

MICROBIOLOGICAL EVALUATION OF THE MUSCLE SURFACE OF LAMB CARCASSES DURING SLAUGHTER PROCESSES IN THE SLAUGHTERHOUSE

AVALIAÇÃO MICROBIOLÓGICA DA SUPERFÍCIE MUSCULAR DE CARÇAÇAS OVINAS DURANTE AS OPERAÇÕES DE ABATE EM MATADOURO

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SUMMARY

In order to evaluate the hygienic-sanitary conditions of lamb carcasses for human consumption, this study aimed at quantifying populations of indicator microorganisms, such as: mesophiles and psychrotrophs, molds and yeasts, *Escherichia coli*, *Staphylococcus* spp. and at identifying pathogenic microorganisms (*Salmonella* sp. and *Listeria* spp.). The study was conducted in one lamb slaughterhouse located in the State of São Paulo. Swab samples were collected from muscle surface of forequarter and hindquarter regions of 30 half-carcasses after skinning, evisceration and washing processes. Population counts were between the following values in log₁₀: 2.00 ± 0.32 and 2.59 ± 0.76 UFC/cm² for mesophiles; 1.52 ± 0.98 and 2.35 ± 1.17 UFC/cm² for psychrotrophs; 0.75 ± 0.87 and 1.23 ± 0.97 UFC/cm² for molds and yeasts; 0.00 ± 0.00 and 0.31 ± 0.84 NMP/cm² for *Escherichia coli* and 1.75 ± 0.71 and 1.95 ± 0.68 UFC/cm² for *Staphylococcus* spp. *Salmonella* sp. and *Listeria* spp. were not detected from any of the sampled points. These results indicate the necessity to improve the hygienic-sanitary conditions.

KEY-WORDS: Carcasses. Lamb. Indicator microorganisms. Hygiene.

RESUMO

Objetivando avaliar as condições higiênico-sanitárias de carcaças ovinas destinadas à alimentação humana, o estudo consistiu em realizar a quantificação das populações de microrganismos indicadores: heterotróficos mesófilos e psicrotróficos, bolores e leveduras, *Escherichia coli*, *Staphylococcus* spp. e a identificação de microrganismos patogênicos (*Salmonella* sp. e *Listeria* spp.). O estudo foi desenvolvido em matadouro-frigorífico de ovinos, situado no interior do Estado de São Paulo. Foram colhidas amostras, através de swabs, da superfície muscular das regiões dianteira e traseira de 30 meias carcaças ovinas, após as etapas de esfolagem, evisceração e lavagem. As populações encontradas estiveram entre os seguintes valores em log₁₀: 2,00 ± 0,32 e 2,59 ± 0,76 UFC/cm² para mesófilos; 1,52 ± 0,98 e 2,35 ± 1,17 UFC/cm² para psicrotróficos; 0,75 ± 0,87 e 1,23 ± 0,97 UFC/cm² para bolores e leveduras; 0,00 ± 0,00 e 0,31 ± 0,84 NMP/cm² para *Escherichia coli* e 1,75 ± 0,71 e 1,95 ± 0,68 UFC/cm² para *Staphylococcus* spp. *Salmonella* sp. e *Listeria* spp. não foram detectadas em nenhum dos pontos amostrados. Os resultados indicam a necessidade da melhoria dos cuidados higiênico-sanitários adotados.

PALAVRAS-CHAVE: Carcaças. Ovinos. Microrganismos indicadores. Higiene.

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INTRODUCTION

Among different animal species used as food by humans, lamb deserves attention. In recent years, lamb is available in supermarkets, butcher shops and upscale restaurants in large metropolitan areas, thus breaking the paradigm of lamb consumption only in small rural towns. This growing demand for lamb products increased the number of entrepreneurs willing to invest in this activity. Therefore, if the agricultural industry and the technologies already installed are able to meet the requirements of the diverse segments of the production chain, the sheep industry will have a great socio-economic impact in Brazil.

The average consumption of lamb/person/year in Brazil is still low. While official statistics show a consumption of 0.7 kg/person/year, this number in countries with long tradition in sheep farming can reach up to 32.5 kg/person/year. Despite this fact, the import of mutton in Brazil, increased from 2.3 thousand tons in 1992 to 14.7 thousand tons in 2000, a growth of over 600% (SIMPLÍCIO & SIMPLÍCIO, 2006). This increasing lamb production and demand requires rigorous hygienic-sanitary conditions during processing, since the slaughterhouses are responsible for providing a safe product from the view point of public health.

The production of good quality meat depends on the control exercised over the physical, chemical and biological processes that permeate all stages of the food chain. Therefore, determining the numbers and types of microorganisms on the surface of carcasses is a matter of public health, both to evaluate the effectiveness of the hygienic-sanitary conditions during slaughter processes and to assess quality characteristics, including shelf life of meat and meat products.

Due to this gradual increase of lamb consumption in Brazil and to few existing data about microbiological characteristics of lamb carcass, the present study aims to evaluate the hygienic-sanitary conditions of carcasses of freshly slaughtered sheep by quantifying microbial indicators and verifying the presence of *Salmonella* sp. and *Listeria* spp.

MATERIAL AND METHODS

Sampling

This study was conducted in a slaughterhouse located in the State of São Paulo. Swab samples were collected from an area of 20 cm² of 30 half-carcasses of 30 animals on the muscular surface of forequarter and hindquarter (APHA, 2001). The samples were obtained after skinning (A), evisceration (B) and washing processes (C). The swabs from each sampling point of the half carcasses were stored in vials containing 20 mL of 0.1% peptone water.

Microbiological analysis

Swabs were homogenized during 20 minutes (Colworth 400 Stomach) in the swab transport solution and diluted up to 10⁻¹ and 10⁻². Subsequently, the

following microorganisms were counted: mesophilic, psychrotrophic, molds and yeasts, as well as *Escherichia coli* and *Staphylococcus* spp. These dilutions were not used to isolate *Salmonella* sp. and *Listeria* spp.

Mesophiles, psychrotrophs, molds and yeasts counts

Total aerobic mesophiles and psychrotrophs counts were determined according to the pour plate technique (APHA, 2001; ICMSF, 2000) on plate count agar (PCA, Oxoid) and incubated at 35°C and 7°C for 48h and 10 days, respectively. Molds and yeasts were determined in malt extract agar (MEA, Oxoid) whose pH was adjusted to 3.5 by adding a 10% sterile solution of tartaric acid and incubated between 22 and 25°C for 5 days (APHA, 2001).

Escherichia coli

The most probable number (MPN) was determined according to multiple tube technique (APHA, 2001), using sodium lauryl sulfate tryptose broth at 35°C for 24-48h for the presumptive test, followed by inoculation and incubation in brilliant green bile broth at 2%, 35°C for 24-48h. Positive cultures in the latter were grown further in EC broth at 45°C for 24h. To confirm *E. coli*, positive cultures in EC broth were plated on methylene blue eosin agar (BEM, Oxoid) at 35°C, for 24h. The colonies with the characteristic metallic sheen were transferred to tubes containing nutrient agar, stained by Gram and submitted to biochemical tests IMViC (Indole, methyl red, Voges-Proskauer and citrate tests).

Staphylococcus spp.

Staphylococcus spp. count was performed according to the spread plate technique (APHA, 2001; ICMSF, 2000) using Baird-Parker agar (Oxoid) and incubated at 35°C for 24-48h. For the coagulase free test, the strains were inoculated in brain heart infusion broth (BHI, Difco) and incubated at 35°C, for 24h. After that, the cultures were inoculated in tubes containing rabbit plasma diluted 1:5. The tubes were incubated in water bath at 37°C and the readings were performed after 1, 2, 3, 4 and 24 hours.

Salmonella sp.

Salmonella was isolated according to APHA (2001). The swabs were pre-enriched in the carrier solution at 35°C, for 24h. Selective enrichment was done in selenite cystine broth (Oxoid) and Rappaport-Vassiliadis broth (Oxoid) and incubated at 37°C for 24h. The cultures were plated on brilliant green agar (Merieux) and McConkey (Oxoid) followed by incubation at 37°C for 24h. Suspected colonies were submitted to biochemical tests.

Listeria spp.

Listeria spp. was detected by the technique recommended by the Health Protection Branch of Canada, cited by Silva et al. (1998), which consists of a primary enrichment of 10 mL of the swab carrier solution with 90 mL of *Listeria* enrichment broth

(LEB, Difco) and incubation at 30°C for 48h. Simultaneously, the culture in enrichment broth was plated on modified Oxford agar (MOX, Difco) and lithium chloride phenylethanol moxalactam (LPM, Difco), followed by incubation at 35°C for 48 horas. Suspected colonies were submitted to biochemical tests.

Statistical analysis

Data from the population of mesophiles, psychrotrophs, molds and yeasts and *Staphylococcus* spp. were transformed into $\log_{10}(\text{UFC}/\text{cm}^2+1)$ and submitted to variance analysis in split-plots and the means were compared by Tukey test.

Data from *Escherichia coli* were analyzed using the Friedman test to compare the steps for each region and the Wilcoxon test to compare the regions of each step.

Significance level adopted was $P<0.05$. Statistical analysis were performed by the SAS program (Statistical Analysis System).

RESULTS AND DISCUSSION

Population means and standard deviation of the populations of mesophilic heterotrophic microorganisms are presented in Table 1. Results show no statistically significant differences between the three processing stages studied for the forequarter. However, the results for the hindquarter were significantly different when comparing skinning and evisceration with washing. Significant differences are also observed between skinning and evisceration in the hindquarter compared to forequarter.

Kelly et al. (1981) evaluated the mesophilic population by comparing the results after the evisceration and spray washing of lamb carcasses. After evisceration, the initial values were between 3.29 and 4.22 \log_{10} UFC/cm²; after the spray washing step with chlorinated water and temperature higher than 57°C, a significant reduction of 0.5 \log_{10} UFC/cm² was observed; and when water temperature was $\geq 80^\circ\text{C}$, the reduction observed was $\geq 1,0$ \log_{10} UFC/cm². The values obtained in this study after evisceration are lower than those reported by these authors. Although

counts decreased after carcass washing, results were not significantly different. However, in the present study the carcasses were spray washed with chlorinate water at room temperature, which may explain the difference in the population numbers.

Sierra et al. (1997) reported higher values compared to the results of this study, for all processing stages, while studying the mesophilic populations of 4 slaughterhouses in Ireland. After skinning, evisceration and washing processes, mean population varied between 4.5 and 5.41 \log_{10} UFC/cm²; 4.52 and 5.01 \log_{10} UFC/cm²; 4.63 and 4.88 \log_{10} UFC/cm² for the different slaughterhouses studied. Similar to the present study, the authors did not find significant differences in population counts between slaughter stages. Therefore, they concluded that maximum contamination occurred once the area was exposed and did not change during the process.

After the skinning, Gill & Baker (1998) reported a mean value of 2.64 ± 0.82 \log_{10} UFC/cm², close to the value reported here for the forequarter, 2.59 ± 0.76 \log_{10} UFC/cm². However, these authors reported a population increase after evisceration (3.28 ± 0.58 \log_{10} UFC/cm²) that decreased after washing (2.97 ± 0.36 \log_{10} UFC/cm²), suggesting a contamination source other than the skin and hair during skinning that the washing contributes to decrease. Likewise, in the present study, there was significant decrease in the population number of the hindquarter after washing, but there was no significant difference among processing stages for the forequarter.

Regarding the comparison between the results from hindquarter and forequarter, there was significant difference after skinning and evisceration, showing a higher count from forequarter for both processes. This higher contamination of the forequarter may have occurred for any of the following reasons: at the time of skinning the carcasses remained close to the ground at the slaughterers height, consequently susceptible to the spills from the soil; also during skinning the carcasses were close to each other, which increased the probability of a carcass with skin to touch another one that had already been skinned and the slaughterers proximity to the carcasses.

Table 1 - Means and standard deviation of the mesophiles population in $\log_{10}(\text{UFC}/\text{cm}^2 + 1)$ found on the muscle surface of lamb carcasses according to the region and slaughter stage.

Step	Region (mean \pm SD)	
	Forequarter $\log_{10}(\text{UFC}/\text{cm}^2+1)$	Hindquarter $\log_{10}(\text{UFC}/\text{cm}^2+1)$
After skinning	2.59 \pm 0.76 a	2.02 \pm 0.60 b
After evisceration	2.38 \pm 0.88 a	2.04 \pm 0.73 b
After washing	2.20 \pm 0.50 a	2.00 \pm 0.32 a

^aMeans followed by different letters in the rows differ by Tukey ($P<0.05$).

Table 2 - Means and standard deviation of the psychrotrophs population in \log_{10} (UFC/cm² +1) found on the muscle surface of lamb carcasses according to the region and slaughter stage.

Step	Region (mean \pm SD)	
	Forequarter \log_{10} (UFC/cm ² +1)	Hindquarter \log_{10} (UFC/cm ² +1)
After skinning	2.35 \pm 1.17 a	1.52 \pm 0.98 b
After evisceration	2.17 \pm 1.19 a	1.89 \pm 1.14 a
After washing	2.05 \pm 1.00 a	2.03 \pm 0.99 a

^aMeans followed by different letters in the rows differ by Tukey (P<0.05).

Table 3 - Means and standard deviation of the population of molds and yeasts in \log_{10} (UFC/cm² +1) found on the muscle surface of lamb carcasses according to the region and slaughter stage.

Step	Region (mean \pm SD)	
	Forequarter \log_{10} (UFC/cm ² +1)	Hindquarter \log_{10} (UFC/cm ² +1)
After skinning	1.16 \pm 1.05 a	1.03 \pm 0.88 a
After evisceration	1.23 \pm 0.97 a	0.90 \pm 1.02 a
After washing	0.75 \pm 0.87 a	1.06 \pm 0.91 a

^aMeans followed by different letters in the rows differ by Tukey (P<0.05).

In this study, the highest population mean was 2.59 \pm 0.76 \log_{10} UFC/cm², indicating that the contact between muscular surface and wool was restricted to few areas, since Bell & Hathaway (1996) considered that the contact between these two surfaces is evident when mesophilic populations are above 4.4 \log_{10} UFC/cm².

The results for the psychrotrophic populations are shown in Table 2. Significant statistical difference was observed between the forequarter and hindquarter after skinning, a large population difference can be seen between these two regions. This population count was the highest mean observed for the forequarter. The remaining stages were not significantly different.

The psychrotrophic populations showed no statistically significant difference between slaughtering stages, but the populations of forequarter and hindquarter were significantly different after skinning, and the first was higher compared to the second. The arguments used to explain these differences in the mesophilic populations still hold here. The same pattern was observed after evisceration with no significant difference. A gradual decrease of population counts was observed in the forequarter region, and the lowest value was recorded after the washing. However, population counts increased in the hindquarter after each processing stage.

The lack of specific legislation for this class of microorganisms and the wide variation among results reported in the literature makes the comparison of the percentages of mesophilic and psychrotrophic population found in this study difficult. Therefore, we chose to evaluate the meat quality by comparing the psychrotrophic population means to the deterioration

values determined by Prieto et al. (1991). According to these authors, meat deterioration becomes evident when the psychrotrophic population is between 7 and 8 \log_{10} UFC/cm². In this study, the lowest value found for psychrotrophic population was 1.52 \pm 0.98 \log_{10} UFC/cm² and the highest one was 2.35 \pm 1.17 \log_{10} UFC/cm² for the hindquarter and forequarter, respectively, after skinning. Therefore, the populations found in this study were much lower than the minimum required to cause meat deterioration.

Table 3 presents the results for populations of yeasts and molds. It can be seen that there were no significant differences between the stages. The forequarter displayed the highest value while the hindquarter displayed the lowest mean after evisceration. The forequarter, after washing, displayed the lowest value and standard deviation between the two studied regions.

Dillon & Board (1989) affirm that yeasts account for only 0.5 to 1.3% of the total population of aerobes, and reported yeast values between 0.07 and 3.41 \log_{10} UFC/cm² for a population of mesophiles between 5.23 and 5.36 \log_{10} UFC/cm². In this study, we found molds and yeasts in all carcasses. The lowest and highest values found were \log_{10} 0.75 \pm 0.87 and 1.23 \pm 0.97 UFC/cm² both in the forequarter after washing and evisceration, respectively. These values are lower than those reported by the authors, showing lower carcass contamination in this study. However, this fact does not exclude the need for extra care during the skinning of carcass at slaughter, since not only the slaughterhouses constitute a source of contamination

Table 4 - Means and standard deviation of the population of *Escherichia coli* in \log_{10} (UFC/cm² +1) found on the muscle surface of lamb carcasses according to the region and slaughter stage.

Step	Region (mean \pm SD)	
	Forequarter \log_{10} (UFC/cm ² +1)	Hindquarter \log_{10} (UFC/cm ² +1)
After skinning	0.31 \pm 0.84 a	0.03 \pm 0.16 a
After evisceration	0.08 \pm 0.21 a	0.10 \pm 0.25 a
After washing	0.07 \pm 0.00 a	0.00 \pm 0.00 a

^a Means followed by different letters in the rows differ by Tukey (P<0.05).

Table 5 - Means and standard deviation of the population of *Staphylococcus spp.* in \log_{10} (UFC/cm² +1) found on the muscle surface of lamb carcasses according to the region and slaughter stage.

Carcass region	\log_{10} (UFC/cm ² +1) (mean \pm SD)
Forequarter	1.95 \pm 0.68
Hindquarter	1.75 \pm 0.71

but also the live animal and especially, the contaminants found on the skin, hair or wool (Empey & Scott & Ayres cited by DILLON & BOARD, 1991).

Brazilian rules does not establish a microbiological pattern for this class of microorganisms, therefore, it is always fundamental to adopt techniques which can increasingly contribute to reduce contamination. Although the populations of these microorganisms are not considered of great importance in the deterioration of red meat, the growth of yeasts is favored in meat products that contain preservatives such as sulfite (DILLON & BOARD, 1991). Varnam & Sutherland (1995) attributed to yeast a role on the deterioration of retail meat stored at 0°C.

Counts of *Escherichia coli* are represented in Table 4. The highest value was found on the forequarter and the lowest on the hindquarter after skinning. Although not significantly different, a gradual reduction of these population counts was observed in the forequarter. After skinning, the main contamination of the muscle surface with fecal material can occur due to the rupture of the gastrointestinal tract or prior contact with contaminated surfaces such as equipment, wool or workers' hands (GILL et al. 1995; BELL & HATHAWAY, 1996).

Results for population counts reported in the literature are sometimes similar to this study, but also much higher. While studying the population of enterobacteria on lamb carcass, Sierra et al. (1997) reported results between \log_{10} 0,43 and 2,1 UFC/cm². We did not find even one mean value close to the minimum reported by these authors. A higher population of *E. coli* was observed after skinning on forequarter and there was a reduction during the processing, were the *E. coli* population almost reached a negative value on the hindquarter, in the third stage of the flowchart.

Staphylococcus spp. counts were performed only after washing and the results are shown in Table 5. These counts were only conducted for the last processing stage because of the greater importance of its presence in the final product regarding consumer health, since the genus *Staphylococcus* provides information on the likely presence of *Staphylococcus aureus*.

Despite how important it is to determine the presence of *Staphylococcus* in food, there is no rule established for meat "in natura" in Brazil. Therefore, the values found in this study were evaluated according to the population of *Staphylococcus spp.* found in literature and the presence or absence of *Staphylococcus coagulase positive*.

Widders et al. (1995) found a mean population of approximately 1.7 \log_{10} UFC/cm². Sierra et al. (1995) reported a mean count of 2.19 \log_{10} UFC/cm² of *Staphylococcus coagulase negative*. It can be seen in Table 5 that the mean value found was 1.95 \pm 0.68 \log_{10} UFC/cm² and 1.75 \pm 0.71 \log_{10} UFC/cm² for the forequarter and hindquarter, respectively. Therefore, the values were close to those cited. Still, *Staphylococcus coagulase positive* was detected in only two carcasses (one from the hindquarter and the other from the forequarter) corresponding to 6.67% of total samples. On the other hand, Phillips et al. (2001) found *Staphylococcus coagulase positive* in 24.1% of the lamb carcasses analyzed. The presence of *Staphylococcus coagulase positive* in only one sample can be considered a good result, since Desmarchelier et al. (1999) mentioned that counts of coagulase positive staphylococci in foods is not a specific technique, but proved to be an effective indicator of the contamination degree with potentially pathogenic strains. However, it is important to notice the presence of coagulase-negative *Staphylococcus enterotoxin producers*.

Salmonella sp. and *Listeria* spp. were not found in any of the areas studied. Martineli et al. (2009) while studying lamb carcasses in Brazil, also obtained similar results for the occurrence of these bacteria after washing. According to RDC nº12 (BRASIL, 2001), meat "in natura" must have absence of *Salmonella* sp. in 25 grams.

Regarding the genus *Listeria*, *L. monocytogenes* has been isolated from a variety of meat products including raw and processed meat, fermented sausages and poultry products. The occurrence of this microorganism has shown prevalence from 0.0 to 92% in fresh meat and 3 to 13% in products ready for consumption (AL-SHEDDY et al., 1995). From the data presented and the pathogenicity of this microorganism, it is important to have control over it from the animal production place to the slaughterhouse, regardless of low prevalence in some cases.

CONCLUSIONS

Although the population values found for the categories of the studied microorganisms were lower compared to research work conducted in other countries, their occurrence indicates the importance of improving manufacturing practices, since there is the possibility of microorganism multiplication during storage, contributing to an early deterioration of the product that may also act as a vehicle for the transmission of pathogens. Still, the washing of the carcasses with chlorinated water was not effective to reduce bacterial contamination.

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