Original Article

**Listeria monocytogenes** in expansion tank milk assessed in Alagoas state counties, Brazil

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**ABSTRACT**

Foodborne diseases represent a global public health issue and dairy products are closely related to it, since the quality of the milk produced in several Brazilian regions is unsatisfactory due to the presence of microorganisms in it. *Listeria monocytogenes* is a serious problem linked to food safety and, when it comes to milk, it represents a potential danger because it can withstand food storage temperatures, among other characteristics. The significant participation of Alagoas State in this dairy production sector and the importance of producing safe food led to the aim of the present study, namely: investigating *Listeria monocytogenes* in expansion tank milk in Alagoas State counties. Milk samples were collected from tanks in 30 milk-processing unit suppliers. Next, they were taken to the Meat and Milk Inspection Laboratory (LICAL - UFRPE). The ISO 11290-1: 1996 / Amd.1: 2004 method, with adaptations, was used in the analyses. Bluish colonies, with or without halo formation, were identified according to their morpho-tintorial and biochemical characteristics. *Listeria monocytogenes* were detected in 20% of the samples (6/30), and such rate represents a public health risk. Thus, monitoring this microorganism and mastitis in the herd, as well as pre- and post-dipping, equipment and storage tank sanitation, and milk collection through refrigerated trucks at the appropriate time is a relevant procedure to prevent contamination and to assure safe food provision to the population.

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**INTRODUCTION**

According to the World Health Organization (WHO, 2017), one in ten individuals worldwide falls ill every year due to foodborne illnesses (DTA). Ministry of Health (BRASIL, 2017) data show that 7.170 food outbreak cases were reported in the country from 2000 to June 2017, and milk and its derivatives accounted for 2.8% of them. As far as patients are concerned, there were approximately one thousand individuals presenting some symptom related to these diseases.

*Listeria monocytogenes* stands out as a psychotrophic pathogen capable of multiplying under temperature between -0.4°C to 50°C with wide distribution in the environment. This microorganism can grow under anaerobic conditions and is heat tolerant, besides being able to tolerate successive freezing and thawing processes (HARTMANN et al., 2009; LIU, 2006).

Data on listeriosis in humans were recorded in Europe and they highlighted the need of studies related to its presence and survival in food, as well as of research about its reflect on the food production chain (BOTSARIS, 2016; EFSA, 2015). This microorganism can be difficult to be eliminated during food processing due to its ability to form biofilms and to become more resistant to materials used in cleaning and sanitation (GRANDI, 2015).

The RDC n. 12/01 of the National Health Surveillance Agency - ANVISA (BRASIL, 2001) in Brazil establishes

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the microbiological standards for food, but does not define the *L. monocytogenes* limit in raw milk, cheese. According to official recommendation, it must be *L. monocytogenes* absence in every 25g of randomly sampled food.

Despite being sterile in the mammary gland of healthy animals, milk can be contaminated by pathogenic microorganisms at milking. In addition, the presence of *L. monocytogenes* in the gastrointestinal tract of several animals can contaminate the milk and its derivatives. The surface of equipment used in milking and storage tanks, as well as the external surface of ceilings and udder are among the main milk contaminant sources (FUSCO; QUERO, 2014; MOLINERI et al., 2012).

Northeast Region milk production accounted for 5.1% of the inspected national production in 2016, and this number represented 1.18 billion liters of milk; by March 2017 more than 10,000 liters were produced in Alagoas (CONAB, 2017). However, these results do not demonstrate the reality of the productive chain in the country, which faces obstacles to milk production, mainly due to hygiene and sanitation conditions. The quality of the raw milk produced in several Brazilian regions remains unsatisfactory because of microorganism-multiplication rates (FREITAS et al., 2005; ZENI et al., 2013).

Accordingly, the aim of the present study was to investigate *Listeria monocytogenes* in expansion tank milk assessed in Alagoas State due to the relevance of milk production in the country, as well as to assess Alagoas relevance in the daily production sector, as well as the importance of providing safe food.

**MATERIAL AND METHODS**

Thirty milk samples from cattle herds were collected in thirty farms located in ten counties of the dairy Alagoas State: Mar Vermelho, Viçosa, Chã Preta, Minador do Negrão, Palmeira dos Índios, Cajueiro, Major Isidoro, Arapiraca, Jacaré dos Homens and Monteirópolis. These farms use mechanical milking and supply raw milk to dairy manufacturers under federal inspection. Fifty (50) mL of milk were aseptically collected according to Embrapa's Milk Cattle Technical Circular 92 (BRITO et al., 2007) with the aid of a stainless steel sink and sterile Falcon-type flasks. The samples were collected directly from the refrigeration tanks through direct property expansion. Next, they were taken to the Meat and Milk Inspection Laboratory (LICAL - UFRPE) in isothermal boxes containing recyclable ice at approximately 4 °C. The ISO 11290-1: 1996 / Amd.1: 2004 (ISO, 2004) method, with adaptations were used to isolate the microorganisms. The milk samples were homogenized and 25 mL of each one was transferred to covered flasks with 225 mL of Demi Fraser pre-enrichment broth (Acumedia) supplemented with 1g of pure Iron Citrate and Ammonium III (Vetec) and incubated for 24 hours at 30°C. Then, 0.1 mL of the mixture from each flask was transferred to covered tubes containing 10 mL of Fraser (Merck) enrichment broth supplemented with 0.5 g of pure Iron Citrate and Ammonium III (Vetec). Next the tubes were incubated for 24 hours at 37 °C. The cultures were grew in Petri dishes containing Chromocult *Listeria* Selective agar supplemented with 5mg Amphotererin B (Cristália) and with 10mg Ceftazidime (ABL), according to Ottaviani and Agosti - Aloa (Merck). The plates were incubated for 48 hours at 37°C, in order to check whether blue colonies with, or without, halo formation would develop, since these colonies characterize *Listeria* spp. and *Listeria monocytogenes*, respectively. Colonies suggestive of *Listeria* spp. and *Listeria monocytogenes* were identified according to their morpho-tintorial (Gram staining) and biochemical characteristics through the catalase, motility and CAMP tests, according to Barrow; Feltham (1993). The American Type Culture Collection (ATCC 19115), which was supplied by the National Agricultural and Livestock Laboratory - LANAGRO-PE, Ministry of Agriculture, Livestock and Supply - MAPA, was used as positive *L. monocytogenes* control. It was kept in stock agar and renewed in a monthly basis.

**RESULTS AND DISCUSSION**

*Listeria* spp. was found in 23.3% of the samples (7/30), whereas 76.7% of them (23/30) did not present suggestive colonies of it.

None of the tanks were subjected to proper sanitation; tanks in 66.6% (20/30) of the properties were extremely dirty and presented hygienic-sanitary conditions unsuitable for milk storage. Positive *Listeria* spp. and *Listeria monocytogenes* samples came from tanks where the hygienic-sanitary conditions were the most critical. Inadequate hygienic-sanitary practices may promote biofilm formation in storage tanks, fact that favors the persistence of this microorganism at the production site (FRANCO et al., 2000; HAÜN, 2004).

However, milk production features in the country impair the development of this activity, since it is mostly conducted by small producers who usually do not have much money to invest in improvements, who have low technical knowledge, lack the necessary sanitary control over the animals and have poor hygiene practices during milking (NERO; VIÇOSA; PEREIRA, 2009).

Another factor likely related to *Listeria* spp. and *L. monocytogenes* in milk is mastitis. According to Ivanek; Gröh; Wiedmann (2006), ruminants can perpetuate *L. monocytogenes* cycles; moreover, they found that high bacterial loads from rural environments can represent a source of these pathogens introduction in the dairy production chain.
Almeida; Pereira; Costa (2013) found contamination by these microorganisms in 10% of the samples in a study on the microbiological quality of milk produced in the Mearim / MA milk basin. This result shows values lower than the one found in the present study. Contrary to these values, Gelsinski; Baseggio (2013) found no positive Listeria spp. samples in raw milk stored under refrigeration in rural properties of Santa Catarina-SC. Araújo (2015) evaluated the microbiological quality of raw milk produced in the Zona da Mata and Agreste of Alagoas State. The collection was performed during milking. He found Listeria spp. in 83.33% of the assessed samples. Catão; Celbalos (2001) also found Listeria spp. in 73.3% of the samples when they investigated the microbiological quality of fresh milk in Paraíba State.

According to Miguel et al. (2014), milk can carry pathogenic microorganisms when it is obtained, or processed, under unsatisfactory hygienic conditions. In addition, bad sanitation can promote the multiplication of deteriorators capable of diminishing milk quality and shelf-life and, consequently, the quality and self-life of its derivatives. According to Barancelli et al. (2011), Listeria spp. in raw milk is worrisome, mainly when it is contaminated by species at risk to public health such as Listeria monocytogenes, because this milk is used in dairy production without any heat treatment.

L. monocytogenes was detected in 20% of samples (6/30) in the present study. Lower scores were recorded by Mansouri-Najand et al. (2015), who evaluated the prevalence of Listeria monocytogenes in raw milk from Iranian tanks and found 5% positivity in the samples. Waak; Tham; Danielsson-Tham (2002) evaluated the prevalence of L. monocytogenes in farm tanks in Sweden and recorded positive result in 1% of the samples.

A similar result was found by Botsaris et al. (2016), who studied the prevalence of Listeria monocytogenes in tanks in Cyprus and found the microorganism in 0.98% of the samples. Agostini et al. (2012) evaluated samples of fresh milk collected from Vale do Taquari region, RS and did not observe contamination by L. monocytogenes. Likewise, Arcuri et al. (2006) investigated this microorganism in samples from refrigerated tank milk in Southeastern Minas Gerais State and in North Rio de Janeiro State; they did not record positive results.

However, the comparison of Listeria spp. and Listeria monocytogenes occurrence results can be hampered by the variety of used analytical methods, as well as by the differences in culture media, in the sampling patterns adopted for each case and in the recorded results, which can show different detection levels. In addition, data differences can be explained by the differences between geographical areas and by the geographic specificity of the distribution of genus Listeria representatives (BOTSARIS, 2016). According to Barancelli et al. (2011), the amount of assessed samples can influence the presence, or absence, of L. monocytogenes. Thus, according to the analysis of few samples, the absence of L. monocytogenes does not necessarily mean that the microorganism is not present in the batch, although one or two positive samples may represent high-occurrence percentage. In addition, it is worth emphasizing that by comparing occurrence data, it is necessary to take into consideration that there was considerable improvement in the L. monocytogenes isolation methods in the 1990s, fact that facilitated its identification.

This microorganism detection in food can be influenced either by the presence of a large population of competitive microbiota, by low pathogen counting or by the interference of inhibitory food components (NORTON, 2000). According to Meyer-Brosseta et al. (2003), L. monocytogenes at low concentrations in food accounts for less than 10 CFU / mL in milk since its growth may be inhibited by other microorganisms found in this product. L. monocytogenes was not detected in the raw milk samples analyzed in a study conducted by Padilha et al. (2001). However, the microbiological analysis revealed other gram positive and gram negative microorganisms (Enterobacter, Citrobacter, Erwinia, Klebsiella, Serratia, Pseudomonas, Aeromonas and Bacillus) in it. The authors associated the non-detection of Listeria monocytogenes with their competition with other microorganisms found in the samples. Contaminated raw milk can be a source of cross-contamination of dairy products processed under environmental contamination (ARCURI et al., 2006). Waak; Tham; Danielsson-Tham (2002) reported the occurrence of Listeria spp. in farm milk tanks and milk storage silos of a dairy industry in Sweden. Chambel et al. (2007) assessed the occurrence of this microorganism in swab samples collected from dairy products in Portugal and found L. monocytogenes in 40% of the tested samples.

The presence of Listeria spp. and Listeria monocytogenes in pasteurized milk may indicate inefficient pasteurization processes or post-processing contamination, or yet the occurrence of both phenomena (ALMEIDA; PEREIRA; COSTA, 2013). Accordingly, milk from healthy cows subjected to adequate hygienic conditions, and its immediate cooling to the appropriate temperature, are fundamental and primary measures to guarantee the quality and safety of milk and of its derivatives (ARCURI et al., 2006).

According to the current study, 96.6% of the tanks (29/30) in the assessed properties comply the Normative Instruction 62/11 of the Ministry of Agriculture, Livestock and Supply (BRASIL, 2011) when it comes to milk storage temperature (4°C). On the other hand, 3.4% (1/30) of the storage temperature was below the recommended (4°C). According to Barancelli et al. (2011), L. monocytogenes control becomes difficult because pathogen control is traditionally focused on milk
hygiene and pasteurization; however, such measures are not necessarily sufficient to prevent this microorganism, because if manufacturing practices are not good enough it is possible finding recontamination. Rodrigues; Sá; Melo (2017) state that it is necessary to identify the diseases affecting the population and the presence of pathogens in food in order to develop risk-mitigation measures.

CONCLUSION

Listeria monocytogenes in milk samples from the assessed expansion tanks represents risk to public health; thus, it is worth monitoring this microorganism and mastitis in herds, as well as pre- and post-dipping, equipment and the storage tank sanitation, and milk collection through refrigerated trucks at the appropriate time. Such practice can help preventing the contamination of safe food essential for population provision.

REFERENCES

AGOSTINI, C. et al. Detecção de Listeria monocytogenes pela técnica da Reação em cadeia da Polimerase (PCR) em amostras de leite bovino in natura. Revista do Instituto de Laticínios Cândido Tostes, v. 7, n. 389, p. 15-20, 2012.

ALMEIDA, V.M.; PEREIRA, L.S.; COSTA, F.N. Listeria spp., coliformes, bactérias mesófilas e psicrotróficas no leite in natura e pasteurizado tipo C. Revista do Instituto Adolfo Lutz, v. 72, n. 1, p. 104-9, 2013.

ARAÚJO, B. F. O. Qualidade microbiológica e contagem de células somáticas de leite cru de vacas mestiças produzida no Zona da Mata e Agreste do Estado de Alagoas. Dissertação (Mestrado), Universidade Federal de Alagoas, Rio Largo, AL, 2015. 48p.

ARCURI, E. F. et al. Qualidade microbiológica do leite refrigerado nas fazendas. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v.58, n.3, p.440-446, 2006.

BARANCELLI, G.V. et al. Listeria monocytogenes: ocorrência em produtos lácteos e suas implicações em saúde pública. Arquivos do Instituto Biológico, v. 78, n.1, p.155-68, 2011.

BARROW, G.I.; FELTHAM. R.KA. Manual for the Identification of Medical Bacteria. 3 ed. Cambridge: Cambridge Univ. Press, 1993.

BOTSARIS, G. et al. Prevalence of listeria spp. and listeria Monocytogenes in cattle farms in Cyprus Using bulk tank milk samples. Journal of Food Safety, 00 (2016) 00-00 VC, Wiley Periodicals, Inc., 2016.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 62, de 29 de Dezembro de 2011. Aprova o Regulamento Técnico de Produção, Identidade e Qualidade do Leite tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel, em conformidade com os Anexos desta Instrução Normativa. Diário Oficial da União: Brasília, 2011.

BRASIL. Ministério da Saúde. Resolução nº 12, de 02 de janeiro de 2001. Dispõe sobre o Regulamento técnico dos padrões microbiológicos para alimentos. Diário Oficial da União, Brasília, 03 jan. 2001.

BRASIL. Ministério da Saúde. Surtos de doenças transmitidas por alimentos no Brasil. 2017. Brasília, 2017.

BRITO, J. R. F.; SOUZA, G. N.; FARIA, C. G.; MORAES, L. C. D. Procedimentos para coleta e envio de amostras de leite para determinação da composição e das contagens de células somáticas e de bactérias. Circular técnica 92 Embrapa gado de leite. 2007.

CATAÔ, R. M. R.; CEBALLOS, B. S. O. Listeria spp., coliformes totais e fecais e E. coli no leite cru e pasteurizado de uma indústria de laticínios, no estado da Paraíba (Brasil). Ciência e Tecnologia de Alimentos, v. 21, n.3, p. 281-287, 2001.

CHAMBEL, L. et al. Occurrence and persistence of Listeria spp. in the environment of ewe and cow's milk cheese dairies in Portugal unveiled by an integrated analysis of identification, typing and spatial-temporal mapping along production cycle. International Journal of Food Microbiology, v. 116, n.1, p.52-63, 2007.

CONAB. Companhia Nacional de Abastecimento. Conjuntura Mensal, Leite e Derivados. Brasília-DF, junho de 2017.

EFSA. European Food Safety Authority. European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal, v. 13, n. 1, p. 3991, 2015.

FRANCO, R.M. et al. Avaliação da qualidade higiênico-saúde de leite pasteurizado tipo C, leite de cabra pasteurizado congelado e iogurtes. Higiene Alimentar, v.14, n.68/69, p.70-77, 2000.

FREITAS, M.F.L. et al. Perfil de sensibilidade antimicrobiana in vitro de Staphylococcus coagulase positivos isolados de leite de vacas com mastite no agreste do estado de Pernambuco. Arquivos do Instituto Biológico, v. 72, n. 2, p. 171 – 177, 2005.

FUSCO, V.; QUIERO, G. M. Culture-dependent and culture-independent nucleic-acid based methods used in the microbial safety assessment of milk and dairy products. Comprehensive Reviews in Food Science and Food Safety, v. 13, p. 493-537, 2014.

GELINSKI, J. L. N; BASEGGIO, P. Avaliação da Presença de Listeria spp., Coliformes totais e contagem bacteriana total em leite cru armazenado sob refrigeração em propriedades rurais do município de Rio das Antas, Sc. Seminário de Iniciação Científica e Seminário Integrado de Ensino, Pesquisa e Extensão, v.3, n. 1, 2013.

GRANDI, A. Z. Influência de moléculas antioxidantes produzidas por Escherichia coli na formação de biofilme por Listeria monocytogenes. 2015. 88f. Tese (Doutorado), Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, São Paulo, SP, 2015.

HARTMANN, W. Qualidade microbiológica do leite cru produzido na Região Oeste do Paraná e ocorrência de Listeria monocytogenes. Ars Veterinaria, v.25, n.2, 072-078, 2009.

HAIN, M. A. D. Avaliação da eficiência de um esterilizador a plasma na inativação de Pseudomonas fluorescens. 2004. 71f. Dissertação (Mestrado), Universidade Estadual de Campinas, São Paulo, SP, 2004.

ISO. International Standard Organization ISO 11290-1:1996/Amd.1:2004 Microbiological of food and animal feeding stuffs – Horizontal method for the detection and enumeration of Listeria monocytogenes – part 1: detection method. AMENDMENT 1: Modification of the isolation media and the hemolysis test, and inclusion of precision data. Geneva: ISO, 2004. 4 p.

IVANEK, R.; GRÖHN, Y.T.; WIEDMANN, M. Listeria monocytogenes in multiple habitats and host populations: review of available data for mathematical modeling. Foodborne Pathogens and Disease, v.3, n.4, p.319-336, 2006.
LIU, D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *Journal of Medical Microbiology*, v. 55, p. 645-659, 2006.

MANSOURI-NAJAND, L. et al. Prevalence of *Listeria monocytogenes* in raw milk in Kerman, Iran. *Veterinary Research Forum*, v. 6, n. 3, p. 223 – 226, 2015.

MEYER-BROSETA, S. et al. Estimation of low bacterial concentration: *Listeria monocytogenes* in raw milk. *International Journal of Food Microbiology*, v. 80, n.1, p.1-15, 2003.

MIGUEL, E. M. et al. Formação de biofilmes em trocadores de calor e seus efeitos em leite e derivados. *Revista do Instituto de Laticínios Cândido Tostes*, v. 69, n. 1, p 53-63, 2014.

MOLINERI, A. I. et al. Association between milking practices and psychrotrophic bacterial counts in bulk tank milk. *Revista Argentina de Microbiologia*, v. 44, p. 187-194, 2012.

NERO, L. A.; VIÇOSA, G. N.; PEREIRA, F. E. V. Qualidade microbiológica do leite determinada por características de produção. *Ciência e Tecnologia de Alimentos*, v. 29, n. 2, p. 386-390, 2009.

NORTON, D.M.; McCAMEY, M.; BOOR, K.J.; WIEDMANN, M. Application of the BAX for screening/genus Listeria polymerase chain reaction system for monitoring Listeria species in cold-smoked fish and in the smoked fish processing environment. *Journal Food Protection*, v. 63, p.:343 - 346, 2000.

PADILHA, M.R.F. et al. Pesquisa de bactérias patogênicas em leite pasteurizado tipo C comercializado na cidade do Recife, Pernambuco, Brasil. *Revista da Sociedade Brasileira de Medicina Tropical*, v. 34, n. 2, p. 161-71, 2001.

RODRIGUES, C.S.; SÁ, C.V.G.C.; MELO, C.B. An overview of *Listeria monocytogenes* contamination in ready to eat meat, dairy and fishery foods. *Ciência Rural*, v.47, n. 2, e20160721, 2017.

WAACK, E.; THAM, W.; DANIELSSON-THAM, M. Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks. *Applied and Environmental Microbiology*, v. 68, n. 7, p. 3366-3370, 2002.

World Health Organization. *The burden of foodborne diseases is substantial* Disponível em: <http://www.who.int/foodsafety/areas_work/foodborne-diseases/ferginfographics.pdf?ua=1> Acesso em 10. jul. 2017.

ZENI, M.P. et al. Influência dos microrganismos psicrotróficos sobre a qualidade do leite refrigerado para produção de UHT. *Unoesc & Ciência - ACET*, v. 4, n. 1, p. 61-70, 2013.