools and blades may have contributed to the contamination of the liver surfaces. In addition, the presence of *Salmonella* spp. in 10% of the blades suggests that blades may also play an important role in the contamination of liver surfaces with *Salmonella* spp. However, it should not be forgotten that staff hands may also play an important role in the contamination of carcasses and offal.

There are limited studies on the *Salmonella* spp. prevalence of lymph nodes in small animal carcasses. El-Tom et al. (9) detected *Salmonella* spp. in 3 out of 78 mesenteric lymph nodes collected

from goat carcasses. Hanlon et al. (11) collected mesenteric (223) and subiliac (223) lymph nodes from sheep and goat carcasses during 14 months, and they found *Salmonella* spp. prevalence as 5.8% in mesenteric and 7.6% in subiliac lymph nodes, respectively. It is almost impossible to use the mesenteric lymph nodes in the production of ground meat, however, removal of subiliac and prescapular lymph nodes from carcass can be forgotten; and they may probably be used in the production of ground meat. In the present study, *Salmonella* spp. was not found in any of the subiliac and prescapular lymph nodes collected from 100 carcasses (Table 3). The reason of these differences among the results may be depend on the condition of the farms where animals are come from and health/disease situations of the animals brought to the slaughterhouse. It has been also noted that the prevalence of *Salmonella* spp. in animals shows seasonal differences (5).

Conclusions

Consequently, the presence of high amount of *Enterobacteriaceae* and *E. coli* on the surfaces of blades and livers, and the detection of *Salmonella* spp. on the surfaces of the blades and livers show that staff should pay extra attention to the “Good Hygiene Practices” and Food Safety Management Systems (such as HACCP) applied in slaughterhouses. In this context, disinfection of blades with the hot water of $\geq 82^\circ\text{C}$, taking care of the sanitation of the surfaces that are contact with livers, and washing liver surfaces with the clean water in slaughterhouse can significantly reduce the microbial load on these surfaces. Although this study was conducted in one slaughterhouse, the samples were collected as many number as possible, and the study was continued for 4 months to reflect the general microbiological status of small animal carcasses and the surfaces contacting with offal in a slaughterhouse. The data of this study can be helpful in the microbiological risk assessment of small animal carcass and offal, and it may show the microbiological loads of the some contamination sources of the carcasses and offal.

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OCENA MIKROBIOLOŠKE OBREMENTIVE TRUPOV MALIH PREŽVEKOVALCEV, JETER IN NEKATERIH BEZGAVK TER ORODIJ IN NOŽEV V KLAVNICI

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Povzetek: Namen študije je bil določiti mikrobiološko obremenitev trupov malih živali, bezgavk na trupih, celotne površine jeter, jetrnih bezgavk in nekaterih orodij, ki prihajajo v stik s trupom ter drobovjem. Pregledanih je bilo 630 vzorcev trupel malih živali, jeter, bezgavk, jetrnih bezgavk, nožev in orodij za klavnice (mize iz nerjavečega jekla, plastični zaboji, zaboji za drobovino). Ugotavljali smo prisotnost mezofilnih aerobnih bakterij, *Enterobacteriaceae* ter število bakterij *Escheria coli* in *Salmonella spp.* Povprečna skupna količina aerobnih mezofilnih bakterij (TAMB), *Enterobacteriaceae* in *E. coli* je bila 3,6, 0,6 in 0,1 log₁₀ CFU/cm². Najbolj onesnaženo področje pri vzorčenju trupov je bilo na boku trupov. Povprečno število TAMB, *Enterobacteriaceae* in *E. coli* na površinah jeter je bilo 6,0, 3,7 in 2,9 log₁₀ CFU/jetra. Povprečno število TAMB, *Enterobacteriaceae* in *E. coli* na nožih je bilo 6,3, 2,9 in 2,1 log₁₀ log₁₀ CFU/rezilo, povprečno število TAMB, *Enterobacteriaceae* in *E. coli* na klavniških površinah pa 5,1 in 1,6, 0,5 log₁₀ CFU/cm². *Salmonello spp.* smo odkrili v 4 odstotkih vzorcev jeter in na 10 odstotkih nožev. Prisotnost salmonele na površini jeter in rezil ter veliko število bakterij *E. coli* na jetrih, rezilih in orodju kažejo na to, da te bakterije lahko predstavljajo tveganje za javno zdravje. Osebe bi morale dodatno pozornost nameniti „dobri higijenski praksi“ in sistemom upravljanja varne hrane (na primer HACCP), ki se uporablja v klavnicah.

Ključne besede: trup zaklanih živali; jetra; limfni vozli; mikrobiološko onesnaženje; *Enterobacteriaceae*; *Escheria coli*; *Salmonella spp.*