Association Between Milking Practices and Psychrotrophic Bacteria Counts on Bulk Tank Milk

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Key words: Psychrotrophic bacteria, bulk tank milk, risk factors, sizing, fundamental

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Page No.: 13-18
Volume: 8, Issue 2, 2014
ISSN: 1993-5277
Research Journal of Dairy Sciences

Abstract: The objective of this work was to determine on-farm risk factors for psychrotrophs counts in bulk tank milk of dairy farms from Argentina. Raw milk from bulk tank was sampled and Total Psychrotrophics bacteria Count (TPC), Proteolitic Psychrotrophic bacteria Count (PPC) and Lipolitic Psychrotrophic bacteria Count (LPC) were used to assess the bacteriological quality of bulk tank milk (dependent or outcome variables). At the same time, a survey was performed asking about infrastructure conditions, equipment and milking management (independent variables). Bivariate association proofs and logistic regression was used to find association between independent variables and psychrotrophic bacteria counts. Milk cooled in plate heat exchangers or barrel tanks had 16.39 and 10.52 times more likely to have elevated TPC and PPC (up to the level established for high quality milk) than bulk milk cooling tank, respectively. When the milking man does not wash his hands while milking there were 7.81 times more likely to have higher PPC. There was not possible to find association between LPC with any of the independent variables. The only variable associated with TPC and PPC was the type of refrigeration system used at the farm being bulk milk cooling tanks the best to avoid bacterial growth. The results of this study highlight the importance of the type of cooling system used at farm being bulk milk cooling tank the equipment that generates the best microbiological quality. The election of the cooling system to be used at farm and the correct sizing of it are fundamental factors to guarantee a correct microbiological quality of the milk obtained.

INTRODUCTION

Because high-quality dairy products start with high-quality raw milk, there are continuing demands upon producers to improve their raw milk bacteria (Elmoslemany *et al*., 2009ab). Milk contamination could happen from three origins during milking time: milking machine and bulk tank, teat skin and the inside of the mammary gland. Cooling capacity of the bulk tank milk, storage temperature and time of permanence will control or not the development of the bacteria present in milk.

Raw milk storage under cooling conditions is a common practice in Argentina. For that reason, growth of mesophylic bacteria were significantly reduced. However, milk storage under 5°C allows the growth of psychrotrophs bacteria (Reinheimer *et al*., 1990).

Psychrotrophic bacteria found in cool milk include gram positive and gram negative microorganisms. The most commonly occurring psychrotrophs in raw milk are the gram-negative bacteria of which Pseudomonas spp. account for nearly 50% (Jayarao *et al*., 2004).

Generally, high levels of psychrotrophs bacteria in raw milk will contribute significant quantities of heat-stable proteases and lipases (mainly lecithinases and phospholipases) generating important flavor defects, reducing cheese production and jellification of UAT milk (Reinheimer *et al*., 1990).

Psychrotrophs are commonly found in untreated water, soil and vegetation. They are introduced into the milk as a result of contamination of milking equipment or the exterior of the udder and teats from these sources (Elmoslemany *et al*., 2009c).

The objective of this research was to determine on-farm risk factors for psychrotrophs counts in bulk tank milk of dairy farms from Argentina.

MATERIALS AND METHODS

Bulk tank raw milk was collected from 27 dairy farms including in this study based on their predisposition to collaborate with us. Farms were located in different provinces of Argentina: 15 in Santa Fe, three in Córdoba province, four in Entre Ríos province and five in Buenos Aires province.

Bulk tank milk samples were collected according to standard methodology (FIL-IDF 50C: 1995) among different seasons: from November, 2003 to March, 2004 (Summer), from April to august 2004 (Autumn and Winter) and from September to December 2004 (Spring). Samples were refrigerated until transported to the laboratory. Total Psichrotrophic bacteria Count (TPC) (FIL-IDF 101A:1991), Lipolitic Psichrotrophic bacteria Count (LPC) and Proteolitic Psichrotrophic bacteria Count (PPC) were performed.

Herds were classified according to their psychortrophs count (high or low counts), considering the following thresholds for high counts: TPC>50000 CFU mL^{-1} (Murphy and Boor, 1998). There are not a referenced thresholds for LPC and PPC and for this reason the percentile 50 of the data distribution was used (1900 CFU mL^{-1} in both cases).

For collecting data on risk factors, a questionnaire was designed. The questionnaire included the following sections: general farm demographics, infrastructure conditions, equipment and milking management. A copy of the questionnaire is available from the corresponding author upon request. The variables were categorized and considered as independent variables.

Statistical analysis: Data were collected on spreadsheets and merge into a single database using Infostat® (Universidad Católica de Córdoba). A logistic regression analyses in two stages was conducted. In the first stage, the dependent variables (TPC, LPC and PPC) were related to each explanatory variable by means of a univariate analysis (χ^2-test or Fisher test). In a second stage a logistic regression was conducted. Only variables associated with the outcome variables (χ^2-test, $p<0.10$) were included in the full model. The estimation method was maximum likelihood with a convergence criterion of 0.01 for a maximum of 10 interactions.

Variables that showed a significant association with the three types of microorganisms were analyzed with ANOVA or T-Student to determine significant differences with the logarithms of the counts. All statistical analysis were performed using Infostat® (Universidad Católica de Córdoba).

RESULTS

A total of 27 herds were evaluated for on-farm risk factors for high psychortrophs count in bulk tank milk. All dairy farms that participated in the study had some kind of milk cooling system: 60.3% of them had bulk milk cooling tank, 15.5% have barrel kind of cooling system (is a vertical cylinder with the cooling source at the bottom which make it less effective than bulk milk cooling tank at refrigerating milk because of the minor surface of contact) and 24.1% had plate heat exchangers system. About technology applied in the farm: 97.7% had automatic cleaning system of the milking machine and 88% of the milk cooling tank.

Milking routine change according to the farm but some practices are used by most of them. For example, 70.7% of the dairy farms practice the elimination of foremilk; 24.4% wash and dry off teats with towels before milking; 62.2% only wash teats; 57.8% used teat dipping after the milking time and only 4.8% used teat dipping before milking the udder.

Table 1: TPC, LPT and PPC associated variables

	Chi-square (p-values)		
Variables	RPT	RPL	RPP
Season	0.219	0.589	0.846
Dairy area	**0.015**	0.110	**0.062**
Milk room	**0.048**	**0.084**	**0.013**
Machine room	0.463	0.476	0.150
Products store	0.464	0.935	0.935
Bathrooms and dressing rooms	0.788	0.158	0.972
Walls(masonry, concrete, glazed ceramic)	0.125	0.521	0.893
Waiting yard (dust, masonry or concrete)	0.305	0.294	0.283
Milking machine maintenance (<45 days, every four month or every six month/annual)	0.300	0.201	**0.079**
Kind of cleaning (manual or mechanical)	0.528	0.971	0.971
Acid detergents cleaning frequency (2-3 times per week or weekly/every fifteen days)	**0.011**	0.890	0.546
Sanitizer use	0.616	0.175	0.695
Do you swill out the machine?	0.879	0.802	0.511
Cooling tank (plate heat exchangers, barrel or bulk milk cooling tank)	**0.021**	0.465	**0.011**
Cooling tank clean (manual, mechanical or combined)	0.895	0.261	0.708
Cleaning frequency (weekly, 3 times per week or daily)	**0.016**	0.125	0.125
Acid detergent wash of the cooling system (weekly or every 15 days vs. daily or every 2 days)	**0.012**	0.424	0.424
Cooling tank sanitization	0.882	0.408	0.515
Use of heat water to clean	0.555	0.432	0.230
Swill out before milking time	0.826	0.441	0.614
Use of apron	0.593	0.233	0.512
Use of gloves	0.202	0.358	0.662
Hands washing	0.370	0.288	**0.027**
Elimination of foremilk	**0.093**	0.349	**0.042**
Udder wash	0.158	**0.079**	0.201
Teats dry off	0.496	0.254	0.547
Pre dipping	0.568	0.351	0.617
Post dipping	0.466	0.610	0.610
Food in milking room	0.105	0.784	0.784
Water sanitization	0.493	0.356	0.595

Bold values are significant

Bulk tank milk bacteriological quality: TPC showed an average of 66,719 CFU mL^{-1}. The 73.8% of the samples were under 50,000 CFU mL^{-1}, LPC presented an average of 20,690 CFU mL^{-1} and PPC showed in average, 27,153 CFU mL^{-1} and 50% of them had >1.900 CFU mL^{-1}.

Associated factors

Total Psychrotrophic Count (TPC): the variables associated with higher counts of psychrotrophic bacteria were: dairy area (p = 0.015), milk room presence (p = 0.048), milking machine cleaning frequency with acid detergent (p = 0.011), type of milk cooling tank (p = 0.021), milk cooling tank cleaning frequency (p = 0.016), acid detergent frequency to clean-up the tank (p = 0.012) and elimination of foremilk (p = 0.093) (Table 1).

The milk area of Entre Ríos showed higher TPC than Santa Fe and Santa Fe South (p<0.05). There was no significant association between the others provinces (Table 2).

When the milking machine was cleaned weekly with acid detergent (1-3 times per week) the average of TPC was 0.4 log CFU mL^{-1} lower than farms that used the same cleaning system but every 15 days (p<0.05). A similar result was obtained with the cleaning of the cooling tank. When the acid detergent cleaning was used daily the TPC was lower than when the cleaning was made weekly or every 15 days (p<0.05) (Table 2).

Table 2: TPC according to variables that were significant to Chi-square test

Variables	Values
Dairy area	
Santa Fe	3.81^{a}
Santa Fe (South)	3.97^{a}
Córdoba	4.10^{ab}
Buenos Aires	4.24^{ab}
Entre Ríos	4.97^{b}
Milk room	
Yes	4.22^{a}
No	2.61^{b}
Acid detergent frequency of use in milking machine	
Every 15 days	4.24^{a}
Weekly or every 2 days	3.85^{a}
Cooling tank	
Plate heat exchangers or barrel	4.52^{a}
Bulk milk cooling tank	3.96^{a}
Cooling tank cleaning frequency	
Weekly	5.38^{a}
3 times a week or daily	3.96^{b}
Acid detergent frequency of use in cooling tank	
Weekly/every 15 days	4.23^{a}
Daily or every 2 days	3.74^{b}
Elimination of foremilk	
Yes	4.09^{a}
No	3.87^{a}

[a, b]significant to Chi square

Table 3: Logistic regression

Predictive variables	Significance (p-values)	OR (CI95%) only for variables with p<0.05
Total psychrotrophic count		
Dairy Area (Santa Fe)	0.471	
Córdoba	0.998	
Entre Ríos	0.998	
Buenos Aires	1.000	
Santa Fe south	0.100	
Type of cooling tank	0.028	0.061 (0.005-0.744)
Milk room	0.106	
Acid detergent wash frequency of the machine	0.491	
Elimination of foremilk	0.152	
Lipolitic psychrotrophic count		
Milk room	0.075	
Udder wash	0.075	
Proteolitic psychotrophic count		
Dairy Area (Santa Fe)	0.931	
Córdoba	0.365	
Entre Ríos	0.999	
Buenos Aires	0.999	
Santa Fe south	0.916	
Type of cooling system	0.012	0.095 (0.015-0.601)
Hands wash	0.032	0.128 (0.019-0.842)
Milk room	0.518	
Milking machine manteinance	0.344	
Elimination of foremilk	0.343	

According to the kind of cooling tank TPC was different. When the tank was a bulk milk cooling tank, the counts were lower than those registered in plate heat exchangers or barrel kind of tank (p<0.05) (Table 2).

The elimination of foremilk was the only action of milking routine that has an influence on the TPC. Dairy farms that used this practice had higher TPC (p<0.05) (Table 2).

When all the variables are introduced into a logistic regression model only the type of cooling tank was significant (p = 0.028) (Table 3). Storage milk in bulk milk cooling tanks showed an Odds Ratio (OR) of 0.061 (CI95% 0.005-0.774) comparing to other systems (plate heat exchangers or barrel. According to this result, storage milk in plate heat exchangers or barrel tanks had 16.39 (CI95% 1.34-200) more times likely to have TPC>50.000 CFU mL^{-1} than milk storage in bulk milk cooling tanks.

Lipolitic Psychrotrophic Count (LPC): The variables associated to highest levels of LPC were: milk room presence (p = 0.084) and udder wash before milking time (p = 0.079) (Table 1).

Dairy farms that had a separate milk room, independent from milking room had higher LPC than farms without it (p<0.05). Milk obtained from farms that wash udders before milking had higher LPC than those that did not make it (p<0.05) (Table 4).

None of the variables that were significant to Chi square test (p = 0.075) were significant in a logistic regression model (Table 5).

Proteolitic Psychrotrophic Count (PPC): The variables associated with the value used to characterized dairy farms were: dairy area (p = 0.062), milk room presence (p = 0.013), milking machine maintenance (p = 0.079), kind of cooling tank (p = 0.011), milking man washing hands (p = 0.027) and elimination of foremilk before milking time (p = 0.042) (Table 1). Milk samples obtained from Santa Fe, Santa Fe South, Córdoba and Buenos Aires dairy areas has lower PPC than Entre Ríos one (p<0.05) (Table 3).

Table 4: LPC according to variables that were significant to Chi-square test

Variables	Values
Milk room	
Yes	3.14[a]
No	1.98[b]
Udder wash	
Yes	3.29[a]
No	2.88[a]

Table 5: PPC according to variables that were significant to Chi-square test

Variables	Values
Milk room	
Yes	3.29[a]
No	1.54[b]
Milking machine maintenance	
Every four months or every 6 months	2.88[b]
Annual	3.53[a]
Cooling tank	
Plate heat exchangers or barrel	3.60[a]
Bulk milk cooling tank	3.06[a]
Hands wash	
Yes	3.01[a]
No	3.61[a]
Elimination of foremilk	
Yes	3.11[a]
No	3.02[a]

[a, b]significant to Chi square

Milk samples obtained from dairy farms that had a milk room separate from the other areas had higher PPC than farms that did not have it ($p<0.05$) (Table 5).

Dairy farms that make maintenance of the milking machine every four or 6 month had lower PPC than those that did it once a year ($p<0.05$). Another part of the equipment that had importance was the type of cooling tank. Milk storage in plate heat exchangers or barrel tanks had higher PPC than that storage in bulk milk cooling tank ($p<0.05$) (Table 5).

Farms where milking man washed their hands before milking time had lower PPC than farms that did not do it ($p<0.05$). Elimination of foremilk was strongly associated with the category of PPC. However, while comparing averages, no significant statistical difference was observed between farms that did or did not do this practice ($p = 0.8830$).

When the significant variables to Chi square test were included in a logistic regression model only hands washing ($p = 0.032$) and type of milk cooling system ($p = 0.012$) were significant. When the milking man washed his hands during milking routine had an or of 0.095 (CI95% 0.015-0.601) compared with those that did not do it. Milk produced in systems that did not include hands washing as a part of good milking practices had 7.81 times more risk (CI95% 1.18-52.6) of having PPC >1.900 CFU mL^{-1}.

Storage milk in plate heat exchangers or barrel kind of cooling systems had 10.5 times more risk (CI95% 1.66-66.6) to have PPC>1.900 CFU mL^{-1} than storage milk in bulk milk cooling tank.

DISCUSSION

The risk factors for elevated TPC and PPC were very similar. The type of cooling tank was positively associated with both TPC and PPC. Some authors reported that storage milk under or at 4°C do not prevent psychrotrophic bacterial growth when milk arrives to storage with high counts of these microorganisms. Psychrotrophic organisms growth at refrigerating temperatures and they are associated with poor hygiene during milking time and of the milking equipment too, reducing the storage time of milk (Murphy and Boor, 1998). Acid detergent cleaning frequency of the milking machine was associated with high TPC, but there was no chance to find a statistical difference if the cleaning was made weekly or every fifteen days.

Milking machines without appropriate maintenance generally become the cause of high bacteriological counts in milk (Murphy and Boor, 1998). This applies to different surfaces in contact with milk. In this work, lower frequency of milk equipment maintenance was associated with higher levels of PPC.

Bacterial adhesion and colonization of milk contact surfaces are considered important factors for a subsequent product contamination. Some researchers have suggested a possible contamination sequence that includes: the microorganism deposit with the subsequent adhesion to equipment surfaces, the reduction of his number by cleaning mechanisms, survival bacterial proliferation between cleaning times and contamination of milk while passing throw the machine.

On the stainless steel surface of the cooling tank there were found average psychrotrophic counts of 3.93 log CFU mL^{-1}. A significant Pseudomona contamination (most important psychrotrophic microorganism in milk) occurs because of inappropriate cleaning of milking and storage surfaces and of the transportation equipment of milk (Elmoslemany *et al*., 2009a). In the present study we proved that a periodic cleaning of cooling tank (3 times a week or daily) generate lower PPC (approximately, 1.5 log CFU mL^{-1}) than a weekly frequency of cleaning. In the same way acid detergent cleaning frequency (every 2 days or daily) generates lower PPC counts than 1 weekly. This is because this bacterial group has the ability to produce adhesives exopolysaccharides that make biofilms when the milking system stays dirty during long time. This biofilms are resistant to chemical substances used to clean the milking machine and they stay in the machine to contaminate milk.

There was only one variable significantly associated with TPC and PPC and it was the type of cooling system. Those farms that used bulk milk cooling tank showed values of TPC 0.6 log CFU mL^{-1} lower than the others cooling systems. Also the PPC was significantly lower (approximately, 0.6 log CFU mL^{-1}) than the obtained with barrel or plate heat exchangers systems. The presence of cooling systems at the farm doesn't guarantees an adequate bacteriological quality. A wrong size dimensioning of the tank has long cooling times as consequence. Milk stay at middle temperatures for long time allowing important bacteriological development since the beginning of milking until achieve 4°C. These faults are more obvious when daily milk production is high and especially when liters produced by hour are higher. Total bacterial count could be 1.5 times more if the time to get 4°C is 5 h instead of 2.

Milk contamination could come from worker hands, cow's teats and dirty udder (especially by feces, dust or a combination of both). From these factors only milking man hands are associated with PPC. Farms which workers wash their hands among milking time had lower PPC (approximately, 0.6 log CFU mL^{-1}) than those who did not do it. A study made at dairy farms of Santa Fe dairy area reported PPC of 1.44 log CFU cm^{-2} on worker hands; supporting the hypothesis that worker is a contamination source of the final product.

During milking time teats could have variable contamination level according to the place animals are keep between milking times (indoor, pastures or farmyard) and especially depending on climate conditions (Elmoslemany *et al*., 2009b, d). Under pasture conditions dust contamination is more important than teat dirt.

Effective premilking udder hygiene is important for the production of high quality milk and the control of mastitis. Between milking, the teats and udder often become soiled with manure. If the teats were not thoroughly cleaned and dried before milking, this dirt with the associated microorganisms will be transferred into the milk (Elmoslemany *et al.*, 2009a). Several studies (Galton *et al*., 1986) demonstrate that wash and dry of teats generate lower contamination on teats and lower total bacterial counts than wash and does not dry teats or does not wash at all. However, dirty or improperly washed teats and the milking environment (floors, air) are not psychrotrophic bacterial sources. This was corroborated in this study because no significant association was found between wash and dry of teats and pre and post dipping with none of the psychrotrophic groups analyzed. We only found an association between udder wash and LPC but it was not statistical significant.

CONCLUSION

The results of this study highlight the importance of the type of cooling system used at farm being bulk milk cooling tank the equipment that generates the best microbiological quality. The election of the cooling system to be used at farm and the correct sizing of it are fundamental factors to guarantee a correct microbiological quality of the milk obtained.

ACKNOWLEDGEMENTS

This research was financed by National Institute of Agricultural Technology (Project INTA 52:22007). To Mr. Emilio Walter for coordination and taken and dispatched of samples to the laboratory.

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