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Effects of pre-dipping and post-dipping protocol on the incidence of bovine mastitis

Corresponding author Mérik Rocha-Silva Universidade Estadual do Piauí merikrocha@cca.uespi.br

Marislane Resende da Silva Universidade Federal do Piauí

Camila Laryssa Nunes Neves Universidade Estadual do Piauí

Dinnara Silva Universidade Estadual do Piauí

Francisco Araújo Machado Universidade Estadual do Piauí

Ana Paula Santos de Sousa Universidade Estadual do Piauí

Ana Paula da Conceição Lira Universidade Estadual do Piauí

Maurílio Souza dos Santos Universidade Estadual do Piauí

Samira Teixeira Leal de Oliveira Universidade Estadual do Piauí

Lourdes Mariane Lages Pereira Agroleste

Abstract. Part of the mammary gland microbial infections are imperceptible to the milker. The prevention through hygienicsanitary measures is thought as an efficient strategy to avoid mastitis. Evaluating whether the adoption of pre-dipping and post-dipping hygienic managements have significant effects with the use of the black background mug and California Mastitis Tests, individualized by post-dipping. The properties have the same breed, similar food management and common location, both in the rural area of Teresina. The only difference between them is the performance or not of the handlings that include pre-dipping and post-dipping. So, performing the minimum protocols or not caused the treatments to have a completely randomized delineation. Statistical analysis was obtained through the use of the packages "dplyr", "rstatix" and "ggplot2", available in Software R (2022). The compliance verification of the least squares method in relation to the normal distribution of data was performed through the Shapiro Wilk test, using the "byf.shapiro" procedure of the "RVAdeMemoire" package. Data did not present normal or Gausean distribution, so non-parametric statistics were reported. To discriminate the difference, the post-hoc test by Dunn's test with p-value adjustment by Bomferroni's method. It was noted that the right rear teat presented milk without changes in lumps, color, and others in farm "A", that the pre-dipping and post-dipping were performed.

Keywords: Mammary gland; spinning; California Mastitis Test

Introduction

A very common disease in dairy herds is mastitis, an inflammation of the mammary gland, which may present in clinically or subclinically form (imperceptible to the milker). This disease hits herds all over the world, with no restrictions on regions, including Brazil, causing big losses for milk producers (Lopes et al., 2012).

Clinical mastitis presents specific characteristics that are different from the subclinical one, such as: udder edema, high temperature, stiffening of the region, sensitivity and pain when the mammary gland is touched, lumps, pus and other milk changes. On the other hand, the subclinical form does not present macroscopic changes, but changes in the composition and volume of the milk produced; therefore, it did not show visible udder inflammation signs of any kind (Lopes et al., 2012; Langoni et al., 2017).

In Brazil, the incidence of the disease is variable, reaching rates from 11.9% to 58.8%. This great occurrence variability is due to sanitary and environmental variations of herds, characteristics of animals, general daily management and microbial agents. So, mastitis is characterized by complex interactions between genetic and environmental factors, having a multifactorial spectrum (Oliveira et al., 2010).

In the state of Piauí, some breeds of dairy cows, like Holstein Friesian, don't perform at their greatest productive potential, due to climate conditions and lack of suitable facilities. At that region, temperatures are very high.

Prevention takes place through pre-dipping and post-dipping hygienic-sanitary prophylactic treatment, among others. Santos et al. (2021) found that the adoption of pre-dipping technique reduces the number of bacteria.

Abnormalities verification in the mammary glands can be done through tests in the milking environment. One possibility is the black background mug test, that makes it possible to visualize macroscopic changes in the milk. The other is the California Mastitis Test (CMT), a quick and low-cost tool, that helps dairy farmers control bovine mastitis (Oliveira et al., 2019).

Therefore, the objective of this research is to evaluate whether the adoption of hygienic managements of pre-dipping and post-dipping has significant effects on black background mug and CMT tests, individualized by mammary gland.

Methods

This work was carried out with data from 29 lactating cows from Farm "A" and 38 lactating cows from Farm "B". Both private properties located on the outskirts of the region of Teresina – Piauí, Brazil. This research takes part in a Project that was institutionalized and approved by the Animal Use Ethics Committee (CEUA) n.005608/2021-31.

The statistical results are based on two models of mastitis prevention, in which on farm A predipping and post-dipping were used, but on farm B, they weren't. On both, mechanized milking (bucketat-the-foot type) was used to promote the acquisition of milk. The adopted diet was Chopped Capiaçu, Corn Grains, Soybean Meal, Wheat Bran and Vitaminic Mineral Salt; representing respectively 55%, 19.7%, 16.3, 0.07% and 0.016% of the dry matter supplied. However, nutritional requirements were not fully met on both farms, where milk production is based on animals from Girolando breed.

Pre-dipping composition: demineralized water, lactic acid, mint glycolic extract, chlorhexidine digluconate, methyl isothiazolinone, starch propyl betaine, CI 19140 and CI 42090. Post-dipping composition: demineralized water, trichlorocarbanilide, etidronic acid, methyl isothiazolinone, glycerin, brown coloring CI ND and CI 18/16255.

The animals from both farms were subjected to the two main field tests, non-laboratory-based, to indicate abnormalities in the mammary gland. Both farms were visited in the spring, when milk samples were obtained during milking (Figure 1), to be later examined under the black background mug test. The objective was to verify if there would be changes in the milk, such as the presence of lumps, blood and pus.



Figure 1. The black background mug test.

After that, milk jets were collected with a racket for the California Mastitis Test. For each mammary quarter, 2 milliliters of a reagent were added, under circular movements for 20 seconds. Then, it was checked if the formation of gel occurred, with a reduction in the fluidity of the samples. Each mammary gland of each cow was classified as 0, +, ++ or +++ (Barnum and Newbould, 1961), Figure 2.



Figure 2. California mastitis test, a) individuality sampling by ceiling, b) reaction reading.

Statistical analysis was carried out using the "dplyr", "rstatix" and "ggplot2" packages available in Software R (2022).

The compliance verification of the least squares method in relation to the normal distribution of data was performed using the Shapiro Wilk test, through the "byf.shapiro" procedure of the "RVAdeMemoire". Once the normality status was verified, the non-parametric statistics were consulted to discriminate the difference between the post-hoc test and the Dunn's test, with an adjustment of the pvalue for the Bonferroni method.

Results and discussion

Data from the dependent variables "milk production", "black background mug" and "CMT", individualized by mammary gland, arranged by the independent variable farm (A and B), showed a p-value greater than α of 0.05 in 9 of the 10 variables. In "Lumps on Teat", as a result of the black background mug test, where there are only two possible outcomes, it is obviously a binomial distribution, not a normal one. Because of that, the use of Kruskal Wallis was needed instead of ANOVA, for example.

Results show that the variable "Lumps on Teat 1" has a different median for each farm, indicating that there are significant differences between the dependent variables in comparison to the "handling" farms (Table 1). So, differences in the prophylactic management of farm A caused significant changes in the incidence of clinical mastitis in one of the four mammary glands.

With Bonferroni (1936), the results of Kruskal Wallis were maintained, with a significant difference only in one of the mammary glands, in comparison to the presence or absence of lumps when using the black background mug test.

The descriptive analysis of data segmented by farm consisted in approaching each of the nine dependent variables (Groups for teats 1, 2, 3 and 4; CMT for teats 1, 2, 3 and 4; and Milk Production) for median and interquartile range (Table 2) by the Posthoc test and Dunn's test with a p-value adjustment.

The interpretation of results can probably be better assimilated through graphic representation, as shown in the following figures. In Figure 3, the incidence of clinical mastitis is presented side by side.

Two types of tests were applied, one that observed visible physical variations, whose results are individualized by ceilings in the first four graphs of Figure 3, and the CMT test to identify non-visible alterations, whose results are in the next four graphs of the same figure, the ninth graph is based on milk production.

Table 1. Kruskal Wallis non-parametric normality test and the difference between treatments with the Bonf	erroni test.
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Variables	Q. Square	p-value	Bonferroni	p-adjusted
LT1	12,674***	0.0004	3.56***	0.000371
LT 2	0.8116	0.3676	0.901	0.368
LT 3	1.9609	0.1614	1.40	0.161
LT 4	1.7943	0.1804	1.34	0.180
CMT Teat 1	0.0038	0.9505	0.0621	0.950
CMT Teat 2	0.0289	0.865	-0.170	0.865
CMT Teat 3	0.2579	0.6116	-0.508	0.612
CMT Teat 4	0.0054	0.9416	-0.0733	0.942
Production	0.8627	0.353	-0.929	0.353

LT: Lumps on Teat, CMT: California mastitis test. *** p < 0,001; ** p<0,01; *p<0,05

Table 2 . Descriptive Analysis of the Sample Data
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Variable	Farm	Median	Interquartile	Farm	Median	Interquartile
LT 1	А	0	2	В	0,5	1
LT 2	А	1	3	В	1	2
LT 3	А	1	3	В	1	2
LT 4	А	1	3	В	1	2
CMT Teat 1	А	0	0	В	0	1
CMT Teat 2	А	0	0	В	0	1
CMT Teat 3	А	0	0	В	0	0
CMT Teat 4	А	0	0	В	0	0
Production	А	8,5	4,5	В	8,25	2,52

LT: Lumps on Teat, CMT: California mastitis test.

It is noted that the right rear teat showed milk without changes in lumps, color, and others, on farm "A", that proceeds pre-dipping and post-dipping. Also, it was noticed, a low incidence for front mammary glands that expel milk through teats 3 and 4 (c and d of Figure 3). The results of subclinical mastitis, indicate that although the median remains 1 for three of the four mammary glands, greater variations are shown in the gray part of the rectangles that project the maximum and minimum values registered in each treatment.

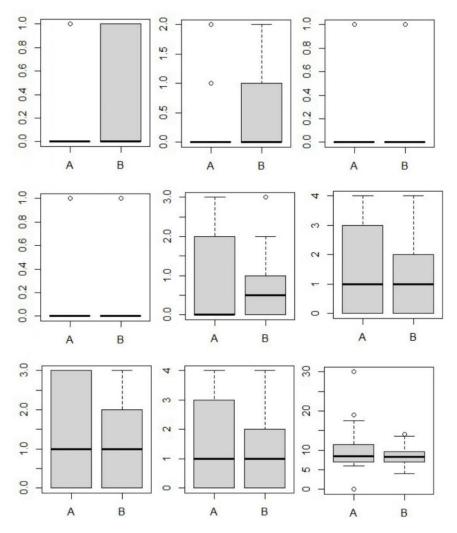


Figure 3. Figure 2. Graphical representation of dependent variables for farm "A" and "B". a) lumps on teat 1, b) lumps on teat 2, c) lumps on teat 3, d) lumps on teat 4, e) CMT teat 1, f) CMT teat 2, g) CMT teat 3, h) CMT teat 4 i) milk production.

Lastly, milk production has similar median on both farms, with no statistically significant variation ($\alpha = 0.05$).

Considering that the values presented in the variables are discrete, an approach such as ordinal categorical, has positive effects on the adoption of pre-dipping (Santos et al., 2021).

For over a century, since the publication of the Journal of Dairy Science, in 1917, an attempt has been made to identify effective procedures for the control of mastitis. In the 1950s and 1960s, tests based on reagents such as CMT increased identification methods, however the problem has not yet been fully solved (Ruegg, 2017). Along the process, human interventions can lead to positive or negative results. Hygienic sanitary protocol can contribute. However, Pyörälä (2002) explains the role of the immune system of cows in the mastitis control process. Besides the humoral defense, the contact with microorganisms that cause mastitis tends to reflect in the specific defense development.

Currently, breeders use intramammary antimicrobials for extreme cases, without regular technical follow-up. Tomanić et al. (2023) that this is the main technique used globally, indicating safer alternatives such as the use of vaccines.

Genetic aspects, even among animals of the same breed, can contribute to the decrease of mastitis incidence rates. Being mastitis the result of a failure in the resistance of the host, bacteria, and environment (Pyörälä, 2002). Then, in this sense, the studied variables contemplate only part of the environmental effects variation, opening a possibility for factors related to the resistance of animals and the profile of microorganisms' strains that affect each property.

Conclusion

Pre-dipping and post-dipping operations help to avoid mastitis in at least one of the mammary glands, however, other measures must also be adopted for an effective prevention.

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