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Full Length Research Paper

Microbiological analysis and somatic cell counts in raw milk from farms of São Paulo State, Brazil

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The objective of the study was to evaluate mesophyle, psychrotroph and somatic cell counts (SCC), and presence of *Staphylococcus aureus* in raw milk produced in 42 dairy farms of the State of São Paulo. Farms were classified according to milk yield in low (< 400 L/day, n = 17), intermediate (400 - 1,000 L/day, n = 8) and high milk production (> 1,000 L/day, n = 17). Mesophylic bacteria counts were lower (p < 0.05) in high production farms, with no differences (p > 0.05) in psychrotrophic and somatic cell counts between the three categories. *S. aureus* was more frequent (p < 0.05) in the milk of individual cows in intermediate production farms. Percentage of samples that did not comply with SCC tolerance limits in grade B milk ranged from 29.4 (low production) to 52.9% (high production). Results indicate that the greatest difficulties for dairy farms to comply with milk quality parameters are related to the adoption of hygiene practices during milking and to failures in milk conservation.

Key words: Milk quality, mesophyles, psychrotrophs, somatic cell counts (SCC), *Staphylococcus aureus*.

INTRODUCTION

Milk and dairy products have an important role in human nutrition, especially in the first year of life, once these products are sources of high quality protein, carbohydrates, fat and mineral salts necessary for growth (Spreer, 1991). Therefore, it is essential to ensure the integrity and intrinsic quality of milk and dairy products for human consumption.

According to Desmasures and Gueguen (1997), the following are characteristics of high quality milk: absence of pathogens, sediments, extraneous material, and extraneous odors and flavors; low somatic cell counts; slightly sweet flavor; no distinctive odor, and compliance with legal standards in terms of minimal concentrations of fat, total solids and nonfat solids. In order to meet these requirements, the product should be controlled throughout the production chain, until it reaches the final consumer (Cousin, 1982). Thus, microbiological examination - such as mesophylic bacteria counts - chemical and physical analyses, and sensorial tests should all be employed in quality control of both raw and pasteurized milk (Caruzo and Oliveira, 1984).

The quality of raw milk delivered to the dairy industry should be ensured by means of hygienic milking of healthy and well-nourished animals, immediate cooling of milk in the farm, and transportation of bulk milk to the industry in insulated tank trucks (Desmasures and Gueguen, 1997). Previous studies demostrated that mesophylic contamination of milk delivered to dairy factories in the state of São Paulo has been historically high in the last decades (Santos and Fonseca, 2002; Guerreiro, 2005; Nero et al., 2005).

Among the standards employed in the evaluation of milk quality, somatic cell counts (SCC) have been widely used recently. Mastitis, the inflammation of the mammary gland in response to bacterial colonization and growth,

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Table 1. Results of mesophylic, psychrotrophic and somatic cell counts obtained in raw bulk mik of dairy farms of São Paulo, Brazil[1].

Milk production (L/day)	Mesophiles (Log CFU/mL)	Psychrotrophs (Log FU/mL)	Somatic cells (Log cells/mL)
< 400 (*n* = 17)	6.5 ± 0.5[a]	3.3 ± 2.3[a]	5.7 ± 0.4[a]
400 – 1,000 (*n* = 8)	6.5 ± 0.9[a]	2.3 ± 1.3[a]	5.8 ± 0.3[a]
>1,000 (*n* = 17)	5.5 ± 0.9[b]	3.4 ± 2.0[a]	5.8 ± 0.2[a]

[1] Results are reported as mean ± standard deviation of duplicate analysis of *n* samples in each category of milk production. [a,b] Values within columns with no common superscript differ significantly ($p < 0.05$).

leads to increased somatic cell counts - blood leukocytes and sloughed epithelial cells (Zecconi and Hahn, 2000). In healthy mammary glands, SSC is generally lower than 3×10^5 cells /mL of milk. This count quickly increases in the presence of bacteria, and may reach 10^6 cells /mL in few hours (Auldist and Hubble, 1998). In dairy cattle, *S. aureus* is frequently associated with subclinical mastitis (Adesiyun et al., 1998) and contamination of milk and dairy products (Zecconi and Hahn, 2000).

International criteria for monitoring milk quality were incorporated to the regulations of the Brazilian Ministry of Agriculture by means of Normative 51/2002 (IN 51) that became in force in Brazil in 2005 (Brasil, 2002). Total plate count (TPC) and SCC are the most commonly used parameters in the evaluation of milk hygiene and quality, and of the prevalence of mastitis in dairy herds. Both parameters are directly associated with milking management and final quality of milk and dairy products.

The objective of the present study was to evaluate the quality of milk produced in grade B dairy farms in the State of São Paulo, Brazil, by means of somatic cell, mesophylic and psychrotrophic counts in bulk tank milk, and somatic cell counts and presence of *Staphylococcus aureus* in the milk of individual cows in these farms.

MATERIALS AND METHODS

The study was conducted in 42 dairy farms located in the regions of São Carlos and Ribeirão Preto, SP, Brazil, from February of 2005 to March of 2006. In each farm, individual milk samples were obtained from all cows showing signs of subclinical mastitis according to the California Mastitis Test (CMT). Duplicate samples (100 mL) were aseptically collected during the first morning milking from the individual meters in the milking machine. At the end of the milking procedure, duplicate bulk milk samples (100 mL) were collected in sterile glass vials. These two hundred eight samples of milk from individual cows and thirty-seven samples of bulk milk aseptically collected were transported to the laboratory in insulated coolers at 4 to 8°C and analyzed on the same day.

Milk production of each farm was recorded and the farm was assigned into one of three categories: low production, when yield was less than 400 L/day, intermediate production, if yield was between 400 and 1,000 L/day, and high production, when yield was greater than 1,000 L/day.

Aerobic and facultative anaerobic mesophylic and psychrotrophic microorganisms in bulk milk were counted using Petrifilm® (3M - USA) and three dilutions: 10^{-3}, 10^{-4} and 10^{-5} for mesophyles, and 10^{-2}, 10^{-3} and 10^{-4} for psychrotrophs. Sampling and analysis were carried out according to the American Public Health Association (1992). Isolation and identification of *S. aureus* followed the methods described by Silva et al. (2001). Samples were plated onto Baird Parker agar (Oxoid) supplemented with egg yolk emulsion and tellurite (1%) (Oxoid). Typical and atypical colonies growing on Baird Parker plates were identified as *S. aureus* using the following tests: coagulase, catalase, DNAse, acetoin production and maltose fermentation (without gas production). Typical colonies are gray to black (reduction of potassium tellurite) surrounded by an opaque zone (breakdown of egg yolk). Atypical colonies are gray to black, and the opaque zone was absent.

Somatic cells in the milk from individual cows and bulk tank raw milk were counted automatically by laser-based flow cytometry (Electronic counter, Somacount 300, Bentley, USA). Quantitative results of microbiological analyses were expressed as colony forming units per mL (CFU/mL) and transformed into log CFU/mL. Results were submitted to one-way ANOVA and treatment means were compared by the Tukey's test, using SAS® General Linear Model (SAS Institute, 2004).

RESULTS AND DISCUSSION

The number of farms classified as low (< 400 L/day), intermediate (400 - 1,000 L/day) and high production (> 1,000 L/day) was respectively 17, 8 and 17. Table 1 shows the results of mesophylic, psychrotrophic and somatic cell counts in bulk tank milk of the 42 dairy farms evaluated and classified according to milk production. Mesophylic counts were lower ($p < 0.05$) in high production farms (> 1,000 L/day), but low and intermediate production farms showed no differences ($p > 0.05$) between them. No differences ($p > 0.05$) in psychrotrophic and somatic cell counts were observed between the categories.

Figure 1 shows the percentage of bulk milk samples that did not comply with IN 51 for grade B milk in terms of mesophylic (500,000 CFU/mL) and somatic cell counts (600,000 cells/mL). Farms of low and intermediate production showed the greatest percentage of samples above the limit determined by the regulation (500,000 CFU/mL) 88.2 and 75.0%, respectively. The opposite was true for SCC: the greatest percentages were

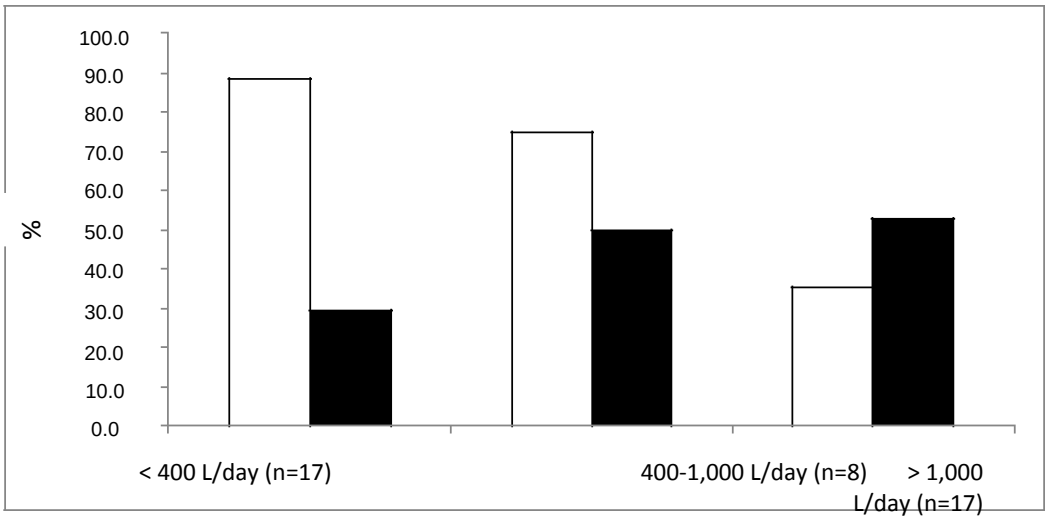

Figure 1. Percentage of milk samples above the tolerance limits adopted in Brazil (500,000 CFU/mL or 600,000 cells/mL) for mesophilic (□) and somatic cell counts (■).

observed in farms of high and intermediate production (52.9 and 50.0%, respectively).

Considering the limit of 500,000 CFU/ mL determined by IN 51 for aerobic mesophylic microorganisms in grade B milk (Brasil, 2002), most bulk milk samples analyzed in the present study did not comply with the official regulation. High mesophylic counts in bulk milk indicate inadequate procedures during milking and cleaning of the equipments (Desmasures and Gueguen, 1997). Therefore, compliance with and inspection of hygiene procedures related to milking routine should not only be an important focus of quality control, but also a commitment of milk producers, mainly small and medium scale ones.

Daily yield of a farm indicates size, importance, and technological level of the enterprise. Only two farms classified as low production (< 400 L/day) still used immersion tanks with icy water to cool milk. The other farmers relied in cooling tanks. Although, immediate cooling does not eliminate microorganisms found in the milk, it decreases their speed of growth. Thus, the faster the temperature drops, the better milk is preserved and the higher its quality when it reaches the industry. In terms of milking system, manual milking in buckets inside covered parlors occurred only in 9 farms; pipeline milking systems predominated. Guerreiro (2005) characterized dairy farms in Minas Gerais based on the levels of contamination of raw milk and observed that initial contamination was high (3.5×10^6 CFU/ml) in the three farms that used mechanical milking, much higher than in the farm that relied on manual milking. This finding indicates that technological level of the milking does not necessarily imply in better microbiological quality of milk. It may function as one more source of contamination.

Nero et al. (2005) evaluated four areas in four dairy States of Brazil and concluded that there are difficulties in complying with the standards determined by IN 51 for the production of refrigerated raw milk. According to the results of these authors, 48.57% bulk tank milk samples showed mesophylic counts above tolerance levels. The present study corroborates these findings, indicating that between 35 and 88% of the farms in the state of São Paulo do not comply with current legal standards for mesophylic counts in raw milk.

IN 51 does not determine a specific threshold for psychrotrophic counts, but the high counts observed in all farms indicate that cooling of milk after milking is inadequate. Santos and Fonseca (2002) consider the contamination of milk by psychrotrophs a critical factor affecting the quality of raw milk. These microorganisms as widely spread in the environment and may be found in the water, soil and the animals. Desmasures and Gueguen (1997) showed that the main sources of psychrotrophs during milking are teat surfaces and milking equipment. However, these sources were not evaluated in the present study.

Table 2 shows the results of somatic cell counts and presence of *S. aureus* in the milk of individual cows. There were no differences ($p > 0.05$) in individual somatic cell counts between the three categories. However, the presence of *S. aureus* was more frequent ($p < 0.05$) in the milk of cows of intermediate production farms (400 - 1,000 L/day). These results indicate serious mastitis problems among lactating cows, and low quality of the milk produced. *S. aureus* was more frequently found (16.9%) in the milk of animals from farms of intermediate production (between 400 and 1,000 L/day). This is important information in terms of public health, because many farms in Sao Paulo may be classified in this range of production and their milk may be a vehicle for this

Table 2. Results of *S. aureus* and somatic cell counts obtained in milk from individual cows in dairy farms of São Paulo, Brazil.

Milk production (L/day)	Number of cows examined	Samples showing *S. aureus*		SCC (Log cells/mL)[1]
		N	%	
< 400	194	12	4.1[b]	5.7 ± 0.7[a]
400 – 1,000	99	12	16.9[a]	5.8 ± 0.5[a]
>1,000	325	9	3.5[b]	5.7 ± 0.7[a]

[1] Results are reported as mean ± standard deviation. [a,b] Values within columns with no common superscript differ significantly ($p < 0.05$).

pathogen. It was also observed that smaller (production < 400 L/day) and larger farms (production > 1.000 L/day) did not show statistical differences when compared with each other, indicating that there were no differences between the rates of isolation of *S. aureus*. Therefore, due to smaller volume of milk produced and better milking conditions, respectively, low and high milk yield could not be considered significant risks in the transmission of *S. aureus* to humans.

SCC in bulk tank and individual cow milk is important in the evaluation of subclinical mastitis rates in the herd; estimation of quantitative and qualitative losses in milk production on the farm and, later on, in dairy factories; indication of milk quality; and implementation of preventive measures and actions for mastitis control. In the present study, high SCC observed in bulk milk samples of all farms in all categories may have been due to the persistence of inadequate hygiene practices during milking, with a consequent increase in the spread of mastitis-causing agents. However, it was observed that mastitis and somatic cell counts are determining factors affecting milk quality, especially in farms producing more than 400 L/day. In low production farms (< 400 L/day) SSC tolerance limits were not respected in 29.4% of the farms, indicating that hygiene practices have a lesser impact on the occurrence of subclinical mastitis in these farms than in mesophylic and psychrotrophic counts.

Aerobic mesophylic counts in bulk milk were lower in farms where yield was more than 1,000 L/day, although *S. aureus* was more frequent in the individual milk of cows from intermediate production farms (400 - 1,000 L/day). Although, there were no differences in somatic cell counts in bulk or individual cow's milks, the high counts obtained indicate difficulties and/or lack of knowledge of the farmers on the correct use of milking management techniques and cleaning of the equipment. Therefore, the greatest difficulties for dairy farms to comply with milk quality parameters are related to the adoption of hygienic practices during milking and failures in the milk conservation. Due to the need to comply with the new regulations as adopted in Brazil for raw milk, educational programs should be addressed to improve milk quality, especially in small and intermediate scale dairy farms.

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