

STRUČNI RAD – PROFESSIONAL PAPER

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**MICROBIOLOGICAL CONTAMINATION OF LAMB
CARCASSES AT ABATTOIRS OF ISTANBUL***
*MIKROBIOLOŠKA KONTAMINACIJA JAGNJEĆIH TRUPOVA U
KLANICAMA ISTANBULA*

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A total of a hundred lamb carcasses were sampled over a 12 month period at abattoirs in Istanbul, Turkey. Each sample examined for total aerobic mesophilic counts (TMC), Enterobacteriaceae count (EC), *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* was obtained from 100 cm² areas on four sides of lamb carcasses using the wet and dry cotton swab technique. The study revealed that total aerobic mesophilic counts in all carcasses ranged between 4.18 and 5.95 log/cm²; Enterobacteriaceae counts between 1.60 and 2.30 CFU/cm². All samples were negative for *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Furthermore *Salmonella* spp. was detected on four carcasses. The data confirms bacteriological monitoring of lamb carcasses as a useful criteria for the verification of slaughter hygiene.

Keywords: Carcass hygiene, abattoir, contamination, 2001/471/EC

Introduction / Uvod

Many potential by harmful microorganism which can all be released from the digestive system or in excretions are present in the hides of healthy cattle and sheep. These organisms include among others *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* which are responsible for foodborne illnesses in humans.

Salmonella spp. is a common cause of foodborne outbreaks and infection is usually acquired by ingestion of contaminated food items, such as meat, poultry, eggs, milk and vegetables [11]. Typical symptoms of illnesses associated with salmonellosis include nausea, vomiting and diarrhea; additional complica-

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tions associated with infection include septicemia or reactive arthritis [18]. Approximately 31% of all food-related deaths are caused by *Salmonella spp.* infections in the United States every year.

Listeria monocytogenes is a pathogen that causes severe illnesses, listeriosis, in populations at risk [9]. Listeriosis, a fatal infection (up to 30%) in human and animal species, has severe consequences such as abortion, septicemia, meningitis and encephalitis [12]. It has been isolated from raw and pasteurized milk, soft cheese, ice cream, raw meat, poultry, raw fish, ready to eat products, fermented sausages, vegetables, food processing plants and soil [16]. In the United States, listeriosis is responsible for 3.8% of foodborne illness – related hospitalization and 27.6% of foodborne disease – related deaths [13].

Escherichia coli O157:H7 is one of the most important neurotoxin producing enterohemorrhagic *E. coli*. It rarely produces clinical disease in animals but is associated with hemolytic uremic syndrome, thrombocytic thrombocytopenic purpura and hemorrhagic colitis in human [14]. *E. coli* O157:H7 bacteria is believed to mostly live in the intestines of cattle [5] but has also been found in the intestines of sheep, pigs, deer and goats [4]. An estimated 73.000 cases of infection and 61 deaths occur in the United States each year.

Contamination of these microorganism from the hide to the carcass is unavoidable due to the nature of hide removal. Contamination can occur by direct contact between the hide and the carcass, or by contact between the carcass and operatives' hands, clothes, tools or factory equipment that had previously been in contact with the hide.

At the same time, 2001/471/EC, which was introduced by the EU, relies on the use of total viable counts and Enterobacteriaceae as indicators of carcass hygiene and fecal contamination [2].

With the implementation of Hazard Analysis Critical Control Point (HACCP) and Quality Management (QM) systems at abattoirs there are increasing requirements for the microbiological sampling of carcasses. Carcass contamination during the slaughter process results in hygiene deficiencies which cannot be compensated for even by the most rigorous hygiene measures during latter processing states of the raw material. This underlines the great significance of slaughter hygiene. Therefore, verification of the efficiency of slaughter hygiene by microbiological examination of carcasses is desirable [20]. This study examined the microbial contamination of lamb carcasses surfaces after slaughter to determine the acceptability of carcass hygiene levels and prevalence of the foodborne pathogens *Salmonella spp.*, *E. coli* O157:H7 and *Listeria monocytogenes*.

Materials and methods / Materijal i metode rada

Plant Process / Proces u klanici

Twenty-eight visits were made to two different abattoirs in Istanbul. These abattoirs were established with government collaboration. Each of the abattoirs consist of a lairage, slaughter floor, dressing cradle, chilling room, cutting room and veterinary service office. One of these slaughterhouses has a capacity of 2000-2500 sheep/day, 200-250 cattle/day and the other's capacity is 200-250 sheep/day, 30-35 cattle/day. All required equipment such as rail system, stunning devices, restraining equipment, stainless steel holding bins and tables were provided. In this survey, a hundred carcasses were sampled. No more than four carcasses were sampled at each abattoir per visit. The carcasses were randomly selected from a batch of four consecutive lambs to avoid any large differences in carcass contamination.

Carcass Sampling / Uzorkovanje trupova

The sampling was performed within 2 hours at postmortem. All samples were taken from the left halves of the carcasses at four parts; the brisket, the breast, the neck and the flank using double swab technique. A 100 cm² sampling area was delineated using a sterile stainless steel template and using a wet swab, moistened in 0.1% peptone + 0.85% NaCl diluent and a dry swab. The handles of the swabs were broken off, leaving the swab tips in the tubes, containing 10 ml of maximum recovery diluent (MRD). The swabs were transported to the laboratory at 4°C.

Microbiological Analysis / Mikrobiološka analiza

After the samples were delivered to the laboratory, each tube containing the swabs was homogenized for 2 minutes in 100 ml of MRD. Samples were examined within 6 hours after sampling.

Total Mesophilic Aerobic Count (TMC) / Ukupan broj aerobnih mezofila (TMC)

Diluted samples were plated in duplicate onto plate count agar (Merck 1.05463). Plate count agar plates were incubated at 37°C. The colonies grown in plate count agar (PCA) were determined as TMC and the observed colonies were enumerated after 48 h from incubation.

Enterobacteriaceae Count (EC) / Broj Enterobacteriaceae (EC)

For detection of Enterobacteriaceae, ISO 5552 was used. Diluted samples plated in duplicate on to violet red bile dextrose agar (VRBDA, Merck 1.10275). VRBDA plates were incubated at 37°C at 24 h. Enterobacteriaceae colonies were enumerated on VRBDA agar, and they were observed as dark red colo-

nies that are 1-2 mm or larger in diameter and surrounded by a zone of precipitated bile acids.

Detection of Escherichia coli O157:H7 / Nalaz Escherichia coli O157:H7

For detection of *E. coli* O157:H7, the FDA method was used [1]. Each MRD tube that contains a sample swab was added into bottles made of borosilicate glass containing Modified EC broth (Oxoid CM 990) with novobiocin supplement (Oxoid SR0181E), and the bottles were incubated for 24 hours at 37°C. This part was the enrichment step of the inoculation procedure for *E. coli* O157:H7.

The inoculum was vortexed and was inoculated with loop to the Sorbitol Mac Conkey Agar (Merck 1.09207.0500) with Cefixime Tellurite supplement (Merck 1.09202)

After the incubation period at 35°C for 18 hours, the colourless or smoggy grey colonies were determined as suspicious bacteria. Typical colonies that are growth were taken and inoculated to the Tryptic Soy Agar (Merck 1.05458.0500) with 0.6% yeast extract (Merck 1.03753.0500). After the incubation of the colonies at 37°C for 12 hours, *E. coli* O157 the latex agglutination test (Oxoid DR 0120) was applied to the grown colonies for recovery.

Detection of Listeria monocytogenes / Nalaz Listeria monocytogenes

For detection of *Listeria monocytogenes*, the FDA method was used [1]. Each pepton water tube that contained a sample swab was added into the bottles containing Fraser broth (Merck 1.10398) with Half Fraser Listeria Supplement (Merck 1.10399) and was added into the bottles containing Fraser broth (Merck 1.10398) with Fraser Listeria Supplement (Merck 1.10399). Half Fraser Broth bottles were incubated for 24 hours at 30°C. Fraser Broth bottles were incubated for 24-48 hours at 35-37°C. This part was the enrichment step of the inoculation procedure for *Listeria monocytogenes*.

The inoculum was vortexed and was inoculated with loop to the OXA (Merck 1.07004) and PALCAM agar (Merck 1.11755). After the incubation period at 35°C for 24-48 hours, black-brown colonies on the OXA and grey-brown green colonies with a black halo on the PALCAM agar were determined as suspicious bacteria.

Five or more typical colonies that are growth on the OXA and PALCAM agar were taken and inoculated to the Tryptic Soy Agar (Merck 1.05458.0500) with 0,6% yeast extract (Merck 1.03753.0500). TSAYE plates were incubated at 30°C for 24-48 hours for recovery. For identification procedure, the FDA methods was used.

For serology, the CAMP test was used. To perform the test, streak a beta-hemolytic *Staphylococcus aureus* and a *Rhodococcus equi* culture in parallel and diametrically opposite each other on a sheep blood agar plate. Streak several test cultures parallel to one another, but at right angles to and between the *S. aureus* and *R. equi* streaks. After incubation at 35°C for 24-48 h, examine the plates for hemolysis.

Detection of Salmonella spp. / Nalaz Salmonella spp.

After enrichment of the buffered peptone water for 24 h at 37°C, 0,1 ml was transferred to 10 ml Rappaport Vassiliadis broth (RV, Oxoid) and 10 ml transferred to 100 ml selenite cysteine broth (Merck 1.07709). The broths were incubated at 42°C and 37°C, respectively, for 24 h. Aliquots from each broth were spread, in duplicate, onto salmonella-shigella agar (SS, Merck 1.07667) and brilliant green phenol red lactose agar (BGPRA, Merck 1.07236) plates, and incubated at 37°C for 24 h. After plating, the selenite cysteine broths were returned to the incubator for a further 24 h, and plated out as described above. Suspicious colonies on either SS or BGPRA were confirmed using Gram staining, catalase, oxidase, urease, indole, simmons citrate, the Voges-Proskauer test, methyl red test, glucose (TSI), lysine decarboxylase and semi indole motility tests, poly O and H antisera (Prolab) and Api 20E kits (Biomerieux).

Results / Rezultati rada

Slaughtering is an open process with many opportunities for the contamination of the carcass with potentially pathogenic bacteria. Microbial contamination results in reduced shelf life of meat, spoilage of meat and public health hazards. The aim of this study was to examine the microbial contamination of lamb carcass surfaces after slaughter, to determine the acceptability of carcass hygiene levels and prevalence foodborne pathogens at the abattoirs in Istanbul.

A total of a hundred lamb carcasses were sampled over a 12 month period. Each sample was analysed for Total Mesophilic Aerobic Count, Enterobacteriaceae Count, *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. All samples were negative for *Listeria monocytogenes* and *Escherichia coli* O157:H7. Furthermore *Salmonella* spp. was detected on four carcasses. Table 1 shows the EU bacterial performance criteria for lamb carcass surfaces. On the other hand, Table 2 shows the result of TMCs and ECs which are indicators of carcass surface hygiene.

Table 1. EU bacterial performance criteria (log mean CFU cm⁻²) for lamb carcasses /
Tabela 1.T EU kriterijumi za performanse bakterija (log SV CFU cm⁻²) za jagnjeće trupove

Bacteria / Bakterija	Acceptable range / Prihvatljiv raspon	Marginal range / Marginalni raspon	Unacceptable range / Neprihvatljiv raspon
Total viable counts / Ukupno održivih	3,5	3,5 – 5,0	5,0
Enterobacteriaceae / Enterobacteriaceae	1,5	1,5 – 2,5	2,5

In this work TMCs and ECs results are transformed to log₁₀ values. The study revealed that total plate counts in 93% and 7% of the carcasses ranged

from 4.18-4.99 and 5.10-5.95 CFU/cm² respectively. The EC was detected on all of the carcasses and ranged between 1.60-2.30 CFU/cm².

Table 2. Variation in Total Mesophilic Aerobic Count and Enterobacteriaceae Count /
Tabela 2. Varijacije u ukupnom broju aerobnih mezofila i broju Enterobacteriaceae

Criteria / Kriterijumi	Total viable counts (CFU cm ⁻²) / Ukupno održivih (CFU cm ⁻²)			Enterobacteriaceae (CFU cm ⁻²) / Enterobacteriaceae (CFU cm ⁻²)		
Range / Raspon	3,5	3,5 – 5,0	5,0	1,5	1,5 – 2,5	2,5
100 ^a	0 ^b	93	7	0	100	0

^a Number of analysed samples / Broj analiziranih uzoraka

^b Percentage of samples / Procenat uzoraka

Discussion / Diskusija

EU 2001/471/EC requires for microbiological data to be obtained from the carcasses before chilling using an excision or swabbing method, with a view to determine the hygiene status within HACCP. According to the decision performance criteria, the TMCs and the ECs should be performed [15]. But no pathogen performance standards. However red meat is a significant source of bacteria which frequently cause foodborne illnesses in human.

A major survey for *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. on beef carcasses in Northern Ireland was studied. Only *Salmonella* spp. was isolated from three carcasses of 780, compared with the present survey, four carcasses of 100 but in a British survey of cattle carcasses (n=29), *Listeria monocytogenes* was found 7% positive [10]. In another study in the UK, 1500 carcasses were analysed and *E. coli* O157:H7 was isolated from 21 (1.4%) carcasses [3]. In France, *E. coli* O157:H7 was present in 1 (0.4%) of 225 carcasses [7]. *E. coli* O157:H7 was not isolated from carcasses in the Netherlands [8], in Germany, in the USA, and in Turkey [6].

At the same time, studies for determining the acceptability of carcass hygiene have been conducted some time ago. In New Zealand, 772 lamb carcasses were analysed and had a mean TMC of 3.35/cm², while in Canada, another study found that sheep carcasses had log₁₀ TMC/cm² at the shoulder, loin and leg of 2.81; 2.80 and 2.56 respectively [19]. In South Australian, Phillips *et al.* [7], sampled 917 carcasses and had a mean TMC of 3.54/cm², EC of 0.16/cm², compared with the present survey of 4.18-5.95 and 1.60-2.30 CFU/cm² respectively.

In conclusion, the main sources of the contamination for the red meat are microorganism on the hides. During the evisceration and dressing processes, they are transferred to the carcass surfaces directly and indirectly. Also, the contamination can be spread from the contaminated carcasses to other carcasses on

the monorail system. The extent of contamination at hide removal can be reduced using improved hygienic practices which include slaughter and personnel hygiene. Another way to reduce or to take control of microbial carcass contamination is carcass decontamination treatments after dressing or hide decontamination before the dressing. They are widely used in the US but not permitted in the EU. The result of this study indicates that there is a potential risk of contamination of meat and that the veterinary supervision at slaughterhouses is not efficient in Turkey.

Literatura / References

1. Anonymous: Food and Drug Administration. Bacteriological Analytical Manual. 8th Edition. AOAC International, USA, 1995. - 2. Anonymous: Commission decision of 8 June 2001 (2001/471/EC). Official Journal of the European Communities L165, 48-53, 2001. - 3. Chapman P. A., Cerdan Malo A. T., Ellin M., Ashton R., Harkin M. A.: *Escherichia coli* O157 in cattle and sheep at products in South Yorkshire, UK. Int. J. Food Microbiol., 64, 139-150, 2001. - 4. Elder R. O., Keen J. E., Siragusa G. R., Barkocy-Gallagher G. A., Koohmaraie M., Laegreid W. W.: Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci., 97, 7, 2999-3003, 2000. - 5. Fu Z., Rogelj S., Kieft T. L.: Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real time PCR. Int. J. Food Microbiol., 99, 1, 47-57, 2005. - 6. Gun H., Yilmaz A., Turker S., Tanlasi A., Yilmaz H.: Contamination of bovine carcasses and abattoir environment by *Escherichia coli* O157:H7 in Istanbul. Int. J. Food Microbiol., 89, 339-344, 2003. - 7. Guyon R., Dorey F., Malas J. P., Grimont F., Foret J., Rouviere B., Collobert J. F.: Superficial contamination of bovine carcasses by *Escherichia coli* O157:H7 in a slaughterhouse in Normandy (France). Meat Sci., 58, 329-331, 2001. - 8. Heuvelink A. E., Roessink G. L., Bosboom K., Boer E. D.: Zero tolerance for faecal contamination of carcasses as a tool in the control of O157 VTEC infections. Int. J. Food Microbiol., 66(1-2), 13-20, 2001. - 9. Jofre, A., Martin, B., Garriga, M., Hugas M., Pla M., Rodriguez – Lazaro, D., Aymerich, T.: Simultaneous detection of *Listeria monocytogenes* and *Salmonella* by multiplex PCR in cooked ham. Food Microbiol., 22(1), 109-115, 2005. - 10. Madden R. H., Espie W. E., Moran L., McBridge J., Scates P.: Occurrence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* and *Campylobacter* spp. on beef carcasses in Northern Ireland. Meat Sci., 58, 343-346, 2001. - 11. Matsuoka D. M., Costa S. F., Mangini C., Almeida G. M. D., Bento C. N., Van Der Heijden I. M., Soares R. E., Gobara S., Tavora L. G. F., Levin A. S.: A nosocomial outbreak of *Salmonella enteridis* associated with lyophilized enteral nutrition. J. Hosp. Infect., 58, 122-127, 2004. - 12. McLauchlin J., Mitchell R. T., Smerdon W. J., Jewell K.: *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. Int. J. Food Microbiol., 92, 1, 15-33, 2004. - 13. Mead P. S., Slutsker L., Dietz V., Mc Caig L. F., Bresee S., Shapiro C., Griffin P. M., Tauxe R. V.: Food related illness and death in the United States. Emerg. Infect. Dis., 5, 607-625, 1999. - 14. Murphy R. Y., Beard B. L., Martin E. M., Keener A. E., Osaili T.: Predicting process lethality of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in ground, formulated and formed beef/turkey links cooked in an air impingement oven. Food Microbiol., 21, 5, 493-499, 2004. - 15. Pearce R. A., Bolton D. J., Sheridan J. J., McDowell D. A., Blair I. S., Harrington D.: Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. Int. J. Food Microbiol., 90, 331-339, 2004. - 16. Pellicer K. E., Copes J. A., Nosetto E. O., Echeverria M. G.: Characterization of *Listeria* spp. isolated from ready to eat products in Argentina using SDS-PAGE and restriction endonuclease. Food Res. Int., 37, 10, 1013-1019, 2004. - 17. Phillips D., Sumner J., Alexander J., Dutton K.: Microbiological quality of Australian sheep meat. J. Food Prot. 64, 697-700, 2001. - 18. Qiongzhen L., Sherwood J. S., Logue C. M.: The prevalence of *Listeria*, *Salmonella*, *Escherichia coli* and *E. coli* O157:H7 on bison carcasses during processing. Food Micro-

biol., 21, 6, 791-799, 2004. - 19. Sumner J., Petrenas E., Dean P., Dowsett P., West G., Wiering G., Raven G.: Microbial contamination on beef and sheep carcasses in South Australia. Int. J. Food Microbiol., 81, 3, 255-260, 2003. - 20. Untermann F., Stephan R., Dura U., Hofer M., Heimann P.: Reliability and practicability of bacteriological monitoring of beef carcass contamination and their rating within a hygiene quality control programme of abattoirs. Int. J. Food Microbiol., 34, 67-77, 1997.

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МИКРОБИОЛОШКА КОНТАМИНАЦИЈА ЈАГНЈЕЋИХ ТРУПОВА У КЛАНИЦАМА
ИСТАНБУЛА

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Uzorkovano je ukupno sto jagnjećih trupova u toku perioda od dvanaest meseci u klanicama u Istanbulu u Turskoj. Svaki uzorak koji je ispitivan na ukupan broj aerobnih mezofila (TMC), broj Enterobacteriaceae (EC), *Salmonella* spp., *Escherichia coli* 0157:H7 i *Listeria monocytogenes* uzet je sa površine od 100 cm² na četiri strane jagnjećih trupova koristeći tehniku vlažnih i suvih pamučnih štapića. Ispitivanja su pokazala da je ukupan broj aerobnih mezofila kod svih trupova iznosio između 4,18 i 5,95 log/cm²; broj Enterobacteriaceae bio je između 1,60 i 2,30 log/cm². Svi uzorci bili su negativni na *Escherichia coli* 0157:H7 i *Listeria monocytogenes*. Osim toga, *Salmonella* spp. je ustanovljena na četiri trupa. Ovi podaci potvrđuju da je bakteriološko praćenje jagnjećih trupova koristan kriterijum za utvrđivanje zdravstvene higijene u klanici.

Ključne reči: zdravstvena ispravnost trupa, klanica, kontaminacija, 2001/471/EC

РУССКИЙ

МИКРОБИОЛОГИЧЕСКАЯ КОНТАМИНАЦИЯ ЯГНЯЧЬИХ ТУЛОВИЩ В
СКОТОБОЙНЯХ СТАМБУЛА

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Образчиковано совокупно 100 ягнячьих туловищ в течение периода от 12 месяцев в скотобойнях в Стамбуле, Турция. Каждый образчки испытыван на совокупное число аэробных мезофилл (ТМС), число Enterobacteriaceae (EC), *Salmonella* spp., *Escherichia coli* 0157:H7 и *Listeria monocytogenes* взят с поверхности от 100 см² на четыре стороны ягнячьих туловищ, пользуя технику влажных и сухих хлопковых палочек. Испытания показала, что совокупное число аэробных мезофили у всех туловищ составляло между 4,18 и 5,95 log/см²; число Enterobacteriaceae было между 1,60 и 2,30 log/см². Все образчики были отрицательные на *Escherichia coli* 0157:H7 и Листерия моноцитогенес. Кроме того, *Salmonella* spp. установлена на четыре туловища. Эти данные подтверждают, что бактериологическая слежка ягнячьих туловищ полезный критерий для утверждения здравоохранительной гигиены в скотобойне.

Ключевые слова: здравоохранительная исправность туловища, скотобойня, контаминация, 2001/471/EC