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Physicochemical and microbiological quality of refrigerated raw bovine milk commercialized in Presidente Médici, Rondônia state, Western Amazon, Brazil

Qualidade físico-química e microbiológica de leite bovino cru refrigerado comercializado em Presidente Médici, estado de Rondônia, Amazônia Ocidental, Brasil

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ABSTRACT: The aimed of the study was to evaluate the quality of raw milk from some suppliers in rural and urban areas and to test the hypothesis that it is unsafe for direct consumption. The study was conducted with suppliers in rural and urban areas in Presidente Médici municipality, Rondônia state. The method of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), was used, which consists of titrating a portion of the sample with a sodium hydroxide solution of known concentration, using phenolphthalein as an indicator. According to Brazilian legislation and literature, stability of Alizarol, foreign substances, Chloride, Hypochlorite, Hydrogen Peroxide and Colostrum were analyzed. Microbiological analysis, standard plate count (SPC) and quantification of thermotolerant coliforms were also performed using the most probable number (MPN) technique. Values above the limits established by legislation were found, mainly for temperature and titleable acidity, in addition to stability results against Alizarol and the presence of colostrum. There were high microbial contents with regard to SPC, and, in this case, the presence of pathogenic microorganisms cannot be disregarded. The presence of thermotolerant coliforms may indicate probable contact of milk with feces. Therefore, it is considered that the samples analyzed here are not safe for ingestion in the raw form.

KEY WORDS: Food microbiology; Food security; Products inspection of products of animal origin; Raw milk quality; Thermotolerant coliforms.

RESUMO: O objetivo do estudo foi avaliar a qualidade do leite cru de alguns fornecedores da zona rural e urbana e testar a hipótese de que não são seguros para o consumo direto. O estudo foi conduzido em fornecedores da zona rural e urbana do município de Presidente Médici, estado de Rondônia. Empregou-se o método do Ministério da Agricultura Pecuária e Abastecimento (MAPA), que consiste na titulação de uma porção da amostra por uma solução hidróxido de sódio de concentração conhecida, utilizando como indicador a fenolftaleína. Conforme a legislação brasileira e a literatura, foram analisados estabilidade do Alizarol, substâncias estranhas, Cloreto, Hipoclorito, peróxido de Hidrogênio e Colostro. Foram realizadas também análises microbiológicas, contagem Padrão em Placa (CPP) e quantificação dos coliformes termotolerantes através da técnica do número mais provável (NMP). Foram encontrados valores acima dos limites estabelecidos pela legislação, principalmente para temperatura e acidez titulável, além dos resultados de estabilidade frente ao Alizarol e da presença de colostro. Houve altos teores microbianos no que se refere à CPP, e, nesse caso, a presença de microrganismos patogênicos não pode ser desconsiderada. A presença de coliformes termotolerantes pode indicar provável contato do leite com fezes. Portanto, considera-se que as amostras aqui analisadas não são seguras para ingestão na forma crua.

PALAVRAS-CHAVE: Coliformes termotolerantes; Inspeção de produtos de origem animal; Microbiologia de alimentos; Qualidade do leite cru; Seguridade alimentar.

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INTRODUCTION

Bovine milk is an important and very present item in diet of the Brazilian population, containing a rich nutritional composition, with fats, carbohydrates, proteins, vitamins and minerals, available every day at the Brazilian table (GALLIER et al., 2010). However, according to a report by the ASSOCIAÇÃO BRASILEIRA DO LEITE LONGA VIDA (ABLV, 2021), was marked by a retraction in the national milk market. As a result of the economic climate and unfavorable conditions in year 2021, the total apparent per capita consumption of dairy products was one of the lowest in the last decade. The period of the coronavirus (COVID-19) pandemic caused an increase in costs of dairy production, compromising consumption (CORTEZ et al., 2022).

The Brazilian milk production in year 2021 was 33.69 billion liters (ABLV, 2021), with Rondônia state participating with 3.07% of this production, 8th place in the national ranking (HOTT et al., 2021). Presidente Médici municipality is in 30th place in dairy production in Rondônia state, with 34,019,000 liters per year, that is, of high social and economic relevance for the region (GASPAROTTO et al., 2021). Milk, due to its nutritional composition and high water activity (aW), is highly perishable, therefore, one of the precautions to taken right after it is obtained is rapid cooling. Normative Instruction No. 77 at Ministério da Agricultura, Pecuária e Abastecimento (MAPA, 2018a) of November 26, 2018, established a temperature of 4º C for storage of raw milk, before its processing. The informal commercialization of raw milk is a great alternative for family-based farming. However, this sale is prohibited by Decree Law No. 66,183 of November 5, 1970, due to lack of sanitary inspection of the product.

An example of fraudulent substances added to milk are the so-called density reconstituents that, according to Tronco (2013), disguise the addition of water. These include starch and sodium chloride. Milks with high microbial load present acid pH, which is diagnosed through the platform tests performed during reception (ABRANTES et al, 2014). Sodium hydroxide, for example, can be illegally used to neutralize this acidity. On the other hand, in order to paralyze this microbial activity, conservative substances such as hypochlorite and hydrogen peroxide can be illegitimately added.

In this way, the consumption of *in natura* milk can cause problems to human health, as it may contain a high microbial count or have undergone the addition of foreign substances to its composition. Therefore, the hygienic-sanitary control of milk is essential, from its acquisition to its packaging (SANTANA et al., 2021). In addition, it is essential that quality control be carried out through physicochemical and microbiological analyzes in order to ensure the minimum quality requirements required by IN No. 77 (MAPA, 2018a). Milk quality is defined by chemical composition parameters – protein, fat, lactose, minerals and vitamins. Quality and hygiene requirements for raw milk are established for the protection of human health and the preservation of nutritious farms for this food. Based on IN No. 76 (MAPA, 2018b), physicochemical and sensory characteristics, such as odor, must be evaluated. Although the legislation does not impose a taste test, some dairies do to check if sucrose has been added.

After milking, in addition to care with temperature and hygiene, it is essential that the quality control of the milk is carried out through physicochemical and microbiological analyzes in order to ensure the minimum quality requirements required by IN No. 77 (MAPA, 2018a). The physicochemical composition of milk may change due to handling and health of animals on rural farms (MORAIS et al., 2020). However, microbiological quality depends on hygiene during milking of animals, storage temperature and transport of milk from farms to dairy (SANTANA et al., 2021). The immediate refrigeration of bovine milk still on farm and bulk transport were implemented with the aim of improving the quality of raw milk and, consequently, of its derivatives. However, when refrigerated for prolonged periods it can compromise the quality of raw milk due to possibility of selection of psychrotrophic proteolytic bacteria, which are spoilage. Then, the need for the use of milk preservation methods arises. The methods carried out by milk processing industries are pasteurization, the use of ultra-high temperatures and the reduction of water activity. The use of preservatives is prohibited by Normative Instruction No. 76 of November 26, 2018.

Given the overview presented, the aimed was to evaluate the quality of raw bovine milk from some rural and urban suppliers and test the hypothesis that they are not safe for immediate consumption.

MATERIAL AND METHODS

Study area

Presidente Médici municipality, Rondônia state, has 1,758,465 km² (Figure 1). Its population, according to IBGE (2010), was 24,446 inhabitants. Its limits are: to the north, Ji-Paraná; to the south, Castanheiras and Nova Brasilândia do Oeste; to the east, Ministro Andreazza and Cacoal; to the west, Alvorada do Oeste (IBGE, 2016). In this municipality, the population has access to raw milk. This milk is transported from producing farms to points of sale in 50L gallons, on motorcycles or automobiles. Distribution is daily and the product is marketed in bulk. Sales occur next to cooling tanks that receive the freshly expressed milk and are located in specific locations in Presidente Médici. In this case, the buyer takes his packaging.

The cooled raw milks were analyzed monthly, from October 2019 to June 2020, making a total of five monthly samples, and the suppliers were always the same. Approximately 40 km from the urban area, the collections took place in the Settlements Chico Mendes 1 and 2. Settlement 1 has three Rural villages called Agrovilas and Settlement 2 has two, and in each Agrovila a sample was collected monthly. In the urban area, raw milk available at three points of sale in Presidente Médici city was analyzed, namely, in two cooling tanks (named F and G) and in a bakery (point H), making up three monthly samples. The samples were always obtained from the same suppliers and collected from October 2019 to June 2020, making a total of 27 samples.

The tanks in the rural area were named A, B, C and D. The tanks had a capacity for one thousand liters of milk each, remained in a covered, closed place, except for tank C, which was next to corral. In the case of tanks A, B and D, the floor was tiled. Tanks C and E were on a cement floor. However, tank F was in a rural farm, in a covered place and protected from solar radiation, although it had thermostat problems, which caused extreme cooling of the product, close to freezing. Then, the milk remained in 50 liters cans, at room temperature, all morning. In fact, according to the person in charge of the tank, the consumer who purchased this milk appreciated the fact that it had a higher temperature, as it generated the sensation of purchasing a "fresh product". Tank G was located in the edicule of a residence whose owner was responsible for it. The tank remained in a partially open area, exposed to the sun from 6 am to 10 am. In the tank bakery H, the milk was stored in a 20 liters plastic bucket accommodated in a horizontal freezer where other food products were also stored.

Obtaining and processing samples

The samples were transported in isothermal containers with ice, as recommended by Manual of Procedures for Laboratories of the MAPA (2021) and sent for immediate analysis to Laboratório de Análises Físico-Químicas e Microbiológicas

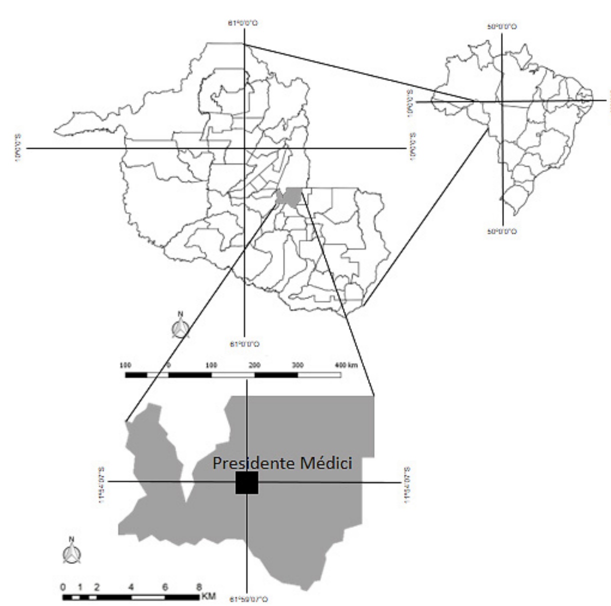

Figure 1. Geographic location of Presidente Médici municipality, Rondônia state.

(LAFQM), Universidade Federal de Rondônia (UNIR), Presidente Médici University Campus, RO. The work area was disinfected with 70% ethanol. All utensils and instruments used were previously sterilized in an autoclave, sterilization oven or flamed at the time of use. The temperature of the milk in the cooling tanks was measured using the thermometers provided by the refrigerators.

Official method of the MAPA was used, which consists of titrating a portion of the sample with a sodium hydroxide solution of known concentration, using phenolphthalein as an indicator (MILAGRES et al., 2012). For this study, the result was expressed in g lactic acid/100 g. The method of Instituto Adolfo Lutz (IAL, 2008) was also used. The principle of the method is based on the fact that milk is an emulsion of fat in water and its density provides information on amount of fat contained in it. The reading was made using the thermolactodensimeter, which was introduced into the sample in a 250mL beaker, taking the reading at the milk level, in the upper meniscus.

For stability to Alizarol, the technique consists of mixing, in a test tube, 2 mL of milk and 2 mL of 0.1% alizarin solution in 68% alcohol (TRONCO, 2013). This test is a quick determination of milk acidity by colorimetry. Alizarin is the indicator, being used as a solution containing alcohol/alizarol 72% v/v, as recommended by IN No. nº 77 (MAPA, 2018b), and the interpretation of the results was made from Art. No. 60 of the same: Brick red color without lumps or with few very fine lumps: milk with normal acidity and stability to alcohol 72% v/v; Yellow or light brown color, both with lumps: milk with high acidity and not stable to alcohol 72% v/v; Lilac to violet color: milk with an alkaline reaction resulting in the presence of mastitis or neutralizers.

Concerning the detection of foreign substances, the methodologies used here are qualitative, that is, they indicate the presence of foreign substances without quantifying them. To verify starch, the method used was described by IAL (2008). If starch was present, a bluish color would develop after heating the sample and subsequent addition of iodine solution. Heating promotes the opening of the helical chain of the starch molecule, allowing the adsorption of iodine with the development of the characteristic color after cooling. 10 mL of fluid milk were heated in a test tube in a water bath until boiling for 5 minutes. Cooling was performed in running water and two drops of Lugol's solution were added and it was observed if there was formation of the color indicating positivity.

To verify chloride, the official method described by MAPA was used, which is based on the reaction of silver nitrate with a molar concentration between 0.095 and 0.105 mol/L with chlorides in the presence of potassium chromate 5% (m/v) as indicator (MILAGRES et al., 2012). The samples were homogenized at room temperature by shaking or inverting the vials 5 to 6 times. When the sample showed lumps of cream, it was heated from 38 to 40° C in a water bath and then cooled to room temperature with occasional stirring. If yellow coloration with absence of red precipitates is observed, the result is positive.

To detect hypochlorite, the methodology used was the one mentioned by Tronco (2013). Hypochlorite can detected by adding 0.5 mL of 75% potassium iodide solution to 5 mL of milk, with consequent formation of potassium chloride. Thus, iodine is released, causing a yellow color, characterizing positivity. To detect hydrogen peroxide, the official method described by MAPA was used, which consists of adding 1% alcoholic guaiacol solution to raw milk. In the presence of the enzyme peroxidase (a natural component of milk) and hydrogen peroxide (a foreign substance), guaiacol is oxidized, causing the formation of a salmon color (MILAGRES et al., 2012).

To detect sodium hydroxide, it was done according to method described by Tronco (2013) so that 2 mL of raw milk in a test tube received the addition of 3 drops of 2% alizarin solution, causing the appearance of a violet color in the presence of sodium hydroxide. To study Colostrum, according to the technique described by Tronco (2013) it is summarized in placing, in a test tube, 5 mL of milk with 5 mL of alcohol at 60º GL and mixing them by shaking. If the clotted milk forms flakes, it indicates that it is colostral milk. This test is based on the dehydrating action of alcohol on milk particles with pH or acidic medium and follows the same principle already mentioned in the alcohol test.

Microbiological analysis

Standard Plate counting (SPC) the surface plating method was used, according to methodology described by Farias et al. (2014). The milk was diluted in sterile saline solution and each dilution was inoculated in sterile Nutrient Agar culture medium at 35º C. The result was expressed in mL Colony Forming Units (CFU/mL).

For quantification of thermotolerant coliforms, the most likely number (MPN) technique was used, also known as the multiple tube method by Jesus et al. (2017). Lactose broth was used to accelerate the growth of coliforms that were present, and a portion of the broth contained in the positive tubes for this analysis was inoculated into *Escherichia coli* - EC broth, a confirming medium for the presence of thermotolerant coliforms.

Data Organization and Statistical Analysis

The data obtained were stored and organized in Epi info™ software, version 3.5.3 - 2011 (OS: MS-Windows, Programming language C Sharp). Data obtained were stored, tabulated and transformed into information, using electronic spreadsheets. To compare the averages followed by standard deviation, the Shapiro-Wilk test (α=0.05) was first carried out to verify the normality of the data. Then, the Kruskal-Wallis test (α=0.05) was applied. The correlation between temperature, acidity and standard plate count was conducted. For this, the Spearman's correlation (ρ) method was used. The program used for data organization and analysis was the Jamovi software, version 1.2.27 (JAMOVI, 2020) in R statistical language, made available by Universidade Federal do Pará (UFPA).

RESULTS AND DISCUSSION

Temperature variable (Table 1), it was found that, considering the entire collection period, 83.3% of the samples were outside the specification of 4º C of IN No. 76 (MAPA, 2018a). Tank D, for example, during the first five months of collection, had a malfunctioning agitator, which certainly affected the temperature of the milk. However, all samples collected at this point were in non-compliance regarding temperature. The maximum temperature detected reached 30.8º C in tank E.

Reasons for temperatures being high in most samples were related to factors such as the fact that at the time of

Table 1. Temperature values (°C) of raw milk from rural and urban areas of Presidente Médici municipality from October 2019 to June 2020.

Milk tanks	Months of sample collection								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rural area									
A	2.4	3.0	5.0	6.2	7.9	14.4	17.3	10.6	7.3
B	30.0	3.2	19.1	12.8	7.1	26.0	18.1	10.4	29.0
C	20.0	4.1	8.8	10.6	4.0	9.3	7.3	9.2	12.7
D	13.3	21.0	27.2	20.5	15.7	8.7	13.6	7.0	8.1
E	14.0	10.9	30.8	11.0	11.4	3.7	23.7	6.0	8.3
Urban area									
F	25.3	22.00	33.0	31.0	1.5	6.0	0.9	30.0	23.0
G	3.9	6.4	4.3	2.9	4.3	3.4	3.5	3.9	6.0
H	30.0	14.00	18.0	14.0	22.0	24.0	23.0	21.0	20.0

Reference value: 4º C.

collection the cooled milk had been added to freshly expressed milk, or an electrical problem in the tank (tank D). The refrigeration of raw milk is closely related to the maintenance of product quality.

In the study by Santos et al. (2009) 11.76% of 34 samples of raw milk collected from cooling tanks were above 4º C. Therefore, the authors observed a lower percentage of non-compliant samples than in this study. Concerning the relative density of the samples (Table 2), the values ranged 1.030 to 1.035 g/mL (Table 2). According to IN No. 76, raw and good quality milk must have a relative density, at 15° C, between 1.028 g/mL and 1.034 g/mL. In cases where the values are below this range, the addition of water should be suspected, while the higher results suggest the addition of reconstituting agents or skimming (MAPA, 2018a). In the current study, 4.44% of the samples showed a relative density value slightly above normality, while the others remained within the standard established by MAPA, indicating, in these samples, the absence of fraud, by adding water, reconstituting agent or skimming.

For the titratable acidity variable, the indices ranged 0.11 g to 0.25 g of Lactic acid 100 mL (Table 3), with only 11.11% of the samples in compliance. Of the 88.89% that were out of the standard, 12.50% had rates below the limit and 87.50% above.

Values below 0.14 g of Lactic acid 100mL are typical of alkaline milks, which may indicate that they come from cows with mastitis, late-lactation milk, retention milk or water-smeared milk (OLIVEIRA et al., 2020). However, values between 0.19 and 0.20 g are slightly acidic, indicating early lactation milk, milk with colostrum or at beginning of the fermentation process. Acidic milks may indicate high microbial contamination and storage at inappropriate temperature before processing. Milks with values 0.22 g do not resist heating to 100º C and with values equal to or greater than 0.24 g do not resist the pasteurization process (72º C) (OLIVEIRA et al., 2020).

Values obtained here corroborate those of study by SILVEIRA et al. (2014) whose values ranged 0.14 to 0.33 g

Table 2. Density values 15° C (g/mL) of raw milk from rural and urban areas of Presidente Médici municipality from October 2019 to June 2020.

Milk tanks	Months of sample collection								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rural area									
A	1.033	1.034	1.035	1.033	1.031	1.033	1.032	1.033	1.033
B	1.032	1.033	1.035	1.034	1.032	1.030	1.032	1.034	1.031
C	1.032	1.033	1.034	1.034	1.031	1.032	1.031	1.033	1.031
D	1.033	1.033	1.034	1.033	1.031	1.032	1.033	1.032	1.032
E	1.034	1.033	1.034	1.034	1.031	1.030	1.033	1.033	1.032
Urban area									
F	1.033	1.031	1.034	1.031	1.031	1.030	1.030	1.027	1.032
G	1.034	1.038	1.034	1.031	1.030	1.032	1.031	1.022	1.031
H	1.031	1.033	1.035	1.035	1.031	1.031	1.031	1.023	1.031

Reference value: between 1.028 and 1.034.

Table 3. Values of titratable acidity (g of Lactic acid/100 g) of raw milk from rural and urban areas of Presidente Médici municipality from October 2019 to June 2020.

Milk tanks	Months of sample collection								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rural area									
A	0.20	0.22	0.20	0.17	0.20	0.20	0.20	0.19	0.11
B	0.20	0.21	0.20	0.20	0.21	0.20	0.20	0.19	0.11
C	0.20	0.22	0.25	0.21	0.20	0.20	0.25	0.20	0.11
D	0.20	0.25	0.23	0.18	0.18	0.21	0.2	0.18	0.12
E	0.21	0.21	0.22	0.20	0.19	0.20	0.22	0.18	0.11
Urban area									
F	0.24	0.25	0.26	0.20	0.19	0.21	0.21	0.10	0.11
G	0.20	0.22	0.22	0.19	0.18	0.19	0.22	0.09	0.11
H	0.24	0.27	0.24	0.22	0.23	0.19	0.25	0.10	0.11

Reference value: between 0.14 and 0.18 grams of lactic acid per 100mL.

of Lactic acid 100mL in the analyzed informal raw milk samples. Of this total, 80% of the samples showed acidity greater than 0.18 g of lactic acid 100mL. In only two samples the average values obtained were within the standards of IN No. 76 MAPA. Regarding stability to alizarol, the indices demonstrated that 15.55% of the samples showed a positive result, with five samples in the same month April 2020 and two in November 2019, 84.45% of the samples were in compliance for this parameter.

For Gasparotto et al. (2020) this test is a simple and quick way to assess the resistance of milk to pasteurized. Alizarin is a colorimetric pH indicator, in which if there is formation of lumps, it will indicate casein instability. The minimum alcohol content recommended by IN No. 76 MAPA in this test is 72º GL or 72%, and only alcohol or alizarol can used. Milk can unstable in two situations: the first, due to acidity of microbiological origin, the second, because the milk is unstable non-acidic (LINA). To differentiate them, it is necessary to carry out the boiling test inside the farm, so that if the milk coagulates during this process, it is a case of microbiological acidity, if it does not, it is a case of LINA, which indicates a false positive, because when exposed to the Dornic test, it demonstrates that even with the loss of casein instability indicated in the alcohol test, it does not present high titratable acidity.

This case arises from an alteration whose causes are not clearly defined, because although the milk is not acidic, it is believed that it would not resist thermal processing (RANGEL et al., 2014). Samples that did not comply with the reference value fit into the first situation, due to acidity of microbiological origin, since their titratable acidity values were high.

Concerning the investigation of foreign substances, none of the samples evaluated presented a positive result for starch, chloride, hydrogen peroxide, hypochlorite and sodium hydroxide. In a study carried out by Gasparotto et al. (2020) no sample evaluated showed a positive result for analysis of chlorine, hypochlorite, hydrogen peroxide and neutralizers. Also in the study of these authors, for the analysis of density reconstituents, it was observed that all samples obtained negative results for the presence of starch, however, it was verified the presence of formaldehyde and nitrate in 13 and 40% of the samples, respectively, and the majority of raw milk samples that showed results outside the physicochemical standards were associated with the occurrence of these substances.

The colostrum results indicated that only samples B and C, only in the month of August 2019, were positive for its presence. According to Cortez et al. (2022) milk samples containing colostrum or from inflamed breast tissues can result in positive results in the alizarol test. After calving, for 8 to 10 days, the cow secretes colostrum, a yellowish liquid with an acidic taste and high density, which coagulates when it is boiled and in the alcohol-alizarol test. Colostrum, which should only be used by the calf, as it contains substances essential to health and favors the elimination of the first feces, and should not even be mixed with normal milk, as it makes pasteurization difficult (SOUZA et al., 2021).

It is worth mentioning that the consumption of colostrum in human diet, in Brazil, is no longer prohibited through Decree No. 9013 RIISPOA, there is even a search for alternatives for its use Entidade Autárquica de Assistência Técnica e Extensão Rural (EMATER, 2017). However, the presence of the same in milk intended for processing in industry remains prohibited. Concerning the standard plate count (CPP), the indices obtained, shown in Table 4, indicate that all were out of the standard, with quarterly averages ranging from 6.39 log to 7.29 log, well above the legal limit of 4, 47 Log CFU/mL, from IN No. 76 MAPA. With the exception of the samples from the out to ten quarters ($p<0.05$), the other quarters did not showed differences ($p>0.05$) in the standard count between the samples. These results indicate that hygiene was lacking at some point in the production chain of these raw milks, from milking to storage in cooling tanks. Allied to this, temperatures above the limit of 4º C contribute to increase in microbial counts. It is worth mentioning that tanks A, B and C, in most of the collections, were in a poor state of conservation, with a dirty floor; open doors, facilitating the entry of dust where the tank remains; presence of dogs very close to site, some even inside the tank storage house.

The reality regarding the milking of cows in many farms in the research region contradicts the recommendations of Embrapa Gado de Leite (2017) that there is hygiene during milking. There are open pens and cleaning at the time of milk

Table 4. Standard Plate Count - SPC (Log CFU/100mL) of raw milk from rural and urban areas of Presidente Médici municipality during the months of collection.

Milk tanks	Quarterly collections		
	Quarterly Geometric Average (Oct to Dec)	Quarterly Geometric Average (Jan to Mar)	Quarterly Geometric Average (Apr to Jun)
Rural area A	6.39 ± 0.40[b]	6.97 ± 0.56[a]	7.15 ± 0.58[a]
B	6.69 ± 0.42[b]	6.77 ± 0.54[a]	7.26 ± 0.58[a]
C	6.90 ± 0.43[b]	6.88 ± 0.56[a]	6.71 ± 0.54[a]
D	7.17 ± 0.45[a]	6.48 ± 0.52[a]	7.07 ± 0.57[a]
E Urban area	6.75 ± 0.42[a]	7.15 ± 0.58[a]	7.29 ± 0.59[a]
F	7.41 ± 0.44[b]	8.21 ± 0.67[a]	8.10 ± 0.59[a]
G	6.94 ± 0.42[b]	7.57 ± 0.61[b]	8.04 ± 0.59[a]
H	7.00 ± 0.42[b]	7.33 ± 0.59[b]	8.42 ± 0.61[a]

Reference value: 4.47 Log CFU/mL; Means followed by different letters (a,b) between columns are different according to Kruskal-Wallis test ($p<0.05$).

removal is not done correctly. Hygienic milking provides milk with better microbiological quality and contributes to reduction of mastitis. In this way, the milking place needs to covered, clean and ventilated. Therefore, high bacterial counts indicate poor hygienic-sanitary conditions during milking and/or problems in milk cooling. SPC results below 4.3 log CFU/mL reflect good hygiene practices in obtaining milk (RIBAS et al., 2016).

In the research conducted by Piana et al. (2014) the authors followed the microbiological quality of milk produced by twelve dairy herds located in seven municipalities of Zona da Mata region, from Minas Gerais state. They observed high geometric averages in SPC in milk from all producers. Some of them did not adopt good production practices, nor did they show interest in changing their routine, such as meeting a specific time for milk delivery to the cooling tank.

Table 5 shown the comparison between the samples regarding the values of temperature, relative density and acidity. For temperature variable, there was a statistical difference ($p<0.05$) between the samples, although the relative density and acidity means showed no difference ($p>0.05$).

Table 5. Statistical comparison of the values of temperature, relative density and acidity of raw milk from rural and urban areas of Presidente Médici municipality from October 2019 to June 2020.

Amostras	Variables		
	Temperature	Relative density	Acidity[1]
Rural area			
A	8.230 ± 2.425^{b}	1.033 ± 0.032^{a}	0.188 ± 0.007^{a}
B	17.300 ± 5.098^{a}	1.033 ± 0.032^{a}	0.191 ± 0.007^{a}
C	9.560 ± 2.817^{b}	1.032 ± 0.032^{a}	0.204 ± 0.007^{a}
D	15.011 ± 4.424^{a}	1.033 ± 0.032^{a}	0.194 ± 0.007^{a}
E	13.311 ± 3.923^{ab}	1.033 ± 0.032^{a}	0.193 ± 0.007^{a}
Urban area			
F	11.111 ± 2.833^{a}	1.039 ± 0.038^{a}	0.211 ± 0.130^{a}
G	12.136 ± 3.095^{a}	1.033 ± 0.038^{a}	0.201 ± 0.120^{a}
H	13.333 ± 3.400^{a}	1.030 ± 0.038^{a}	0.210 ± 0.130^{a}

Averages followed by different letters (a,b,c) between the lines are different according to Kruskal-Wallis test ($p<0.05$). [1]Lactic acid values (g/100 mL). Put the units of the other variables.

Table 6 showes correlation between the variables temperature, relative density, acidity and standard plate count. With the exception of the variables relative density x temperature (0.33) and relative density x acidity (0.35), the others showed a significant positive correlation and, therefore, it is inferred that there was interference between these quality variables.

Figure 2 represents correlation between the variables temperature, titleable acidity and standard plate count, so that as the temperature increases, the values of the other correlated variables increase. Although the legislation on raw milk does not provide for limits on thermotolerant coliforms, their presence may indicate probable contamination of milk with feces of homeotherms.

For this group of microorganisms, the indeces are shown in Table 7. In 71% of the samples they were not detected, being represented as <3.0 MPN/mL. When comparing samples from same tank over the months, there were significant differences: tank A - only in the month of Oct 2019; tank B - in the months of Oct 2019 and Feb 2020; tank C – in the months of Oct 2019, Jan, Feb, May 2020; tank D – in the months of Oct Nov 2019, Feb May 2020 and tank E – in the months of Oct 2019 and Mar 2020. Taking into account the collection periods, with the exception of the months of December 2019, April and June 2020, in the others the samples showed a statistical difference between them ($p<0.05$).

These results for thermotolerants agree with the results obtained by Sousa et al. (2021) whose results showed that 40% of the samples were positive for this group. In the study carried out by Dias et al. (2015) 68.7% of the samples demonstrated high results for thermotolerants, reaching >1100 MPN/mL. Also, in the study carried out by Farias, Croisfelt and Baffi (2014), thermotolerant coliforms were quantified in raw, unrefrigerated, refrigerated and pasteurized raw milk.

The results reached 1100 MPN/mL for unrefrigerated raw milk, 7 MPN/mL for refrigerated raw milk and not detected in pasteurized milk. In this way, it is observed that the temperature influences the multiplication of thermotolerant coliforms, as the milk exposed to room temperature showed a significantly high result, while the refrigerated, even contaminated, contained much lower values. The results indicated that pasteurization destroyed this group, shown the importance of processing.

Table 6. Spearman's correlation coefficients (ρ) of the averages of temperature, relative density and acidity of raw milk brands from rural and urban areas of Presidente Médici muncipality from October 2019 to June 2020.

Variables	Temperature	Relative density	Acidity[1]	SPC[2]
Temperature	-	0.33	0.52	0.57
Relative density	0.33	-	0.35	0.30
[1]Acidity	0.52	0.35	-	0.70
2SPC	0.57	0.30	0.70	-

The darker the color, the strongly is correlation; [1]Lactic acid values (g/100 mL). [2]Standard Plate Count (Log UFC/100mL). Put the units of the other variables.

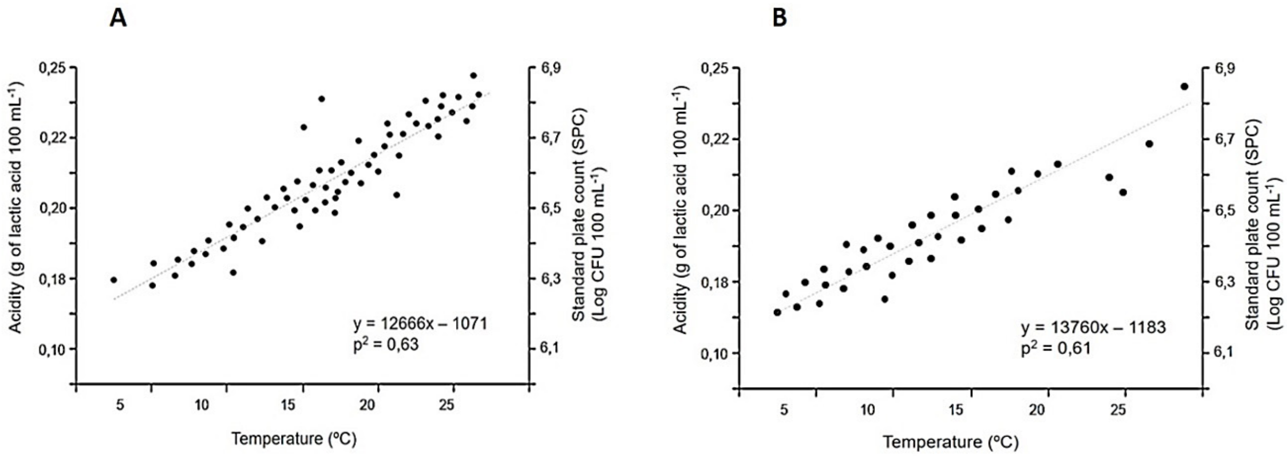

Figure 2. Correlation between the variables temperature, acidity and standard plate count (SPC) of raw milk from urban (A) and rural (B) areas of Presidente Médici municipality from October 2019 to June 2020.

Table 7. Most likely number (MLN/mL) of thermotolerant coliforms in raw milk from rural and urban areas of Presidente Médici municipality from October 2019 to June 2020.

Milk tanks	Months of sample collections								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rural area									
A	7.4 ± 1.4^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}
B	35.0 ± 6.5^{b}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	30000.0 ± 5700.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}
C	75.0 ± 14.3^{b}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	3000.0 ± 660.0^{a}	7200.0 ± 1368.0^{b}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	7200.0 ± 1296.0^{ab}	<3.0 ± 0.0^{a}
D	15.0 ± 2.9^{ab}	3600.0 ± 612.0^{a}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	30000.0 ± 5700.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	610000.0 ± 109800.0^{a}	<3.0 ± 0.0^{a}
E	23.0 ± 4.4^{b}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{ab}	30000.0 ± 6600.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}
Urban area									
F	17.2 ± 3.29^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}
G	18.3 ± 3.50^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}
H	16.7 ± 3.19^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{b}	<3.0 ± 0.0^{a}	<3.0 ± 0.0^{ab}	<3.0 ± 0.0^{a}

Averages followed by different letters (a,b) between the lines are different according to Kruskal-Wallis test ($p<0.05$).

CONCLUSION

Because some samples are non-compliant with the legislation, they are considered unsafe for ingestion in raw form. There may be a lack of information on the part of producers regarding good practices in the milk production chain.

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