PREVALENCE OF Listeria monocytogenes IN FRESH CHILLED BEEF PRODUCED IN THE STATE OF MATO GROSSO, BRAZIL

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RESUMO – Listeria monocytogenes é o agente causador da listeriose, enfermidade de origem alimentar que pode levar à bacteremia e meningite. O Brasil é o maior exportador de carne bovina, com fornecimento para mais de 100 países e Mato Grosso o maior produtor de carne do país. Nesse contexto, objetivou-se estimar a prevalência de L. monocytogenes em carne bovina in natura resfriada produzida no estado de Mato Grosso, Brasil. A amostragem foi calculada e um total de 50 amostras de carne bovina produzida por 13 diferentes matadouros frigoríficos foram submetidas à análise microbiológica, a análise qualitativa imunoenzimática automatizada e a PCR em tempo real (q-PCR). A prevalência de L. monocytogenes em carne bovina in natura resfriada produzida em Mato Grosso é de 14% e matadouros frigoríficos habilitados à exportação também possuem a presença do patógeno podendo gerar riscos à saúde pública, sofrerem futuras restrições no comércio internacional e prejuízos econômicos.

ABSTRACT – Listeria monocytogenes is the causative agent of listeriosis, a foodborne illness that can lead to bacteremia and meningitis. Brazil is the largest beef exporter, supplying over 100 countries, and Mato Grosso is the largest producer in the country. In this context, the aim of the present study was to estimate the prevalence of L. monocytogenes in fresh chilled beef produced in the state of Mato Grosso, Brazil. The sample number was calculated and a total of 50 beef samples produced by 13 different slaughterhouses were subjected to microbiological analyses, qualitative analyses automated enzymatic immunoassay and real-time PCR (q-PCR). The prevalence of L. monocytogenes in beef produced in Mato Grosso is of 14% and slaughterhouses authorized to export this product also show the presence of this pathogen, which may pose risks to public health, suffering future restrictions on international trade and economic losses.

PALAVRAS-CHAVE: Listeria spp; carne vermelha; microbiologia, mini-VIDAS®, q-PCR.

KEYWORDS: Listeria spp; red meat; microbiology; mini-VIDAS®, q-PCR.
1. INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis, a foodborne illness that causes listeriosis, which may lead to bacteremia and meningitis, affecting, mainly, the elderly, newborns, pregnant women and immunocompromised individuals (Gianfranceschi et al., 2014). Contaminated foods are the most common sources of this microorganism, whose presence has been reported in many different foods, such as crude and/or pasteurized milk, cheese, meat, fish and meat products (SALUDES et al., 2015). Human listeriosis is a high mortality disease (20-30%), both undiagnosed and subnotified in Brazil (Silva et al., 2007), reinforcing the need to identify sources of infection, the possible foods involved and the profile of isolated strains, to then evaluate the impact of Listeria monocytogenes on public health and the meat market.

Currently, Brazil is the second largest producer of bovine meat, being surpassed only by the USA. However, Brasil is the main beef exporter, supplying over 100 countries, with an export volume of 7.5 million tons per year (ABIEC, 2015). The estimates for 2016 are for a profit of about US$ 7.5 billion and volume of approximately 1.76 million tons (ABIEC 2016). In this scenario, in 2015, the state of Mato Grosso registered the highest production volume within all Brazilian states, responsible for 15.64% (1.17 million tons) of all beef protein produced in the country (IMEA, 2015).

Although Brazilian beef exports are increasing, many product embargoes by importing countries are still in place, such as the occurrence of 2013, when Russia suspended imports of beef processing units after observing the presence of L. monocytogenes in meat lots imported from Brazil (Affa Sindical, 2013). Although cases regarding restrictions regarding Brazilian meat have occurred, the Brazilian legislation does not impose tolerance limits for this pathogen in meats and meat-derived products, and only demands regarding the absence L. monocytogenes in ready-to-eat meat (Brasil, 2009) and high and very high water content cheeses are in place (Brasil, 2001).

Considering the great potential for export, the Brazilian slaughterhouses that process meat must meet the requirements of consumer markets (WTO, 2013). International requirements for food trade are mainly based on quality and safety aspects, primarily identified by microbiological characteristics observed along the food production chain and the final products (Neeliah, 2013). In this sense, the monitoring of hygiene indicator microorganisms and estimations on the prevalence of pathogens is critical for quality assurance and safety of produced food, and subsequent maintenance of international trade.

In this context, the present study aimed to estimate the prevalence of L. monocytogenes in fresh chilled beef produced in the state of Mato Grosso, Brazil.

2. MATERIAL AND METHODS

To calculate the sample size, the sample calculation for finite populations was applied (Bolfarine and Bussab, 2005), considering a confidence level of 99%, margin of error of 5 % and expected prevalence of 16 % aording to the worldwide prevalence established by Jay et al. (1996). Thus, a total of 50 samples of fresh beef were evaluated, from 13 different slaughterhouses with federal or state inspection services, with six of these being qualified for export.

Samples were received chilled in the laboratory between 1 to 8 °C, with an average weight of 1-2 kg, consisting of meat cuts palette, chuck, sirloin, rib steak, sirloin, rump, sirloin, lizard and filet mignon, packaged as sold. The analyses took place from August 2015 to February 2016 in a laboratory that meets the quality requirements of the ISO/IEC17025:2005 ABNT standard, with an implemented
quality management system, skilled technical team, with evidence in proficiency testing using certified reference materials, calibrated equipment and analytical controls.

Research and identification of *L. monocytogenes* was performed by the bacteriological method (ISO 11290-1:1996/Am 1:2004) with an automated qualitative enzyme immunoassay analysis system (mini VIDAS, BIOMERIEUX®) and real-time PCR (q PCR) (Moura, 2016). An aliquot of 25 g of each meat sample was subjected to primary and secondary enrichment steps in selective half-fraser and fraser broths, plating in Modified Oxford Agar (MOX) and Listeria Agar (ALOA) and colony isolation on Tryptone agar, soy and yeast extract (TSYEA). Suspected and isolated colonies were subjected to confirmation of being *L. monocytogenes* in morphology (Gram), biochemical (motility test, catalase, use of xylose and rhamnose carbohydrates, CAMP-test and API Listeria® BIOMERIEUX®) and q-PCR (hlyA gene) assays. Fraser broths, after incubation for 48 hours ± 2 hours at 37°C were examined using a Mini-lives LMO2® system (BIOMERIEUX®) for the EnzymeLinked Fluorescent Assay technique (ELFA) according to the manufacturer's recommendations.

The DNA from all suspected isolated colonies was obtained by thermal lysis, quantitated by a fluorimetry QUBIT 2.0 kit (Invitrogen®) and submitted to q-PCR, consisting in the amplification of a fragment of HlyA gene, with primers and specific probe, (FhlyA 5’ - AAGAAGTNATTAGTTTAAACAAATTACTATAACG-3’, R1hlyA 5’ - AACTGCTTTTAGTNAACGCTTTGC-3’ and TaqMan MGB PhlyA FAM-TGAACTACANGACCTTCC-MGB) (Invitrogen®). The qPCR was conducted with 2x TaqMan Universal PCR Master Mix (Invitrogen®), 600 mM of each primer and 200 mM of TaqMan MGB (Invitrogen®) and 40 ng DNA. The amplification conditions were adjusted for an initial denaturation at 50°C for 2 min, followed by 45 ciclos at 95°C for 10 min, 95°C for 15 s and 60°C for 1 min. The reactions were conducted in a 7500 Real Time PCR System thermocycler (Applied Biosystems®).

For purposes of calculating the prevalence of *L. monocytogenes*, the combination of all the results obtained in the microbiological methods, enzyme-linked immunosorbent and q –PCR assays were considered.

### 3. RESULTS AND DISCUSSION

Of the 50 analyzed samples, 18 (36%) were contaminated by *Listeria* spp. and 7 (14%) showed the presence of *L. monocytogenes*. The prevalence of 14% (7/50) was determined by the combined results obtained in the bacteriological assays considered the "gold standard" method, mini VIDAS (BIOMERIEUX®), and real-time PCR of the DNA of suspect isolated colonies. The mini VIDAS method showed a sensitivity of 57.14 % and a specificity of 100%, while the qPCR technique achieved 100% sensitivity and specificity in the identification of the isolated colonies previously identified in by biochemistry assays.

The presence of *L. monocytogenes* was been identified in four industries, two of them certified for export, while meat contamination by *Listeria* spp was observed in 9 of the 13 studied industries. The occurrence of 36% (18/50) of *Listeria* spp is close to the results found by Kasnowski (2004) and Andrade et al. (2014), that observed an occurrence of *Listeria* spp of 41.62 % and 45.7% in whole pieces of beef sirloin and ground beef, respectively.

The presence of other species belonging to the Listeria genus in the industrial environment may indicate that these locations show favorable conditions for the development of these microorganisms. Saunders and Wiedman (2007) believe that this is due to high DNA homology DNA, that makes them very similar phenotypically and, thus, leads to both presenting the same ecology. This is of great importance, since the presence of any species of the genus may be indicative of inefficient cleaning, providing a favorable environment for bacterial persistence.
The prevalence of 14% *L. monocytogenes* in beef from Mato Grosso (Table 1) is higher than that found by Andrade et al. (2014), which determined a prevalence of 11.4% in ground beef in the Federal District and less than that found in Chile between 2008 and 2012, where prevalence was of 23% (Saludes et al., 2015). This highlights the possibility of cross-contamination during preparation of ready-to-eat food.

Table 1. *L. monocytogenes* prevalence in fresh chilled beef produced in Mato Grosso/Brazil, from August 2015 to February 2016.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th><em>Listeria spp</em></th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 11290-1:1996/Amd 1:2004</td>
<td>50 meat samples</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Mini-VIDAS, LMO2 BIOMERIEUX®</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Real Time PCR</td>
<td>27 colonies</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td><strong>Prevalence</strong></td>
<td><strong>50</strong></td>
<td><strong>36%</strong></td>
<td><strong>14%</strong></td>
</tr>
</tbody>
</table>

The detection of this bacterium in meat can be explained due to its wide dissemination in nature, since it may be present in vegetation, soil, food, human and animal feces, sewage and water effluents, as well as its ability to form biofilms and remain for years in slaughterhouses as a recurring source of contamination (D’Ovidio, 2008; Zhu et al, 2012; Andrade et al., 2014; Monteiro, 2015).

The prevalence of *L. monocytogenes* in beef in the state of Mato Grosso is significant and requires specific regulatory action, to ensure consumer health, avoid the restrictions of foreign trade regarding products produced in the state and encourage epidemiological studies to demonstrate the actual occurrence and causes of human listeriosis in Brazil.

### 4. CONCLUSION

The prevalence of *Listeria monocytogenes* in fresh chilled beef produced in Mato Grosso is of 14% and slaughterhouses certified to export beef also showed the presence of this pathogen, which may pose risks to public health, suffering future restrictions on international trade and economic losses.

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6. REFERÊNCIAS BIBLIOGRÁFICAS


