RESEARCH ON DETERMINATION OF SHEEP CARCASSES DEGREE AND CONTAMINATION DURING SLAUGHTER

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Abstract

Meat quality is influenced by the slaughter of animals, especially the hygiene conditions in which killing is carried out.

During slaughter, sheep carcasses can become contaminated with microorganisms directly by blowing air during evisceration and storage.

The study was conducted on 80 samples of lamb carcasses and 40 muscle samples from regions of adult sheep. We perfomed qualitive and quantitative bacteriological examination. The purpose of this study was to show the dergee of bacteriological contamination of surface and profile of adult sheep and lamb carcasses, obtained at the slaughterhouse. Samples were colected from the cervical region, chest, and of the external biceps femoris muscles from the lambs carcasses (after evisceration and cold storage) by swabbing an area of 100 cm^2, with a sterile gauze pad that has been moistened with peptone water.

Samples from adult sheep were collected from the biceps femoris inside muscles (after skinning and before chiling) by cutting a 1 cm^3 surfaces using a sterile scalpel.

For lambs, depending on the region housing, NTG ranged from 5.6 to 6.5 log cfu/cm^2 after evisceration and from 3.1 to 4.3 log cfu/cm^2 after chilling. Bacteriological profile was represented by changes in Gram - and Gram + strains. Gram - strains detected are: 82% coliforms, 43% E. coli, 37% Proteus spp and 29% Pseudomonas spp.

Gram + strains detected are: Staphylococcus spp, Bacillus and Clostridium spp.

For adult sheep, in different medias culture were developed: coliforms (Enterobacteriaceae lactose- positive, Enterobacteriaceae lactose- negative), colonies of Clostridium perfringens and colonies of Lysteria monocytogenes.

Key words: mutton, bacteriological exam, health

INTRODUCTION

Sheep meat is an important food in the human food needed. The world production of about 50 milion tons annualy, representing 10% of sheep meat, ranking fourth after beef and veal meat, poultry and after pig meat. If in some countries like Australia and New Zealand sheep meat consumption is 40-42%, in our country, consumption is quite low, representing about 10-12% of total meat consumption.

Bacteriological inspection has a particular importance in assessing the degree of innocuity meat. The most important sources of contamination of carcasses are the feces, gastric contents, wool or animal from the environment by contact with different surfaces or unhealthy environments.

MATERIALS AND METHODS

The study was conducted on 80 samples collected from carcasses of lambs slaughtered in environmental conditions, 40 samples were taken after evisceration stage and 40 samples after cooling, of the neck, chest and the outside of the thigh.

Samples from lambs was collected by wiping a surface of 100 cm^2 with a sterile swab. The area was demarcated by a sterile template. After harvest, swabs were placed in salines tubes. The initial tests were carried on serial dilutions up to 10^{-6} with sterile saline.

Total aerobic mesophilic plate count was determined for 10^{-4} and 10^{-5} dilution on nutrient agar by incorporating 0,1 ml inoculum. For each dilution were made two plates.

Seeded plates were incubated at 37^0C for 24 hours. The microorganisms was determinate by using as indicators of specific selective media. To determine the probable number of *E. coli* was used ANSVA 78323/1998 standard. According to this standard are seeded series of three test tubes containing selective enrichment medium and Durham tubes with the sample and its decimal dilutions. This medium is represented by the tryptone broth and lauryl sulfate. Tubes thus prepared incubated at 37^0C for 24 hours.

After this interval, each tube of gas release was observed, seeded the second selective enrichment medium, several EC Broth. The tubes are incubated at 45^0C for 24 horus. From each tube considered positive for *E. coli* culture was taken and inoculated on a selective medium, respectively Levine. In this environment the presence of the species *E. coli* colonies was confirmed by the appearance of slightly raised, dark purple color with metallic luster and golden green reflections. Simultaneously, in each tube with EC who has made medium clearance gas seeded one tryptone water tube which is incubate at 45^0C for 48 hours. After incubation tubes with tryptone water, added 0,5 ml Kovacs reagent it identify the presence of indole. The positive tubes were examined in UV light and the fluorescence were positive confirmed for E. coli. It is positive considered if the culture area is a red ring. The calculation of most probable number of positive tubes containing culture considered using Mac Grady table.

For detection of coliform bacteria was using the method of working according to ISO 4832/1992 STAS. To detect strains of Proteus spp and Pseudomonas spp were used in specific environments.

The isolation of *Staphylococcus spp* strains was performed on special media: Vogel-Johnoson and Baird-Parker, 24 hours at 37^0C and Bacillus spp strains were isolated from egg yolk agar and blog agar.

To identify the species of *Clostridium perfringens* was used SN EN13401/1999. Under this standard, it was transferred with sterile pipette, 1 ml sample, respectively dilutions 10^{-4}, 10^{-5}, 10^{-6} in the center of two Petri dishes, then poured 10 ml of TSC agar (sulphite triptoses cicloserine) maintained at 45^0C and was thoroughly mixed with inoculums easily turning every box. When solidified medium was poured another layer of 10 ml of the same agar and was allowed to solidify. Incubation was made in anaerostat at 37^0C, 24 hours. On each case were counted black colonies, suspecting the precence of *Clostridium perfringens* strains.

Biochemical confirmation was taking five characteristic colonies of TSC environment and passing on LS medium (lactose-sulfite). Each selected colony was inoculated into thioglycolate liquid medium, making it the anaerostat incubation at 37^0C for 24 hours, the tubes which showed gas production in less than one quarter of the height and appeared black were considered positive.

Samples from adult sheep are represented by 40 samples of 1 cm^3 cutting from biceps femoris inside muscle.

The initial tests were carrid serial dilutions up to 10^{-6} with sterile saline. Total mesophilic aerobic plate count was determined for 10^{-6} dilution on Bacterial Count Agar medium by incorporating 0,1 ml inoculums. For each dilution was performed by a plate. Seeded plates were incubated at 37^0C for 24 hours. Microorganisms was determinate using as indicators specific selective media. To determine the probable number of E. coli was used SR EN ISO 16649/2007. Inoculation was done on Levine medium. Tubes thus prepared was incubated at 37^0C for 48 hours. The presence of the *E. coli* colonies species was confirmed by the appearance of slightly raised, dark purple with bright golden-green metallic reflections.

Isolation of *Staphylococcus spp* strains was performed on special media: Rapaport Vassiliadis and Mach Conchi and XLD agar.

To identify strains of *Listeria monocytogenes* was performed on the

primary enrichment demi-Fraser medium, Fraser medium secondary enrichment, incubated at 37^0C, 48 hours and is done by potting ribbed chromogenic agar medium according to SR EN ISO11290/2000.

The identification is made by the emergence of green with blue halo colonies around training.

To identification of *Clostridium* species was used SR ISO 7937/2005. Under this standard it was transferred to sterile pipette, 1 ml of sample, respectively 10^{-6} dilutions in test tubes were then poured 10 ml SPS agar (Sulfadiaxin, Polymyxin, Sulfate) maintained at 45^0C and was mixed well with inoculums slightly rotating each tube. Incubation was done in anaerostat at 37^0C/24 hours. It were counted black colonies on each case os suspected of Clostridium perfringens strains.

Biochemical confirmation was taking five characteristic colonies of TSC environment and passing on LS medium (lactose-sulfite). Each selected colony was inoculated into thioglycolate liquid medium, incubation being made in anaerostat at 45^0C for 24 hours.

Identification: After the first 5 hours of incubation it was observed, noting a release of gas and is suspected contamination with *Clostridium perfringens*.

Colonies were finally identified by lenticular black *Clostridium* with H2S formation).

RESULTS AND DISCUSSIONS

The microflora carcass evalution and to identify steps of the slaughter process, during they may be contaminated, are important in establishing preventive and corrective measures by which progress can be microflora controlled.

The total number of germs (NTG), led the process of slaughtering lambs, provided values ranging from 5,6 to 6,5 log cfu/cm^2 cooling stage. The highest values were recorded after the evisceration step outside of the thigh region, and the lowest on the neck after cooling (Table 1).

Duffy et al. (2001), in a study of lamb carcasses, obtained 4,2-4,5 cfu/cm^2 values after cooling step and Zweifwl and Stephan (2003) obtained lower values, 2,5-3,8 cfu/cm^2.

Bhandare et al. (2007) in a comparative study has obtained 6,06 and 5,13 cfu/cm^2 values after evisceration on cooling stages of carcasses in the slaughterhouse and higher values of 6,4 to 6,1 cfu/cm^2 after the same stages during the slaughter in the traditional system.

Table 1 NTG/cm^2 values in different regions of lamb carcasses during slaughter

Slaughter phase	Carcass region	Numb. of examined samples	NTG (log CFU/cm^2)		
			Minimum value	Maximum value	Media
Evisceration	Neck	11	5.5	6.2	5.8
	Chest	15	5.4	6.5	6.05
	The outer thigh	17	6.1	6.6	6.6
Cooling	Neck	11	2	2.7	2.3
	Chest	15	3.4	5.2	4.3
	The outer thigh	16	4.0	4.8	4.8

Table 2 The microflora of lambs carcasses contamination slaughtered in the traditional system

Microflora of contamination	Gender	Numb. of examined samples+	%	Numb. of examined samples-	%
Gram -	*Coliforms*	69	82	16	18
	E. coli	36	43	49	57
	Proteus spp.	31	37	54	63
	Pseudomonas spp.	24	29	61	71
Gram +	*Staphzlococcus spp.*	17	21	68	79
	Clostridium spp.	11	13	74	87
	Bacillus spp.	6	8,2	79	91,8

The high values of NTG after evisceration of this operation is explained by specific carcass weight may come in contact with gastrointestinal and even its content, which is more common situation when the operation is poorly performed.

The bacterial profile showed a varied microflora, dominated by faeces. The highest values have been fecal coliforms (82%), followed by *E. coli* (43%), *Proteus spp.* (37%) and *Pseudomonas spp* (29%). The Gram+ microflora showed lower values: *Staphylococcus spp*. (21%), *Clostridium spp* (13%) and *Bacillus spp* (8,2%). Bacteriological profile is shown in table 2.

Philips et al. (2004) in a study of lamb carcasses at the slaughterhouse, has detected generic *E. coli* (79%) and *Staphylococcus spp.* (32%) contamination. Nerasimha et al. (1992) identified strains of the genera *Micrococcus, Staphylococcus* and faecal coliforms as the predominant microflora of slaughter.

S. Bhandare S. et al. (2007), obtain different data depending on the type of slaughter: *Staphylococcus spp* (50%), *Bacillus spp*. (9%), *Clostridium spp.* (20%), *E. coli* (20%), coliforms (46%), *Pseudomonas spp*. (20%) in carcasses obtained after slaughtering and *Staphylococcus spp* on carcasses surface are sanitation indicators and shows non stages of slaughter and hygiene conditions in particular.

For samples collected from adult sheep after inoculation of dilutions performed on the Bacterial Count Agar medium, there was a lush growth of mesophilic bacteria in samples collected before refrigeration, NTG was detected over the maximum allowed. The results for samples collected after skinning have an average of 82.500 germs/g of sample, placing the values rendered by law applicable.

The coliforms and *E. coli* are two microbiological genres used to assess the state of food hygiene and technological areas.

Escherichia coli, a species classified as coliforms, which includes the potentially pathogenic strains reached a higher number of colonies for samples before refrigeration compared with those taken after skinning. E. coli was identified with the cult following media: Levine (green colonies with metalic sheen), XLD (Xylose, Lysine, Deoxycholic) – Salmonella specific medium – negative, E. coli present, yellow colonies, on MachConkye medium lush growth of *E. coli* colonies, large, convex metalic luster, sorbitol positive.

The coliforms distribution is closely correlated with the values shown for total aerobic mesophilic plate count. Regarding the body region examined, coliforms were conistently present in the biceps femoris muscle surface. The coliforms are represented by *E. coli, Enterobacteriaceae, Klebsiella and Proteus* and are present in the following areas: Levine (matte pink, convex colonies), XLD (yellow colonies) and MachConchie (pink bomb coliform colonies, sorbitol positive).

Salmonella spp were not recovered on XLD and MachConchie medium, the samples were identified as coliforms and *Shigella spp.* Skinning samples collected before refrigeration (XLD) and coliform bacteria matte pink bombs, sorbitol negative and a lush colony's development of E. coli, large, convex metallic luster, sorbitol positive for all the samples.

On SPS medium (Sulfadiaxin Polymyxin Sulfate) noted a release of gas and is suspected contamination with *Clostridium perfringens*. It were finally identified lenticular black colonies of *Clostridium* with H_2S formation on Cromogenic agar medium were identified *Listeria monocytogenes* colonies in green with blue halo formation around, noting a slow colony samples collected before refrigeration.

On interpretation of these results we can conclude that the positive values (exceeding the maximum limits allowed by applocable law and the values enshrined in HACCP plans) were due to shortcomings in the handling of caecasses, disregard personal hygiene of workers, casual deficiencies which do not involve a matter of routine.

CONCLUSIONS

Although the results of tests carried out shows a relatively favorable situation in terms of microbiological contamination of sheep carcasses and muscle regions

continuously monitor the parameters set by the legislation in force and those enrolled in the HACCP plan is absolutely necessary in order to quickly identify potential sources of contamination and to minimize its effects on consumers, through timely intervention to eliminate and destroy products with high microbiological contamination higher than that stipulated by the legislation.

Microflora knowledge gives data on the state of contamination of meat is also an indicator of health. The study showed that the microbiological steps that contribute most to contamination of sheep carcasses in the slaghterhouse are bleeding and evisceration, which requires special steps to reduce the contamination of carcasses at slaughterhouses.

The results obtained allowed to establish a consistent positive correlation between the total mesophilic number of aerobic bacteria and hygiene status indicators (*Eserichia coli* and coliforms).

The study of lamb carcasses contamination revealed that the microbiological of carcasses slaughtered in the traditional system is gutting.

The most contaminated region in part of thigh (6,6 cfu/cm^2) after evisceration, and the neck is less contaminated after cooling (2,0 cfu/cm^2). The NTG ranged from 5,6 to 6,5 cfu/cm^2 stage from 3,1 to 4,3 cfu/cm^2 after cooling step.

The contamination microflora of both species was the Gram – (coliforms, *E. coli, Proteus spp., Pseudomonas spp*.) and Gram + species (*Staphylococcus spp., Clostridium spp., Bacillus spp.).*

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