

Presence of *Listeria monocytogenes* and *Salmonella* spp. in lamb meat commercialized in Uruguaiana, Rio Grande do Sul, Brazil

Presença de *Listeria monocytogenes* e *Salmonella* spp. em carne de cordeiro comercializada em Uruguaiana, Rio Grande do Sul, Brasil

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Abstract

The aim of this study was to evaluate the hygienic-sanitary quality of lamb meat sold in the city of Uruguaiana, Rio Grande do Sul (RS) by counting the indicator microorganisms and detecting pathogens such as *Salmonella* spp. and *Listeria monocytogenes*. Thirty-nine lamb meat samples were collected from 10 commercial establishments in Uruguaiana. The samples were subjected to counts of aerobic mesophilic microorganisms and enterobacteria, and to the detection of *Salmonella* spp. and *L. monocytogenes*, all following standard methods. The average counts of mesophilic microorganisms and enterobacteria were 6.08 log CFU/g (minimum 4.07 and max 6.87) and 4.73 log CFU/g (minimum 0 and max 5.88), respectively. For pathogens, *L. monocytogenes* was isolated from five samples (12.82%), with three samples in the same location. Only two samples (5.13%) were positive for *Salmonella* spp. The results demonstrated unsatisfactory hygienic-sanitary conditions because high counts of pathogens such as *Salmonella* spp. and *L. monocytogenes*. The counts of enterobacteria showed poor hygiene conditions during the various stages of production. The results also indicated fecal contamination, as *Salmonella* spp. and *L. monocytogenes* are present in the intestinal tract of both humans and animals. The high count of mesophilic microorganisms obtained could be owing to contaminated raw material or unsatisfactory processing, including unsanitary conditions and the inappropriate use of binomial time/temperature during storage.

Keywords: contamination, Lamb meat, *Listeria monocytogenes*, *Salmonella* spp.

Resumo

O objetivo foi avaliar a qualidade higiênico-sanitária da carne de cordeiro comercializada na cidade de Uruguaiana, Rio Grande do Sul (RS) através da contagem dos microrganismos indicadores e detecção de patógenos como *Salmonella* spp. e *Listeria monocytogenes*. Trinta e nove amostras de carne de cordeiro foram coletadas em 10 estabelecimentos comerciais em Uruguaiana. As amostras foram submetidas a contagens de microrganismos aeróbios mesófilos e enterobactérias e à detecção de *Salmonella* spp. e *L. monocytogenes*, todas seguindo metodologias padrão. As contagens médias de microrganismos mesófilos e enterobactérias foram 6,08 log UFC/g (mínimo 4,07 e máximo 6,87) e 4,73 log UFC/g (mínimo 0 e máximo 5,88), respectivamente. Para os patógenos, *L. monocytogenes* foi isolado de cinco amostras (12,82%), com três amostras no mesmo local. Apenas duas (5,13%) foram positivas para *Salmonella* spp. Os resultados demonstraram condições higiênico-sanitárias insatisfatórias, baseado nas altas contagens de patógenos como *Salmonella* spp. e *L. monocytogenes*. As contagens de enterobactérias indicaram más condições de higiene durante as várias etapas de produção. Os resultados também indicaram contaminação fecal, uma vez que *Salmonella* spp. e *L. monocytogenes* estão presentes no trato intestinal de humanos e animais. A alta contagem de microrganismos mesófilos obtidos pode ser devida a matéria-prima contaminada ou processamento insatisfatório, incluindo condições insalubres e uso inadequado do binômio tempo/temperatura durante o armazenamento.

Palavras-chave: contaminação, carne de cordeiro, *Listeria monocytogenes*, *Salmonella* spp.

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Introduction

Although lamb meat represents only a small portion of meat production in the world, sheep production has economic and social relevance in the countries that produce it (Ponnampalam et al., 2016). The state of Rio Grande do Sul (RS) is the second largest lamb meat producer in the country with 3.2 million heads. The municipality of Uruguaiana is localized in the western frontier Region, occupies a prominent position in livestock farming with a herd of 164,723 animals (Instituto Brasileiro de Geografia e Estatística, 2018).

Despite the importance of lamb meat production in RS, the consumption of this meat is lower compared to other types. It is estimated that the Brazilian *per capita* consumption of lamb is 0.62 kg per year, compared to 44 kg of poultry, 35 kg of beef, and 15 kg of pork. The lower consumption of lamb is due to its lesser availability and the lack of appropriate cuts for daily preparation, as compared to other kinds of meat (Andrade et al., 2016; Brasil, 2018a; Sorio & Rasi, 2010).

Low-quality lamb products are the result of inadequate planning, high production costs, poor sanitary inspections of lamb, butcher shops, and supermarkets, and variations in pricing, slaughtering, and obtaining lamb carcasses, all of which expose consumers to hazards (Sorio & Rasi, 2010). In the Brazil, the informal lamb slaughter market may exceed 90%. In Rio Grande do Sul, the informality rate is estimated at 85% (Brasil, 2018a).

Obtaining and selling lamb meat in unsanitary conditions results in contamination by pathogenic microorganisms such as *Salmonella* spp. and *Listeria monocytogenes*; these may either be present in feces and wool or may contaminate the meat during obtaining, storing, and processing. *Salmonella* spp. is the primary cause of foodborne infections in Brazil as well as the world. *Listeria monocytogenes* is closely related to industrial environments, especially those in which the cleaning of surfaces and utensils is deficient (Brasil, 2018b; González et al., 2019; Melero et al., 2019; Viana et al., 2020).

The ability to form biofilm by foodborne pathogens is a major concern for the food industry since biofilms protect the bacteria from sanitizers and others environmental factors. *Listeria monocytogenes* and *Salmonella* spp. are able to form biofilms and remain for a long time favoring food contamination (Shi & Zhu, 2009; Steenackers et al., 2012; Villa-Rojas et al., 2017).

Adults with listeriosis can develop meningoencephalitis and children with severe septicemia. Pregnant women, although more susceptible to listeriosis, present a condition like a cold, but fetuses generally do not survive by having an abortion or death before birth and babies who are born alive can develop bacteremia or meningitis (Franco & Landgraf, 2008; Silva et al., 2017).

In humans, the symptoms associated with salmonellosis vary from according to the strain involved and the resistance of the host. Generally, clinical symptoms are characteristic of a febrile gastroenteritis that is defined diarrhea, stomach pain, fever (above 40°C), headache, vomiting, nausea and malaise (Pereira et al., 2010).

Despite the consumption of lamb meat by the population of Rio Grande do Sul, there is limited data in the national literature that evaluate the hygienic-sanitary quality of lamb meat, especially in the western frontier region of Rio Grande do Sul. Therefore, the present study aimed to evaluate the hygienic-sanitary quality of lamb meat sold in the city of Uruguaiana by counts of indicator microorganisms and the detection of pathogens such as *Salmonella* spp. and *L. monocytogenes*.

Materials and methods

Ethical aspects

This study was exempted from approval by a research ethics committee because it is a study involving food sold in retail establishments.

Sample collection

Thirty-nine samples of chilled lamb meat were collected from 10 commercial establishments, including butcher shops and supermarkets, in Uruguaiana, RS, Brazil. During the collection of samples, it was not possible to determine whether the cuts came from carcasses certified by sanitary inspection (municipal, state, or federal inspection service). The samples were stored in an isothermal box containing recyclable ice, sent to the laboratory for analysis, and kept under

refrigeration (4.0 °C) until the moment of microbiological analysis. The samples were examined to count indicator microorganisms (mesophiles and enterobacteria) and detect *Salmonella* spp. and *L. monocytogenes* (Figure 1).

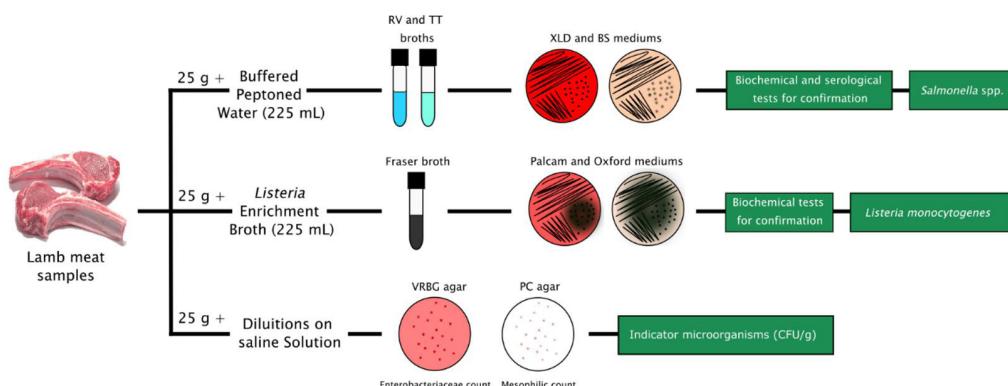


Figure 1. Scheme of microbiological analyzes performed on lamb meat samples.

Counting of indicator microorganisms

The preparation, weighing, and dilution of the samples was done based on the recommendations provided by the BAM-USDA (U.S. Food and Drug Administration, 2003). The indicator counting methodology was established by the Ministry of Agriculture, Livestock, and Food Supply (Brasil, 2003). After weighing approximately 25.0 g of each sample in sterile plastic bags, 225.0 mL of 0.9% saline solution 0.9% (SS) was added, and serial dilutions were performed in tubes containing 9.0 mL saline solution.

For the mesophilic count, 1.0 mL of the dilutions were added to Petri dishes with 15.0 mL of plate count agar, which was incubated at 36 ± 1 °C for 48 h after homogenization and solidification of the culture medium. After incubation, the plates were counted, and the results were expressed in log CFU/g.

For the Enterobacteriaceae count, 1.0 mL of the dilutions were pipetted into sterile Petri dishes, 15.0 mL of violet red bile glucose agar was added, and a new addition of 10.0 mL of agar was performed (overlay) after homogenization and solidification. The plates were incubated at 36 ± 1 °C for 24 h. After incubation, the plates were counted, and the results were expressed in log CFU/g.

Detection of *Listeria monocytogenes*

Listeria monocytogenes was detected as described by Pagotto et al. (2001). Twenty-five grams of lamb meat was weighed in sterile plastic bags, and 225.0 mL of *Listeria*-selective enrichment broth (LEB) was added. After homogenization, the mixture was incubated at 30 ± 1 °C for 48 h. After this period, 0.1 mL of LEB was transferred to a tube containing 10.0 mL of Fraser broth, which was incubated at 35 ± 1 °C for 48 h. Then, aliquots of Fraser broth were cultivated on the surface of Palcam and Oxford agar plates, and both were incubated at 35 ± 1 °C for 24-48 h. Characteristic colonies were cultivated on plates containing soybean trypticase agar with 0.6% yeast extract and incubated at 30 ± 1 °C for 24-48 h to verify the purity. Purified isolates were subjected to tests of catalase production, fermentation of carbohydrates (xylose, rhamnose, and mannitol), production of β-hemolysis, and motility. The results were expressed in the presence or absence of *L. monocytogenes* in 25.0 g of the sample.

Detection of *Salmonella* spp.

Salmonella spp. detection methodology followed the recommendation of the BAM-USDA (U.S. Food and Drug Administration, 2014) with some modifications. After weighing 25.0 g of lamb meat in sterile bags, 225.0 mL of buffered peptone water (BPW) was added. Next, the samples were incubated at 35 ± 1 °C for 24 h after homogenization. From the incubated buffered peptone

water, 0.1 mL and 1.0 mL were transferred to tubes containing 10.0 mL of Rappaport-Vassiliadis broth (RV) and 10.0 mL of tetrathionate broth (TT), respectively. The RV and TT were incubated for 24 h at 42 ± 1 °C, and aliquots were cultivated on plates with bismuth sulfite agar and xylose lysine deoxycholate agar and incubated at 35 ± 1 °C for 24 h. From the plates with typical colonies, biochemical (lysine iron agar, triple sugar iron agar, and IMViC test) and serological (polyvalent anti-*Salmonella* spp. serum) tests were performed to confirm the presence or absence of *Salmonella* spp. in 25.0 g of sample.

Results

Among the 39 lamb meat samples, the average count for Enterobacteriaceae was $4.73 \log \text{CFU/g}$ (minimum 0.0 and maximum $5.88 \log \text{CFU/g}$). The average mesophilic microorganisms count was $6.08 \log \text{CFU/g}$ (minimum 4.07 and maximum $6.87 \log \text{CFU/g}$). Of the analyzed samples, *L. monocytogenes* was isolated from 5 (12.82%). *Listeria monocytogenes*-positive samples were collected from three different commercial establishments, with three of the samples from the same place. *Salmonella* spp. were isolated from 2 (5.13%) of the 39 samples (Figure 2).

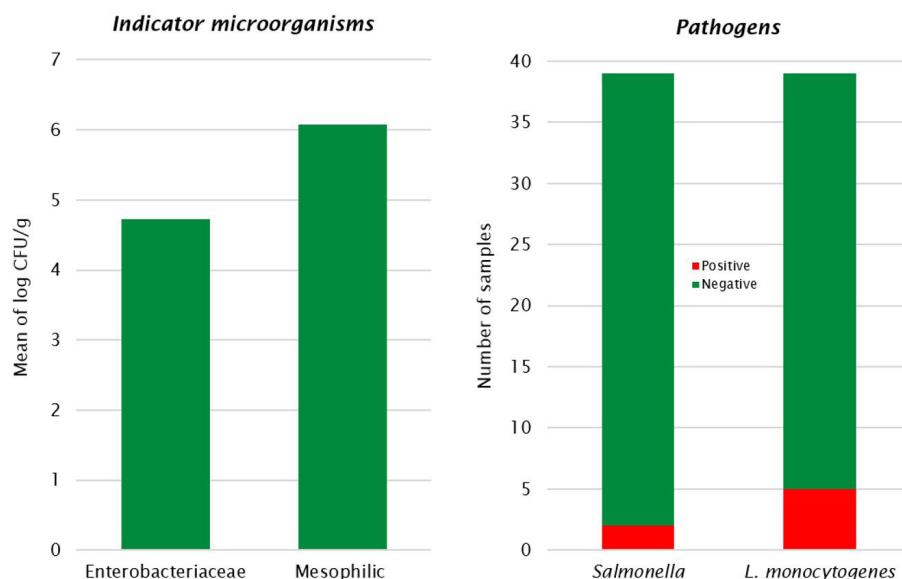


Figure 2. Results of counts of indicators microorganisms and detection of pathogens.

Discussion

Although there are no specific parameters in the legislation for Enterobacteriaceae, the high counts obtained are noteworthy and denote poor hygiene conditions during the various stages of obtaining, handling, and storing lamb carcasses. After the slaughter of the animals, several operations are performed until finally obtaining the carcasses, cuts, and byproducts, such as skinning, eviscerating, processing, packaging, and storing, among others. These many processing phases favor the development of a highly variable microbiota when due care is not taken concerning hygiene (Matos et al., 2013).

According to Normative Instruction 60 of the National Health Surveillance Agency of the Brazilian Ministry of Health (ANVISA), the microbiological limit for aerobic mesophilic microorganisms is $5 \log \text{CFU/g}$ (Brasil, 2019). The high count of mesophilic microorganisms obtained in samples averaged $6.08 \log \text{CFU/g}$ and could be caused by the use of contaminated raw material or unsanitary processing or by the inappropriate use of the time/temperature binomial during storage (Franco & Landgraf 2008). The presence of enterobacteria indicates mainly fecal contamination because these microorganisms are present in the intestinal tracts of humans and animals.

Contamination can occur in handling while obtaining the carcass or in commercial establishments as well as failures in slaughter, mainly in the evisceration stage, with rupture of intestinal loops and leakage of the gastrointestinal contents (Diyantoro & Wardhana, 2019; Røssvoll et al., 2018).

The fact that there is no documentation that the cuts came from carcasses certified by a veterinary sanitary inspection service can be a determining factor for the results obtained. In addition, the lack of sanitary inspection during slaughter can facilitate the transmission of pathogenic microorganisms, exposing the population that will consume this food to hazards. Foodborne disease outbreaks involving lamb meat have been reported in several countries such as Australia, the United States, Wales, and England (Centers for Disease Control and Prevention, 2020; Food Standards Agency, 2018; Greig et al., 2001).

The presence of *L. monocytogenes* in the slaughterhouses and meat processing environments is widely cited in the literature (Autio et al., 2000; D'Ostuni et al., 2016; Kalchayanand et al., 2007); however, there are few reports related to the occurrence of this pathogen in the lamb production chain (Antoniollo et al., 2003; Fernandes et al. 2009). In Iran, Zarei et al. (2013) detected *L. monocytogenes* in 4.3% of the lamb meat samples analyzed, which was lower than what was found in this study. Similarly, Antoniollo et al. (2003) found the pathogen in 4.3% of samples from carcasses slaughtered in Pelotas, Brazil. Martineli et al. (2009) and Sierra et al. (1997) did not detect *L. monocytogenes* in their studies in São Paulo, Brazil, and Dublin, Ireland, respectively.

Listeria monocytogenes is a pathogen, with a wide distribution, that can form biofilms, making its presence frequent and permanent, regardless of the point of the production chain (industry or commerce), especially when the conditions of hygiene and sanitization of equipment and utensils are precarious. This is more worrying with regard to butcher shops and supermarkets as the rotation of inspection is not possible here, unlike the industry, and this causes hygiene practices to be overlooked (Colagiorgi et al., 2017; Franco & Landgraf, 2008).

In the Brazil, Barros et al. (2004) verified the presence of *L. monocytogenes* in meat grinders, knives, and facilities, demonstrating that failures in the hygiene process are the leading causes of its persistence in the environment. Another point that must be considered is the psychrotrophic characteristic of *L. monocytogenes* (Franco & Landgraf 2008), especially that it can survive for long periods at low temperatures, such as cutting rooms and cold rooms. These factors explain the frequency of isolation found in the present study. In addition, issues related to the lack of hygiene in obtaining and especially in the processing and storage of meat in butcher shops led to the isolation of *L. monocytogenes* in three samples from the same establishment.

According to ANVISA (Brasil, 2019), *Salmonella* spp. must be absent in 25.0 g of food; however, during the study, there was the presence of the pathogen. Superior results were found by Fernandes et al. (2009), who found *Salmonella* spp. in 30.8% samples from public markets in Recife, Pernambuco, Brazil. Yang et al. (2010) isolated *Salmonella* spp. in 20% of the samples of lamb meat sold in Shaanxi, China. In the New Zealand, Wong et al. (2007) demonstrated a lower frequency (1%). In the USA, a large study involving the Department of Agriculture and some Universities analyzed 2,548 skin samples and lamb carcasses before and after evisceration in large slaughterhouses. The prevalence of *Salmonella* spp. was 14.4% for skin samples, 4.3% for carcasses before evisceration, and 1.8% after evisceration (Kalchayanand et al., 2007).

These frequencies of *Salmonella* spp. are the result of poor sanitary conditions, mainly during obtaining and cutting the carcass. Dainty & Mackey (1992) reported that contamination during lamb slaughter could come from several sources, such as the animal's skin, feces, intestinal contents, and the hands of employees. These risks increase proportionally as technological slaughter operations are neglected. Specifically, during lamb slaughter, wool has a fundamental role in the contamination of the carcass (Humphrey, 2000). This was demonstrated in a study by Edrington et al. (2009), who analyzed samples of wool and feces from lambs under confinement and found that 50% of wool was contaminated with *Salmonella* spp.

Conclusions

The lamb meat sold in the city of Uruguaiana, RS showed unsatisfactory hygienic-sanitary quality owing to the counts of indicator microorganisms and the presence of *L. monocytogenes* and *Salmonella* spp. The results obtained reinforce the need for more efficient monitoring through sanitary inspection, both in slaughter and in trade, to prevent cases of foodborne diseases in consumers. In addition, this study may contribute with scientific data on the safety of lamb meat marketed in Rio Grande do Sul, especially in the region studied, since few data in the literature report the presence of pathogens in this type of food in the region.

Ethics statement

This study was exempted from approval by a research ethics committee because it is a study involving food sold in retail establishments.

Financial support

None.

Conflict of interests

No conflict of interests declared concerning the publication of this article.

Authors' contributions

VMS, EARS, LET and JGP - Development of methodology and writing the initial draft. ANCES and JGP - Preparation, writing the final draft, review and editing the manuscript.

Availability of complementary results

None

The study was carried out at Food Inspection Lab - Lab.IPOA, Faculdade de Medicina Veterinária, Universidade Federal do Pampa - UNIPAMPA, Uruguaiana, RS, Brasil.

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