An Acad Bras Cienc (2020) 92(suppl.1): e20180557 DOI 10.1590/0001-3765202020180557
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Identification of *Listeria monocytogenes* in cattle meat using biochemical methods and amplification of the hemolysin gene

ALESSANDRA S. SILVA, ELIZABETH A.A. DUARTE, THIAGO A.S. DE OLIVEIRA & NORMA S. EVANGELISTA-BARRETO

Abstract: In Brazil and in other countries of the world, studies have been conducted to identify *Listeria monocytogenes* in cattle meat that is preferably consumed undercooked and, when marketed without meeting strict phytosanitary requirements, may cause outbreaks of listeriosis. In the such, foodborne outbreaks, the methods used for the detection of the pathogen and the efficiency associated with them are crucial for the proper assessment. In this study, we used the techniques biochemical and molecular for identification of the *L. monocytogenes* isolated from 30 samples of the fresh beef, marketed in ten butchers' shop of the free-fair from a municipality from the Bahia, Brazil. The results obtained from biochemical tests (catalase, motility, β-hemolysis and carbohydrate fermentation), as well as PCR analysis for the *hly* gene (hemolysin production is an important factor in the pathogenesis of listeriosis) revealed that 50% of butchers shops presented bovine meat contaminated with bacteria of the *Listeria* sp. and confirmed that 54.16% of the analyzed meat samples were positive for *L. monocytogenes*. This study highlights the importance of microbiological surveillance in free-fair to minimize the exposure of consumers to this foodborne pathogen.

Key words: Foodborne diseases, Listeriosis, meat products, *hly* A gene.

INTRODUCTION

Listeria monocytogenes a foodborne pathogen regarded as a serious public health concern, is associated with severe infections such as septicemia, encephalitis, meningitis and abortion. These infections predominantly affect vulnerable populations such as elderly people, pregnant women, newborns, immunocompromised individuals, etc., and are associated with high hospital admission rates and mortality (Mead et al. 1999, Liu et al. 2007, Parihar et al. 2008, Fox et al. 2011). Mode of transmission for listeriosis are varied and include ingestion of contaminated food and water, as well as direct transmission between humans and animals (Liu et al. 2007, Law et al. 2015).

The ability to survive in adverse conditions such as low temperatures and pH, high concentrations of salt and multiplication in cooling temperatures causes this bacterium to be related as a threat to public health (Mohamed et al. 2016), being cited in various foods processed as cheeses and dairy products (Barancelli et al. 2014, Lee et al. 2017), milk and milk productions (Usman et al. 2016), meat products and ready-to-eat fish (Rodrigues et al. 2017). Food consumption, as well as direct transmission between humans and animals represent the dynamics of listeriosis (Steckler et al. 2018). Insofar as of the contamination of bovine meat by *L. monocytogenes* is concerned, free markets (free-fair) are regarded as very important as large

quantities of beef are commercially sold here. In Brazil, as well as in other parts of the world, several studies have been conducted aimed at identifying the presence of *L. monocytogenes* in cattle meat that is preferably consumed undercooked and which, when marketed without meeting strict phytosanitary requirements, may cause outbreaks of listeriosis (Stavru et al. 2011, Välimaa et al. 2015).

The genus *Listeria* is composed of eight species: *L. monocytogenes, L. innocua*, *L. ivanovii, L. seeligeri, L. welshimeri, L. marthii, L. rocouurtiae* and *L. grayi* (ITIS Global 2018). With respect to pathogenicity, *L. monocytogenes* is reported to cause infections in humans, as well as other animals whereas *L. ivanovii* (*L. monocytogenes* serotype 5) causes infections in mammals such as the ungulates (Vázquez-Boland et al. 2001, Liu 2006). *Listeria monocytogenes* is a small Gram-positive rod with rounded ends; it does not produce spores or form capsules and is mobile when cultivated between 20-25 °C and immobile or minimally mobile at 37 °C (CFSAN 2009). As these bacteria are resistant to large variations in pH, temperature as well salt concentrations, they are capable of propagating in a variety of environments including food substrates (Jadhav et al. 2012, Zhu et al. 2012).

The presence of *L. monocytogenes* in food can be detected by conventional methodologies such as microbiological analyses, which are based on the tests developed by the U.S. Department of Agriculture (USDA). These tests are usually utilized for the analysis of meat, meat products, and surface swabs along with other similar methods adopted by the Food and Drug Administration - FDA, United States of America (Silva et al. 2010). In addition, molecular methods based on the amplification of specific gene regions, especially those encoding virulence genes that play an important role in bacterial pathogenicity and food-borne infection are widely applied until today. Biochemical testing of *Listeria* species remains time-consuming (taking up to 6 days to finalize a result) and costly. Furthermore, as biochemical tests measure the phenotypic characteristics of *Listeria* bacteria, their performance can be influenced by external factors that affect bacterial growth and metabolic mechanisms (Liu 2006).

International epidemiological surveys have revealed that contaminated animal products are among the most common food items implicated in outbreaks of *L. monocytogenes* (Uchima et al. 2008). In 2008, an outbreak of listeriosis in Canada caused by the consumption of processed meat resulted in 53 confirmed cases of the infection along with 6-suspected cases and 20 deaths (Warriner & Namvar 2009). In Brazil, where the Program for the Control of *Listeria monocytogenes* in Products of Animal Origin Ready for Consumption (formulated by the Ministry of Agriculture and Supply) has been in force since 2009 (Brasil 2009), data regarding the prevalence of this pathogen is still scarce largely due to the underreporting of cases.

Beef, one of the most commonly consumed animal products in Brazil, is an important source of protein and micronutrients such as iron, vitamin B complex and zinc (Oliveira et al. 2013). It is regarded as a highly perishable food item because the chemical composition of the meat, which is rich in nutrients and has a high water content and favorable pH, promotes the rapid proliferation of microorganisms (Moura et al. 2015). Contamination and multiplication of pathogens in meat is facilitated at multiple levels starting with the unhygienic and unsanitary conditions that prevail during slaughtering up to the processing of the product and exhibition for sale. Wherein the meat is traded in open fairs and exposed to conditions such as high temperature, exposure to insects, rodents, and

other vectors of disease (Almeida et al. 2011, Barros & Violante 2014).

The free-fair is a place for buying, selling and exchanging goods especially food items such as fruits, vegetables, and meats. The dominating characteristic of these types of markets is the simple physical and structural layout accompanied by inadequate or absence of facilities for temperature regulation and other measures for maintaining hygienic and sanitary conditions required for the marketed products (Almeida et al. 2011). Such practices are commonly observed in the city from the Jiquiriçá, Bahia, Brazil, where the free-fair is usually held on Saturdays when the rural population travels to the urban center for buying and trading purposes.

The commercial sale of beef in open markets (free-fair) is a common practice in third world countries including Bahia, which is an important state in the Northeast of Brazil. This reinforces the importance of this study as the first report of the type, since we did isolate and identification by biochemical and molecular approach, *L. monocytogenes* in butcher shopping's from one free-fair of Bahia, Brazil.

MATERIALS AND METHODS

Samples of fresh beef (300 g) were collected in the 10 butcher's shop from one free-fair from the Jiquiriçá, Bahia, Brazil. Three consecutive collections were performed in the 90-days interval, which totaled 30 samples (n = 30). The beef samples were conditioned and transported under refrigeration for immediate processing in laboratory.

For the isolation of *L. monocytogenes*, 25 g of each meat sample was homogenized in 225 mL of UVM Medium (Acumedia, Lansing, MI, USA) and incubated at 30 °C for 24 h. Subsequent to the incubation, aliquots of 0.1 mL each were transferred to tubes containing 10 mL of Fraser broth (Acumedia, Lansing, MI, USA) and incubated at 35 °C for 26 h. The tubes that exhibited darkening (due to esculin hydrolysis) were selected for plating by exhaustion in Palcam Agar (Acumedia, Lansing, MI, USA); these plates were incubated at 35 °C for 48 h. The colonies that exhibited a greenish gray morphology with a concave center surrounded by a black halo (result of hydrolysis of esculin) were subjected to Gram staining and biochemical testing for catalase activity, motility, β-hemolysis and the fermentation of carbohydrates (dextrose, xylose, rhamnose, mannitol and maltose) (Silva et al. 2010). The samples that were Gram-positive and exhibiting mobility were positive for the catalase and β-hemolysis tests, fermented dextrose, maltose and rhamnose, while not fermenting xylose or mannitol were regarded as confirmed isolates of *L. monocytogenes*.

The genomic DNA was extracted from the *in vitro* culture using the UltraClean® Microbial DNA Isolation kit (MOBIO, South, USA) following the manufacturer's recommendations. DNA integrity and quantity were verified using the Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, California, EUA). PCR amplifications were performed using the LM1 (5' CCTAAGACGCCAATCGAA 3') and LM2 (5' AAGCGCTTGCAACTGCTC 3') primers previously described by Border et al. (1990); primers LL5 (5' AACCTATCCAGGTGCTC 3') and LL6 (5' CTGTAAGCCATTTCGTC 3') were used for specific amplification of the region between position 622 and 639 of the *hly* A gene, which is regarded as specific for the identification of *L. monocytogenes* (Thomas et al. 1991, Herman et al. 1995). The composition and concentration of the PCR reaction were as follows: 2.0 ng DNA (from the sample); 0.3 μL Taq DNA polymerase; buffer solution (10x); 7.0 mM $MgCl_2$; 1.5 pmol/μL dNTPs; 1.5 pmol/μL of each primer; 0.4 U of Taq

DNA polymerase and ultrapure water to final volume of 50 µL. The Veriti Thermal Cycler PCR (App. Biosystems, São Paulo, Brazil) was used for amplification using previously reported run conditions (Border et al. 1990, Herman et al. 1995). The amplified products were applied on 1% agarose gel for electrophoresis, stained with ethidium bromide and visualized using ultraviolet light. The amplicons were then purified using the Illustra® GFX PCR DNA and Gel Band Purification kit (GE Healthcare Life Sciences, São Paulo, Brazil).

Subsequently, nucleotide identification was performed using the ABI-Prism 3500 automated sequencer (Applied Biosystems, California, EUA). The sequences generated in this work were manually edited using Geneious software (version 9.1.6) (Kearse et al. 2012). Then, the query sequences were combined with “sequences from type” (*hly* A gene) of the *Listeria monocytogenes* downloaded from GenBank. The taxonomic identity of the isolates was verified by cross checking with the GenBank database using BLAST program of NCBI (http://www.ncbi.nlm.nih.gov) and RDB - Ribosomal Database Project (https://rdp.cme.msu.edu/) (Cole et al. 2014).

RESULTS

The occurrence of contamination of *Listeria* sp. in butcher’s shops from free-fair from the Jiquiriçá, Bahia, Brazil was 50% (5/10). Biochemical tests (catalase, motility, β-hemolysis, and carbohydrate fermentation), allowed the isolation of 24 strains of *Listeria* sp. (Table I). Hemolysin production was verified by the hemolysis B test, which allows characterizing the samples as probable species: *L. monocytogenes*, *L. seeligeri* and *L. ivanovii*, as described by Ryser & Donnelly 2001, Holt et al. 1994.

PCR assays with 24 isolates of *Listeria* sp. they were conducted with amplification of the hly gene that allowed the obtaining of amplicons that were sequenced and aligned with species of the type of *Listeria monocytogenes* (ATCC 15313 and NCTC10357), both deposited in the GenBank (Table II). In addition, the sequences obtained in 13 isolates form a monophyletic group with other bacteria taxonomically related to *L. monocytogenes*.

DISCUSSION

L. monocytogenes contamination of beef can be attributed to inadequate sanitary control during processing or commercialization (Brasil 2010). *L. monocytogenes* is common in the intestinal tract of the animal, as well as in nature, bacteria can easily contaminate carcasses and cuts of meat (Mantilla et al. 2007). Hence it can be concluded that the presence of *L. monocytogenes* in the beef samples analyzed in this study confirm the unhygienic and unsanitary conditions prevalent at the site where they were collected. This is a reality that is common to most free-fairs in Brazil (Barros & Violante 2014, Matos et al. 2015).

Microbiological contamination of free-trade beef by *L. monocytogenes* is a serious public health threat as listeriosis is often associated with the consumption of meat and meat cooked briefly by the people belonging to the vulnerable sections of the society (Sergelidis & Abrahim 2009). The risk factor for the dissemination of infection is heightened by the availability of barbecued meat on skewers (fast snacks) in free-fair and public markets and the preference of most people for meat cooked briefly because of its superior texture and flavor. In addition, *L. monocytogenes* contamination can occur even after receiving heat treatment, and this pathogen persists in the refrigerated environment,

Table I. **Microbiological contamination of beef by *Listeria* sp. at the free-fair from the Jiquiriçá, Bahia, Brazil.**

Sample of the meat/Isolates	Butcher's Shop										Total
	BS1	BS2	BS3	BS4	BS5	BS6	BS7	BS8	BS9	BS10	
N° of contaminated samples (positive for *Listeria* sp.)	2(3)	0(3)	0(3)	0(3)	0(3)	1(3)	2(3)	0(3)	2(3)	1(3)	8(30) **26.66%**
N° of isolated of *Listeria* sp.	4	-	-	-	-	3	5	-	8	4	24

Total (%): percentage of fresh meat samples contaminated by *Listeria* sp. from the total of 30 samples collected (n = 30). Score (-): not contaminated with *Listeria* sp.

Table II. **Identification of the isolates of *Listeria monocytogenes* collected from fresh beef samples commercialized in the free-fair from the Jiquiriçá, Bahia, Brazil.**

Isolated (sample/meat)*	Amplicon size [1]	Query cover	Identities[2]	Taxonomy	Accession number[3]
M1, A8, M3, M10, M5	466	100%	98%	*Listeria monocytogenes*	LT906436.1; AY750900.1
M6, M7, M2, M9	674		98%		
M4, M11, M12, M13	773		97%		

***M: number assigned to the isolates by samples meat from the 10 Butcher's Shop analyzed (BS1, BS2, ..., BS10). [1]The amplicons were sequenced in both orientations and the query fragments presented corresponded to the sequence obtained. [2]"e-values" were zero for the isolates. [3]Access numbers that correspond to the descriptive, 'sequences from type', of the taxonomy are indicated in the previous column.**

utensils and the environment for a long period of time (several months), promoting pathogenic propagation in food (Barancelli et al. 2014).

The propensity of *L. monocytogenes* for infecting and propagating in food and on other non-nutritive surfaces is related to its ability to form biofilms and to biotransfer; both these characteristics confer adhesive property and grant protection to the microorganism (Oliveira et al. 2010). The biofilms thus formed are viable for months or even years; this allows for the occurrence of recurrent contamination of food (Markkula et al. 2005). Certain additional characteristics of *L. monocytogenes* such as its ability to multiply in the presence of environmental acids, cold refrigerated conditions, high concentrations of sodium chloride and other conditions that are usually averse to growth of other pathogenic bacteria, further aid in the propagation and perpetuation of the contamination (Oliveira et al. 2010). The exposure to these stress situations and the composition of meat-based foods potentiate the expression of the virulence gene listeriolysin in the bacterium, which in turn increases the risk of listeriosis upon consumption of the item (Olesen et al. 2010). In addition, meat and its derivatives confer greater risk of cross-contamination, due to the hygienic sanitary conditions of the commercial places (supermarkets and free markets) and the habit of consumption (undercooked). In this sense, the presence of *Listeria monocytogenes* in meat, sausage, parsley and dairy products, has been reported in Brazil since the 1990s, according to studies realized by Destro et al. (1991).

The study results indicate that the biochemical tests conducted by us were effective for the identification of *L. monocytogenes* as were the molecular tests involving amplification

of two separate regions from listeriolysin (*hly* A) gene. In this regard, it should be noted that one of the major problems in using the traditional methods for identification of *L. monocytogenes* is the time involved as conventional microbiological analysis can take up to 14 days to complete and requires higher quantity and quality of the samples, when compared to the molecular methods. In contrast, molecular methods present advantages when compared to the classical (biochemical) methods for two reasons: *i)* PCR methods are accurate and less expensive when compared to traditional methods that are often limiting factors, since they require bacterial enrichment and culture media and growth conditions favorable (temperature, pH, luminosity); *ii)* PCR methods are faster and more reproducible, key factors for immediate identification, diagnosis and therapy (treatment) that are fundamental to avoid outbreaks and public health problems and in some cases environmental impacts (river discharge, fish contamination, for example). Just as it is important for diagnosis and treatment of listeriosis (Liu et al. 2007).

Inadvertently some PCR reagents may lead to false negative results, for example (UNG) uracil-DNA-glycosylase (Bacich et al. 2011), DMSO and BSA. This is study theses reagents were not used. In addition, false positive results not were observed in agarose gel electrophoresis, when compared to positive control (including DNA template, strain with target gene). The qualitative PCR results (present bands that correspondents' base pairs for the primers used) were confirmed by sequencing of amplicons.

Based on the results of this study it is logical to propose that rapid tests such as PCR should also be used by food-exporting industries, especially those involved in exporting highly perishable items such as beef, for legal release and compliance with the laws and regulations of the countries for which they are intended. This is crucial as Brazil exports meat and its derivatives to several countries, such as the European Community, which test for the absence of *L. monocytogenes* in 1 g or 25 g of the imported product (Codex Alimentarius International Food Standards 2007). Thus, the PCR technique used in our study has the potential to be useful for monitoring the spread of *L. monocytogenes* in the Hazard Analysis and Critical Control Points (HACCP) plan for monitoring the quality of food products (Santos et al. 2006).

In Brazil, although the implementation of food safety systems is still a challenge in the area of food production, this is necessary to ensure food safety because it reduces populations of indicator microorganisms and should be implemented in small and medium-sized food industries (Cusato et al. 2013). The implementation of good manufacturing practices (GMP) and standard operating sanitation (POPS) procedures is considered the first step in minimizing food-borne diseases in the food production chain (Cusato et al. 2014).

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for their financial support. Elizabeth Amélia Alves Duarte received a pos-doctoral scholarship from Programa Nacional de Pós-Doutorado da CAPES (PNPD/CAPES) associated with the Programa de Pós-Graduação em Microbiologia from the Universidade Federal do Recôncavo da Bahia (UFRB).

REFERENCES

ALMEIDA RB, DINIZ WJS, SILVA PTV, ANDRADE LP, DINIZ WPS, LEAL JBG & BRANDESPIM DF. 2011. Condições higiênico-sanitárias da comercialização de carnes em feiras livres de Paranatama, PE. Alim Nutr 22: 585-592.

BACICH DJ, SOBEK KM, CUMMINGS JL, ATWOOD AA & O'KEEFE DS. 2011. False negative results from using common PCR reagents. BMC Res Notes 4: 1-7.

BARANCELLI GV, CAMARGO TM, GAGLIARDI NG, PORTO E, SOUZA RA, CAMPIONI F, FALCÃO JP, HOFER E, CRUZ AG & OLIVEIRA CAF. 2014. Pulsed-field gel electrophoresis characterization of *Listeria monocytogenes* isolates from cheese manufacturing plants in São Paulo, Brazil. Int J Food Microbiol 173: 21-29.

BARROS LSS & VIOLANTE PC. 2014. Microbiologia da carne bovina "in natura" comercializada nas feiras livres do Recôncavo baiano. Rev Bras Hig e Sanidade Anim 8: 185-197.

BORDER PM, HOWARD JJ, PLASTOW GS & SIGGENS KW. 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett Appl Microbiol 11: 158-162.

BRASIL. 2009. Ministério da Saúde. Sistema de informações hospitalares. Disponível em http://portalsaude.saude.gov.br/portalsaude/area/11/biblioteca.html. [Acessado em 12 aug 2015].

BRASIL. 2010. Ministério da Saúde. Secretaria de Vigilância em Saúde-SVS. Manual integrado de prevenção e controle de doenças transmitidas por alimentos. Brasília, DF, 136 p.

CFSAN-CENTER FOR FOOD SAFETY & APPLIED NUTRITION. 2009. "Bad Bug Book: Foodborne pathogenic microorganisms and natural toxins handbook" 2nd ed., U.S. Food and Drug Administration (FDA). Available in http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf. [Accessed on 16 July 2016].

CODEX ALIMENTARIUS INTERNATIONAL FOOD STANDARDS. 2007. Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL 61/2007. Available in http://www.codexalimentarius.net. [Accessed on 12 Nov 2016].

COLE JR, WANG Q, FISH JA, CHAI B, MCGARRELL DM, SUN Y, BROWN CT, PORRAS-ALFARO A, KUSKE CR & TIEDJE JM. 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucl Acids Res 42: 633-642.

CUSATO S, GAMEIRO AH, CORASSIN CH, SANT'ANA AS, CRUZ AG, FARIA JAF & DE OLIVEIRA CAF. 2013. Food safety systems in a small dairy factory: implementation, major challenges and assessment of systems' performances. Foodborne Pathog Dis 10: 6-12.

CUSATO S, GAMEIRO AH, SANT'ANA AS, CORASSIN CH, CRUZ AG & DE OLIVEIRA CAF. 2014. Assessing the costs involved in the implementation of GMP and HACCP in a small dairy factory. Qual Assur Saf Crop 6: 135-139.

DESTRO MT, SERRANO AM & KABUKI. 1991. Isolation of *Listeria* species from some Brazilian meat and dairy products. Food Control 2: 110-112.

FOX E, HUNT K, O'BRIEN M & JORDAN K. 2011. *Listeria monocytogenes* in Irish farmhouse cheese processing environments. Int J Food Microbiol 145: 539-545.

HERMAN LM, DE BLOCK JH & MOERMANS RJ. 1995. Direct detection of *Listeria monocytogenes* in 25 milliliters of raw milk by a two-step PCR with nested primers. Appl Environ Microbiol 61: 817-819.

HOLT JG, KRIEG NR, SNEATH PHA, STALEY JT & WILLIAMS ST. 1994. Bergey's Manual of Determinative Bacteriology, 9th ed., Baltimore: Williams & Wilkins, 787 p.

ITIS GLOBAL. 2018. The Integrated Taxonomic Information System (version Jun 2017). Available in http://www.catalogueoflife.org/annual-checklist/2018/ (Accessed on August 2018).

JADHAV S, BHAVE M & PALOMBO EA. 2012. Methods used for the detection and subtyping of *Listeria monocytogenes*. J Microbiol Methods 88: 327-341.

KEARSE M ET AL. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

LAW JWF, MUTALIB NSA, CHAN KG & LEE LH. 2015. An insight into the isolation, enumeration, and molecular detection of *Listeria monocytogenes* in food. Front Microbiol 6: 1-15.

LEE SH, BARANCELLI GV, DE CAMARGO TM, CORASSIN CH, ROSIM RE, DA CRUZ AG, CAPPATO LP & DE OLIVEIRA CA. 2017. Biofilm-producing ability of *Listeria monocytogenes* isolates from Brazilian cheese processing plants. Food Res Int 91: 88-91.

LIU D. 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes* an important foodborne pathogen. J Med Microbiol 55: 645-659.

LIU D, LAWRENCE ML, AUSTIN FW & AINSWORTH AJ. 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. J Microbiol Methods 71: 133-140.

MANTILLA SPS, FRANCO RM, OLIVEIRA LAT, SANTOS EB & GOUVÊA R. 2007. Importância da *Listeria monocytogenes* em alimentos de origem animal. Rev FZVA 14: 180-192.

MARKKULA A, AUTIO T, LUNDÉN J & KORKEALA H. 2005. Raw and processed fish show identical *Listeria monocytogenes* genotypes with pulsed-field gel electrophoresis. J Food Prot 68: 1228-1231.

MATOS JC, BENVINDO LRS, SILVA TO & CARVALHO LMF. 2015. Condições higiênico-sanitárias de feiras livres: uma revisão integrativa. Rev Eletrônica Gestão & Saúde 6: 2884-2893.

MEAD PS, SLUTSKER L, DIETZ V, MCCAIG LF, BRESEE JS, SHAPIRO C, GRIFFIN PM & TAUXE RV. 1999. Food-related illness and death in the United States. Emerg Infect Dis 5: 607-625.

MOHAMED Y, REDA WW, ABDEL-MOEIN K, EL-RAZIK KAA, BARAKAT AMA, EL FADALY HA, HASSANAIN NA & HEGAZI AG. 2016. Prevalence and phylogenetic characterization of *Listeria monocytogenes* isolated from processed meat marketed in Egypt. J Gen Eng Biotec 14: 119-123.

MOURA JWF, MEDEIROS FM, ALVES MGM & BATISTA ASM. 2015. Fatores influenciadores na qualidade da carne suína. Rev Cient Prod Anim 17: 18-29.

OLESEN I, TRORSEN L & JESPERSEN L. 2010. Relative transcription of *Listeria monocytogenes* virulence genes in liver pates with varying NaCl content. Int J Food Microbiol 141: 560-568.

OLIVEIRA JD, SILVA TRS & CORREIA MGS. 2013. Fatores determinantes da qualidade nutricional da carne bovina. Cad Grad - Cienc Hum e Sociais 1: 37-46.

OLIVEIRA MM, BRUGNERA DF, ALVES E & PICCOLI RP. 2010. Biofilm formation by *Listeria monocytogenes* on stainless steel surface and biotransfer potential. Braz J Microbiol 4: 97-106.

PARIHAR VS ET AL. 2008. Characterization of human invasive isolates of *Listeria monocytogenes* in Sweden 1986-2007. Foodborne Pathog Dis 5: 755-761.

RODRIGUES CS, DE SÁ CVGC & DE MELO CB. 2017. An overview of *Listeria monocytogenes* contamination in ready to eat meat, dairy and fishery foods. Cienc Rural 47: 1-8.

RYSER ET & DONNELLY CW. 2001. *Listeria*. In: Compendium of methods for the microbiological examination of foods. 4., Washington. APHA. DOWNES FP, ITO K, p. 343-363.

SANTOS LAG, PINTO PSA, MORAES MP, VANETTI MCD, BEVILACQUA PD, PINTO MS & DIAS FS. 2006. Pesquisa molecular e convencional de *Listeria monocytogenes* para o controle de qualidade da carne suína. Rev Ceres 53: 481-486.

SERGELIDIS D & ABRAHIM A. 2009. Adaptative response of *Listeria monocytogenes* to heat and its impact on food safety. Food Control 20: 1-10.

SILVA N, JUNQUEIRA VCA, SILVEIRA NFA, TANIWAKI MH, SANTOS RFS & GOMES RAR. 2010. Manual de métodos de análise microbiológica de alimentos e água. 4ª ed., São Paulo: Varela, 624 p.

STAVRU F, BOUILLAUD F, SARTORI A, RICQUIER D & COSSART P. 2011. *Listeria monocytogenes* transiently alters mitochondrial dynamics during infection. PNAS 108: 3612-3617.

STECKLER AJ, CARDENAS-ALVAREZ MX, RAMSETT MKT, DYER N & BERGHOLZ TM. 2018. Genetic characterization of *Listeria monocytogenes* from ruminant listeriosis from different geographical regions in the U.S. Vet Microbiol 215: 93-97.

THOMAS EJ, KING GK, BURCHAK J & GANNON VPJ. 1991. Sensitive and specific detection of *Listeria monocytogenes* in milk and ground beef with the polymerase chain reaction. Appl Environ Microbiol 57: 2576-2580.

UCHIMA CA, CASTRO MFPM, GALLO CR, REZENDE ACB, BENATO ER & PENTEADO AL. 2008. Incidence and growth of *Listeria monocytogenes* in persimmon (*Diospyros kaki*) fruit. Int J Food Microbiol 126: 235-239.

USMAN UB, KWAGA JKP, KABIR J, OLONITOLA OS, RADU S & BANDE F. 2016. Molecular characterization and phylogenetic analysis of *Listeria monocytogenes* isolated from milk and milk products in Kaduna, Nigeria. Can J Infect Dis Med Microbiol 2016: 1-7.

VÄLIMAA AL, TILSALA TA & VIRTANEN E. 2015. Rapid detection and identification methods for *Listeria monocytogenes* in the food chain - A review. Food Control 55: 103-114.

VÁZQUEZ-BOLAND JA, KUHN M, BERCHE P, CHAKRABORTY T, DOMÍNGUEZ-BERNAL G, GOEBEL W, GONZÁLEZ-ZORN B, WEHLAND J & KREFT J. 2001. *Listeria pathogenesis* and molecular virulence determinants. Clin Microbiol Rev 14: 584-640.

WARRINER K & NAMVAR A. 2009. What is the hysteria with *Listeria*? Trends Food Sci Technol 20: 245-254.

ZHU L, FENG X, ZHANG L, ZHU R & LUO X. 2012. Prevalence and serotype of *Listeria monocytogenes* contamination in Chinese beef processing plants. Foodborne Pathog Dis 9: 556-560.

How to cite

SILVA AS, DUARTE EAA, DE OLIVEIRA TAS & EVANGELISTA-BARRETO NS. 2020. Identification of *Listeria monocytogenes* in cattle meat using biochemical methods and amplification of the hemolysin gene. An Acad Bras Cienc 92: e20180557 DOI 10.1590/0001-3765202020180557.

Manuscript received on June 5, 2018; accepted for publication on October 11, 2018

ALESSANDRA S. SILVA[1]
https://orcid.org/0000-0001-6403-0653

ELIZABETH A.A. DUARTE[2]
https://orcid.org/0000-0002-8667-502X

THIAGO A.S. DE OLIVEIRA[2]
https://orcid.org/0000-0002-6582-7434

NORMA S. EVANGELISTA-BARRETO[1]
https://orcid.org/0000-0001-5960-0957

[1]Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Barbosa, 710, Centro, 44380-000 Cruz das Almas, BA, Brazil

[2]Programa de Pós-Graduação em Biotecnologia, Faculdade Maria Milza, BR 101, 44350-000 Governador Mangabeira, BA, Brazil

Correspondence to: **Elizabeth Amélia Alves Duarte**
E-mail: elizabethaad@gmail.com

Author contributions

Alessandra Santana Silva, performed the experiments and analyzed the data. Thiago Alves Santos de Oliveira, analyzed the data and wrote the paper. Elizabeth Amélia Alves Duarte and Norma Suely Evangelista-Barreto conceived, designed the experiments and wrote the paper.