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Association between milking practices and psychrotrophic bacterial counts in bulk tank milk

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ABSTRACT

The objective of this work was to determine on-farm risk factors for psychrotrophic bacterial counts in bulk tank milk from dairy farms in Argentina. Raw milk samples from bulk tanks of 27 dairy farms were examined for total psychrotrophic counts (TPC), proteolytic psychrotrophic counts (PPC) and lipolytic psychrotrophic counts (LPC) (dependent or outcome variables). A survey recording infrastructure conditions, milking equipment and milking management (independent variables) was performed. Bivariate association proofs and logistic regression analyses were used to determine association between independent variables and psychrotrophic bacterial counts. Milk cooled in plate heat exchangers or barrel tanks were 16.39 and 10.52 times more likely to yield TPC and PPC above the standard established for high quality milk compared with milk cooled in bulk tanks, respectively. Periodic cleaning of cooling tanks (3 times a week or daily) was associated with lower TPC (approximately 1.5 log CFU/ml) than weekly cleaning frequency and farms where milkers did not wash their hands during milking time were 7.81 times more likely to have higher PPC. No association was found between LPC and any of the independent variables. The only variable associated with TPC and PPC in a logistic regression model was the refrigeration system used on the farm. Dairy farms that possessed bulk milk cooling tanks yielded the lowest bacterial counts. Results of this study highlight the importance of both the type of cooling system used on the farm and its adequate hygienic maintenance for obtaining low pshychrotrophic counts at dairy farm.

Key words: psychrotrophic bacteria, bulk tank milk, risk factors

RESUMEN

Asociación entre prácticas de ordeño y recuento de organismos psicrótrofos en leche de tanque de frío. El objetivo del trabajo fue determinar los factores de riesgo para altos recuentos de organismos psicrótrofos en leche de tanques de tambos de la Argentina. Se examinaron muestras de leche cruda de tanques de frío de 27 tambos, y se realizó el recuento de organismos psicrótrofos totales (PT), de psicrótrofos proteolíticos (PP) y de psicrótrofos lipolíticos (PL) (variables dependientes). Se realizó una encuesta para registrar las condiciones de infraestructura, el equipo de ordeño y las prácticas de ordeño (variables independientes). Se utilizaron pruebas bivariadas de asociación y regresión logística para determinar la asociación entre las variables independientes y los recuentos de organismos psicrótrofos. La leche enfriada en sistemas de placas de intercambio o tanques tipo cuba tuvo una probabilidad mayor de dar recuentos elevados de PT y PP (16,39 y 10,52) comparada con la enfriada en tanques tipo “panza fría”. La limpieza periódica del equipo de frío (3 veces por semana o diariamente) se asoció con bajos recuentos de PT (aproximadamente 1,5 log de UFC/ml). Los tambos cuyos ordeñadores no se higienizaban las manos durante el ordeño tuvieron una probabilidad 7,81 veces mayor de tener recuentos elevados de PP. No se encontró asociación entre el recuento de PL y las variables independientes. La única variable asociada con los recuentos de PT y PP en el modelo de regresión logística fue el sistema de enfriamiento utilizado en el tambo. El tipo de sistema de refrigeración usado y su adecuado mantenimiento higiénico son importantes para la obtención de leche con baja carga de organismos psicrótrofos en el tambo.

Palabras clave: bacterias psicrótrofas, leche de tanque, factores de riesgo

INTRODUCTION

Bacterial contamination of bulk tank milk (BTM) decreases both the shelf life of fluid milk and the quality of dairy products. Hygienic management practices during milking time are directed to keep BTM bacterial counts at acceptable levels (17). Sources of BTM bacterial contamination during milking time are the surfaces of milking equipment, the external surface of teats and udder skin, and mastitis pathogens from within the udder (16, 17). The cooling capacity of the bulk tank, storage temperature and time of permanence aim to control the growth of bacteria present in milk (8, 16).

Raw milk storage under cooling conditions became a common practice in Argentina during the 1990 decade; only refrigerated farm milk is currently accepted by dairy industries. This practice led to a significant reduction of mesophilic bacterial counts (14, 19); however, milk storage under 5 °C allows the growth of psychrotrophic bacteria (16). Psychrotrophic organisms present in cool milk include gram-positive and gram-negative bacteria. The most commonly occurring psychrotrophs in raw milk are gram-negative bacteria, of which *Pseudomonas* spp. account for more than 50 % of bacterial genera (15, 18). High levels of psychrotrophic bacteria in raw milk can yield significant quantities of heat-stable proteases and lipases, mainly lecithinases and phospholipases, generating important flavor defects, reducing cheese yield and causing ultra high temperature (UHT) milk jellification (15, 18).

Psychrotrophs are commonly found in untreated water, soil and vegetables and are introduced into the milk as a result of contamination of milking equipment or the exterior of the udder from these sources (2, 16). There is only little information dealing with the presence of psychrotrophic bacteria in BTM in Argentina (6) or bulk-collected raw milk (18). Risk factors associated with the presence of these organisms in BTM at farm level (*i.e.* dairy farm infrastructure conditions, milking procedures and mastitis control practices (5, 7), milker hygiene (20), milking equipment maintenance (16, 17) and cleaning (4, 12) have not been explored. The objective of this study was to determine on-farm risk factors for psychrotrophs counts in BTM fromdairy farms in Argentina.

MATERIALS AND METHODS

Dairy farms

Dairy farms included in the present study were among 45 farms stratified on the basis of milk protein and fat concentration. Of those dairy farms, 27 were included based on their willingness to cooperate in the study (14). Farms were located in different provinces of Argentina: 15 in Santa Fe, three in Córdoba, four in Entre Ríos, and five in Buenos Aires.

All dairy farms that participated in the study had a milk cooling system: 60.3 % of them had bulk milk cooling tanks, 15.5 % have barrel cooling systems (*i.e.* a vertical cylinder with the cooling source at the bottom) and 24.1% had plate heat exchanger systems. Regarding the cleaning capabilities, 97.7 % of the farms had an automatic cleaning system for the milking machine and 88 % for the milk cooling tank.

Milking routine procedures varied among the farms; however, some practices were carried out by most of them. Briefly, fore stripping was practised in 70.7 % of the dairy farms; washing teats in 62.2 %, post-dipping with a teat disinfectant after removing the milking units in 57.8 %. Washing and drying of teats with paper towels before milking was carried out in 24.4 %, and pre-dipping with a teat disinfectant before attaching the milking units in only 4.8 % of the farms.

Collection and processing of BTM samples

BTM samples were collected by trained personnel according to standard methodology (11) in 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 kept at a temperature below 6 °C during transport to the laboratory. Total psychrotrophic bacterial count (TPC) (10), lipolytic psychrotrophic bacterial count (LPC) and proteolytic psychrotrophic bacterial count (PPC) were performed using standard methodology (1).

Herds were classified according to their psychrotrophic bacterial counts (high or low), considering the following threshold: high counts TPC > 50,000 CFU/ml (16). Since there are not reference threshold values available for LPC and PPC, the 50th percentile of the data distribution was used (1,900 CFU/ml for both cases).

On-farm data collection

For collecting data on the risk factors, a questionnaire was designed. The questionnaire included the following sections:

- general farm infrastructure conditions (*i.e.* existence of a milk room, a machine room, a products store, wall building materials);
- milking procedures and mastitis control (*i.e.* elimination of foremilk, udder wash, teats dryoff, pre and post dipping);
- milkers' hygiene (*i.e.* hand wash, use of apron and gloves);
- equipment maintenance and cleaning (*i.e.* milking machine maintenance, kind of cleaning, acid detergent cleaning frequency, cooling tank) (Table 1).

The questionnaire was completed in a personal interview with the farm owner or farm manager that was carried out by trained personnel. A copy of the questionnaire is available from the corresponding author upon request.

The information about herd size and farm milk production included in the study was not available.

Data analysis

Data were collected on spreadsheets and merged into a single database using Infostat® (Universidad Nacional de Córdoba). The variables were categorized and considered as independent variables. 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 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 log CFU/ml of the three types of microorganisms were analyzed with ANOVA or the Student's T test. All statistical analysis were performed using Infostat® (Universidad Nacional de Córdoba).

Table 1. Association ($p < 0.1$) between farm environment, equipment and milking hygiene factors and total psychrotrophic counts (TPC), lipolytic psychrotrophic counts (LPC) and proteolytic psychrotrophic counts (PPC) in bulk tank milk

Variable	Chi-Square significance		
	TPC	LPC	PPC
Season	0.219	0.589	0.846
Dairy area	**0.015**	0.110	**0.062**
Existence of 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 months or every six months/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
Swill out after cleaning 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 cleaning (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 hot 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
Hand 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 dryoff	0.496	0.254	0.547
Pre-dipping	0.568	0.351	0.617
Post-dipping	0.466	0.610	0.610
Fed cows in milking parlor	0.105	0.784	0.784
Water sanitization	0.493	0.356	0.595

Numbers in bold mean significance differences ($p < 0.1$)

RESULTS

Descriptive statistics

TPC showed an average of 4.82 log CFU/ml, being 73.8 % of the samples below 50,000 CFU/ml (4.69 log CFU/ml). Lipolytic psychrotrophic counts and PPC presented an average of 4.31 log CFU/ml, and 4.43 log CFU/ml, respectively. Fifty per cent of the counts were higher than 1,900 CFU/ml (3.27 log CFU/ml).

Associated factors

Total psychrotrophic counts (TPC): the variables associated with higher counts were dairy area (p = 0.015), existence of milk room (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), frequency of acid detergent used to clean the tank (p = 0.012) and fore stripping (p = 0.093) (Table 1).

The dairy herds from Entre Ríos showed higher TPC than those from Santa Fe and Southern Santa Fe ($p < 0.05$). There was no significant association between the other geographical areas (Table 2).

Dairy farms cleaning the milking system weekly using acid detergent (1 to 3 times per week) had in average a TPC 0.4 log CFU/ml lower than farms using the same cleaning system every 15 days ($p < 0.05$). A similar result was obtained with the cooling tank cleaning frequency; TPC were lower in cases where acid detergent cleaning was used daily than in those cases where cleaning was carried out weekly or every 15 days ($p < 0.05$) (Table 2).

TPC differed regarding the type of cooling tank. TPC were lower for farms that possessed bulk milk cooling tanks, compared with those with plate heat exchangers or barrel type tank ($p < 0.05$) (Table 2). Fore stripping was the only activity of milking routine associated with TPC. Dairy farms using this practice yielded higher TPC ($p < 0.05$) (Table 2). When all the variables were introduced in a logistic regression model, only the type of cooling tank was significant ($p = 0.028$) (Table 5). Milk storage in bulk milk cooling tanks showed an Odds Ratio (OR) of 0.061 (CI95 % 0.005 - 0.774) compared with other systems (plate heat exchangers or barrel). Milk storage in plate heat exchangers or barrel tanks were 16.39 (CI95 % 1.34 – 200) more times likely to yield TPC higher than 50,000 CFU/ml than storage in bulk milk cooling tanks.

Lipolytic psychrotrophic counts (LPC): the variables associated to the highest levels of LPC were milk room existence ($p = 0.084$) and udder washing before milking time ($p = 0.079$) (Table 1). Dairy farms having a separate milk room, independent from the milking

Table 2. Variables associated ($p < 0.1$) with mean log total psychrotrophic counts in bulk tank milk

Variable	Level	log CFU/ml
Dairy area	Santa Fe	3.81$^{(1)}$
	Santa Fe (South)	3.97$^{(1)}$
	Córdoba	4.10$^{(1,2)}$
	Buenos Aires	4.24$^{(1,2)}$
	Entre Ríos	4.97$^{(2)}$
Milk room	Yes	4.22$^{(1)}$
	No	2.61$^{(2)}$
Acid detergent frequency of use in milking machine cleaning	every 15 days	4.24$^{(1)}$
	weekly or every 2 days	3.85$^{(1)}$
Cooling tank	plate heat exchangers or barrel	4.52$^{(1)}$
	bulk milk cooling tank	3.96$^{(2)}$
Cooling tank cleaning frequency	weekly	5.38$^{(1)}$
	3 times a week or daily	3.96$^{(2)}$
Acid detergent frequency of use in cooling tank	weekly or every 15 days	4.23$^{(1)}$
	daily or every 2 days	3.74$^{(2)}$
Elimination of foremilk	Yes	4.09$^{(1)}$
	No	3.87$^{(1)}$

Different superscript numbers indicate a significant difference ($p \leq 0.05$).

Table 3. Variables associated ($p < 0.1$) with mean log lipolytic psychrotrophic counts in bulk tank milk

Variable	Level	log CFU/ml
Milk room	Yes	3.14$^{(1)}$
	No	1.98$^{(2)}$
Udder wash	Yes	3.29$^{(1)}$
	No	2.88$^{(1)}$

Different superscript numbers indicate a significant difference ($p \leq 0.05$).

parlor, had higher LPC than farms without it ($p < 0.05$). Milk obtained from farms that washed udders before milking had higher LPC than those from farms that did not carry out this practice ($p < 0.05$) (Table 3). None of the variables that were significant to the χ^2 test ($p = 0.075$) were significant in a logistic regression model (Table 5).

Proteolytic psychrotrophic counts (PPC): the variables associated to the highest levels of PPC were dairy area ($p = 0.062$), milk room existence ($p = 0.013$), milking machine maintenance ($p = 0.079$), type of cooling tank ($p = 0.011$), hand washing by milker during milking time ($p = 0.027$) and fore stripping ($p = 0.042$) (Table 1). Milk samples obtained from dairy herds belonging to Santa Fe, Southern Santa Fe, Córdoba and Buenos Aires dairy areas yielded lower PPC than those from Entre Ríos ($p < 0.05$) (Table 5). Milk samples from dairy farms having a milk room separated from the other areas yielded higher PPC than those from farms not having a separate milk room ($p <0,05$) (Table 4). Milk samples from dairy farms carrying out maintenance service of the milking machine every four or six month yielded lower PPC than those farms that did it once a year ($p < 0.05$). In addition, dairy farms that cooled milk in plate heat exchangers or barrel tanks yielded higher PPC than those possessing bulk milk cooling tanks ($p < 0.05$) (Table 4). Milk samples from dairy farms whose milkers washed their hands during milking time yielded lower PPC than those where this hygiene practice was not carried out ($p < 0.05$). Fore stripping 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 perform this practice ($p = 0.8830$) (Table 4).

When the significant variables to the χ^2 Test were included in a logistic regression model only washing hands during milking time ($p = 0.032$) and type of milk cooling system ($p = 0.012$) were significant. Washing hands by milkers during milking time had an OR of 0.095 (CI95 % 0.015 – 0.601) compared with those cases where this practice was not carried out. Milk produced in systems that did not include hand washing as a part of the milking practices had 7.81 times more risk (CI95 % 1.18 - 52.6) of yielding PPC higher than 3.27 log CFU/ml. Milk storage in plate heat exchangers or barrel type of cooling systems had 10.5 times more risk (CI95 % 1.66 - 66.6) of having PPC higher than 3.27 log CFU/ml than milk storage in bulk cooling tanks.

DISCUSSION

Few reports deal with the enumeration of psychrotrophic organisms in milk in Argentina. In the present study, 73.8 % of the samples yielded less than 50,000 CFU/ml; while in a study carried out in milk samples from 17 dairy farms located in the Central dairy area of Santa Fe, 79.8 % yielded less than 10,000 CFU/ml (6), indicating that most of the farms had satisfactory bacteriological quality for this group of organisms.

Risk factors associated with high counts of psychrotrophic organisms have been related to cow and milking equipment hygiene (4, 12). In the present study, risk factors for elevated TPC and PPC were very similar. The type of cooling tank was positively associated with both TPC and PPC. Milk samples from farms with bulk milk cooling tank showed TPC values 0.6 log CFU/ml lower than farms using other cooling

Table 4. Variables associated ($p < 0.1$) with mean log PPC in bulk tank milk

Variable	Level	log CFU/ml
Milk room	Yes	$3.29^{(1)}$
	No	$1.54^{(2)}$
Milking machine maintenance	Every four months or every six months	$2.88^{(2)}$
	Annual	$3.53^{(1)}$
Cooling tank	Plate heat exchangers or barrel	$3.60^{(1)}$
	Bulk milk cooling tank	$3.06^{(1)}$
Hand wash	Yes	$3.01^{(1)}$
	No	$3.61^{(1)}$
Elimination of foremilk	Yes	$3.11^{(1)}$
	No	$3.02^{(1)}$

Different superscript numbers indicate a significant difference ($p \leq 0.05$).

Table 5. Final logistic-regression model of TPC, LPC and PPC in bulk tank milk

Predictive variables	Significance (*p*)	OR (CI95 %) only for variables with $p < 0.05$
TPC		
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 system	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	
LPC		
Milk room	0.075	
Udder wash	0.075	
PPC		
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)
Hand washing	0.032	0.128 (0.019 - 0.842)
Milk room	0.518	
Milking machine maintenance	0.344	
Elimination of foremilk	0.343	

TPC: total psychrotrophic counts, LPC: lipolytic psychrotrophic counts, PPC: proteolytic psychrotrophic counts.

systems. In addition, PPC was significantly lower (approximately 0.6 log CFU/ml) than those obtained in farms with barrel or plate heat exchanger systems. Milk storage under or at 4 °C does not prevent psychrotrophic bacterial growth if high counts of these organisms are present before storage (2, 9). The presence of cooling systems on the farm does not guarantee adequate bacteriological quality. Faulty milk refrigeration systems and wrong size dimensioning of bulk tanks lead to long cooling times, keeping milk above optimal cooling temperature from the beginning of milking until reaching 4 °C, thus allowing for significant bacteriological development (8). Not only these conditions allow growth of bacterial groups that will normally not grow in properly refrigerated milk, but also will not prevent growth of typical psychrotrophic organisms (16).

Milking machines without appropriate maintenance generally become the cause of high bacteriological counts in milk (16, 17). Seals and gaskets and all rubber goods should be changed at least annually, since aged rubber is very difficult to clean (17). In the present study, lower frequency of milk equipment maintenance was associated with higher levels of PPC. However, it was not statistically significant in the logistic regression model.

Cleaning and sanitation of the milking equipment have also been identified as a risk factor associated with high bacterial counts (4, 12). Bacterial adhesion and colonization of milk contact surfaces is considered an important factor for subsequent milk contamination (21). A contamination sequence that includes deposit of microorganisms with subsequent adhesion to equipment surfaces, reduction of bacterial numbers by cleaning mechanisms, bacterial proliferation between cleaning times and contamination of milk while passing through the machine has been suggested (13). In the present study periodic cleaning of cooling tanks (3 times a week or daily) was associated with lower TPC (approximately 1.5 log CFU/ml) than weekly cleaning frequency. In addition, acid detergent cleaning frequency (every two days or daily) was associated with lower PPC counts than those obtained by using it once a week. In a recent study, Elmoslemany *et al*. (5) found that manual cleaning of the bulk tank was associated with increased risk of elevated total aerobic and psychrotrophic bacteria. In turn, manual cleaning of the bulk tank was associated with a lower frequency of detergent and acid use, as well as with lower temperature of the cleaning solution (5). Gram-negative noncoliform bacteria, such as *Pseudomonas* and *Serratia* can attach, colonize and grow at low temperatures on the stainless steel surfaces of the milking system (9, 18). Average psychrotrophic counts of 3.93 log CFU/ml were found on the stainless steel surface of the cooling tank (9).

Several sources of milk contamination during milking time have been identified. Cow teats could have variable contamination level according to the place animals are kept between milking times (indoor, pasture or farmyard) and especially depending on climate conditions (3, 4, 7). Under grazing conditions, which predominate in Argentina, dust contamination is considered more relevant than teat dirt originated from cow's bed (23). Effective premilking udder hygiene is important for high quality milk production and mastitis control. Several studies (7, 22, 23) demonstrated that teat washing and drying before milking generated lower contamination on teats and lower total bacterial counts than washing only or not washing at all. In the present study, udder washing was associated with higher LPC; however it was not statistically significant in the logistic regression model. Using water to wash teats without drying was associated with elevated total bacterial and psychrotrophs counts (5).

Milk contamination could also arise from workers' hands. In the present study, lack of handwashing during milking time was associated with PPC. Farms where milkers washed their hands during milking time yielded lower PPC (approximately 0.6 log CFU/ml) than those where this practice was not carried out. A study performed at dairy farms of Santa Fe dairy area (20) reported PPC of 1.44 log CFU/cm^2 on workers' hands; which supports the hypothesis that milkers' hands can be a significant source of milk contamination.

In conclusion, several factors related to milking equipment and milking time hygiene (i.e. the frequent cleaning of cooling tanks), were associated with high psychrotrophic counts in milk. However, only the type of milk refrigeration system was significant after logistic regression analysis, which underscores the importance of both the election of an adequate milking system and its proper maintenance to guarantee an adequate microbiological quality of the milk obtained.

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