

Bacteriological Quality of Raw Milk: A Problem Concerning Many Farmers

Eliandra Mirlei Rossi*, Jessica Fernanda Barreto, Rafael Mueller, Katiussa Cipriani,
Cheila Biazussi, Emanuela Valer, Jéssica Tederke, Djoney Pedro Dorfey

Universidade do Oeste de Santa Catarina-UNOESC, Campus de São Miguel do Oeste-SC, Rua Oiapoc, Santa Catarina, Brazil

Abstract In Brazil, many farmers have been having a hard time trying to meet the standards of raw milk. Thus, this study sought to verify if there was any kind of microbiological contamination in raw milk as well as assessing the hygiene practices held by farmers. One hundred and eighty raw milk samples were collected from sixty different dairy farms. In order, was analyzed sixty water samples and a questionnaire was applied to the farmers. 51.6% (31) of the farms have counting of bacteria above the ones set by Brazilian Law. 66.6% (40) have water contaminated with coliforms. The farmers' daily practices showed that in all dairy farms assessed for this study there are several mistakes taking place during the milking, storing and cleaning processes. Thus, continuous training programs must be offered to farmers to demonstrate the importance of caring about a suitable bacteriological quality in milk production.

Keywords Raw Milk, Contamination, Good practices, Farms

1. Introduction

In recent years, there has been greater interest in increasing milk quality, mainly because the production of this food has taken on a really important role inside Brazilian Economy. Nowadays, the state of Santa Catarina, Brazil, is an icon inside the national agricultural sector and dairy farming has become the highlight of this state. In 2013, the milk production in Santa Catarina reached 2.9 billion liters, meaning 8.4% of the total amount produced in Brazil and it had 6.3% growth compared to 2012. The West region of Santa Catarina (region of our study) is responsible for 73% of all the state production [1].

Information released by the Brazilian Institute of Geography and Statistics – IBGE [1] revealed that the farm of west region of Santa Catarina is responsible for the greatest portion of the state's dairy industry. The region's small farms are extremely important for the local economy, what has supplemented concerns coming from dairy farmers and milk production professionals about the quality of the product offered.

For this purpose, physical, chemical, microbiological, hygiene and sanitary aspects have been the parameters used to check and determine the quality of the raw milk produced in farms [2-4].

Several researches have shown that raw milk has very low

microbiological quality, in other words, it has high bacteria counting and this contamination may come from different sources like: milking environment, breast infection, cooling time, animal bacterial microbiota and inappropriate cleaning of udders and milking equipments [5-9].

Dairy is rich in nutrients, what makes it an ideal environment for many microorganisms to develop and, therefore, highly perishable [10]. The growth of microorganisms is unavoidable, unless food undergoes heat, irradiation, cooling and freezing treatments [11].

The microbiological contamination of raw milk is a serious problem worldwide and, for many years, strategies to improve dairy quality have been widely discussed [3, 8, 12-16]. Although raw milk intakes are low, it is used to manufacture several other dairy based products, what, consequently, affects their quality [3, 15].

In Brazil, the Normative Instruction number 62 (IN62) created by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) on September 29th, 2011 focuses on setting improvements needed for the dairy sector and establishes final standards for the quality of refrigerated raw milk [17].

In this context, it is clear that there is a need to implement changes and improvements in the milk's productive chain in order to meet IN62 standards, because most dairy farmers have a hard time meeting the quality standards established in this law [17].

Thus, it is also clear that there is a need to perform studies which will demonstrate the quality of the raw milk produced in farms located in far West Santa Catarina, Brazil, as well as factors that may stand in the way of keeping milk's quality as

* Corresponding author:

eliandra_bio@yahoo.com.br (Eliandra Mirlei Rossi)

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the water used for milking process or even cleaning utensils, for example.

According to Verdier-Metz, Michel, Delbès & Montel [18] and Chye et al. [10], the microbiological quality of raw milk in farms must be monitored as a way to determine what can be improved during the process to ensure a safe and suitable product.

Because of the role the dairy sector plays in the national economy and considering all requirements set by the federal sanitary law [17], this study aims at checking the total count of mesophilic bacteria present in refrigerated raw milk and analyze the microbiological quality of the water used in these farms. Besides that, this study has also assessed cleaning and sanitizing practices held by dairy farmers, because they are vital when determining the microbiological quality of raw milk.

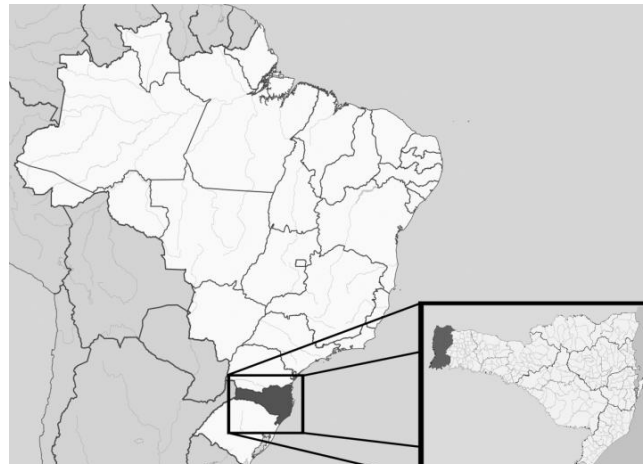


Figure 1. West region of Santa Catarina, Brazil

Water (n: 60) and milk (n:180) samples were collected straight from the cooling tanks of the farms with sterile bottles and transported in refrigerated boxes to the Laboratory of Microbiology of University to microbiological analysis.

2. Materials and Methods

2.1. Sampling

Samples were collected from 60 dairy farms located in the far west region of Santa Catarina, Brazil (figure 1) from September 2014 to December 2015, randomly and in triplicate.

Farm:

Number of lactating cows:

Milk production/day:

Type of milking:

() Channeled (direct to tank) () Mobile milking (manual transfer to tank)

How hygienization is made of breasts of cow?

PRE-DIPPING: () No () Yes

If yes, write:

Product:

Composition:

Concentration recommended by the manufacturer:

Effectively used concentration:

Describe the method used:

Use of towels (paper/clothing) for tear drying:

AFTER-DIPPING: () No () Yes

If yes, write:

Product:

Composition:

Concentration recommended by the manufacturer:

Effectively used concentration:

Describe the method used:

Use of towels (paper/clothing) for tear drying:

Incidence of mastitis: () No () Yes

Milk storage temperature: () below at 4°C () Above at 4°C

Do you analyze water? () No () Yes

Is the tank used to store the water used in milking cleaned frequently? () No () Yes

Figure 2. Questionnaire used to evaluate the daily practices held by dairy farmers

2.2. Microbiological Analysis of Refrigerated Raw Milk and Water Used in the Dairy Farms

The mesophilic bacteria count was performed using refrigerated raw milk according to the methodology described by [19] in which 1ml of the sample was previously diluted in peptone water 0.1% (Merck, Darmstadt) and inoculated in triplicate using standard method agar (PCA, Merck, Darmstadt). The plates used to counting mesophilic bacteria were incubated at 36°C for a period of 48 hours and the results were shown in UFC/ml.

The microbiological analyses of water (total and thermotolerant coliforms and heterotrophic bacteria) followed the methodology set by the Normative Instruction number 62 from August 26th, 2003 from the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA).

This study has used the multiple-tube fermentation technique to access the amount of coliforms present in water. The results were displayed using the Most Probable Number (MPN/100 ml).

Yet, the analysis of heterotrophic bacteria was performed using the Pour Plate technique with Standard Agar (PCA) for the results. The results were displayed CFU/ml.

2.3. Daily Practices Held by Dairy Farmers

The daily practices kept by dairy farmers were assessed through a questionnaire, which focused in questions related to their hygiene habits (figure 2) used during milking and hygiene of equipment.

3. Results and Discussion

3.1. Microbiological Analyses of Refrigerated Raw Milk

The results we found demonstrate that the refrigerated raw milk collected from the 60 farms assessed in this study, 51.6% had their bacterial counting above the standards (table 1) set in IN 62 from December, 2011 [17].

Even though only 48.4% of the properties have had bacterial quality within what is allowed by IN62/2011, all of them were very close to 5.0 log₁₀ CFU/ml. This shows that the quality of the raw milk still a challenge for dairy farmers, mainly because MAPA has been discussing this subject only since 2002 when it published the Normative Instruction 51, lately replaced by IN62/2011.

IN62 was published in 2011 and, since then, MAPA has been trying to make it easier for dairy farmers to adjust to this new law. For this purpose, it has determined different total bacterial counts (TBC) for different time periods established in a schedule. This means that until 07/01/2016 all dairy farmers must produce refrigerated raw milk with TBCs lower than 5.0 log₁₀ CFU/ml. In this study, the bacterial amounts specified for 07/01/2016 were used to discuss the results obtained here, once this was one of the main concerns of the dairy farmers and because most of them were still having high TBC in their refrigerated raw milk.

The bacteriological contamination of refrigerated raw milk is worldwide concern [8, 20] and Brazil is no different [9, 21].

Refrigerated raw milk samples are above acceptable limits, showing that hygiene procedures held during milking and storing processes were inappropriate [9, 22].

According to Verdier-Metz et al. [19], the total bacterial counting in raw milk may change as a result of several factors like: cleaning procedures used for the milking place and cattle udders, appropriate cooling, and others.

Segundo Verraes et al. [4] the bacteriological quality of raw milk can be determined based on several parameters, but the total mesophilic bacteria count has been widely used in several studies, because it determines the general amount of microorganisms present in milk, which include pathogenic and non-pathogenic bacteria. These researchers say although milk naturally owns antimicrobial agents in its composition (lactoferrin, lysozyme and lactoperoxidase), these components are really not relevant, because they are considered limited when controlling microorganism growth in raw milk.

Thus, it is essential to keep milk with low mesophilic bacterial counting [23], because they are built mostly of acidifying microorganisms [24, 25]. Besides being a risk for human health, heterotrophic bacteria may induce irreversible physical and chemical changes in milk through acidification [26, 27], destabilizing soluble proteins and casein micelles, favoring coagulations, changing milk's flavor and smell and reducing its immunological and nutritional value [28].

In the far west region of Santa Catarina, Brazil, most dairy farms are small farms. The daily production of the 60 dairy farms assessed by this study was 365.6 l/day, and most of them (56.6%) produce up to 300 L of milk every day. From these, 56.6% (34) had 5.0 log₁₀ CFU/ml TBC (values established by IN62/2011).

Table 1. Microbiological quality of raw milk and water from 60 farms studied in the west region of Santa Catarina, Brazil

Performed analyzes	Above Standards	Below Standards	General average counting	Maximum and minimum counting	Standards used
Counting bacterial total in raw milk	31 (51.6%)	29 (48.4%)	5.03 log ₁₀ CFU/ml	3.9 to 6.2 log ₁₀ CFU/ml	Normative Instruction number 62 (IN62/2011)
Contamination of water	40 (66.6%)	20 (33.3%)	MPN 266/100 ml# MPN 412/100 ml*	MPN 29 to >1100/100 ml# MPN 3.6 to >1100/100 ml*	Decree 2914/2011

total coliforms

* thermotolerant coliforms

The highest percentage of samples with TBC above standards was found in the group of dairy farmers who produced between 300 and 1.000 l/day (11.1%), because 41.6% of them the TBC amounts were higher than the ones established by IN62/2011.

According to Mhone, Matope & Saidi [29] and Suranindyah *et al.* [8] the high amounts of bacteria found in refrigerated raw milk can be explained by the fact that in small farms there are several hygiene problems occurring during milking and raw milk storing processes. On the other hand, it is important to highlight that other studies like the ones performed by Zweifel, Muehlherr, Ring & Stephan [30] say that new technologies implemented in the milking and milk storing sectors in bigger dairy farms are not always linked to better microbiological conditions. This tells us that keeping good hygiene habits would be the most important method to decrease worries with TBC in the dairy sector.

3.2. Microbiological Quality of the Water used in the Rural Properties

The water, in most dairy farms (66.6%) was contaminated with coliforms (table 1). In most farms (45) the water is not chlorinated and only seven were not contaminated. Of the chlorinated water samples 15 only two of them were contaminated water. This shows us how important is disinfection to maintaining the microbiological quality of water.

The average amount of coliforms were high (table 1) and thirty (75%) of the 40 water samples contaminated with total coliforms were also contaminated with thermotolerant coliforms.

These results demonstrate a high amount of bacteria in the water assessed for this study and enhances the studies previously performed in far West Santa Catarina, Brazil, because other studies like the one developed by Rohden, Rossi, Scapin, Cunha & Sardiglia [31], Malheiros *et al.* [32] and Rossi *et al.* [33] also found a great amount of total and Thermotolerant coliforms in their water samples. This makes clear that the water used in rural properties in far West Santa Catarina, Brazil, has low microbiological quality.

Similar results were found worldwide. We can mention a study performed by Schets *et al.* [34] and Hong, Quiu & Liang [35], for example, who found coliforms in water intended for human consumption.

Water contamination in farms has also appeared in other Brazilian locations like São Paulo, because Amaral, Filho, Rossi Junior, Ferreira & Ludmilla Barros [36] showed that 90% of the water samples taken from water sources, 90% of the ones taken from reservoirs and 96.7% of drinking water were contaminated by total and thermotolerant coliforms. Yet, Soto *et al.* [37] found in a study performed with shallow wells in public schools located in the countryside of Ibiúna/SP that 90% of their water samples were

contaminated with total coliforms and 82% with thermotolerant coliforms, restating that this is not an isolated problem and that it is more common in rural areas where water quality control is less effective.

Having water contaminated just with total coliforms may indicate that this contamination came from organic matter, because this group of microorganisms is used as an environmental contamination parameter [35].

Taking into consideration the fact that the presence of thermotolerant coliforms contamination indicates fecal contamination and perhaps the presence of enteric pathogenic microorganisms, the results found for the wells show that they be a health risk for the ones drinking water from it, mainly because when this water is consumed with no previous treatment, it may pass on several gastrointestinal diseases [36].

Moreover, several studies [6, 39, 40, 41] report that if contaminated water is used to rinse equipments and thin cleaning products, it may contaminate directly or indirectly the milk produced in the farm.

Our study indicates that water contamination may be linked to several factors, but the main one is the lack of cleaning. Only 25 (41.6%) farms clean their water tanks every six months. From these, fifteen were contaminated with coliforms, showing that this microbiological contamination may not come from the water tank itself and that cleaning has not been performed properly.

This information found about the water microbiological quality in dairy properties may suggest that this is the source of raw milk contamination.

These results are similar to the ones found by Perkins *et al.* [42] in Canada, where they have discovered that water has directly influenced the quality of refrigerated raw milk. The fact demonstrates how important is using safe water to clean utensils and equipments used during the milking process and raw milk storage in these properties.

As mentioned above, in the far West region of Santa Catarina water contamination is a serious problem that locals have faced for so long [31-33, 43] and this may be affecting the bacteriological quality of the refrigerated raw milk produced there, because, most of the time, producers deny the fact that water is a relevant factor to improve milk quality.

Most dairy farmers (83.3%) assessed in this study do not analyze their water, because they believe that since water comes from a shallow or a deep well it has great microbiological conditions and they understand it as microorganism free water. These results uncover that farmers do not worry about the quality of water they use in their properties and that it is responsible for changing the microbiological quality of the raw milk they produce every day.

Table 2. Practices held by dairy producers from 60 farms studied in the west region of Santa Catarina, Brazil

Daily practices kept by dairy farmers	Yes	No
Clean the udders of the dairy cattle before (pre-dipping) and after (pos-dipping) the milking process	63.3 %	36.7%
Use mobile milking systems	65%	35%
Clean their water tanks	61.6%	38.4%
Control animals' mastitis	36.6%	63.4%

3.3. Practices Held by Dairy Producers

Several studies [6-9, 13, 16, 19, 30, 44] have reported that daily practices held by dairy farmers have direct effects on milk quality. The results uncovered by our study show that there are many problems changing the microbiological quality of refrigerated raw milk.

Cleaning the udders of the animals before and after milking is one of the requirements set by the Brazilian law (IN62/2011) and, in this study, we have noticed that the most (63.3%) of the dairy farmers clean the udders of the dairy cattle before (pre-dipping) and after (pos-dipping) the milking process (table 2). From these, 26 (68.4%) perform the correct procedures established in IN62/2011 from MAPA.

According to Verdier-Metz et al. [19] cleaning the cows' udders correctly is imperative, because many bacteria are commonly found on the skin of these animals, what makes the udder one of the main sources of microorganisms inoculation in milk. Even though udder disinfection is a widely recommended practice, several studies [7, 8, 19, 21] say the methods used when cleaning and disinfecting udders are, most of the time, incorrect.

This problem has been noticed in several countries (Indonesia, India, France, Canada, Brazil and others). In the far western region of Santa Catarina- SC, a study performed by Maldaner et al. [21] reported that in the two properties assessed by their study the udder cleaning process was performed incorrectly, reinforcing the need for offering continuous training focused on showing dairy how important is to sanitize the udders of the cows in order to have refrigerated milk with low bacterial count.

Besides cleaning the breasts correctly, another criterion recommended by the Brazilian law (IN62) is the mechanization of the milking process. This study has shown that most dairy farmers (65%) use mobile milking systems (table 2). However, 60% (18% with pipeline system and 47% with mobile system) use these systems incorrectly, what entails in high bacteria count most of the time.

In the far Western region of Santa Catarina many dairy producers (no analyzed data) say that after they had installed the milking system in their properties, the total bacterial count (TBC) has increased significantly and this may be assigned to the incorrect sanitizing and use of these systems.

According to Zweifel et al [30], even though different milking technologies decrease the contact with other animals, workers and air, the amount of bacteria found in the milk is not related to the type of technology used in the property, because many properties that use automated milking systems

are producing low bacterial count milk. Thus, these researchers say these technologies (automated milking systems) require special cleaning care; otherwise it may become the factor responsible for decreasing the microbiological quality of refrigerated raw milk.

According to Zweifel et al. [30] in addition to the hygiene conditions of the cooling and storage tanks, it is vital to keep milk correctly refrigerated, because high temperatures facilitate microbiological growth. The most of the farms assessed during the study performed would clean their water tanks correctly, but only 30% of them use different disinfecting products, what helps microorganisms to develop resistance and decreases the antimicrobial resistance of the products used.

Moreover, another factor observed during the study was that 36.6% of the dairy farmers say they control the animals' mastitis (table 2). This can contribute to increase TBC in raw milk, because according to Kalmus et al. [12] mastitis increases the amount of TBC in raw milk and it may be responsible for contaminating this food with several pathogens.

Generally, it has been observed that the biggest problem faced by the dairy farmers still the mistakes they make during the milking and storing processes of raw milk, because besides all mistakes mentioned in this study, it has been noticed that it is very common to use cloths to dry breasts of dairy cattle, incorrect concentration of sanitizers used to disinfect utensils and manual transfer of raw milk to storage and cooling tanks, actions that contribute directly to dairy bacteriological contamination.

4. Conclusions

The results found in this study allow us to conclude that the bacteriological quality of refrigerated raw milk in the far west region of Santa Catarina, Brazil is a huge problem for dairy farmers, because only 48.3% (29) of the farms assessed here had their milk production with TBC amounts within IN62/2011 standards. These results are extremely worrying, because dairy production is one of the main agricultural activities in this region and from July 1st, 2016 onwards, producers who do not keep TBC amounts within law standards will be excluded from this activity.

Another conclusion we had with our study is that the problems affecting directly the microbiological quality of the milk produced by these farms are water microbiological contamination and mistakes made during milking and storing raw milk processes.

Thus, results from this study suggest that training and inquiries must be continuously performed in dairy farms in order to improve the microbiological quality of this product, as well as keeping alive this important source of income for families who live in small properties.

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