Risk factors associated with selected indicators of milk quality in semiarid northeastern Brazil

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ABSTRACT

The aim of this study was to gain information on quality traits, mainly bacterial and somatic cell counts of bulk milk, produced by small- and medium-scale producers in a semiarid northeastern region of Brazil and to identify and characterize possible risk factors associated with those quality traits. A cross-sectional study was performed on 50 farms. Bulk milk samples were collected for bacterial and somatic cell counts. Additionally, information about farm demographics, general management practices, hygiene, and milking procedures was also obtained. Multivariable analysis using logistic regression was performed with predictors previously identified by univariate analysis using a Fisher's Exact test. Aerobic mesophilic bacteria counts varied from 3.59 log to 6.95 log cfu/mL, with geometric mean of 5.27 log cfu/mL. Mean total coliform count was 3.27 log (1.52 log to 5.89 log) most probable number (MPN)/mL, whereas mean thermotolerant coliforms was 2.38 log (1.48 log to 4.75 log) MPN/mL. A high positive correlation was observed between aerobic mesophilic bacteria and coliform counts. Although most farms met the standard for the current regulations for total bacteria (88%) and somatic cell counts (94%), nearly half of the producers (46%) would have problems in achieving the 2012 threshold limit for total bacteria count if no improvement in milk quality occurs. Mean value for staphylococci was 3.99 log (2.31 log to 6.24 log) cfu/mL, and *Staphylococcus aureus* was detected in 33 (66%) farms. Premilking teat-end wash procedure (odds ratio = 0.191) and postmilking teat dip (odds ratio = 0.67) were associated with lower aerobic mesophilic bacteria and *Staphylococcus aureus* counts in bulk milk, respectively. Considering that the farm characteristics in this study are representative of the semiarid northeastern region, these findings encourage

further investigations for supporting intervention measures intended to improve the quality of milk produced by smallholders.

Key words: bacterial count, dairy, smallholder milk producer, somatic cell count

INTRODUCTION

Milk contamination by pathogenic microorganisms is considered a major public health issue worldwide (Nero et al., 2003; Jayarao et al., 2006), especially in developing regions, where raw milk is frequently consumed by the population, including children and the elderly. Marketing of raw milk is illegal in Brazil, but cultural habits and lack of information on public health issues make its consumption a common practice among individuals of low social status.

In addition, the physicochemical and sensory attributes of milk and dairy products are largely affected by microbiological contamination of raw milk (Barbano et al., 2006). Thermo-resistant enzymes and toxins produced by some bacterial species can negatively affect sensory attributes and safety of pasteurized milk and dairy products. Therefore, improvement and monitoring of raw milk quality is a major issue for the development of the milk production chain worldwide.

The Brazilian Ministry of Agriculture (MAPA) has launched a national program for the improvement of milk quality ("Programa Nacional de Melhoria da Qualidade do Leite – PNMQL") and substantial changes in the federal legislation regarding milk have been implemented (Brasil, 2002). This program has established lower tolerance limits for bacterial and SCC in raw milk in the 5 regions of the country. For the northeast region, the limit initially established (until July 2010) for mesophilic aerobic bacteria (cfu/mL) and SCC (cells/mL) was 1,000,000. From July 2010 until July 2012, the maximum threshold was decreased to 750,000 for both parameters. From July 2012 onward, the tolerance limits for mesophilic aerobic bacteria and SCC will be 300,000 cfu/mL and 400,000 cells/mL, re-

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spectively. Currently, information on the baseline data of these values in milk produced in the northeastern Brazil region is lacking.

Milk production in the northeast region increased by 54% between 1998 and 2006 (IBGE, 2008) and is considered one of the most important economic drivers in this part of the country. The dairy industry in this region normally comprises small- and medium-scale family cattle producers using crosses of Zebu (*Bos indicus*) and Holstein (*Bos taurus*) managed extensively or semi-intensively. Although improvements have been observed in recent years, milk productivity is still very low. Budget limitations and lack of technical knowledge are key factors that could jeopardize attainment of the minimal quality requirements established in the current legislation. Knowledge about factors associated with milk quality traits in such production systems could support intervention measures to improve the quality of milk produced in developing regions. Indeed, most scientific information on the factors affecting milk quality available in the literature relates to studies performed in intensive systems using specialized animals and automated milking systems (Barkema et al., 1999; Peeler et al., 2000; Suriyasathaporn et al., 2000; Elmoslemany et al., 2009a).

Because of the lack of comprehensive data needed to establish baseline values on milk quality produced in the region, our aim in this study was to assess microbiological traits and SCC in raw milk yielded in extensive production systems from a semiarid region and to tentatively identify risk factors associated with those traits toward the implementation of control measures intending to improve milk quality.

MATERIALS AND METHODS

Study Design and Milk Sampling

Fifty dairy farms were randomly selected by drawing among members of a dairy cooperative located in Caturité and Boqueirão municipalities in Cariri, a semiarid region of Paraíba State. Both municipalities are major milk suppliers for the cooperative (83 members in Caturité and 91 members in Boqueirão), and all producers (members and nonmembers) supply approximately one-third of the total milk production of the region (15,789,000 L/yr). The milk chain in Paraíba State comprises mainly smallholder producers and is characterized by low production (1,097.92 L/cow per year on average, with 208,324 cows producing 228,724,000 L/yr; IBGE, 2008); therefore, only small-scale (<50 L/d) and medium-scale (50–500 L/d) farms were sampled. The sampling size was restricted to 50 farms because of budget limitations. This sample size allowed detection

of odds ratios (**OR**) of ≥ 9.0 at the 95% level of confidence, given a power of 80% and percentage of farms with high bacterial/SCC group unexposed to a given risk factor of 10%. Samplings were performed during dry and wet seasons from April to September 2008. Duplicate milk samples (500 mL) were collected from refrigerated bulk tanks on farms that had their own individual tanks. Most smallholder producers in the region share collective storage tanks for cooling milk. In such cases, duplicate samples were directly collected from milk buckets from each individual farm just before milk was placed into the collective bulk tank. Sampling procedures followed National Mastitis Council recommendations (NMC, 1999) using previously sterilized material. Samples were kept under refrigeration until analyses, which were performed within 6 h of sampling.

Data Collection

On-farm data on possible risk factors were collected by a questionnaire containing 44 closed-ended and fill-in-the-blank questions organized in 3 sections: (1) farm demographics and characterization; (2) general management practices; and (3) hygiene and milking procedures. A prototype of the questionnaire (Supplementary file; available online at <http://www.journalofdairyscience.org/>) was initially tested on 5 farms located in the survey area and was modified appropriately considering the characteristics of the semi-extensive dairy herds in the region and limitations in the farmer's educational level and communication skills. The questionnaires were completed on the farms during the milking procedure. Whenever possible, the collected information was checked visually. To reduce variations intrinsic to subjective evaluations, such as herd's hygiene status, the questionnaire was applied by the same investigator throughout the survey.

Bacteriological Analysis

Mesophilic aerobic bacteria counts were performed after milk was serially diluted (1:10) in quarter-strength Ringer's solution and plated in plate count agar (Oxoid, Basingstoke, UK), according to protocol described by AOAC (1998). Petri dishes were incubated at 35°C for 48 $\pm$ 2 h.

Total and thermotolerant coliform counts (30/35°C and 45°C, respectively) were obtained using the most probable number (**MPN**) technique according to AOAC (1998). Milk aliquots (1 mL) from each dilution were transferred to a 3-tube series of lauryl sulfate triptose broth (Acumedia, Lansing, MI) with inverted Durham tubes. After incubation at 35°C for 48 h, the MPN of total coliforms per milliliter of milk was confirmed by

transferring aliquots (10 μ L) of positive lauryl sulfate triptose tubes to brilliant green bile broth (Acumedia), which was incubated at 35°C for 48 h. Thermotolerant coliforms (45°C) were determined using *Escherichia coli* broth (Acumedia) incubated at 44.5 $\pm$ 0.1°C for 24 to 48 h in a water bath.

Staphylococci were enumerated in milk according to APHA (1992) with minor modifications. Samples (25 mL) were initially diluted in 225 mL of lactose broth (Acumedia) and then serially diluted (1:10) in quarter-strength Ringer's solution. Aliquots (100 μ L) were transferred to Petri dishes in duplicates and Baird-Parker agar (Acumedia) supplemented with potassium tellurite and egg yolk (Laborclin, Pinhais-PR, Brazil) was added by the pour-plate method. Plates were incubated at 35°C for 24 to 48 h. Colonies showing morphological characteristics were Gram-stained and evaluated for catalase. Presumptive *Staphylococcus aureus* colonies were tested for coagulase in tubes using rabbit plasma and for acetoin production by Voges-Proskauer test.

Qualitative detection of *Salmonella enterica* was performed according to Andrews and Hammack (2007) with minor modifications. Briefly, milk samples (25 g) were pre-enriched in lactose broth (Acumedia) at 1:9 (wt:vol) ratio for 18 h, and then aliquots of 0.1 mL and 1 mL were transferred to Rappaport-Vassiliadis broth and tetrathionate broth (Acumedia), respectively. The Rappaport-Vassiliadis tubes were enriched at 42°C for 48 h and the tetrathionate broth at 35°C for 24 h. Aliquots were streaked onto xylose lysine desoxycholate agar (Acumedia) and Hektoen enteric agar (Acumedia) plates, which were incubated at 35°C for 24 h. Presumptive black colonies were inoculated in triple sugar iron (Acumedia) and lysine iron slants (Acumedia). *Salmonella* was confirmed by slide agglutination test using polysomatic antisera (Probac, Sao Paulo-SP, Brazil).

SCC

Somatic cell count was determined using direct microscopy as suggested by Prescott and Breed (1910), according to recommendations from FDA (2005), with minor modifications. Duplicate circular smears (1 cm²) were produced on glass slides using 10- μ L aliquots. After drying, smears were fixed in methanol for 15 min and then stained with Rosenfeld solution (methylene blue, eosin, and methanol) for 4 min. Afterward, Rosenfeld dye was washed and slides were immersed in a solution containing Rosenfeld and distilled water 1:2 (vol:vol). Smears were then washed with water and dried at room temperature. Counts were performed in 60 microscopic fields using optical microscopy (E-200, Nikon, Tokyo, Japan) at 1,000 $\times$ magnification (immer-

sion objective). Somatic cell numbers were calculated by multiplying the average number of cells in the 60 fields by the microscopic factor (3,183.09) and by the dilution factor (100) for 1 mL of milk.

Data Analyses

Logarithmically transformed data of bacterial counts and SCC were used for statistical analysis. Descriptive statistics using transformed data were used to determine geometric means, minimum, and maximum values. Coefficients of correlation were calculated between microbiological and somatic cell determinants. To test the main hypothesis; that is, the presence of on-farm risk factors significantly associated with milk quality indicators, the thresholds used for categorizing the dairy herds regarding high or low aerobic mesophilic bacteria and high or low SCC in raw milk were 5.48 log (3×10^5) cfu/mL and 5.6 log (4×10^5) cells/mL, respectively. These threshold values were selected based on the regulation limits for both indicators in Brazilian dairy herds to be implemented in 2012. Considering the absences of legislation limits for coliform and staphylococci counts in bulk tank milk, the 75th percentile of observed data was used as threshold.

Univariate analysis by the Fisher Exact test was performed to screen potential risk factors associated with bacterial and somatic cell counts. Only significant unconditional associations ($P < 0.2$) were selected for inclusion in the subsequent multivariable analyses using logistic regression. Predictors were entered stepwise into the logistic regression models by forward selection for aerobic mesophilic bacteria and somatic cell counts. Only variables that were significant at $P < 0.05$ by likelihood ratio were considered in the final models. Odds ratio were estimated with respective confidence intervals at 95%. Tetrachoric correlation among all predictors was determined to examine possible collinearity problems.

RESULTS

Farm Information

The on-farm data for farm demographics and characterization, general management practices, and hygiene and milking procedures are shown in Tables 1, 2, and 3, respectively. The majority of the farms (76%) were semi-intensive and in most of them (66%) milking was performed by a single milker only. The dairy farms had low milk production (68% of the farms yielded <10 L/animal daily), and 62% of the farms had 10 or fewer milking cows. On most farms (86%), the cows were milked manually. Milking room was observed in 20

Table 1. Unconditional associations ($P < 0.2$) between farm demographics and general characteristics of 50 dairy herds in semiarid northeastern Brazil and on-farm predictors for bulk milk quality indicators

Predictor	Category	Herds, n (%)	<i>P</i> -values for bulk milk quality indicators ¹					
			AMB	TCC	TTC	TSS	STA	SCC
Municipality	1	25 (50)	—	—	0.009	—	—	0.069
	2	25 (50)						
Age of the owner (yr)	≤47	24 (48)	0.074	—	—	—	—	0.193
	>47	26 (52)						
Owner's experience in dairy production (yr)	≤20	30 (60)	—	—	—	—	0.191	—
	>20	20 (40)						
Owner's activities other than milk production	Yes	8 (16)	—	—	0.082	—	—	0.026
	No	42 (84)						
Monthly income from dairy production (US\$)	≤1,500	40 (80)	—	0.061	—	—	—	0.010
	>1,500	10 (20)						
Milking room on the farm?	Yes	21 (42)	0.107	—	0.050	—	—	0.002
	No	29 (58)						
Facilities maintained appropriately?	Yes	6 (12)	—	—	—	—	—	—
	No	44 (88)						
Facilities supporting adequate hygienic procedures?	Yes	3 (6)	—	—	—	—	—	—
	No	47 (94)						
Breed	High <i>Bos taurus</i> blood level	28 (56)	—	—	—	0.034	—	—
	Low <i>Bos taurus</i> blood level	22 (44)						
Number of lactating cows	≤10	31 (62)	—	0.092	—	—	—	0.059
	>10	19 (38)						
Total milk yield (kg)	≤50	27 (54)	—	—	0.094	0.162	—	0.045
	>50	23 (46)						
Milk yield per animal (kg)	≤10	34 (68)	0.129	0.158	—	—	—	—
	>10	16 (32)						
Animal species other than dairy cows in the farm?	Yes	5 (10)	—	—	—	—	—	—
	No	45 (90)						
Veterinary assistance on farm?	Yes	11 (22)	0.037	—	—	0.045	0.032	0.017
	No	39 (78)						
Season at sampling	Dry	8 (16)	0.182	—	—	—	0.082	0.179
	Wet	42 (84)						

¹Indicators: AMB = aerobic mesophilic bacteria; TCC = total coliform count; TTC = thermotolerant coliform; TSS = total staphylococci, STA = *Staphylococcus aureus* count.

(40%) of the farms, whereas milking was usually performed in the holding pens in most of the farms (60%). Veterinary assistance was used in only 11 (22%) of the farms. These characteristics are typically related to the production systems in semiarid northeastern Brazil and allow information to be inferred to similar regions in other states in northeastern Brazil.

Microbiological Findings

Plate counts for mesophilic aerobic bacteria varied from 3.59 log (3×10^5) to 6.95 log (8.8×10^6) cfu/mL, with geometric mean of 5.27 (2.1×10^5) cfu/mL. The upper thresholds for aerobic mesophilic bacteria established by the Brazilian Ministry of Agriculture (Brasil, 2002) until and after 2012 are 7.5×10^5 and 3.0×10^5 SCC/mL, respectively.

The mean total coliform count (35°C) was 3.27 log (2.6×10^3) MPN/mL and ranged from 1.52 (33) to 5.89 (7.8×10^5) MPN/mL, whereas the mean thermotolerant coliform count (44.5°C) was 2.38 (3.8×10^2) MPN/mL, ranging from 1.48 (30) to 4.75 (5.6×10^4) MPN/mL. A positive correlation was observed between

mesophilic aerobic bacteria and coliform counts ($r = 0.78$) and between mesophilic aerobic bacteria and fecal coliforms ($r = 0.85$).

The mean value for staphylococci was 3.99 (1.3×10^4) cfu/mL, ranging from 2.31 (2.1×10^2) to 6.24 (1.7×10^6) cfu/mL. However, no correlation ($r = -0.11$) was observed between staphylococci counts and SCC. *Staphylococcus aureus* was detected in 33 (66%) farms. In those farms, a mean value of 3.7 log (7.1×10^3) cfu/mL was detected and the maximal count was 5.3 log (2.1×10^5) cfu/mL. *Salmonella enterica* was detected in only one sample at a prevalence of 2%.

SCC

Somatic cells ranged from 4.63 log (4.3×10^4) to 6.18 log (1.5×10^6) cells/mL with a mean value of 5.38 log (2.4×10^5) cells/mL. Forty-seven (94%) farms presented bulk milk SCC below the maximal threshold (7.5×10^5 cells/mL) of the current Brazilian regulation (Brasil, 2002). If the 2012 scenario is hypothetically considered (i.e., when the threshold will decrease to 4.0×10^5 cells/mL), 9 (18%) of the farms would not

Table 2. Unconditional associations ($P < 0.2$) between general management practices of 50 dairy herds in semiarid northeastern Brazil and on-farm predictors for bulk milk quality indicators

Predictor	Category	Herds, n (%)	<i>P</i> -values for bulk milk quality indicators ¹					
			AMB	TCC	TTC	TSS	STA	SCC
Production system?	Semi-extensive	38 (76)	—	—	—	—	—	—
	Extensive	12 (24)						
Concentrate feeding?	Yes	37 (74)	0.163	—	—	—	—	—
	No	13 (26)						
Feeding cows after milking?	Yes	10 (20)	—	—	0.179	0.062	0.046	—
	No	40 (80)						
SCC analysis performed routinely?	Yes	4 (08)	—	—	—	—	—	0.144
	No	46 (92)						
CMT ² performed routinely?	Yes	2 (04)	—	—	—	—	—	—
	No	48 (96)						
Deworming performed routinely?	Yes	37 (74)	0.170	—	—	0.144	—	—
	No	13 (26)						
Ectoparasite-related problems?	Yes	46 (92)	—	—	0.038	0.029	—	0.144
	No	4 (08)						
Mastitis-related problems?	Yes	28 (56)	—	0.018	—	—	—	0.139
	No	22 (44)						
Drying cow management?	Yes	32 (64)	—	—	—	—	—	—
	No	18 (36)						
Dry cow therapy?	Yes	11 (22)	—	—	—	—	0.184	0.017
	No	39 (78)						
Early weaning?	Yes	14 (28)	—	—	0.198	—	—	0.057
	No	36 (72)						
Milking system	Manual milking	33 (66)	0.077	0.166	—	—	0.126	0.100
	Mechanic milking	17 (24)						
Milk place	Milking room	20 (40)	—	—	0.035	—	—	0.002
	Holding pen	30 (60)						
Milkings per day	= 1	4 (08)	—	—	—	—	—	—
	>1	42 (92)						
Number of milkers	= 1	33 (66)	—	—	—	—	—	0.101
	>1	17 (34)						

¹Indicators: AMB = aerobic mesophilic bacteria; TCC = total coliform count; TTC = thermotolerant coliforms; TSS = total staphylococci, STA = *Staphylococcus aureus* count.

²CMT = California Mastitis Test.

meet the legislative threshold breakpoint for acceptable quality.

Risk Factor Analyses

Univariate analyses performed to screen candidate predictors associated with milk traits indicated unconditional associations (Tables 1, 2, and 3). Demographic predictors linked to bacterial counts were producer's age ($P = 0.07$), presence of milking room on farm ($P = 0.11$), milk yield per animal ($P = 0.13$), and veterinary assistance ($P = 0.04$). Unconditional associations for total coliforms included owner's income ($P = 0.061$), number of lactating cows ($P = 0.092$), and milk yield per animal ($P = 0.158$). For thermotolerant coliforms, municipality ($P = 0.021$), owner's activities other than milk production ($P = 0.082$), milking room on farm ($P = 0.050$), and total milk yield ($P = 0.094$) were selected. Unconditional associations with total staphylococci counts included breed ($P = 0.034$), total milk yield ($P = 0.162$), and veterinary assistance ($P = 0.045$). For

Staph. aureus, selected predictors were season ($P = 0.173$), owner's experience (0.191), and veterinary assistance ($P = 0.032$). Regarding associations with SCC, the predictors were municipality ($P = 0.07$), producer's age ($P = 0.19$), owner's activities other than milk production ($P = 0.026$), income from dairy production ($P = 0.010$), milking room on the farm ($P = 0.00$), number of lactating cows ($P = 0.06$), total milk yield ($P = 0.05$), and veterinary assistance ($P = 0.02$).

Considering general management practices, use of concentrate ($P = 0.016$), deworming ($P = 0.17$), and milking system ($P = 0.08$) were statistically associated with aerobic mesophilic bacteria. Predictors for total coliforms were mastitis-related problems reported by the farmer ($P = 0.018$) and milking system ($P = 0.166$). For thermotolerant coliforms, feeding cows after milking ($P = 0.179$), ectoparasite-related problems ($P = 0.038$), early weaning ($P = 0.198$), and milking place ($P = 0.035$) were selected for further analysis. Predictors associated with total staphylococci counts were feeding cows after milking ($P = 0.062$), deworming ($P =$

Table 3. Unconditional associations between hygiene and milking procedures of 50 dairy herds in semiarid northeastern Brazil and on-farm predictors for raw milk quality indicators

Predictor	Category	Herd, n (%)	<i>P</i> -values for raw milk quality indicators ¹					
			AMB	TCC	TTC	TSS	STA	SCC
Premilking teat-end wash?	Yes	21 (42)	0.008	—	—	—	0.041	0.021
	No	29 (58)						
Strip cup test before milking?	Yes	10 (20)	0.066	0.061	—	—	0.046	0.010
	No	40 (80)						
Premilking teat-end dip?	Yes	16 (32)	—	—	—	—	0.172	0.103
	No	34 (68)						
Postmilking teat-end dip?	Yes	14 (28)	0.030	0.098	—	0.111	0.010	0.009
	No	36 (72)						
Teat-end drying after cleanliness	Disposable paper tissue	17 (34)	0.022	—	—	—	0.134	0.000
	Fabric tissue or nondrying	33 (66)						
Cleaned buckets with filter for milk collection?	Yes	38 (76)	—	—	—	—	—	0.065
	No	12 (24)						
Milker's personal hygiene	Adequate	04 (08)	—	0.145	—	—	—	0.016
	Inadequate	46 (92)						
Cleanliness and appropriate use of milking accessories	Adequate	10 (20)	—	—	—	—	—	0.065
	Inadequate	40 (80)						
Milking order according to CMT? ²	Yes	25 (50)	—	0.056	—	—	—	0.069
	No	25 (50)						
Time elapsed between milking and refrigeration (min)	≤90	25 (50)	—	0.180	0.110	0.085	0.048	—
	>90	25 (50)						
Water reservoir on farm?	Yes	20 (40)	—	—	—	—	—	—
	No	30 (60)						
Water treatment on farm?	Yes	08 (16)	—	—	—	—	—	—
	No	42 (84)						
Cleanliness of milking area	Adequate	06 (12)	—	0.148	0.141	—	—	0.063
	Inadequate	44 (88)						
Cleanliness of the barn	Adequate	03 (06)	—	0.144	0.139	—	—	0.080
	Inadequate	47 (94)						

¹Indicators: AMB = aerobic mesophilic bacteria; TCC = total coliform count; TTC = thermotolerant coliforms; TSS = total staphylococci, STA = *Staphylococcus aureus* count.

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0.144), and ectoparasite-related problems ($P = 0.029$). Screened variables for *Staphylococcus aureus* counts included feeding animals after milking ($P = 0.046$), milking system ($P = 0.126$), and medicated drying ($P = 0.184$). For somatic cells, the selected variables were routine cell counts ($P = 0.14$), ectoparasite-related problems ($P = 0.14$), mastitis-related problems ($P = 0.14$), medicated drying of cows ($P = 0.02$), early weaning ($P = 0.06$), milk system ($P = 0.10$), milking place ($P = 0.00$), and number of milkers ($P = 0.10$).

Regarding hygiene and milking procedures, teat-wash ($P = 0.001$), strip-cup test ($P = 0.07$), post-dip ($P = 0.03$), and teat-end dry after cleanliness ($P = 0.022$) were selected as predictors for aerobic mesophilic bacteria. For total coliforms, significant variables were strip-cup test ($P = 0.061$), cleanliness of milking area ($P = 0.148$), post-dip ($P = 0.098$), cleanliness of barn ($P = 0.144$), milker's personal hygiene ($P = 0.145$), milking order ($P = 0.056$), and time elapsed until milk refrigeration ($P = 0.180$). For thermotolerant coliforms, cleanliness of the milking area ($P = 0.141$), cleanliness of the barn ($P = 0.139$), and time elapsed between

milking and refrigeration ($P = 0.110$) were selected. Predictors associated with total staphylococci were post-dip (0.111) and time elapsed between milking and refrigeration ($P = 0.085$). Teat-end wash (0.041), strip-cup test ($P = 0.046$), pre-dip ($P = 0.172$), post-dip ($P = 0.010$), teat-end drying after wash ($P = 0.134$), and time elapsed between milking and milk refrigeration ($P = 0.048$) were screened predictors for *Staphylococcus aureus*. For somatic cells, only 3 variables were not selected ($P > 0.2$): time elapsed between milking and milk refrigeration, on-farm water treatment, and on-farm water reservoir.

Table 4 presents the risk factors ($P < 0.05$) associated with bacterial counts and SCC determined by the multivariable analyses. According to the final logistic regression models, teat-end wash procedure was the only predictor ($P < 0.05$) associated with reduction of aerobic mesophilic bacteria in raw milk produced by smallholders in the semiarid region. Increased total coliform counts in raw milk were associated with mastitis-related problems reported by the farmer, whereas thermotolerant coliform count variations in raw milk

Table 4. Final logistic regression model for on-farm predictors associated with aerobic mesophilic bacteria, total coliform, thermotolerant coliform, total staphylococci, *Staphylococcus aureus*, and SCC in bulk tank milk in the semiarid region of Paraíba State, Brazil

Predictor	β coefficient	SE of β	Odds ratio	CI
Aerobic mesophilic bacteria				
Teat-end wash before milking	-1.656	0.640	0.191	0.056–0.653
Intercept	1.163	0.512		
Total coliforms count				
Mastitis-related problems	2.031	0.853	5.54	1.27–24.1
Milker's personal hygiene	-2.692	1.454	0.14	0.01–1.6
Intercept	0.466	0.445		
Thermotolerant coliforms				
Municipality	-2.037	0.843	0.13	0.025–0.680
Intercept	2.442	0.737		
Total staphylococci				
Ectoparasite-related problems	-2.657	1.218	0.07	0.009–0.576
Intercept	21.203	12,118.63		
<i>Staphylococcus aureus</i>				
Post-dip	-20.510	10,742.02	0.667	0.529–0.840
Intercept	21.203	10,742.02		
SCC				
Teat-end drying with paper tissue	3.635	1.182	28.4	3.87–193.84
Intercept	-0.581	-0.623		

were explained only by the municipality. According to the final model, ectoparasite-related problems reported by the producers were significantly associated with lower staphylococci counts. The absence of post-dip procedure was the only risk factor linked to higher *Staph. aureus* contamination in raw milk. The logistic model for somatic cells included 2 significant ($P < 0.05$) variables: disposable paper tissue for teat-end drying before milking and milking performed by more than one milker. The use of paper towel for teat-end drying was associated with a higher (OR = 4.41) risk for high SCC in milk.

DISCUSSION

Although the number of farms in the region that currently meet the legislative threshold for aerobic mesophilic bacteria is high (88%), caution is warranted in interpreting these findings because a more stringent threshold (3.0×10^5 SCC/mL) will be defined by legislation in 2012. Considering this scenario, only 27 (54%) farms would meet the legislative standard if no decrease occurs in total aerobic counts for raw milk in the region until 2012. Therefore, these findings strongly indicate the need to implement intervention measures to improve the microbiological quality of milk in the region. This is obviously a challenging issue because the milk value chain comprises mostly smallholder producers with considerable educational and economic limitations. Microbiological quality of bulk tank milk varies among different studies performed in Brazil. According to previous reports carried out in southern states, the frequency of farms meeting legislative standards re-

garding total bacterial counts in bulk tank milk ranged from 18 to 83% (Nero et al., 2005; Arcuri et al., 2006).

Total coliform counts $>1.0 \times 10^3$ MPN/mL are considered indicative of hygiene-related problems in milk production (Murphy, 1997), although no limit exists for coliform organisms in the current Brazilian legislation for bulk tank milk. The present study showed that 29 (58%) and 18 (36%) bulk tank milk samples had counts exceeding 1.0×10^3 MPN/mL for total and fecal coliforms, respectively. The present findings could indicate possible hygiene problems in milking procedures in most assessed farms, because fecal coliforms such as *E. coli* might contaminate bulk milk through intramammary secretion or fecal contamination in the milking equipment (Van Kessel et al., 2004). Higher coliform counts also indicate possible contamination by pathogenic bacteria of public health significance.

A high positive coefficient of correlation was observed between aerobic mesophilic bacteria and total coliforms ($r = 0.78$) and between aerobic mesophilic bacteria and fecal coliforms ($r = 0.85$). These findings are similar to a previous report (Arcuri et al., 2006) and therefore indicate that aerobic mesophil counts could be effectively used as the single microbiological hygienic indicator for mesophilic aerobic bacteria in raw milk.

Staphylococci organisms are major mastitis-causing agents in cattle worldwide and in Brazil (Souto et al., 2008; Whist et al., 2009). Thus, the high number of milk samples (66%) harboring *Staph. aureus* could be indicative of mastitis in the investigated herds. Considering that mastitis is associated with increased somatic cells in milk (Barbano et al., 2006), a high correlation between staphylococci counts and SCC would be ex-

pected. However, this was not observed in the present study ($r = -0.11$). Furthermore, small correlations between SCC and other bacterial counts (aerobic mesophilic bacteria and coliforms) have been observed in the present study, which corroborate a previous report (Jayarao et al., 2004).

Salmonella isolation rate (2%) was very similar to the results previously reported in many countries. It was also similar to the frequency of 2.6% noted by Van Kessel et al. (2004) in the United States as part of the National Animal Health Monitoring System (NAHMS) Dairy Survey. However, a higher contamination rate (11.8%) was observed in bulk milk samples in the NAHMS 2002 survey (Karns et al., 2005) when samples were analyzed by real-time PCR. Therefore, further studies on the methods used to identify *Salmonella* in milk and dairy products are required to evaluate the real importance of milk as a *Salmonella* vehicle. Moreover, such information is very important for the development of monitoring programs intended to control and reduce this pathogen in the dairy production chain.

Although SCC ranged widely in the investigated farms, high SCC, indicative of mastitis-associated problems, seemed to be a problem for certain smallholder farms only. In this sense, monitoring somatic cells in bulk tank milk in the region is needed to identify farms with mastitis-related problems and to define strategies for control measures.

The large variations observed for bacterial counts and SCC in bulk tank milk samples could suggest putative risk factors associated with bulk tank milk quality. Therefore, logistic regression models were attempted and possible risk factors associated with microbiological indicators and SCC in raw milk have been identified (Table 4). The teat-wash procedure before milking was the single factor retained in the model for aerobic mesophilic bacteria, which could explain 54% of the variation. According to this model, teat washing before milking was significantly associated with lower aerobic mesophilic contamination in raw milk (OR = 0.191, 95% CI: 0.056 to 0.653). It is known that udder cleanliness before milking is crucial to reduce microbial contamination in milk (Magnusson et al., 2006; Elmoslemany et al., 2010). In addition, poor teat-end cleanliness was significantly associated with high bacterial counts in bulk milk (Elmoslemany et al., 2009b). In view of the scientific knowledge available and the high plausibility of this variable to be considered a major factor associated with microbial contamination in milk, the present findings confirm that udder hygiene plays an important role in the quality of bulk tank milk. However, high collinearity among the predictors was observed. Among the other 11 significant predictors unconditionally as-

sociated with aerobic mesophilic bacteria, teat wash was significantly correlated with 6 variables by the tetrachoric correlation test. This could explain the fact that although many unconditional associations were detected by the univariate analyses, only one factor was retained in the final model. However, the present findings suggest that premilking teat-end wash could help smallholder producers reduce aerobic mesophilic bacteria counts in raw milk. Detailed information about premilking teat cleaning and disinfection was recently published (Elmoslemany et al., 2010).

The results of the present investigation corroborated a previous report indicating a high correlation between coliform and total bacterial counts (Pantoja et al., 2009). In the present study, high coliform counts were significantly associated with herds having mastitis-related problems reported previously by the farmer. The model explained 77.6% of the variation but the high correlation between predictors and the limitations of the cross-sectional studies in determining causality make it difficult to reach conclusions about the real role of these predictors. Again, mastitis-related problems were correlated with 7 out of 12 predictors. This association could be biologically plausible because some microorganisms in raw milk could also be mastitis-causing agents. Furthermore, a significant association between coliform and SCC has been described (Pantoja et al., 2009). In the mentioned study, coliforms increased 4.3% for every 10,000 cells/mL. On the other hand, no significant coefficient of correlation ($P > 0.05$) was observed in the present study between somatic cell and coliform counts when tested by tetrachoric correlation test.

According to the logistic regression model, a higher risk of milk contamination by thermotolerant coliforms was associated with milk produced in the municipality of Caturité. Dairy herds in both regions, Caturité and Boqueirão, are very similar, which makes this association very difficult to be explained. This result could be due to factors not covered in the questionnaire. Only limited information about the cleaning procedures of bulk tank was available and this could have a major effect on the microbiological findings, including coliforms.

Thermotolerant coliforms are related mainly to fecal coliforms, a potential agent of environmental mastitis. Theoretically, it makes sense to associate higher levels of thermotolerant coliforms in milk from herds with very poor hygienic procedures. However, many possible hygienic predictors identified in the univariate analysis were not retained in the final model, such as cleanliness of milking area, cleanliness of the barn, time elapsed between milking and refrigeration and milking place. The high collinearity among predictors could be related

to this problem. Although the municipality has not been retained in the final model for somatic cells, this variable was also a predictor (Table 1) associated with somatic cells. Tetrachoric correlation between thermotolerant coliforms and somatic cells was not significant.

Curiously, a lower risk of staphylococcal contamination was observed in farms where problems associated with ectoparasites have been reported by the farmer. This might be a confounding variable because control of ectoparasites could be related to a large number of variables. On the other hand, post-dip was significantly associated with *Staph. aureus* reduction in raw milk in the present study. This model explained 76% of the data variability. Although premilking udder hygiene procedures are known to play a major role in the microbial quality of bulk tank milk (Elmoslemny et al., 2010), postmilking teat dip is also important for mastitis control purposes. In this sense, the findings of the present study could corroborate the benefits of post-dip in reducing *Staph. aureus* and consequently preventing contagious mastitis.

In regard to SCC, drying of teat-ends with disposable paper towels was the only variable retained in the final model as a risk factor associated with SCC >400,000 cells/mL. We strongly believe this might a confounding association because it lacks biological plausibility. In recent years, much information has been published about the importance of detailed premilking management practices and especially pre-dip followed by teat-end drying (Elmoslemny et al., 2010) in reducing somatic cells in bulk milk. The small sample size of the present study should also be considered a major problem for multivariable analysis, mainly because of the absence of variation between herds. It is crucial, however, to highlight the limitations of cross-sectional studies in determining causality in complex diseases such as mastitis. As noted earlier, the results of this study must be seen as preliminary data on milk quality indicators for semi-intensive dairy herds in semiarid northeastern Brazil. Among the models for quality indicators presented in this investigation, we identified risk factors associated with aerobic mesophilic bacteria and *Staph. aureus* contamination in bulk tank milk. Therefore, the present findings encourage further research, including more complex study designs and larger sample sizes, to identify risk factors associated with milk quality in semi-intensive production systems in developing areas. We strongly believe that extension activities toward implementation measures intending to improve on-farm milk quality directly depend on the precise detection of priorities for smallholders and scientific information that is still lacking in those regions.

CONCLUSIONS

This study provides baseline data for the current milk quality assessment in Paraíba State in northeastern Brazil. The current findings show high microbiological contamination in bulk tank milk. Because of upcoming stricter legislative standards, intervention measures are urgently needed on some farms to enable them to meet the standards enforced after 2012. However, it is expected that the new legislative standards will remain a challenge in the semiarid regions of northeastern Brazil, where the milk value chain comprises smallholder producers facing educational and economic limitations. Absence of premilking teat-end wash and postmilking teat dip were identified as potential risk factors for aerobic mesophilic bacteria and *Staph. aureus* contamination in bulk milk. Even considering the limitations of this study, the present findings suggest that milking hygiene procedures could be successfully used to improve bulk milk quality in the region. Further investigations are encouraged to use larger samples sizes and other study designs.

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