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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 the 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.

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 -0.4ºC to 50ºC 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 Amphotericin 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; HAUN, 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; Gröhn; 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, Gelinski; 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; Ceballos (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, it 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-Broseta 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.

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