Effect of operation practices of raw milk in small production systems on microbiological quality in Tlaxcala, Mexico

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The aim of this study was to evaluate the effect of operation practices of raw milk in dairy small production systems, first obtained under traditional management (Phase 1) and implementing by training a sanitation technique (Phase 2) on microbiological quality in three different counties near Zahuapan river in Tlaxcala, when sanitization technique was applied in operation practices microbiological quality was improved.

Keywords Raw milk, sanitization, microbial quality

1. Introduction

The Dairy Family Production System (DFPS) is part of the Mexican dairy sector that contributes 10% on domestic production (1), DFPS are important because it provides raw material to produce dairy products in small industries or at home (2), which are distributed in local markets. Approximately 170, 000 dairy farms nationwide have an economic influence; in addition, they provide basic food and contribute to income generation (3). Moreover, this DFPS use family labor, which is an important source of employment which is characterized by small work areas of surrounding land near farms, where other animal species, fodder and crops residues are used (4). Milking is done manually and in some cases, with machines, lacking an adequate milking routine (5), with adapted and non-functional facilities (6); these characteristics become determinant in milk quality.

In practical terms, milk quality comes from the milking of healthy and well-fed cows, lacking adulterants, physical, chemical and biological contamination (7), therefore, overall quality is summarized in the fulfillment of nutritional, organoleptic and hygiene expectations. However, when unsuitable production management and transformation practices appears, these expectations are modified, due to milk nutrient richness makes it an ideal culture medium for many microorganisms (8) and therefore an excellent vehicle for human disease transmission, both zoonotic (brucellosis, tuberculosis), or those caused by pathogenic microorganisms including *Salmonella* sp., *Listeria monocytogenes* and *Escherichia coli* O157: H7 (9,10). Furthermore, there are other microorganisms (total coliforms, aerobic mesophilic bacteria, molds and yeasts, among others) that affect organoleptic and physical characteristics of the product, reducing its shelf life (11).

For DFPS, milk quality (physical, chemical, adulterating, microbiological and sanitary quality) is unattractive since not receive an additional payment for this concept, the producer priority is to sell largest milk volumes covering the minimum requirements (lipid content and acidity) from buyers, in addition, current regulations are not mandatory. Given the characteristics mentioned above, DFPS is in the public eye due its limited production of raw milk, which is mostly collected to produce cheeses that are distributed in local markets and could lead to consumer's latent risk, since the product is not subjected to any thermal process for hygienic quality assessment to ensure its safety. The implementation in DFPS for good milking practices leads to the execution of different activities that contribute to the fulfillment of the minimum hygienic milk requirements. These requirements include the provision of an adequate milking infrastructure, producers training and motivation to carry out the activities, as well as an optimal state of instruments, cleaning material and healthy animals (12).

On the other hand, Zahuapan river crosses 14 counties in Tlaxcala, Mexico, river basin irrigates 5,853 hectares. The main crops are maize and forage that are the cattle food support whose production is near 34 million of milk liters from DFPS in al Tlaxcala state (13). In this region, there are several factors that influence the production and milk, including the use of wastewater for forage production. Therefore, the aim of these chapter is to evaluate the microbiological milk quality produced in DFPS of three counties located near the Zahuapan river under traditional management and to evaluate the implementation of the pre-milking cleaning, to demonstrate that there are alternatives in management during the production process that allow to obtain milk with a higher standard of hygienic quality.

2. Materials and methods

2.1 Sample collection

The population of this study comprises DFPS form the most important productive counties near the Zahuapan river in the state of Tlaxcala, Mexico. Counties were selected according to grain, forage and milk production, corresponding to Tlaxco (Site 1 = S1; n=50), Atlangatepec (Site 2 = S2; n=35) and Tetlatlahuca (Site 3 = S3; n=43).

2.2 Phase 1: Initial microbiological characterization

At this stage, DFPS traditional management, generally lacking pre-milking procedures, were evaluated. At the end of milking, 100 ml milk samples were collected in sterile bottles per unit of production and transported at a temperature of 4-8 ° C for later analysis to the Laboratory Unit of Colegio de Posgraduados Puebla, Mexico.

2.3 Phase 2: Food safety and sanitation training program

A food safety and sanitation training program (Table 1) was developed for 27 DFPS members, highlighting issues related to the importance of risks and implications of milk producing and sanitary quality, in addition producers were trained to incorporation of pre-milking cleaning practices.

Table 1 Content of Food safety and sanitation training program to DFPS members of 3 counties near Zahuapan river in Tlaxcala, Mexico.

Training Program	Subjects	Participants
Food safety	Importance of milk hygienic quality. Factors implicated in milk hygienic quality. Benefits of milk production with sanitary quality Good handling and milking practices Discussion pannel	27 DFPS members
Sanitation	Udder washing technique	

In udder washing technique participants practiced washing udders with drinking water and liquid soap, followed by drying with disposable towels and disinfected with a solution of 5% sodium hypochlorite, executing the subsequent drying. Training program effectiveness was evaluated by sampling after milking at the same conditions in Phase 1.

2.4 Microbial analyses

Five microbiological parameters were determined: total coliform bacteria (TC), mesophilic aerobic bacteria (MAB), *Staphylococcus aureus*, molds and yeasts, and *Salmonella* sp (14). Decimal solutions 10^{-1} to 10^{-5} were made with phosphate-buffered solution as established by the Official Mexican Standard NOM-110-SSA1- 1994 (15) for the preparation and dilution of samples for microbiological analysis. Standard plate count agar and violet red bile agar (BD-Bioxon, Mexico) were used for TC and MAB. Baird Parker agar (Difco, Mexico), coagulase and thermonuclease were used to isolate and identify *S. aureus.* For molds and yeasts PDA medium (BD-Bioxon, Mexico) were grown. For *Salmonella* sp. of pre-enrichment lactose broth (BD Bioxon, Mexico) was used and tetrathionate broth (BD-Bioxon, Mexico) for selective enrichment, from selectively enriched medium was streaked onto selective xylose lysine deoxycholate (XLD) agar (BD bioxon, Mexico). *Salmonella* suspected colonies from XLD agar medium were used for biochemical confirmation (TSI agar, LIA agar, MIO agar, and Simons citrate agar)

2.5 Statistical analyses

Microbiological counts were expressed in log (CFU/mL) were analyzed under a completely randomized design with a factorial arrangement (3^2), using SPSS version 21.0 (IBM Corp, Armond, NY) statistical software. Values are presented as mean ± standard deviation. A Tukey multiple test was performed to compare means ($p < 0.05$).

3. Results and discussion

Milk quality is conditioned by different factors including handling, which as documented has a direct influence on microbiological counts. In Table 2, bacterial load of raw milk in Phase 1 for each site (S1, S2, S3) are presented. TC counts were set between 3.57-5.92 Log cycles, for MAB 6-6.69; for *Staphylococcus aureus* the results ranged between 4.76-4.98 Log cycles, finally for molds and yeasts the averages were 2.17-4.38 Log cycles (1.5x102 and 2.4x104 cfu / ml). In addition, it was observed that there were no significant differences ($p < 0.05$) for CT, BMA and *Staphylococcus aureu*s counts, except for molds and yeasts estimates, demonstrating that management is homogeneous in all DFPS,

characterized by deficient materials or no pre-milking cleaning (16), improper facilities for obtaining the product (17), and a determining factor was hand-milking since milk showed a higher microbial density, specially with higher counts of *Staphylococcus aureu*s (18).

Table 2. Analyses of microbial parameters [Log(CFU/ml) mean ± standard deviation] in raw milk from three different counties near Zahuapan river, Tlaxcala, Mexico during Phase 1.

Microbial parameters	S1	S2	S3	*Limits
TC	5.92±1.62[a]	3.57±2.83[a]	4.77±1.13[a]	1.3
MAB	6.69±0.67[a]	6.19±1.54[a]	6.00±1.03[a]	5
Staphylococcus aureus	4.98±1.02[a]	4.96±1.10[a]	4.76±1.07[a]	1
Molds and yeasts	4.38±0.63[a]	4.22±1.38[a]	2.17±1.63[b]	2.6

Different letters within the same row for each microbiological parameter indicates significant difference ($p < 0.05$).
* According to NOM- 243-SSA1-2010.

Official Mexican Standard (NOM-243-SSA1-2010) establishes milk sanitary specifications. In Phase 1, the mean values obtained in TC and MAB exceed those limits, shows a high contamination due to poor management practices at milking. Arcuri et al. (8) in Brazil (n = 24) and Fulya (19) in Turkey (n = 100) reported similar results, they associate them with a possible cow udder infection, milking materials, stables and storage unhygienic conditions, poor cleaning practices developed by workers; While in Sudan (n = 120) they showed that seasons also influences in high microbiological content (20). For molds and yeasts the standard stipulates that the count should be less than 500 cfu/ml; however, results exceed the limits; however, in addition to associating it with other factors like high content of TC and MAB, it also attributes it to the poorly preserved forage storage (19). Presence of these microorganisms in milk and milk products is important because they not only modify organoleptic characteristics, but also produce toxins in consumer's intestinal tract (20).

Table 3 shows the microbiological analyses during Phase 2, it is observed that there was a considerable reduction of the bacterial load (0.69-1.04 Log cycles for TC, 3.32-4.95 for BMA, and 2.58-3.04 for *Staphylococcus aureus*); for molds and yeasts counts were between 0.47-1.11 Log cycles, no significant differences were found between sites ($p < 0.05$) for this Phase. In both study phases *Salmonella* sp. was negative. Consequently, the implementation of food safety and sanitation training program to DFPS results on a significant ($p < 0.05$) decrease on microbial loads, thus achieving minimum safety requirements in all parameters except for *Staphylococcus aureus*, demonstrating that hygiene it was not enough practice to obtain an allowable count in this variable, because this microorganism is associated with animal health mainly related to udder and mastitis problems (21,22).

Table 3. Analyses of microbial parameters [Log(CFU/ml) mean ± standard deviation] in raw milk from three different counties near Zahuapan river, Tlaxcala, Mexico during Phase 2.

Microbial parameters	S1	S2	S3	*Limits
TC	1.02±1.28[a]	0.71±1.08[a]	0.62±0.96[a]	1.3
MAB	4.24±1.85[a]	4.95±1.59[a]	3.33±1.64[a]	5
Staphylococcus aureus	2.69±2.02[a]	2.58±1.94[a]	3.05±1.80[a]	1
Molds and yeasts	1.12±1.69[a]	NG	0.53±1.05[a]	2.6

Different letters within the same row for each microbiological parameter indicates significant difference ($p < 0.05$).
* According to NOM- 243-SSA1-2010. NG= No Growth.

Microbial counts obtained in Phase 1 and 2 are shown in Figure 1, shows that pre-milking cleaning, microbial load considerably reduced, being affected TC and molds and yeasts (83.7% and 84.8% of Log cycles reduction respectively); however, although the decrease of MAB was lower (33.9%), it was found to be within the maximum permissible limits; therefore pre-milking hygiene becomes a tool that contributes to obtaining milk with acceptable hygienic quality.

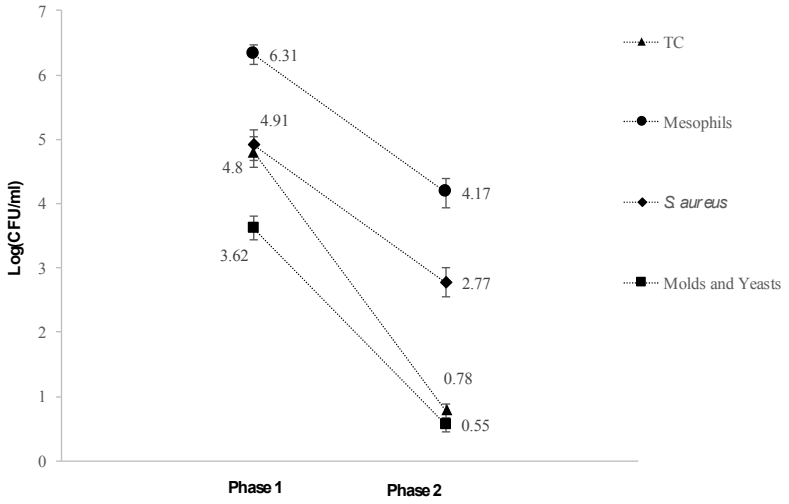

Figure 1. Comparison of microbial load in Log(CFU/ml) in Phase 1 and Phase 1 by study variable in raw milk from three different counties near Zahuapan river, Tlaxcala, Mexico.

4. Conclusion

The study showed that milk traditionally produced in DFPS is deficient, making it a consumer risk; however, training programs to DFPS members about the importance of producing quality milk and its implications, and the implementation of basic hygiene procedures can generate milk with acceptable quality to the dairy chain (producers). In addition, it is necessary to spread the importance of providing a product that does not have a potential risk to the consumers.

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