Revista MVZ Córdoba
ISSN: 0122-0268
ISSN: 1909-0544
revistamvz@gmail.com
Universidad de Córdoba
Colombia

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Revista MVZ Córdoba, vol. 23, no. 3, 2018
Universidad de Córdoba, Colombia
Available in: http://www.redalyc.org/articulo.oa?id=69357037010
DOI: https://doi.org/10.21897/rmvz.1375

Originales

Listeria spp., in churn storage of raw cow's milk in Tunja - Boyacá

Listeria spp., en cantinas de almacenamiento de leche cruda de vaca en Tunja – Boyacá

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DOI: https://doi.org/10.21897/rmvz.1375
Redalyc: http://www.redalyc.org/articulo.oa?id=69357037010

Received: 07 August 2017
Accepted: 04 June 2018

Abstract:

Objective. To determine the relationship between the type of detergent, time use of the churn and the type of water used to wash storage churns and the presence of *Listeria spp*., in samples collected from storage churns of raw cow's milk, in farms of Tunja-Boyacá-Colombia. **Materials and methods**. 293 samples were collected by non-probabilistic sampling at convenience, in a period of time of 9 months. Isolation of *Listeria spp*., was performed by microbiological methods and species identification using biochemical tests. A questionnaire was applied to assess the associated factors (the type of detergent, time use of the churn and the type of water used to wash storage churns). **Results**. The prevalence of L. monocytogenes was 2.7% (n=8). No statistically significant association was found between the variables related to cleaning of churns and the presence of *L. monocytogenes*. **Conclusions.** The existence of L. monocytogenes in raw milk was demonstrated, being the prevalence found lower than those reported in national and international studies. It is evidenced the circulation of species of Listeria in the dairy production chain in Boyacá. On the other hand, it is the first overview of *L. monocytogenes* and *L. ivanovii* showing the need for the implementation of control measures in the dairy industry.

Keywords: Dairy products, food inspection, food safety, food storage, Gram-positive bacteria.

Resumen:

Objetivo. Determinar la relación entre el tipo de detergente, el tiempo de uso de la cantina y el tipo de agua utilizada para lavado de las cantinas y la presencia de *Listeria spp*., en muestras recolectadas a partir de cantinas de almacenamiento de leche cruda de vaca, en fincas del municipio de Tunja-Boyacá-Colombia. **Materiales y métodos.** Se recolectaron 293 muestras mediante muestreo no probabilístico a conveniencia, en un periodo de nueve meses. El aislamiento de *Listeria spp*., se realizó por métodos microbiológicos y la identificación de especies utilizando pruebas bioquímicas. Se aplicó un cuestionario para evaluar los factores asociados (tipo de detergente, tipo de agua y tiempo de uso de las cantinas). **Resultados.** La prevalencia de *L. monocytogenes* fue de 2.7% (n=8). No se encontró asociación estadísticamente significativa entre las variables relacionadas con la limpieza de las cantinas y la presencia de *L. monocytogenes*. **Conclusiones.** Se demostró la existencia de L. monocytogenes en leche cruda, siendo la prevalencia encontrada inferior a las reportadas en estudios nacionales e internacionales. Se evidencia la circulación de especies de Listeria en la cadena productiva láctea del departamento de Boyacá. Por otra parte, es el primer panorama de *L. monocytogenes* y *L. ivanovii* mostrando la necesidad de la implementación de medidas de control en la industria láctea.

Palabras clave: Almacenamiento de alimento, bacterias Gram positivas, inocuidad de los alimentos, inspección de alimentos, productos lácteos.

INTRODUCTION

Food security is an important characteristic for dairy customers; pasteurization protects the consumers from potential dangers when they consume raw milk, for instance foodborne diseases (1).

Listeriosis considered as a foodborne disease is caused by *Listeria monocytogenes*, from this disease numerous outbreaks have been reported (2) mainly caused by human contamination with Listeria's pathogenic strains, by consuming raw milk, derivatives or by the ingestion of processed contaminated food post-processing (3). The food cross contamination is produced due to the access of *L. monocytogenes* through the clothing, footwear, hands and the worker's tools, as well as through the equipments and materials used in their processing; mostly for the capacity that this bacteria has to develop biofilms on inert surfaces when the washing and disinfection process are unsuitable (4,5), being able to persist in the environment over long periods of time, for even more than 10 years.

To identify the source of contamination of raw milk with *Listeria spp*., the storage tanks must be reviewed since the presence or absence of the bacteria in the milk might be determined by the farm's storage conditions and hygienic environments. Considering the above, the high consumption of raw milk, the low public health's reports of this pathogen in the state and the use of churns for the storage, this paper was raised with the objective of identifying the relationship between the type of detergent, the churn's use of time, and the type of water used to wash the churns and the presence of *Listeria spp*., in collected samples from raw milk churns, in some farms of Tunja, Boyaca, Colombia.

MATERIALS AND METHODS

Type of study. It was developed a quantitative, descriptive and cross-sectional study.

Sample. The sample size calculated was 300, in total, 293 samples of raw cow's milk from the dairy livestock in Tunja. The 7 remaining samples were excluded due to the fact that they did not meet the conditions established by the protocol for the isolation of *Listeria* (insufficient volume, < 25ml). The collection was carried out between October 2014 and June 2015.

Samples collection. It was made under aseptic conditions directly from the storage churn of the farms included in the study; the churns were transported by means of refrigeration to the Universidad de Boyacá Microbiology Laboratory, where they were stored at 4°C until the processing. At the same time of the collection, a questionnaire was applied in order to inquire into the water, type of detergent and time that the churn is used.

Laboratory methods. The samples were processed in this way: A pre-enrichment was made, for which, 25 ml of sample were inoculated in 255 ml of enriched Fraser broth (Oxoid®), it was homogenized by shaking at 150 rpm during 2 minutes (Laboratory shaker incubator 10 - 500 rpm IKA - KS 4000) and they were incubated at 4°C for 15 days. After 15 days, they were spread in enriched PALCAM agar (Oxoid®) and they were incubated at 37°C during 24 hours, ending with their respective reading. For the processing of each batch, it was included both a negative control (pasteurized milk sample) and a positive control (*L. monocytogenes* ATCC 7744). The positive presumptive colonies were tested qualitatively by means of the immunological rapid test (Merck®) for detection of *Listeria monocytogenes* in food. When the rapid test was positive the presence of Listeria was confirmed by the following methods: Gram staining, mobility at 25°C catalase, hemolysis, rhamnose and xylose acid production, nitrate reduction; once the presence of Listeria was confirmed, the biochemical characterization was carried out by using the kit (Oxoid®), which allowed the identification of the different species of Listeria. The analysis of the questionnaires was done with the program SPPS® version 20.

Ethical aspects. Milk samples were collected with veterinary assistance. The investigation was approved by the Bioethics Committee at Universidad de Boyacá, according to the memo CB085 set in March, 2014.

RESULTS

The prevalence of microorganisms isolated in the 293 samples analyzed was 32.76%, of which only 2.7%, which corresponds to *L. monocytogenes*, are important in public health. The 67.24%, that is 197 samples, were negative for microorganism as can be appreciated on table 1.

TABLE 1

Table 1. Microorganisms isolated from storage churns of raw milk in Tunja-Boyacá

Isolated microorganisms	Frequency	Prevalence (%)
Bacillus brevis	3	1.02
Bacillus megaterium	11	3.75
Corynebacterium aquaticum	15	5.12
Enterococcus faecium	8	2.73
Enterococcus raffinosus	2	0.68
Enterococcus solitarius	15	5.12
Lactococcus lactis	1	0.34
Listeria grayi	1	0.34
Listeria ivanovii	3	1.02
Listeria monocytogenes	8	2.73
Listeria seeligeri	21	7.17
Listeria welshimeri	3	1.02
Streptococcus intermedius	3	1.02
Streptococcus pyogenes	3	0.34
Negatives	197	67.24
Total samples	293	

Regarding to the type of component used for the cleaning, there is no difference in using a degreasing agent (51%) and a detergent one (49%). In terms of the type of detergent, from the 144 farms that reported using it, 75% (n=108) used alkaline detergent and 25% (n=36) acid detergent. On the other hand, when analyzing the period of use of the churns, it was found that 70% IC 95% (64.75% - 75.24%) of the respondents expressed that they were using the churns one year ago or less and 30% IC 95% (24.75% - 35.24%), declared that they were already using the same churns over a year. In the 35% (n=103) of the cases, the owners of the farms used aqueduct, a 10% (n=29) used treated water, but others employed rainwater 23% (n=67) or river/stream water, 32% (n= 94).

When analyzing the relationship between the detergent used for cleaning the churns (2=2.445, p=0.118), the period of use (2=0.224, p=0.636), the type of water (2=0.087, p=0.767) and the presence of the microorganism, any statistically significant association was found as can be seen on table 2.

TABLE 2

Table 2. Factors related to the use of churns versus positivity for *Listeria monocytogenes*.

Variable		PL +	PL -	IC (95%)	Total	c2 Fisher	Value p	OR - IC
Type of detergent	Degreasing	2	147	45.27% - 56.72%	149 (51%)	2.445	0.118	0.293 (0.056 - 1.526)
	Detergent*	6	138	43.27% - 54.72%	144 (49%)			
Time of use of the churns	Less than or equal to one year	5	200	64.75% - 75.24%	205 (70%)	0.224	0.636	0.692 80.154 - 3.103)
	Greater than one year	3	85	24.75% - 35.24%	88 (30%)			
Time of water used to wash the churns	Treated or aqueduct water	4	128	39.30% - 50.69%	132 (45%)	0.087	0.767	1.244 (0.293 - 5.280)
	Rain or river water	4	157	49.30% - 60.69%	161 (55%)			

*Alkaline or acid; PL=Presence of Listeria monocytogenes;

DISCUSSION

Isolation of *Listeria spp*., from samples of different origins, sometimes could be extensive and difficult, since it grows slowly and there are different factors that disable its growing, among them, a high level of milk microbial contamination, a presence of residual of chemical substances and inhibitors specifically released by the autochthonous microbiota present in the samples analyzed. Within the genres that are part of this microbiota are: *Enterococcus* and *Lactobacillus*, which interfere during the phase in which the enrichment broth Listeria is used for its recovery, inhibiting its growth competitiveness (6). Likewise, the *L. monocytogenes* usually appears in the milk and the derivatives on the low concentrations (6). In this study, microbiota was also isolated, specially the bacteria from the Enterococcus (Table 1), a factor that is possibly the cause of the low prevalence of this.

Since the last century and up to this date, no study has been conducted whose research object lies in determining the relationship between the use of detergents and the presence of *L. monocytogenes*. A pilot study was conducted in 1989 to test the efficiency of several disinfectants or detergents against three strains of *L. monocytogenes* and one strain of *L. innocua* using water and raw milk, and synergistic effects were observed between the active agents and the matrix. It was evident that products containing iodine, peroxide or quaternary ammonium as active agents demonstrated to be efficient, even at relatively low concentrations (7).

In a research carried out in three farms where the presence of the bacterium was evidenced, it was possible to control its growth by continuously washing the churns with an alkaline detergent (8).

Several sanitizing and disinfecting agents used in the food industry have demonstrated to be effective against L. monocytogenes cells in suspension. However, the formation of biofilms on surfaces and the presence of organic matter in them reduces the effectiveness of disinfectants (9,10).

The time of use of the churn is a factor related to the formation of biofilms. In this regard, the literature mentions that one of the main problems in the food industry is the survival of pathogenic or altering microorganisms derived from insufficient disinfection of surfaces or instruments in contact with food (11).

The presence of biofilms in pipes, equipment and materials is a very common issue because they can be formed on any surface, including plastic, glass, wood, metal and food. Since these formations may contain pathogenic microorganisms and have a higher resistance to disinfection, probabilities of product

contamination increase and thus, the risk of food infections; for this reason, the presence of biofilms in the contact surfaces of the food industry constitutes an obvious risk to human health (12).

In the storage tanks, as well as in the raw milk production devices without sufficient cleaning have origin the microbial community, some of these bacteria are able to generate biofilms that allow the growth of pathogens like *L. monocytogenes*, which can cause continuous contamination of food processing plants. In the year 2013, Weiler et al., analyzed different strains of L. monocytogenes found in raw milk, which were biofilm forming, they also analyzed their stage of formation evidenced the time of use of milk production and storage devices (13).

This becomes a critical point for the dairy industry; bearing in mind that the quality of the milk offered depends directly on the product that is originally obtained, this means that the quality of the product offered to the consumer is conditioned to the control applied to the raw milk that is obtained in farms (14).

To conclude, the prevalence obtained (2.7%) in the study from the analysis of raw cow milk stored in churns evidences the presence of the microorganism where it was found not only the species *L. monocytogenes* but also other species of Listeria. In this paper, no association between the type of detergent used, the time of used of the churn and the type of water used to wash the churns regarding the presence of *L. monocytogenes* was found. The results of this study prove that themicrobiological quality of the milk produced in the dairy strip of Tunja is not what it was expected, which is probably directly related to the food security, since raw milk is the raw material for dairy products such as cheese and yogurt.

Some limitations were displayed in this document in relation with the sample size and some further studies are suggested to be done determining the presence of the microorganism directly from the nipple.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Acknowledgements

The authors thank the Rectory of the University of Boyacá and the Faculty of Veterinary Medicine and Animal Science of the Pedagogical and Technological University of Colombia for financial support.

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