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Microbiological counting in lamb carcasses from an abattoir in São Paulo, Brazil

Contagens microbiológicas em carcaças ovinas de um abatedouro de São Paulo, Brasil

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ABSTRACT

The consumption of lamb meat in Brazil has increased in the last years but little information about the microbiological quality of this product is available. To evaluate the hygienic-sanitary conditions of lamb carcasses, the quantification of microorganism populations indicators (mesophiles and psychrotrophs; total and thermotolerant coliforms; *Escherichia coli*; moulds and yeasts) and the pathogenic microorganisms identification (*Salmonella* sp. and *Listeria* spp.) were performed. A total of 60 lamb carcasses were sampled from one abattoir in São Paulo. Swab samples were collected from three points (forequarter, back and hindquarter) on the muscle surface after carcasses final washing. Statistical analysis consisted of descriptive evaluation of the results whose counts were grouped by intervals of microorganism populations. Counts ranged from 1.0×10^1 to 8.0×10^4 colony-forming unit cm^{-2} (CFU cm^{-2}) for mesophiles; 1.0×10^0 to 4.4×10^4 CFU cm^{-2} for psychrotrophs; $< 1.0 \times 10^0$ to 4.4×10^4 CFU cm^{-2} for moulds and yeasts; < 0.3 to > 32.0 most probable number/ cm^2 (MPN cm^{-2}) for total and thermotolerant coliforms and *Escherichia coli*. *Salmonella* sp. and *Listeria* spp. were not found in any of the carcasses. Most carcasses presented low counts for all microorganisms. Overall results may be explained by the small size of the industry where the study was taken. Results suggest that good microbiological quality lamb meat is possible to be obtained, but improvement in hygienic-sanitary conditions is still required.

Key words: carcasses, lamb, indicator and pathogenic microorganisms, hygiene.

RESUMO

O consumo de carne ovina tem aumentado nos últimos anos, no Brasil. Entretanto, pouca informação sobre a qualidade desse produto está disponível. Com o intuito de

avaliar as condições higiênico-sanitárias das carcaças ovinas, foram realizadas a quantificação das populações de microrganismos indicadores (mesófilos e psicrotróficos; coliformes totais e termotolerantes; *Escherichia coli*; bolores e leveduras) e a identificação de microrganismos patogênicos (*Salmonella* sp. and *Listeria* spp.). Um total de 60 carcaças foram amostradas em um frigorífico, em São Paulo, entre fevereiro e dezembro de 2006. Suabes foram coletados em três pontos (dianteiro, lombo e traseiro), na superfície muscular das carcaças, após a lavagem final destas. A análise estatística consistiu na avaliação descritiva dos resultados cujas contagens foram agrupadas em intervalos populacionais. As contagens variaram de $1,0 \times 10^1$ a $8,0 \times 10^4$ UFC cm^{-2} para mesófilos; de $1,0 \times 10^0$ a $4,4 \times 10^4$ UFC cm^{-2} para psicrotróficos; de $< 1,0 \times 10^0$ a $4,4 \times 10^4$ UFC cm^{-2} para bolores e leveduras; de $< 0,3$ a $> 32,0$ NMP cm^{-2} para coliformes totais e termotolerantes; e *Escherichia coli*. *Salmonella* sp. e *Listeria* spp. não foram detectadas em nenhuma das amostras coletadas nas carcaças. A maioria das carcaças apresentou baixas contagens para todas as categorias de microrganismos. Os resultados encontrados podem ser explicados pelo pequeno tamanho da indústria onde o trabalho foi realizado e sugerem que carne ovina de boa qualidade microbiológica pode ser obtida. No entanto, melhorias nas condições higiênico-sanitárias ainda são necessárias.

Palavras-chave: carcaças, ovino, microrganismos indicadores e patogênicos, higiene.

INTRODUCTION

Ovine meat is consumed and commercialized in almost all countries around the world, even though the demand for lamb meat is strongly influenced by

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cultural habits. Since the importation of these animals increased at about seven times from 1992 to 2000 in Brazil (SIMPLÍCIO & SIMPLÍCIO, 2006), rigorous hygienic-sanitary conditions during the whole process of slaughter, handling and storage are required.

Microbiological quality of fresh meat has been receiving attention all over the world due to the occurrence of significant outbreaks of foodborne illness affecting consumers. Quality maintenance is important not only for consumer health protection but also to assure uniformity in fresh meat shelf-life. Premature deterioration is a hindrance to beef and lamb marketing due to color, flavour, texture and aspect changes (WIDDERS et al., 1995).

Meat and its products are highly perishable and therefore adequate handling must be adopted during processing. Deterioration begins just after slaughtering as a result of microbiological, physical and chemical processes (HEDRICK et al., 1994).

As soon as muscle tissue is exposed it may be contaminated by pathogens and deteriorative bacteria from the hide's normal microbiota (staphylococci, micrococci, pseudomonads, molds and yeasts) as well as from telluric and faecal microorganisms. Hooves, hide, hair, fleece and guts are considered the most important sources of microbial contamination on flayed carcasses (NEWTON et al., 1978; KELLY et al., 1982; BELL & HATHWAY, 1996). Other sources of potential contamination in abattoirs include equipments, operatives' clothing and hands, air, water, walls and doors (HEDRICK et al., 1994; SIERRA et al., 1995a).

Although data about cases of foodborne illness in Brazil are scarce, the incidence of foodborne disease may be underestimated. According to TAUXE (2002) even in developed countries such as the USA, the occurrence of such diseases is significant, despite the technological advances in food production and control.

The consumption of lamb meat in Brazil has been increasing in the last years, but little information about the microbiological quality of this product is available. Herein it was aimed to evaluate the hygienic-sanitary conditions of freshly dressed lamb carcasses by quantifying indicator microorganisms (mesophiles and psychrotrophs; total and thermotolerant coliforms; *Escherichia coli* and moulds and yeasts) and also verifying the presence of *Salmonella* sp. and *Listeria* spp. Samples were swabbed according to APHA (2001) after carcasses final washing.

MATERIALS AND METHODS

Sampling procedures

This study was done within a six month period in one abattoir in São Paulo, Brazil. The abattoir had distinct areas for slaughtering, handling and cold storage of the meat. Six visits were made to the abattoir and ten carcasses per visit were analyzed, given a total of 60 carcasses.

After bleeding, skinning and eviscerating processes, the carcasses were washed with pressurized chlorinated water, and then analyzed after final washing. Surface areas, 20cm² on the forequarter (fore arm), back (loin) and hindquarter (rump), were sampled by swabbing technique (APHA, 2001). Swabs from the three points of each carcass were dipped into the same flask with 60 ml of 0.1% peptone water. Samples were transported in refrigerated containers (at 4°C) to the laboratory within 2h after collection. Samples were cultured on the same day.

Microbiological analysis

Swabs were homogenized for 2min (Colworth 400 Stomach) in swab transport solution (0.1% peptone water) and diluted to obtain 10⁻¹ and 10⁻² solutions. This procedure was done for counting the following microorganisms: mesophiles, psychrotrophs, moulds and yeasts, total coliforms, thermotolerant coliforms and *Escherichia coli* counts.

These dilutions were not applied for the isolation of *Salmonella* sp. and *Listeria* spp.

Mesophiles, psychrotrophs, moulds 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, Oxford) and incubated at 35°C for 48h and at 7°C for 10 days, respectively. Moulds and yeasts were determined on malt extract agar (MEA, Oxoid) which pH was adjusted to 3.5 by adding a sterile 10% tartaric acid solution and incubated at 22-25°C for 5 days according to APHA (2001).

Total Coliforms (TC), Thermotolerant Coliforms (TTC) and *Escherichia coli*

Determination of the most probable number (MPN) of coliforms was done according to the APHA (2001) method in a three-tube series from each dilution. The MPN of TC was calculated by using lauryl sulphate tryptose broth at 35°C for 24-48h for the presumptive test and brilliant green bile broth 2% at 35°C for 24-48h for the confirmative test. The MPN of TTC was calculated by using EC broth at 45°C for 24h. For *E.*

coli confirmation, gas positive cultures from EC broth were plated onto eosin methylene blue agar (EMB) agar (Oxoid) at 35°C for 24h. Characteristic colonies with a metallic sheen were transferred to nutrient agar tubes, Gram-stained and subjected to IMViC (Indole, Methyl red test, Voges-Proskauer test, and citrate production) biochemical tests.

Salmonella

Salmonella isolation was done according to APHA (2001). Pre-enrichment step was performed by incubating the swabs in transport solution at 35-37°C for 24h. After that, selective enrichment step was performed with selenite-cystine broth (Oxoid) and Rappaport-Vassiliadis broth (Oxoid) and incubated at 35-37°C for 2h. The cultures were streaked onto brilliant green agar (Merieux) and McConkey (Oxoid) agar followed by incubation at 37°C for 24h. Suspected colonies were subjected to biochemical tests.

Listeria

The method recommended by Health Protection Branch of Canada and cited in SILVA et al. (1998) was done for the detection of the genus *Listeria*. Primary enrichment consisted to adding 10mL of swab transport solution into 90mL of *Listeria* enrichment broth (LEB, Difco) and incubation at 30°C for 48h. Secondary enrichment was done by using Fraser broth and, simultaneously, streaking onto plates of modified Oxford agar (OXA, Difco) and lithium chloride-phenylethanol-moxalactam agar (LPM, Difco) following incubation at 35°C for 48h. Suspected colonies were subjected to biochemical tests.

Statistical analysis

Statistical analysis consisted of descriptive evaluation of the results that were grouped by intervals of microorganism populations.

RESULTS AND DISCUSSION

In this study, the counts of mesophiles were distributed in five different population intervals; table 1 shows the mesophiles counts in CFU cm⁻² that were obtained for each interval.

In the literature, reported results from aerobic plate counts (APC) for sheep carcasses after non-destructive sampling are between 1.2 x 10² and 1.0 x 10⁵ CFU cm⁻². However, the comparison of results from different studies is difficult because of factors such as different sampling sites, treatments adopted in the carcasses, evaluation methods (ZWEIFEL & STEPHAN, 2003) and different techniques used for

Table 1 - Mesophiles counts in CFU cm⁻² for five population intervals.

Population interval (CFU/cm ²)	-----Samples-----	
	Number	%
1.0 x 10 ¹ 1.0 x 10 ²	8	13.3
1.0 x 10 ² 1.0 x 10 ³	26	43.3
1.0 x 10 ³ 3.2 x 10 ³	15	25.0
3.2 x 10 ³ 1.0 x 10 ⁴	8	13.3
1.0 x 10 ⁴ 8.0 x 10 ⁴	3	5.0

collecting samples (PEARCE & BOLTON, 2005). According to BELL & HATHAWAY (1996), mesophiles population higher than 2.5 x 10⁴ CFU cm⁻² can be considered as an indicative of direct contact between fleece and carcass. In our study, most of sampled carcasses (49/81.7%) showed counts below 3.2 x 10³ CFU cm⁻².

Some scientific studies presented mesophiles counts similar to the present work. In Ireland, the lowest counts were between 1.9 x 10² and 7.9 x 10² CFU cm⁻² for the thorax, shoulder/neck, breast/brisket and flank areas (PEARCE & BOLTON, 2005). Also in Ireland, KELLY et al. (1980) found values between 6.6 x 10³ and 1.6 x 10⁴ CFU cm⁻² on the crutch/hind leg while sites with the lowest bacterial number ranged from 1.3 x 10³ to 2.6 x 10³ CFU cm⁻² on the carcass dorsal region. In Ireland SIERRA et al (1997) found counts similar but also higher to the present work ranging from 1.2 x 10³ to 2.5 x 10⁵ CFU cm⁻² on the neck, leg and flank.

There is a consensus among researchers that aerobic spoilage of raw meat becomes evident when the level of bacteria reaches 1.0 x 10⁷ (off-odour) and 1.0 x 10⁸ (slime) cells cm⁻² (PRIETO et al., 1991). Although 49 out of 60 of the carcasses (81.7%) have presented a population much lower than that, an improvement of the slaughter hygiene techniques is still required such as adequate sterilization of knives and greater care during pelt removal in order to avoid contact between fleece and muscle surface.

Table 2 shows the most probable number of total coliforms (TC), thermotolerant coliforms (TTC) and *Escherichia coli* counts per cm² where the counts were divided in four population intervals and in the lowest and highest counts. The maximum count was > 3.2 x 10¹ MPN cm⁻² which was found in two out of 30 carcasses for the three categories of microorganisms. Faecal contamination of dressed carcasses may occur as a consequence of direct contact with either faecal material or surfaces that had previous contact with faeces, e. g. fleece or operative hands (BELL & HATHAWAY, 1996).

Table 2 - Total coliforms (TC), thermotolerant coliforms (TTC) and *Escherichia coli* counts in MPN cm⁻² in four population intervals and in the lowest and highest counts.

Population interval (MPN cm ⁻²)	Samples					
	TC		TTC		<i>E. coli</i>	
	Number	%	Number	%	Number	%
< 0.3	22	36.7	23	38.4	25	41.7
0.3 x 10 ⁰ + 5.0 x 10 ⁰	26	43.4	28	46.7	26	43.3
5.0 x 10 ¹ + 1.0 x 10 ¹	6	10.0	3	5.0	4	6.7
1.0 x 10 ¹ + 2.0 x 10 ¹	2	3.3	2	3.3	1	1.7
2.0 x 10 ¹ + 3.2 x 10 ¹	2	3.3	2	3.3	2	3.3
> 3.2 x 10 ¹	2	3.3	2	3.3	2	3.3

In a study with 470 lamb carcasses in Australia, VANDERLINDE et al. (1999) found averages of 2.3×10^1 MPN cm⁻² for *E. coli* and 3.8×10^1 MPN cm⁻² for total coliforms from samples collected on the loin, flank and brisket regions. PEARCE & BOLTON (2005) found counts ranging from 9.8×10^1 and 1.0×10^2 CFU cm⁻² for enterobacteria in samples collected from thorax, shoulder/neck, breast/brisket and flank. Nevertheless, in the present study, as observed in table 2, most samples (56 carcasses, 93.4%) presented a population lower than 2.0×10^1 MPN cm⁻² for the three enterobacteria.

BISS & HATHWAY (1996) compared *E. coli* population in sites cleaned after pelt removal with sites visually contaminated with faecal material. In the first, counts were between 9.1×10^0 and 3.2×10^1 CFU cm⁻² while the latter presented mean count 1.0×10^3 CFU cm⁻². Table 2 shows that none of the samples presented a population similar to that found in visibly faeces-contaminated areas, which indicates that appropriated measures to avoid visceral rupture or knives contact and soiled fleece with muscular surface was taken.

Results referring to psychrotrophs, and moulds and yeasts, presented in table 3, were divided in four population intervals and lowest counts. It is observed that for both categories the major amount of

carcass presented counts between 1.0×10^2 and 1.0×10^3 CFU cm⁻². Only a minority of samples had counts higher than 1.0×10^4 CFU cm⁻².

Determination of the psychrotrophs population on meat surface is used to verify the quality of cold storage because of their capacity to multiply under refrigeration temperatures (INGRAM & ROBERTS, 1976; SIERRA et al., 1995a; ZWEIFEL & STEPHAN, 2003).

From the 60 carcasses sampled, 44 (73.3%) presented population counts inferior than 1.0×10^3 CFU cm⁻². The remaining 16 carcasses (26.7%) presented counts above 1.0×10^3 CFU cm⁻² (Table 3). PRIETO et al. (1991) studied the multiplication of psychrotrophs on lamb carcasses during their shelf life and found a mean population of 8.9×10^7 CFU cm⁻² related to meat deterioration. The current study found a maximum population value of 4.4×10^4 CFU cm⁻², which is much lower than that found by PRIETO et al (1991). Based on this, it can be suggested that the storage of this products is safe for longer period.

In this study, moulds and yeasts were found in all carcasses. Most samples (52/86.7%) presented population up to 1.0×10^3 CFU cm⁻² while a few (8/13.3%) were above that (Table 3). These results show similarity with the populations found by DILLON & BOARD

Table 3 - Psychrotrophs and moulds and yeasts counts in CFU cm⁻² for four population intervals and lowest counts.

Population interval (CFU cm ⁻²)	Samples			
	Psychrotrophs		Moulds and Yeasts	
	Number	%	Number	%
< 1.0×10^1	-	-	3	5.0
1.0×10^1 + 1.0×10^2	5	8.3	21	35.0
1.0×10^2 + 1.0×10^3	39	65.0	28	46.7
1.0×10^3 + 1.0×10^4	13	21.7	6	10.0
1.0×10^4 + 4.4×10^4	3	5.0	2	3.3

(1989) in the United Kingdom which were between 8.5×10^{-1} and 2.3×10^3 CFU cm^{-2} on the carcass back region. The verified results do not eliminate the necessity of greater care during pelt removal process because the live animal and particularly its hide, hair or fleece represents the main source of introducing microbial contaminants into the abattoir (DILLON & BOARD, 1991).

Salmonella was not found in the sampled carcasses. In Spain SIERRA et al. (1995b) isolated this bacterium in 10% of freshly dressed lamb carcasses studied in samples collected from the neck, leg and flank regions. These authors suggested that the structure of the abattoirs and slaughtering practices may influence contamination with certain *Enterobacteriaceae*.

An official resolution, RDC 12 (BRASIL, 2001), established by the National Sanitary Vigilance Agency determines that *Salmonella* sp. must be absent in meat *in natura*. The fact that salmonella was not isolated in this study suggests that the contact of fleece and gastrointestinal contents with muscle was controlled or reduced and cross contamination was probably avoided. In addition, it must be considered that tested animals could not present salmonella in their faeces.

As *Salmonella*, the genus *Listeria* was not isolated in this study. This finding is similar to that found by SIERRA et al. (1997) who did not isolate them from samples collected from neck, leg and flank regions on lamb carcasses. Lowry and Tiong, cited by SIERRA et al. (1997), detected the presence of this microorganism in 30% of the examined lamb carcasses. Except for *Salmonella* sp., the Brazilian legislation does not establish other microorganism classes to evaluate the hygienic-sanitary conditions of meat *in natura*.

Differently from our results, ANTONIOLLO et al. (2003) in Brazil isolated *Listeria* spp. from 69 carcass surface samples (neck, shoulder blade, flank, and posterior rump) both after washing as well as after 24h of refrigerated storage. According to these authors, the absence of some *Listeria* species in lamb carcasses may be related to the feeding from natural pastures instead of silage. In the current study it was not possible to establish a connection between the silage consumption and *Listeria* presence due to the lack of that information. The absence of this microorganism can also be related to the fact that not all animals carry microorganisms of this genus in their faeces. One of the possible contributing factors for low populations of the analyzed microorganisms found in this study may be the small size of the industry where it was performed, which has a reduced daily number of slaughtering and improved hygiene conditions.

CONCLUSIONS

Results indicate that it is possible to obtain lamb carcasses of good microbiological quality if adequate good manufacturing practices are performed. However, a regime of permanent improvement in hygienic-sanitary conditions must be adopted by the abattoir in order to maximize the quality of meat, despite of not finding pathogenic microorganisms on the evaluated carcasses.

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