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PREVALENCE OF MASTITIS AND CHARACTERIZATION OF MILKING MANAGEMENT OF DAIRY COWS IN NORTHWESTERN SAO PAULO STATE

João Vitor Stefanin Fuzatti,
Universidade Brasil. Campus Fernandópolis, SP, Brazil,
https://orcid.org/0000-0002-0996-0556

Richer Costa Camargo,
Universidade Brasil. Campus Fernandópolis, SP, Brazil,
https://orcid.org/0000-0002-2773-3533

Michel dos Santos Pinto,
Universidade Brasil. Campus Fernandópolis, SP, Brazil,
https://orcid.org/0000-0002-6146-9553

Kedma Lorena da Silva Souza,
Universidade Brasil. Campus Fernandópolis, SP, Brazil,
https://orcid.org/0000-0002-8064-4644

Danila Fernanda Rodrigues Frias,
Universidade Brasil. Campus Fernandópolis, SP, Brazil,
https://orcid.org/0000-0001-8621-3338

Corresponding author:
danila.frias@universidadebrasil.edu.br

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Abstract

The lack of application of good practices in milking management can lead to the occurrence of one of the main diseases affecting the Brazilian dairy herd—mastitis. This study was developed to examine the prevalence of clinical and subclinical mastitis as well as to evaluate the contribution of milking management to this prevalence, thereby providing bases for a better control of mastitis in dairy herds at Fernandopolis region, Sao Paulo State, Brazil The study involved nine farms with 223 lactating cows. All cows were evaluated by the strip-cup test and the California Mastitis Test (CMT). In addition, the employees' conduct during milking; the physical structure of the farm; animal handling; and dry cow treatment were observed. Results were tabulated and evaluated for the development of a flowchart of good practices. Subclinical mastitis was detected in 30.5% of the farms; 66.7% perform mechanical milking and 89% have a cooling tank, but 77.7% do not sanitize the equipment after milking. Pre- and post-dipping were not performed by 89%; 66.6% do not adopt a milking line; and none of the farms realized mastitis detection tests. In conclusion, subclinical mastitis is present in the evaluated herds. By observing the management adopted on each farm, the main critical points were found to be related to failures in handling and in milking and equipment hygiene. The implementation of a flowchart of good milking practices is extremely important for the producer, since the main method to avert and control infection is prevention.

Keywords Flowchart, mastitis, milking hygiene, prevention

PREVALÊNCIA DE MASTITE E CARACTERIZAÇÃO DO MANEJO DE ORDENHA DE BOVINOS LEITEIROS DA REGIÃO DE FERNANDÓPOLIS, SÃO PAULO

Resumo

A falta da aplicação de boas práticas no manejo de ordenha pode resultar na ocorrência de uma das principais doenças que acomete o rebanho leiteiro no Brasil, a mastite. O objetivo deste trabalho foi avaliar a prevalência de mastite clínica e subclínica bem como avaliar a contribuição do manejo da ordenha nessa prevalência para que, dessa forma, se tenham subsídios que proporcionem um melhor controle da mastite em rebanhos leiteiros da região de Fernandópolis, São Paulo. Participaram da pesquisa 9 propriedades rurais, com 223 vacas em lactação. Todas as vacas em lactação foram avaliadas por meio da realização do teste da caneca de fundo escuro e CMT ("California Mastitis Test"). Além disso foi realizada observação da conduta do funcionário durante a ordenha, estrutura física da propriedade, manejo com os animais e tratamento das vacas secas. Os resultados obtidos foram tabulados, avaliados para elaboração de um fluxograma de ações de boas práticas. A prevalência de mastite subclínica detectada foi de 30,5%, sendo que, 66,7% possuíam ordenha mecânica e 89% tanque de expansão, mas 77,7% não efetuavam higiene dos equipamentos após ordenha. O pré e o pós-dipping não eram realizados por 89%, sendo que, 66,6% não faziam linha de ordenha e ninguém realizava testes de detecção de mastite. Conclui-se que a mastite subclínica está presente nos rebanhos avaliados. Pode-se observar o manejo adotado em cada propriedade e elencar os principais pontos críticos sendo eles relacionados principalmente a falhas de manejo e higiene de ordenha e equipamentos. A implantação do fluxograma de boas práticas de ordenha é de extrema importância para o produtor, uma vez que, o principal método para evitar e controlar a infecção é a prevenção.

Palavras-chave Fluxograma, higiene de ordenha, mastite, prevenção

INTRODUCTION

In Brazil, dairy cattle farming is a widespread and growing activity in several States (VENTURINI, 2014). According to data from the last Agricultural Census, 75% of dairy farms in Sao Paulo are familiar system production, which are responsible for 50.6% of the total milk produced in the State. Fernandopolis, an important municipality in the dairy farming sector, located in the northwest region of Sao Paulo, had a total milk production of 5,144 (x1000 L) per year (IBGE, 2017).

Drops in production and changes in the milk composition are characteristics that determine the occurrence of mastitis. Different infectious agents, such as viruses, fungi and, especially, bacteria, are known to be the main causes of this disease. The presence of clear signs of this pathology classifies it as clinical mastitis. On the other hand, when additional tests are required for its detection, the disease is classified as subclinical (SILVA and NOGUEIRA, 2010).

To reduce the number of microorganisms that can be transfered to milk and thus ensure its microbiological quality, several measures must be adopted during the milking process. The observation of factors related to the operation and hygiene of the milking machine and the milker's hand, hygiene practices and teat injuries prevents the transmission of contagious pathogenic microorganisms from infected to non-infected animals during the process (SANTOS and FONSECA, 2019).

To aiming an economically acceptable level of prevalence, each farm must implement a disease control program. Identifying the necessary factors for the occurrence of mammary gland infections is an important step for the program, which should be based on four fundamental aspects, namely, 1- susceptibility: nutrition analysis, selection of more resistant animals and milking hygiene; 2- the source of infection: diagnosis, treatment or culling; 3- transmission routes: milking hygiene methods and the environment; and 4- awareness of the problem among producers and health education for milker's (SILVA and NOGUEIRA, 2010).

The duration of infections in cows is a factor that influences the level of infection in the herd; the faster they are eliminated, the more successful the control program will be. Therefore, control and prevention measures must be associated with treatment measures so that mastitis is successfully eliminated from the farm.

In this scenario, the present study was conducted to examine the prevalence of

clinical and subclinical mastitis as well as to evaluate the contribution of milking management to this prevalence, aiming to provide bases for a better control of mastitis in dairy herds in the northwest region of Sao Paulo State.

MATERIAL AND METHODS

The research project that originated this study was approved by the Ethics Committee on Animal Use (CEUA) at Universidade Brasil (approval no. IC18-19/005). The study involved nine dairy farms enrolled in the CATI milk program in the region of Fernandopolis, Northwestern Sao Paulo, which had milk production as the basis of their family economy.

In total, the evaluated herds consisted of 223 lactating cows, which were between five and seven years old, two and three months in milk and reared in an extensive system. In November 2019, all animals were evaluated by the strip-cup test and CMT, on all mammary quarters, to determine the prevalence of clinical and subclinical mastitis.

To detect clinical mastitis, the strip-cup test was performed. After previously restraining the cow in the milking line and cleaning the milker's hands, the first three jets of milk were directed to the strip cup. Then, the appearance of the milk was evaluated for alterations such as yellowish color and presence of lumps or pus. On the farms where the cow was milked with the calf at the foot, the calf was held close to the mother and the test was carried out before the suckle.

For the detection of subclinical mastitis, the CMT was performed following the strip-cup test, always by the same researcher. The teats were cleaned with a disinfectant solution and dried with a paper towel. Then, the milk was collected from each teat until reaching the mark contained in the racket used in the test (2 mL of milk).

Afterwards, the reagent was added up to the second mark in the paddle (2 mL of reagent) and the solution was homogenized for 20 s. The test result is based on gel formation, where the more affected the quarter is, the thicker the mixture will be. Table 1 displays the interpretation of results.

Table 1. Interpretation of California Mastitis Test

Interpretation	Reaction
No gel formation	Negative (-)
Slight precipitation	Traces (TR)
Gel formation	Weak positive (+)
Thicker gel with central nipple	Positive (++)
Very thick gel adhered to the bottom of the paddle	Strong positive (+++)

Source: Oliveira et al. (2015). Adapted.

According to Philpot and Nickerson (1991), the score found in CMT can indicate the SCC (Somatic Cell Count) of each animal. Table 2 shows this reference.

Table 2. Relationship between the CMT result and SCC.

CMT Scores	SCC
0	0 to 200,000
Traces	150,000 to 500,000
+	400,000 to 1,500,000
++	800,000 to 5,000,000
+++	>5,000,000

The percentage of mammary quarters affected by mastitis is also very important, since the higher the number of quarters infected, the higher the SCC/mL of milk, and consequently, the lower the amount of milk produced by the animal (Table 3).

Table 3. Percentage of infected mammary quarters and milk production loss

Infected mammary quarters (%)	Milk Production loss (%)
6	0
16	6
32	18
48	29

Source: Nacional Mastitis Council, 1996. Adapted.

The researcher would observe the conduct of employees during milking; the physical structure of the farm; the handling of the animals before, during and after milking; and dry cow treatment by monitoring these practices on the studied farms. These data were recorded as a checklist, which contained all the mentioned elements.

All results were tabulated and evaluated using descriptive statistics.

RESULTS AND DISCUSSION

In total, all farms participating in the study included 223 lactating animals. Table 4 describes the data for the strip-cup test and CMT results.

Table 4. Results obtained with the strip-cup test and CMT in dairy herds in the northwest region of Sao Paulo, 2019

Farm	N. Lactating cows	N. Cows Clinical mastitis (%)	N. Cows Subclinical Mastitis (%)
1	8	0 (0%)	3 (37.5%)
2	29	0 (0%)	5 (17.2%)
3	24	1 (4.2%)	8 (33.3%)
4	17	0 (0%)	3 (17.6%)
5	37	1 (2.7%)	11 (29.7%)
6	28	0 (0%)	10 (35.7%)
7	26	0 (0%)	8 (30.8%)
8	33	2 (6%)	13 (39.4%)
9	21	0 (0%)	7 (33.3%)
TOTAL	223	4 (1.8%)	68 (30.5%)

Four animals were positive for clinical mastitis, corresponding to 1.8% of the analyzed lactating-cow herd. In their study, Melo et al. (2019), revealed a 15.25% prevalence of clinical mastitis in the municipality of Lagoa Grande - MG, Brazil, whereas Damasceno et al. (2020) mentioned a 66.67% prevalence of clinical mastitis in herds in the South of Minas Gerais. These values are well above those found in the present study, which may indicate that the local management of control, prevention and treatment of clinical mastitis is being carried out effectively on the evaluated farms.

Subclinical mastitis, in turn, was detected in 68 animals, representing 30.5% of the lactating cows. This result is similar to that found by Melo et al. (2019), who detected subclinical mastitis in 28.75% of the animals. The high occurrence of subclinical mastitis may be related to faulty hygiene practices by the milker and ineffective cleaning of the cow teats and milking equipment (SILVA et al, 2019). In addition, the presentation of the disease, i.e., without apparent symptoms, can make it go unnoticed by producers and employees, thus facilitating its dissemination through the herd.

An important finding was the number of teats whose function was compromised due to the occurrence of clinical mastitis (2.5%). The disease damages the mammary gland tissue, which is replaced by fibrous tissue, causing the loss of the parenchyma.

Table 5 presents data referring to subclinical mastitis related to the CMT score.

Table 5. CMT scores found in cows from dairy herds in the northwest region of Sao Paulo, 2019.

Farm	Lactating cows	Score 0 cows	Score + cows	Score ++ cows	Score +++ cows
1	8	5	3	0	0
2	29	24	5	0	0
3	24	15	8	0	0
4	17	14	3	0	0
5	37	25	5	6	0
6	28	18	5	5	0
7	26	18	6	2	0
8	33	18	8	3	2
9	21	14	4	1	2
Total	223	151	47	17	4

The California Mastitis Test is mainly indicated for the detection of subclinical mastitis in the herd. Additionally, it can be used to determine which mammary quarter is affected, detect infections related to the dry period, evaluate the treatment of the dry cow and also identify the cow with the highest somatic cell count (SCC) in the

herd (SANTOS and FONSECA, 2019).

By analyzing Table 5 and relating it to Table 2, we observe that 151 cows (67.7%) did not have subclinical mastitis, which could suggest an average count of 100,000 SCC per animal. On the other hand, 47 cows (21%) were +, and thus had an average SCC of 950,000. Seventeen cows (7.6%) were ++ and had an average SCC of 2,900,000, and four (1.8%) were + ++, with an average SCC greater than 5,000,000.

The farms included in this study did not sell milk directly to the dairy industry, but participated in a community tank and sold the milk through a company outsourced to a regional dairy factory.

Thus, the milk from farms with a higher somatic cell count was mixed with that of farms that produced better-quality milk, which led to a decrease in the quality of the milk from those which had stricter care in terms of product quality.

Based on the number of evaluated cows without clinical mastitis (219) and considering the average SCC suggested by Philpot and Nickerson (1991), according to the data obtained with CMT, the average projection for the community tank is approximately 590,000 cells/mL. Normative Instruction 76, of 2018, states that, for refrigerated raw milk, the quarterly geometric mean concentration should not exceed 500,000 cells mL^{-1} (BRASIL, 2018). It is believed that the milk from these farms did not meet the specifications of the legislation in force in the month of evaluation.

Table 6 shows the percentage of quarters affected by mastitis, per farm.

Table 6. Mammary quarters affected by clinical and subclinical mastitis in cows from dairy herds in the northwest region of Sao Paulo, 2019.

Farm	Lactating cows	N. Functional teats	N. Teats affected	% Teats affected
1	8	32	6	18,8
2	29	115	10	8.7
3	24	96	13	13.5
4	17	68	4	5.9
5	37	145	20	13.8
6	28	109	17	15.6
7	26	97	8	7.2
8	33	127	26	20.5
9	21	81	7	8.6
mean	24.77	96.66	12.33	12.51
CV	33.05	32.70	56.04	39.27
TOTAL	223	870	111	

* CV: coefficient of variation.

By comparing Table 3 with Table 6, we note that except for farm 4, all others are having milk production losses due to the occurrence of mastitis. Following the

projection made by the National Mastitis Council (1996), of 6 to 16% of affected quarters, production losses will be, on average, 6%. The average number of affected mammary quarters found on the evaluated farms was 12.7%, that is, a production loss of approximately 4.1%.

However, according to Oliviera (2013), when SCC values exceed 200,000 cells/ mL (above 6% of the affected teats in the herd), there may be a drop of approximately 2.5% in production for every 100,000 cells/mL added. If we compare this statement with the previously made projection of 590,000 cells in the community tank, losses could be even greater, reaching approximately 10% of production.

Mastitis results in the destruction of the mammary parenchyma, causing loss of function of the secretory tissue, and directly affects the quality of milk. This culminates in great losses related to the decline in production on the dairy farm and by the dairy industry (LANGONI, 2017).

Table 7 describes the observations on the characterization and milking practices performed on the farms.

Table 7. Characterization of farms and milking practices in dairy herds in the northwest region of São Paulo, 2019.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Milking type	Hnd	Mec	Hnd	Hnd	Mec	Mec	Mec	Mec	Mec
N of daily milkings	1	1	1	1	1	1	1	1	1
Impermeable floor	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes
Pre-dipping	No	No	No	No	No	No	No	Yes	No
Post-dipping	No	No	No	No	No	No	No	Yes	No
Strip cup	No	No	No	No	No	No	No	No	No
CMT	No	No	No	No	No	No	No	No	No
Cooling	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Milking line	No	Yes	No	No	No	No	Yes	Yes	No
Use of disinfectants	No	Yes	No	No	No	No	No	Yes	No
Post-milking feeding	No	No	No	No	No	No	Yes	Yes	No
Calf at food	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Treatment of dry cows	No	No	No	No	No	Yes	No	Yes	No

* Hnd: by hand, Mec: mechanical.

As regards the milking type, 66.7% performed mechanical milking with a bucket at the foot and 33.3% hand milking; 89% milked with the calf at the foot; 66.7% had an impermeable floor and 89% owned a bulk tank. As in this study, Marques and Leaes (2019) detected the predominance of mechanical milking machines with a bucket at the feet. This may be related to the high cost of a piped milking system, as

the study was carried out with small producers.

Another fact also mentioned by Marques and Leaes (2019), which the present findings corroborate, was the presence of a bulk tank on practically all farms. This fact may be related to the requirement of NI 76, of 2018, which determines that the milk must reach a maximum temperature of 4.0 °C, within three hours (BRASIL, 2018).

During the visits, the milker's behavior was also observed by analyzing essential practices for the prevention and control of mastitis. Of the farms participating in the study, 89% did not perform pre-dipping and post-dipping; 66.6% did not make a milking line, and those that did it were only for cows with clinical mastitis; and 100% did not perform the strip-cup test or CMT.

Marques and Leaes (2019) stated that all farm owners said they followed Good Milking Practices, in their survey. However, pre-dipping was not performed by 70% of them, post-dipping by 60% and CMT by 40% of producers; only the strip-cup test was performed by 100% of the study participants.

The practice of pre-dipping is one of the main factors for preventing mastitis—especially among environmental factors—, as it creates a protective barrier to the teat.

Like pre-dipping, post-dipping is very important, as it removes the film of milk that remains on the teat after milking, in addition to sealing the entrance of the mammary quarter sphincter and thus helping to prevent the penetration of microorganisms through the teat canal (ZSCHÖCK et al., 2011). A study carried out in Guaranhuns - PE, Brazil, revealed that the high prevalence of SCC in herds is associated with negligence in hygienic management, especially non-performance of pre-dipping (OLIVEIRA JUNIOR et al., 2012).

This study evidenced the importance of pre-dipping and post-dipping, as practically all farms that had a prevalence of subclinical mastitis above 30% and occurrence of clinical mastitis did not perform the aforementioned practices.

The strip-cup test is also an important practice, as it detects cases of clinical mastitis, allowing decisions to be made to minimize milk contamination.

The California Mastitis Test, which reveals the occurrence of subclinical mastitis, is not being performed on the studied farms. This may be an important factor that explains the 30.5% incidence of cows positive for subclinical mastitis found in this study.

The use of a milking line should be implemented on the farm, and based on

the diagnosis of mastitis. Accordingly, the milking sequence should be as follows: primiparous cows without mastitis; multiparous cows that have never had mastitis; cows that have already had mastitis but are healed; cows with subclinical mastitis and cows with clinical mastitis. In this way, there is no transmission of pathogens to healthy cows during milking (FLORIÃO, 2013). Tischer et al. (2018) and Silva et al. (2019) reported that the farms evaluated in their study did not have a milking line, which the present findings corroborate.

Only one farm (P8) performed most of the procedures indicated for good milking practices. Nevertheless, the farm had a high number of cases of subclinical mastitis, which may be related to non-performance of the CMT test and, consequently, non-adoption of a milking line, which caused high dissemination in the herd.

Mistakes in handling procedures during milking or lack thereof are a crucial factor for the occurrence of mastitis. Hygiene of the teats (pre- and post-dipping), milking equipment and in milking; applying the strip-cup test and CMT; establishing a milking line; and culling cows with chronic mastitis are key measures to eliminate cases of mastitis in a herd (ALMEIDA et al., 2015; SILVA et al., 2019).

In the evaluation of the cleaning of milking equipment with disinfectants, 77.7% of the farms did not sanitize the equipment during and after milking. This fact may also be related to the high prevalence of subclinical mastitis and the presence of clinical mastitis in the herds, since the farms that did not perform this practice had practically the highest rates of prevalence of the disease.

It is important to emphasize that poor quality of raw milk is usually related to a number of factors, but the handling and hygiene in milking combined with proper maintenance and disinfection of the equipment can improve the quality of the product.

The cleaning of milking equipment is just as important as hygiene and handling during milking. Therefore, it must be rinsed in warm water (30 to 41 ^{o}C), then rinsed with chlorinated alkaline detergent between 71 to 74 ^{o}C and finally rinsed with acid (water at room temperature). Only in this way are organic and mineral residues removed from the equipment surfaces.

In their study, Tischer et al. (2018) also highlighted the failures in the process of cleaning the milking equipment on farms in the northwest region of Rio Grande do Sul. The authors stressed that the improper practice of cleaning is a contributing factor

for increasing the total bacterial count (TBC) in milk.

Silva et al. (2019) stated that 92.3% of farmers did not carry out procedures for cleaning rooms and milking equipment as recommended. It is important to point out that failures in these practices contribute to increasing the rate of disease in herds, the incidence of mastitis, the costs of treatment, the appearance of chronic lesions and even culling (BRITO et al., 2014).

A noteworthy fact is that 77.7% of the farms did not provide feed to the cows after milking, causing their vast majority to lie down afterwards. This position favors the penetration of microorganisms into the mammary quarters, as the teat sphincter is open, at that moment. The supply of feed is an important management strategy, as it encourages the animal to stand up after milking (SANTOS and FOSNECA, 2019).

Dry cow treatment must be carried out to prevent the occurrence of mastitis soon after calving. In the present study, 77.7% of the farms did not adopt this practice. Reis et al. (2020) described adoption of dry cow treatment by 50% of the evaluated producers, while 25% only do it when they deem it necessary. The proper drying of the cow, with the use of long-acting intramammary medications, is essential for curing subclinical mastitis and preventing new infections. In the current study, the non-treatment of dry cows may be one of the reasons for the high prevalence of subclinical mastitis in the herds.

Food quality is a very important factor, which is further highlighted in dairy farming because milk is highly perishable and requires special care due to the risk of microbial contamination. Therefore, establishing a flowchart of good milking practices on the farms is an essential practice that meets the current need to provide quality food to increasingly demanding consumers.

Dairy industries routinely perform SCC and SPC (standard plate count) analyses to provide the consumer with a quality product, preventing the release of a food of doubtful quality, contaminated or unsuitable for consumption (SILVA, 2016).

Increases in SCC and SPC may be related to a number of factors, including inadequate management by the farm regarding lack of hygiene in milking and cleaning of the environment; lack of a milking line, since animals with mastitis are milked and this milk has high SCC and pathogens; and high prevalence of mastitis in the herd (CESPEDES et al. 2014; QUINTÃO et al., 2017).

Costa (2017) concluded, in his study, that the control of mastitis is

economically viable when compared with its treatment, as prevention accounted for 17.14% of the farm's revenue in the period, whereas treatment would cost 28.38% of the revenue. Thus, the proper application of good milking management practices can lower production costs, in addition to providing the possibility of productive and economic return with the animal.

In addition to being fundamental for dairy farming in Brazil, the methods of control and prevention of mastitis provide animal welfare. Mastitis is responsible for great economic losses, depending on the type and percentage of positive animals, and many of these infections are due to poor sanitary education and hygiene at the site.

CONCLUSIONS

Subclinical mastitis is present in the evaluated herds due to problems related to milking management, non-performance of the test to detect subclinical mastitis and absence of dry cow treatment, which induce low quality of milk due to a high SCC. The study revealed the management adopted by each farm and made it possible to list major critical points, which are mainly related to failures in handling and hygiene in milking and of the equipment.

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