Listeria monocytogenes IN COW'S MILK PRODUCED BY FAMILY-OWNED DAIRY FARMS

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ABSTRACT

Refrigeration is an important milk preservation method. However, milk quality may deteriorate if the product is refrigerated for long periods, mainly due to the growth of psychrotrophic bacteria. This group of microorganisms includes pathogenic genera, most notably *Listeria monocytogenes.* The detection of this bacterium in food is important, given its pathogenic effects on human and animal health and also its economic relevance. This study focused on detecting the presence of *L. monocytogenes* in milk samples collected at small family-owned dairy farms. Samples were cultivated on PALCAM and ALOA agars for microbiological analysis and a molecular analysis by polymerase chain reaction was performed for the detection of *L. monocytogenes*. Despite the negative results obtained in both these analyses, further studies are recommended to confirm or refute the negligible effect of *L. monocytogenes* on small dairy farms.

Keywords: raw milk, listeriosis, microbiology, molecular biology, diagnosis.

Listeria Monocytogenes EM LEITE DE VACA PRODUZIDO POR PROPRIEDADES LEITEIRAS FAMILIARES

RESUMO

A refrigeração é um método importante de preservação do leite. No entanto, a qualidade do leite pode piorar se o produto for mantido sob refrigeração por longos períodos, principalmente devido ao crescimento de bactérias psicrotróficas. Este grupo de microorganismos inclui gêneros patogênicos, mais notavelmente *Listeria monocytogenes*. A detecção dessa bactéria em alimentos é importante, tendo em vista seus efeitos patogênicos na saúde humana e animal e também sua relevância econômica. Este estudo teve como objetivo detectar a presença de *L. monocytogenes* em amostras de leite coletadas em pequenas fazendas leiteiras de propriedade familiar. As amostras foram cultivadas em ágar PALCAM e ALOA para análise microbiológica e análise molecular por reação em cadeia da polimerase

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para detecção de *L. monocytogenes*. Apesar dos resultados negativos obtidos em ambas as análises, estudos adicionais são recomendados para confirmar ou refutar o efeito insignificante de *L. monocytogenes* em pequenas fazendas leiteiras.

Palavras chaves: leite cru, listeriose, microbiologia, biologia molecular, diagnóstico.

Listeria monocytogenes EN LECHE DE VACA PRODUCIDA POR GRANJAS LECHERAS FAMILIARES

RESUMEN

La refrigeración es un método importante de conservación de la leche. Sin embargo, la calidad de la leche puede deteriorarse si el producto se refrigera durante períodos prolongados, principalmente debido al crecimiento de bacterias psicrotróficas. Este grupo de microorganismos incluye géneros patógenos, más notablemente *Listeria monocytogenes*. La detección de esta bacteria en los alimentos es importante, dados sus efectos patógenos sobre la salud humana y animal y también su relevancia económica. Este estudio se centró en detectar la presencia de *L. monocytogenes* en muestras de leche recolectadas en pequeñas granjas lecheras familiares. Las muestras se cultivaron en agares PALCAM y ALOA para análisis microbiológico y se realizó un análisis molecular por reacción en cadena de la polimerasa para la detección de *L. monocytogenes*. A pesar de los resultados negativos obtenidos en ambos análisis, se recomiendan más estudios para confirmar o refutar el efecto insignificante de *L. monocytogenes* en pequeñas granjas lecheras.

Palabras clave: leche cruda, listeriosis, microbiología, biología molecular, diagnóstico.

INTRODUCTION

Due to its composition and nutritional characteristics, milk is considered an excellent medium for the multiplication of pathogenic microorganisms that can compromise its quality and alter its components (1), making it a viable route of transmission of pathogens and their toxins to humans (2).

Bacterial contamination of raw cow milk may occur inside the animal itself, in the environment, or during handling in the production line. All these situations are directly related to hygienic milk production (3).

An efficient method for preserving raw milk is refrigeration. However, if refrigerated for long periods, the quality of raw milk may deteriorate due to the proliferation of psychrotrophic bacteria (4). These microorganisms can develop at temperatures below 7°C, although this development varies among species (5). Bacterial multiplication is favored by poor water quality, inadequate hygiene procedures and bovine mastitis (6,7,8).

These bacteria may be the cause of different foodborne diseases transmitted through dairy products, raw milk and even pasteurized milk. The group of psychrotrophic pathogens

include *Listeria monocytogenes*, *Yersinia enterocolitica* and some strains of *Bacillus cereus* (9,5).

In addition to *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. ivanovii* have also been reported in food and in the environment (7,10). However, only *L. monocytogenes* is potentially pathogenic for humans. *L. monocytogenes* is divided into 19 serological groups, among which serotypes 1/2a, 1/2b and 4b are the main ones responsible for cases of listeriosis in humans (11).

The continuous surveillance of pathogenic microorganisms and evaluation of hygienic conditions and animal feed quality are considered necessary, given the economic importance of psychrotrophic bacteria and the public health risks posed by their growth and high count in milk samples, especially when stored under refrigeration, as well as the zoonotic potential of *L. monocytogenes* and the risk of contamination of dairy products.

The purpose of this study was to investigate the presence of *Listeria monocytogenes* in raw cow's milk produced by small family-owned dairy farms. To this end, milk samples were isolated on selective PALCAM and ALOA agar media before and after cooling to 4ºC for 48 hours, simulating the cooling conditions in expansion tanks at the farms. Another objective was to detect *L. monocytogenes* based on a molecular technique - polymerase chain reaction (PCR)- in these milk samples.

MATERIAL AND METHODS

Ethics statement

This research was approved by the Ethics Committee on Animal Use of School of Veterinary Medicine and Animal Science, São Paulo State University and is registered under number 91/2016.

Collection of milk samples from dairy farms

A total of 95 milk samples were collected from 19 family-owned dairy farms. The samples were stored in a direct expansion tank located in the state of São Paulo. The 19 dairy farms were selected based on convenience and the farm owners' interests.

The dairy farm owners were asked to answer a simplified questionnaire aimed at evaluating the management and procedures involved in milk production at each farm.

The main economic activity of the 19 farms is milk production from pasture raised dairy cattle. The cows were cross breeds, and the milk samples were collected, mixed, and stored in the direct expansion tank. None of the dairy farms receive technical assistance or quality bonuses. Moreover, they do not apply the California Mastitis Test (CMT), nor do they use milking pipeline systems.

After homogenizing the milk in the milk cans at each farm, samples of 50 mL of cow milk were collected aseptically, using properly sterilized stainless steel ladles, and were stored in sterile 50mL tubes. Sampling was performed twice a week, with a total of five samples collected from each farm. The samples were placed in cooler boxes and transported from the collection site to the Mastitis Research Center at the School of Veterinary Medicine and Animal Science (FMVZ) of UNESP in Botucatu, SP.

At the laboratory, the samples were cultured for the detection of *Listeria monocytogenes* and their DNA was extracted for molecular analysis. They were then stored at 4°C for 48

hours. Subsequently, the procedures for bacterial isolation and bacterial DNA screening of the agent were repeated in order to evaluate the effect of refrigeration on bacterial isolation.

At least one raw milk sample, several bulk tank milk samples and one sample from the expansion tank were incubated for 72 hours at 37°C on 8% bovine blood agar and total bacterial counts (TBC) were made to investigate possible contaminating microbiota.

Detection of Listeria spp. in microbial cultures

Twenty-five mL of each sample were homogenized in 225 mL of listeria enrichment broth (LEB, from Oxoid®) and incubated at 30°C for 4 hours. Subsequently, 0.5% (1.8mL) nalidixic acid, 1% cycloheximide (1.15mL) and 0.5% acriflavine (0.455mL) were added as selective agents and the samples were incubated at 30°C for 48 hours. After 24 and 48 hours, the incubated samples were seeded onto ALOA agar (Sigma®) and PALCAM agar (Oxoid®) using a 10µl disposable inoculating loop, and incubated at 35ºC for up to 48 hours. After that, up to five characteristic colonies (blue, ALOA and black agar with black halos on PALCAM agar) were place in a tube with tryptone soya yeast extract agar (TSYEA) (TSA plus 0.6% yeast extract) and incubated at 35ºC for 24 hours. After this, preliminary identification tests were conducted, such as Gram staining (Gram positive rods), catalase proof (catalase positive), esculin hydrolysis or agar motility in order to observe isolated "umbrella-like" growth patterns.

To evaluate the development of *Listeria* in selective medium using possibly contaminated samples, as well as the behavior of the bacterium at different culture temperatures, all the samples of one of the samplings were cultured in duplicate. After culturing in LEB and adding selective agents, these samples were also cultured on PALCAM and ALOA agars and incubated at 10°C for 15 days.

DNA Extraction

Directly from milk

DNA was extracted directly from raw milk using a commercial Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare®) adapted for the diagnosis of zoonotic diseases at the Laboratory of Applied Molecular Biology of UNESP in Botucatu, SP. DNA extraction was performed on 200µL milk aliquots, using a 1.5mL DNAse and RNAse free microtube, as follows:

The material was centrifuged at 10,000g for 1 minute, after which the fat was removed using a sterile swab. The supernatant was pipetted using sterile DNAse and RNAse free tips. After performing this procedure, the manufacturer's recommendations were followed.

Listeria Enrichment Broth– LEB

Each sample previously incubated in LEB was also subjected to DNA extraction. To this end, 300 µL were pipetted into microcentrifuge tubes for DNA extraction and purification, using acommercial Illustrator Genomic Prep Mini Spin Kit (GE Healthcare®), according to the manufacturer's instructions.

Amplification of nucleic acid (PCR)

The 25 µL volume was composed of 2.5 µL of PCR Buffer 10x (Invitrogen®), 0.75 µM magnesium chloride (Invitrogen®), 200 µL of each dNTP, 1 U of Taq DNA Polymerase, 10 picomoles of *inlA* primer (Rousseaux, Olier, Lemaître, Piveteau, & Guzzo, 2004), ultrapure autoclaved water (qsp) (Milli-Q Plus, Millipore) and 3 µL of the DNA sample.

Incubation was performed in a Geneamp 9700 PCR System (Applied Biosystems), applying initial cycle parameters of 94°C for 5 minutes for initial denaturation, followed by 35 cycles at 94°C for 30s, 60°C for 30s and 72°C for 30s.

The final extension temperature was 72°C applied for 4 minutes. A negative control was used in all the reactions, using ultrapure water in place of nucleic acid. Two standard strains of *Listeria monocytogenes* (ATCC 19.117) were used as positive control.

Preview of PCR amplification products

The products of PCR amplification were subjected to agarose gel electrophoresis on 1.5% Tris EDTA (TBE) and developed with Nancy-520 DNA Gel Stain (Sigma Life Science®). DNA fragments were analyzed by comparing them with 50 or 100 bp DNA markers, and were photographed using an image analyzer (GelDoc-It Imaging System (UVP)).

RESULTS

Despite the growth of blue colonies (Figure A1), in none of the samples was it possible to identify *Listeria monocytogenes*, nor the suggestion of growth of bacteria or black colonies (Figure A2) on ALOA agar, or of the pathogenon PALCAM agar.

Due to the presence of contaminants in the cultures, tests were carried out using standard samples of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* to observe the characteristic development of *L. monocytogenes* in selective media in the presence of other pathogens. No *L. monocytogenes* was identified among the microorganisms developed on either PALCAM agar or ALOA agar.

Considering the high contamination rate of the samples, a total bacterial count (TBC) was made of the milk samples from the bulk and expansion tanks. The sample from the expansion tank and four samples (21%) of the 19 bulk tank samples presented mesophilic aerobic colony counts higher than $3x10^5$ CFU/mL.

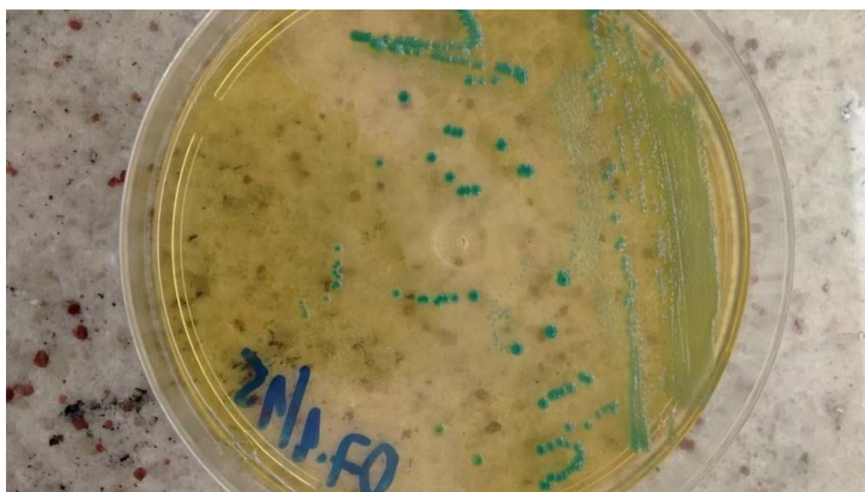

Figure A1. Blue colonies suggesting the presence of *L. monocytogenes* isolated on ALOA agar in samples of cow's milk from Botucatu, SP, 2016-2017.

Aiming to facilitate the selective isolation of *L. monocytogenes* from contaminated milk, samples cultured in LEB were diluted in saline solution to reduce the contaminating microbiota. However, this procedure proved to be unsuccessful.

All the samples were PCR negative for *L. monocytogenes*, regardless of whether the DNA extraction was performed with raw milk or LEB. Positive controls were used for DNA extraction and PCR, and tests were performed using controls cultured in LEB and raw milk prior to extraction. Visualization of the amplified products revealed that all of them yielded positive results.

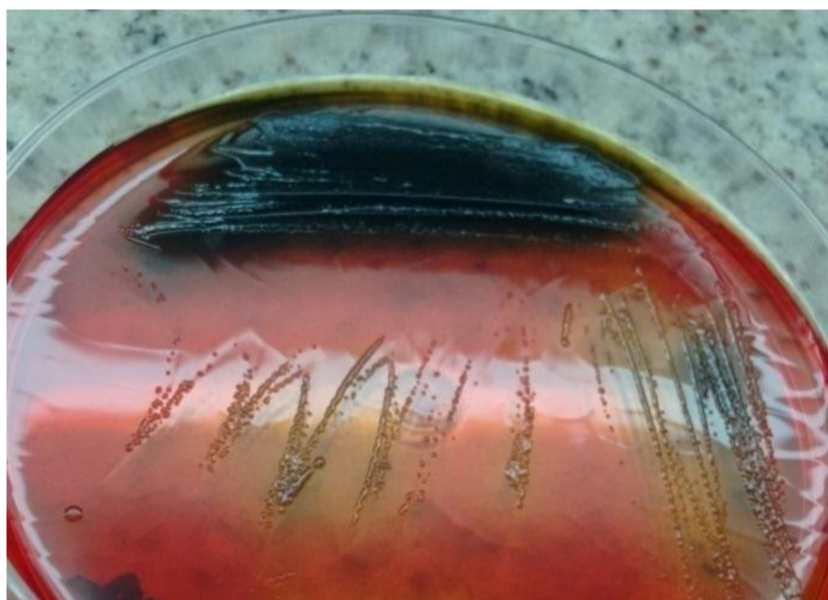

Figure A2. Black colonies suggesting the presence of *L. monocytogenes* isolated on PALCAM agar in cow's milk samples from Botucatu, SP, 2016-2017.

DISCUSSION

In Brazil, listeriosis is a non-notifiable zoonotic disease. Epidemiological data on listeriosis are out of date, and its profile and particularly its etiology are unknown. The literature is limited to a few reports about the detection of pathogens, which confirm the presence of *L. mocytogenes* in foods in Brazil. This study was developed in order to add information on the subject and report the presence of *L. mocytogenes* in a specific food, i.e., milk. The cultures and molecular biology analysis of the 95 samples collected from different dairy farms yielded no positive results.

The dairy farms involved in this study use pasture raising as the main milk production system, although silage has been identified as an important route of transmission of listeriosis among farm animals. Some authors argue that poor grazing and herd grouping may also be risk factors for the transmission of this disease (12,13). The transmission of meningoencephalitis caused by *L. monocytogenes* in ruminants reportedly does not occur in Brazil in association with silage feeding (14,15).

The fact that raw milk is initially stored in bulk milk tanks is a factor of concern, given the storage temperature that this material may reach before it is mixed in expansion tanks,

since it is known that bacterial counts are strongly influenced by temperature. Milk should be stored at dairy farms at temperatures below 4.5ºC (16).

It was observed that at 47% (9/19) of the dairy farms, the milk was stored in milk cans for at least one hour without refrigeration before it reached the community expansion tank. Although *L. monocytogenes* is a psychrotrophic bacterium, the multiplication of mesophilic bacteria that compete with *Listeria* can inhibit its proliferation. In addition, the dairy farms did not receive technical assistance, did not perform pre-dipping or post-dipping, the CMT, and had no milking line, which could favor the proliferation of pathogenic microorganisms commonly present in milk and handling equipment, and that could have negatively influenced the isolation of the pathogen in the milk samples (14).

After improving cow udder and teat hygiene prior to milking, Santana et al. (17) observed a 99% reduction in mesophilic and psychrotrophic aerobic counts. Similarly, Fonseca and Santos (14) also reported a reduction of up to 80% in the total bacterial count of milk, which they attributed to the practice of pre-dipping.

Among the dairy farms involved in this study, 78.2% (15/19) performed manual milking, which hypothetically already decreases the risk of *L. monocytogenes*. The reason for this is that, although it is an environmental microorganism, milk is usually contaminated in the pipelines or equipment of high yield farms, which are materials on which *L. monocytogenes* can form a biofilm (18). The small dairy farms of this study, which had on average 24 lactating cows producing a daily average of 80 liters, used little technified services.

The absence of *L. monocytogenes* can be attributed to the presence of contaminants in the samples, which came from the group of 19 dairy farms and were therefore contaminated with different microorganisms. Moreover, storing the milk in unrefrigerated cans until it reached the expansion tank may also have contributed to this result.

Langoni et al. (19) identified the presence of *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus* sp., *Bacillus* spp. and *Pseudomonas aeruginosa* in a microbiological examination of milk samples from a group of dairy farms in the municipality of Botucatu, SP. Their study revealed a high degree of contamination of milk from dairy farms that use the same production system as the farms evaluated in this study.

In contrast, Catão e Ceballos (20) isolated high proportions of *Listeria* spp., ranging from 66.6% to 86.6%, in samples of raw milk and skim milk. They reported finding high levels of fecal contamination, and a wide diversity of *Listeria* species in the raw milk, predominantly *L. monocytogenes*.

The FDA protocol used in this study did not succeed in inhibiting competing microorganisms. To evaluate this methodology, Besse et al. (21) used Fraser broth to cultivate samples of salmon artificially contaminated with *Listeria* and introduced changes to minimize inhibitory interactions. They reported that, unlike the current practice of 48 hours, 24 hours of incubation of samples naturally contaminated with *Listeria* spp. sufficed to reach the maximum population level. They also demonstrated that this isolation of only 24 hours enabled the optimal recovery of multiple strains of the pathogen, suggesting the possibility of further reducing the enrichment time.

Competitive inhibition has already been described by other authors. Wei et al. (22) reported that the multiplication of *L. monocytogenes* was competitively inhibited by *Yersinia enterocolitica*. Farrag e Marth (23) observed that *P. aeruginosa* may also have an inhibitory effect on *L. monocytogenes*. The findings of this study are corroborated by those of Bang et al. (24), who state that the isolation of *L. monocytogenes* may be hindered by the

multiplication of other bacteria, although they used BHI and TSBYE media, which are not selective for *L. monocytogenes*.

The PCR results obtained from milk samples or LEB were negative. However, PCR yielded positive results in the tests involving samples of either raw milk or LEB contaminated with *L. monocytogenes* ATCC. As discussed previously, contaminating microbiota can reportedly interfere with the multiplication of *Listeria* species (25). The reduction in pathogen multiplication also reduces the amount of DNA available for PCR detection.

Peres et al. (26) did not detect *L. monocytogenes* by PCR in LEB after 24 hours of incubation in samples of whole raw milk or pasteurized skimmed milk. However, after 48 hours of enrichment in this broth, the bacterium was identified by PCR in skim milk, albeit not in whole raw milk.

On the other hand, in the tests carried out in this study, PCR yielded positive results in both pasteurized and whole raw milk samples experimentally contaminated with *L. monocytogenes* ATCC. In Peres et al.'s study (26), the authors observed that the sensitivity of the traditional PCR methodology was reduced in raw milk with high counts of mesophilic aerobes. It is possible that this may also have been the case in our study, given that better results were obtained when the reaction was performed with material obtained directly from the suspect colony in solid medium. This suggests that phenotypic identification tests of *L. monocytogenes* may be replaced by the PCR technique, reducing the time spent in identifying the bacterium.

CONCLUSIONS

It is probable that the high contamination rate found in the cultures of bulk milk samples as well as the TBC found in the community expansion tank negatively affected the isolation and/or molecular detection of *Listeria monocytogenes* in milk samples.

The low microbiological quality and high cellularity of the milk indicate the need to adopt measures for the prevention and control of mastitis in the sampled herds, as well as health education projects aimed at hygienic milk production.

Despite the negative culture and PCR results, the pathogenicity of *L. monocytogenes* to animals and the public health risks it poses justify the continuation of studies aimed at the epidemiological surveillance of the agent in animal products such as milk.

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REFERENCES

1. Merussi GD, Maffei DF, Catanozi MDPLM. Surtos de gastroenterite relacionados ao consumo de laticínios no estado de São Paulo no período de 2000 a 2010. Alimentos Nutr. 2012;23(4):639-45.

2. Guimarães FF, Nóbrega DB, Richini-Pereira VB, Marson PM, Pantoja JCF, Langoni H. Enterotoxin genes in coagulase-negative and coagulase-positive staphylococci isolated from bovine milk. J Dairy Sci. 2013;96(5):2866-72.

3. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC. A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control. 2017;75:1-13.

4. Martins EDS, Reis NEV. Qualidade microbiológica do leite cru em função de medidas profiláticas no manejo de produção. Rev Bras Tecnol Agroind. 2014;8(2):216-22.

5. Sørhaug T, Stepaniak L. Psychrotrophs and their enzymes in milk and dairy products: quality aspects. Trends Food Sci Technol. 1997;8(2):35-41.

6. Eneroth Ǻ, Ahrné, Molin G. Contamination of milk with Gram-negative spoilage bacteria during filling of retail containers. International J Food Microbiol. 2000;57(1/2):99-106.

7. Jay JM. Foodborne listeriosis. In: Jay JM. Modern food microbiology. 6th ed. Gaithersburg: An Aspen Publication; 2000. p.485-504.

8. Murphy SC, Boor KJ. Trouble-shooting sources and causes of high bacteria counts in raw milk. Dairy Food Environ Sanit. 2000;20(8):606-11.

9. Shah NP. Psychrotrophs in milk: a review. Milchwissenschaft. 1994;49(8):432-7.

10. McLauchlin J. The identification of *Listeria* species. Int J Food Microbiol. 1997;38(1):77-81.

11. Barancelli GV, Silva-Cruz JV, Porto E, Oliveira CAF. *Listeria monocytogenes*: ocorrência em produtos lácteos e suas implicações em saúde pública. Arq Inst Biol. 2011;78(1):155-68.

12. George LW. Listeriosis. In: Smith BP. Large animal internal medicine. 3th ed. St. Louis: Mosby; 2002. p.946-9.

13. Varnam AH, Evans MG. Foodborne pathogens: an illustrated text. St. Louis: Mosby Year Book; 1991.

14. Fonseca LFL, Santos MV. Qualidade do leite e controle de mastite. São Paulo: Lemos Editoria; 2000.

15. Schild AL. Listeriose. In: Riet-Correa F, Schild FAL, Lemos RAA, Borges JRJ. Doenças de ruminantes e equídeos. 3a ed. Santa Maria: Palotti; 2007. p.352-7.

16. Bueno VFF, Mesquita AJ, Oliveira AN, Nicolau ES, Neves RBS. Contagem bacteriana total do leite: relação com a composição centesimal e período do ano no Estado de Goiás. Rev Bras Cienc Vet. 2008;15(1):40-4.

17. Santana EHW, Beloti V, Barros MAF, Moraes LB, Gusmão VV, Pereira MS. Contaminação do leite em diferentes pontos do processo de produção: I. Microrganismos aeróbios mesófilos e psicrotróficos. Semin Cienc Agrar. 2001;22(2):145-4.

18. Latorre AA, Van Kessel JS, Karns JS, Zurakowski MJ, Pradhan AK, Boor KJ, et al. Biofilm in milking equipment on a dairy farm as a potential source of bulk tank milk

contamination with *Listeria monocytogenes*. J Dairy Sci. 2010;93(6):2792-802.

19. Langoni H, Sakiyama DTP, Guimarães FF, Menozzi BD, Silva RC. Aspectos citológicos e microbiológicos do leite em propriedades no sistema orgânico de produção. Pesqui Vet Bras. 2009;29(11):881-6.

20. Catão RMR, Ceballos BSO. *Listeria* spp., coliformes totais e fecais e *E. coli* no leite cru e pasteurizado de uma indústria de laticínios, no estado da Paraíba (Brasil). Cienc Tecnol Alimentos. 2000;21(3):281-7.

21. Besse NG, Audinet N, Kérouanton A, Colin P, Kalmokoff M. Evolution of *Listeria populations* in food samples undergoing enrichment culturing. Int J Food Microbiol. 2005;104(2):123-34.

22. Wei QK, Fang TJ, Chen WC. Development and validation of growth model for *Yersinia enterocolitica* in cooked chicken meats packaged under various atmosphere packaging and stored at different temperatures. J Food Prot. 2001;64(7):987-93.

23. Farrag SA, Marth EH. Behavior of *Listeria monocytogenes* when incubated together with *Pseudomonas* species in tryptose broth at 7 and 13°C. J Food Prot. 1989;52(8):536-9.

24. Bang J, Beuchat LR, Song H, Gu MB, Chang H-I, Kim HS, et al. Development of a random genomic DNA microarray for the detection and identification of *Listeria monocytogenes* in milk. Int J Food Microbiol. 2013;161(2):134-41.

25. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and *Listeria monocytogenes*: a review. FEMS Microbiol Rev. 2005;29(5):851-75.

26. Peres ND, Lange CC, Brito MAVP, Brito JRF, Arcuri EF, Cerqueira MMOP. Detecção de *Listeria monocytogenes* pela técnica de PCR em leite contaminado artificialmente. Arq Bras Med Vet Zootec. 2010;62(4):973-9.

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