Prevalence and Risk Factors of Mastitis among Dairy Buffaloes from the Departments of Antioquia and Córdoba, Colombia

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Abstract: *Background*: Mastitis is the most common disease among dairy buffaloes worldwide, and it significantly affects the economic profitability of buffalo farms as well as animal welfare and public health.

Methods: This study was conducted between 2018 and 2019 at the Colombian departments of Antioquia and Córdoba, where 41% of the country's total buffalo population is concentrated. Overall, 1,018 dairy buffaloes, including 603 in Antioquia and 415 in Córdoba, distributed among 11 farms, were assessed in the study. These animals were evaluated using the California mastitis test (CMT) and somatic cell count (SCC) to determine the presence of subclinical mastitis (SM). They were considered positive for SM when the results of CMT were higher than traces and SCC was $\geq$200,000 cells/mL.

Results: The total prevalence of the disease was 7.9%, and microbiological culture was performed on the samples obtained from the SM-positive animals. The main isolated bacterium was coagulase-negative *Staphylococcus*. Furthermore, risk factors affecting milking routine, hygiene, and farm facilities were determined. Manual milking, milking in the barn, non-disinfection of milkers' hands, etc., were identified as risk factors for the disease.

Conclusion: To the best of our knowledge, this is the first large-scale study of mastitis among buffaloes in Colombia.

Keywords: Mastitis, buffaloes, risk factors, hygiene, farms, Colombia.

INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland characterized by physical and chemical changes in the milk that correspond to pathological changes in the mammary tissue [1]. It can present in two ways: 1) subclinical, when the inflammation is not obvious and not easily detectable in the animal or its milk without diagnostic methods; 2) clinical, when the affected quarter exhibits signs of inflammation and there are changes in the appearance of the milk [2].

Mastitis is the most common disease in dairy buffaloes [3] owing to the pendulous udder and long teats of buffaloes that make them susceptible to the disease [4]. Despite these characteristics, this species is less susceptible to mastitis than cattle [5]. Notably, mastitis in buffaloes is important from the perspective of economic losses resulting from decreased milk production and quality, costs of treatment and veterinary services, and early discarding of animals [6,7]. It has been estimated that these economic losses are equivalent to $93 per case of mastitis in the buffalo species [8]. Other implications of mastitis include loss of animal welfare due to inflammatory response and bacterial proliferation, production of metabolites affecting consumer health, changes in organoleptic and compositional qualities of milk, and resistance of some mastitis-causing bacteria to antimicrobials, making mastitis the origin of an important public health problem [9-11].

The population of buffaloes in Colombia has increased by 30% in recent years and is mainly distributed in the departments of Córdoba, Antioquia, and Santander [12]. This increase in the population can be mainly attributed to the advantages related to meat and milk production, adaptation to tropical conditions, and better quality of milk from buffaloes than that from cattle [13,14].

Overall, 6,318 million liters of milk are produced in Colombia per year, of which 3.4% comes from buffaloes, and this product is mainly used for manufacturing dairy products [15]. Hence, optimum product quality and hygiene must be ensured to protect consumer health. One of the ways to improve the quality of milk is to avoid diseases such as mastitis in

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buffaloes, which directly affect its hygienic and sanitary properties.

Subclinical mastitis in buffaloes is a common disease. Recent studies have shown that the global prevalence of the disease is increasing and is estimated at approximately 46% [16]. This is important because, in addition to the economic losses typically attributed to veterinary services, medication costs, and withdrawal time, there may be a decrease in milk production of up to 4 liters per animal per day [17]. Moreover, the disease has significant repercussions on reproduction, wherein the presentation of a case close to the time of service can decrease the pregnancy rate by up to 50% [18].

Previous studies on mastitis among buffaloes in Colombia have indicated the presence of this problem in their herds [19,20]. However, more comprehensive and complete studies on the etiology of the problem and risk factors associated with its presentation in buffalo herds in Colombia remain lacking. Furthermore, to the best of our knowledge, there are no known studies in the departments of Antioquia and Córdoba, where the country's largest buffalo population is found. Therefore, the present study aimed to determine the prevalence of mastitis among buffaloes and to investigate the risk factors associated with its occurrence in buffalo farms in the departments of Antioquia and Córdoba, Colombia.

MATERIALS AND METHODS

Ethical Considerations

This study was approved by the Ethics Committee for Animal Experimentation of the Universidad de Antioquia (Act 113 of October 12, 2017).

Location

This study was conducted in buffalo farms in the department of Antioquia, located in the northeast of Colombia, with a buffalo population of 56,889 animals, and the department of Córdoba, located in the Colombian Caribbean in the northeast of the country, with the largest buffalo population in the country, with 90,760 animals. These two departments (Antioquia and Córdoba) collectively account for 41.37% of the total number of buffaloes in Colombia.

Type of Study and Population

This analytical cross-sectional study was conducted from November 2018 to March 2019. Nonprobability convenience sampling was used, with 11 dual-purpose buffalo farms selected according to producers' availability to participate in the study. In total, 4,072 quarters, corresponding to 1,018 lactating buffaloes, were included in the present study, and their samples were collected.

California Mastitis Test (CMT) and Udder Examination

All lactating buffaloes were examined for the presence of clinical mastitis based on the presence of any signs of inflammation. Subsequently, 2 mL of milk was collected on a clean, dark-bottomed black palette, with four compartments marked by the anatomical distribution of each quarter. The appearance of the milk was then examined for the presence of lumps or blood, and if any of these were found, the sample was considered positive.

For subclinical mastitis (SM), an equal amount of sodium lauryl alkyl sulfate reagent (Nocar®) was added to each of the sample compartments containing samples obtained from the buffaloes exhibiting no changes in the udder or milk appearance to determine the presence of SM. Further, the mixture was homogenized and interpreted according to the recommendations of the National Mastitis Council [21].

Somatic Cell Count (SCC)

Notably, 5 mL of milk sample was collected from each of the positive quarters into sterile containers labeled with the code of the animal and the affected quarter in accordance with the procedures in the study by Ramírez Vásquez *et al*. [22] after obtaining samples from all buffaloes on the farm. SCC was analyzed on the farm using the portable automated DeLaval cell counter (Delaval®). Samples with a count of ≥200,000 cells/mL were refrigerated and transported to the laboratory.

Microbiological Culture and Antimicrobial Susceptibility Test

The samples were processed in the Microbiology Laboratory of the Diagnostic Unit of the Department of Agricultural Sciences of the Universidad de Antioquia in accordance with the method established by the laboratory for the detection of gram-positive and -negative pathogens. In addition, antimicrobial susceptibility was evaluated using the disk diffusion test.

Case Definition

A quarter was considered to have SM if it had a CMT result of traces or greater and an SCC of ≥200,000 cells/mL. A buffalo was classified as SM-positive if it had SM in one or more quarters. For pathogen prevalence, a quarter was considered to be infected when SM was diagnosed, and one or two pathogens were isolated in the milk sample. A buffalo was considered to be infected if it had one or more infected quarters. Quarters not showing a CMT result of trace or greater were not sampled for SCC and were, therefore, considered negative in data analysis.

Questionnaire and Data Collection

For the present study, data on variables such as age, number of births, days in milk, and average milk production were collected from each farm's existing production records. In addition, information was collected via questionnaires provided to the milkers (questionnaires available upon request) and direct observations on the milking routine, hygiene, and facilities to analyze risk factors.

Only variables that were consistently recorded (with few missing values) are presented in this study. The questionnaires were administered by one of the four technicians. Before starting the project, technicians were trained at one of the farms of the Universidad de Antioquia to ensure that the records were consistent.

Analysis of the Information

Databases were developed using Excel® software (Microsoft, Redmond, USA) and exported to Stata® version 15 software (StataCorp, Texas) for statistical analysis. The prevalence of mastitis among buffaloes was calculated by dividing the number of positive cases by the total number of buffaloes included in the study. Initially, data analysis was performed using descriptive statistics with measures of central tendency and frequency distribution analysis. The geometric means and their confidence intervals (CIs) were estimated for SCC. In the univariate analysis, the sample proportions were calculated along with their corresponding 95% CIs for each factor of interest. Further, measures of central tendency and dispersion were used to describe quantitative variables. The nonparametric Mann–Whitney U test was used to determine statistical differences among the variables of milking routine, facilities, and milking hygiene. In the case of variables that did not follow a normal distribution of the quantitative variables considered in each category of qualitative variables, the Shapiro–Wilk test was used with a statistical significance threshold of $\alpha = 5\%$. For the normally distributed quantitative variables considered in each category of the qualitative variables, the t-test was used for the differences in the mean values in independent samples along with their 95% CIs. For the analysis of the association between the qualitative variables and the variable of interest mastitis, the chi-square test of independence was used along with the association measure odds ratio (OR) and its corresponding 95% CI. When the expected frequencies were <5, Fisher's exact test was used instead of the chi-square test.

RESULTS

Information Regarding Farms and Buffaloes

In total, 1,018 buffaloes were included from 11 farms in the departments of Antioquia and Córdoba. From the department of Antioquia, one farm in the northeast, one in Urabá, one in the middle Magdalena, and four in Bajo Cauca provided a total of 603 buffaloes. From the department of Córdoba, four farms in the San Jorge region provided a total of 415 buffaloes. The buffalo farms were located 20–1,080 meters above sea level, and most had no records or lacked information about buffaloes. In 64% of the farms, milking was mainly performed manually, and the remaining farms were divided into small (1–38 animals), medium (39–100 animals), and large (101–246 animals) groups according to the quartiles. Most farms were large, accounting for 45.4% of all farms, whereas small- and medium-sized farms accounted for 27.3% of all farms, respectively (Table **1**).

Most buffaloes were from the Murrah breed or one of its crosses, and their average age was 8.5 ± 3.2 (range, 2–20) years. The average number of births among the evaluated buffaloes was 4.3 ± 2.8 (range, 1–13); the average milk production per buffalo per day was 3.1 ± 1.5 (range, 0.5–9.5) liters; and the average number of days in milk at the time of the visit was 108.4 ± 60.8 (range, 5–299) days (Table **2**). The majority of the animals (50.1%) were in the first trimester of lactation, followed by the second (43.5%) and third (6.4%) trimesters.

Overall, 45.4% of the farms milked buffalo in the barn, 36.4% in the parlor, and the remaining 18.2% in the paddock. Regarding the milking routine, milkers in 54.5% of the 11 farms washed their hands before starting milking, and milkers in only 18.2% of farms washed their hands after milking each buffalo. Notably,

Table 1: General Characteristics of the Buffalo Farms Included in the Study

Farm	Department	Subregion	MASL	Milking type	Availability of records	Number of buffaloes sampled
1	Antioquia	Northeast	1080	Mechanical	Available	15
2	Antioquia	Urabá	20	Manual	NA	100
3	Antioquia	Middle Magdalena	125	Mechanical	Partially available	132
4	Antioquia	Lower Cauca	150	Manual	NA	246
5	Antioquia	Lower Cauca	150	Manual	NA	39
6	Antioquia	Lower Cauca	200	Manual	Available	23
7	Antioquia	Lower Cauca	150	Manual	Partially available	48
8	Córdoba	San Jorge	44	Mechanical	NA	38
9	Córdoba	San Jorge	50	Mechanical	NA	150
10	Córdoba	San Jorge	90	Manual	Available	123
11	Córdoba	San Jorge	120	Manual	Available	104
Total						1018

MASL: Meters above sea level; NA: Not available.

Table 2: Characteristics of Buffaloes Included in the Study

Variable	Number of buffaloes	Median	Standard deviation	Minimum	Maximum
Age	335	8.5	3.2	2	20
Births	247	4.3	2.8	1	13
DIM	375	108.4	60.8	5	299
Production	285	3.1	1.5	0.5	9.5

DIM: days in milk.

udders or teats were washed before milking in 45.5% of the farms. Pre-milking teat dipping was used in 45.5% of buffalo farms, 80% of which used the iodination method for pre-milking teat dipping, whereas the remaining 20% sed the chlorination method. Regarding the material used to dry the pre-milking teat dipping product, 80% of the farms used newspapers, and the remaining 20% used towels. None of the farms used post-milking teat dipping. In all farms, the calf-supported milking and the teat manipulation technique during manual milking were found to be adequate.

Prevalence of Mastitis

No buffalo was found to be positive for clinical mastitis. The overall prevalence of SM in buffaloes was 7.9% and 4.8% based on CMT and SCC, respectively. The Antioquia department had the highest prevalence of SM, with 9% and 5.6% of positive cases based on CMT and SCC, respectively. The prevalence in the Córdoba department was 6.3% and 5.1% based on CMT and SCC, respectively (Table **3**). Among the quarters with at least one result equal to or greater than

Table 3: Prevalence of Clinical and Subclinical Mastitis Determined using CMT and SCC in Buffaloes from the Departments of Antioquia and Córdoba

Mastitis	Antioquia		Córdoba		Total	
	CMT	SCC	CMT	SCC	CMT	SCC
Clinical	0/603	0/603	0/415	0/415	0/1018	0/1018
Subclinical	54/603	34/603	26/415	21/415	80/1018	49/1018
Total	9%	5.6%	6.3%	5.1%	7.9%	4.8%

CMT, California mastitis test; SCC, somatic cell count.

Table 4: Distribution of Buffaloes Positive for Subclinical Mastitis in the Subregions using CMT

Subregion	Department	Buffaloes positive for subclinical mastitis using CMT	%
San Jorge	Córdoba	26	32.5
Lower Cauca	Antioquia	22	27.5
Urabá		17	21.2
Middle Magdalena		10	12.5
Northeast		5	6.3
Total		80	100

CMT, California mastitis test.

traces, 54% corresponded to anterior quarters, whereas 46% corresponded to posterior quarters. Notably, the most commonly affected quarter was the left anterior quarter (n = 28).

The subregion with the highest number of SM-positive buffaloes was San Jorge in Córdoba, followed by the Lower Cauca of Antioquia. The area with the lowest number of subclinical mastitis-positive buffaloes was northeast Antioquia (Table **4**).

SCC

In total, 91 quarters were found to be positive for SM based on CMT, and their SCC geometric mean values with respective confidence intervals were as follows: left anterior quarter, 412,948 cells/mL (95% CI: 264,091–645,758); right anterior quarter, 425,875 cells/mL (95% CI: 236,359–767,350); left posterior quarter, 447,134 cells/mL (95% CI: 239,456–834,928); and right posterior quarter, 462,126 cells/mL (95% CI: 251,648–848,648).

Among the total CMT-positive samples, 63.7% had SCC of $\geq$200,000 cells/mL; at Antioquia, 58% had SCC of $\geq$200,000 cells/mL, whereas at Córdoba, 85% had SCC of $\geq$200,000 cells/mL.

Microbiological Culture and Etiology of Mastitis

Overall, 61 milk samples from 58 buffaloes with SCC of ≥200,000 cells/mL were sent to the laboratory. Bacteria corresponding to 23 buffaloes were not isolated in 38% (23/61) of the samples; hence, these buffaloes were not considered positive for infectious mastitis. At least 1 bacterium was isolated in 62% (38/61) of the samples, corresponding to 35 buffaloes, and 2 bacteria were isolated in 3 samples; hence, these buffaloes were considered positive for infectious mastitis. Among the tests performed in 35 buffaloes positive for infectious mastitis, more than 3 pathogens grew in 10 cultures, corresponding to 7 buffaloes. Therefore, the culture was considered to be contaminated and was not included in the calculation of pathogen prevalence (Table **5**).

Table 5: Results of the Microbiological Cultures of Buffalo Milk

Result	Number of samples	%
Bacteria were not isolated	23	38%
Infectious mastitis	28	46%
Contaminated sample	10	16%
Total	61	100%

The most frequently isolated bacterium from buffalo milk samples was coagulase-negative *Staphylococcus* (CoNS; 36%), followed by *Streptococcus dysgalactiae* (18%) and *Streptococcus agalactiae* (14%) (Table **6**).

Table 6: Prevalence of Bacteria in Buffalo Mastitis

Result	Number of cultures	%
Coagulase-negative *Staphylococcus*	10	36%
Streptococcus dysgalactiae	5	18%
Streptococcus agalactiae	4	14%
Corynebacterium sp.	3	11%
Streptococcus uberis	2	7%
Staphylococcus aureus	2	7%
Enterococcus sp.	1	3.5%
Flavimonas sp.	1	3.5%
Total	28	100%

Risk Factors

The questionnaires comprised 31 questions divided into 3 categories: milking routine, milking hygiene and facilities, and actions of the milkers. In total, 27 factors were qualitative and were evaluated in terms of OR with $p < 0.05$. The remaining four were quantitative; hence, they were evaluated using the Mann–Whitney U and Shapiro–Wilk tests or t-test of mean difference. The same procedure was used for factors inherent to the buffalo, such as age, number of births, and milk production (Table **7**). Among the 1,018 buffaloes, 80 were positive for SM based on CMT, and 49 were positive for SM based on SCC. Notably, the latter were considered positive for SM, and risk factors were analyzed based on their information. Among these, only 21 buffaloes underwent pre-milking teat dipping; therefore, it was impossible to calculate the OR of the associated values (Figure **1**).

For the milking routine, 14 possible risk factors were examined. Table **8** shows the frequency distribution with respective 95% CIs of the individual characteristics of the animals during milking. Owing to the small amount of missing data, OR could not be calculated for three factors (Table **9**).

Table 7: Summary Indicators Related to Buffalo Production according to the Presence of Mastitis

Covariables	SCC								
	Less than 200,000				Equal or greater than 200,000				
	n	Mean (SD)	Median (IQR)	p*	n	Mean (SD)	Median (IQR)	p*	p
Buffalo age	18	9.3 (2.8)	9.6 (2.7)	0.440	12	7.6 (4.6)	7.5 (8.45)	0.095	0.2136**
Number of births	10	4.2 (1.8)	4.0 (2.0)	0.890	12	4.3 (3.6)	4.5 (5)	0.914	0.9170**
Milk production in liters	12	2.6 (1.3)	2.0 (2.0)	0.175	8	3.4 (1.3)	3.2 (1.2)	0.314	0.1918**
Number of milkers	31	4.4 (1.6)	4.0 (2.0)	0.598	49	4.7 (1.7)	4.0 (3.0)	0.112	0.4021**
Number of buffaloes per milker	31	26.6 (9.7)	30.0 (7.0)	0.000	49	28.4 (10.6)	30.0 (9.0)	0.062	0.6504†
Number of milking buffaloes	31	107.8 (61.9)	104.0 (32.0)	0.021	49	127.5 (76.9)	104.0 (50.0)	0.007	0.416†
Contact time between the pre-milking teat dipping product and teat	10	7.0 (2.6)	5.0 (5.0)	0.915	21	8.6 (2.3)	10.0 (5.0)	0.057	0.0991**

*p-value for the Shapiro–Wilk test of normality; **p-value for the t-test for difference in the mean values; †p-value for Mann–Whitney U test.
SCC, somatic cell count; SD, standard deviation; IQR, interquartile range.

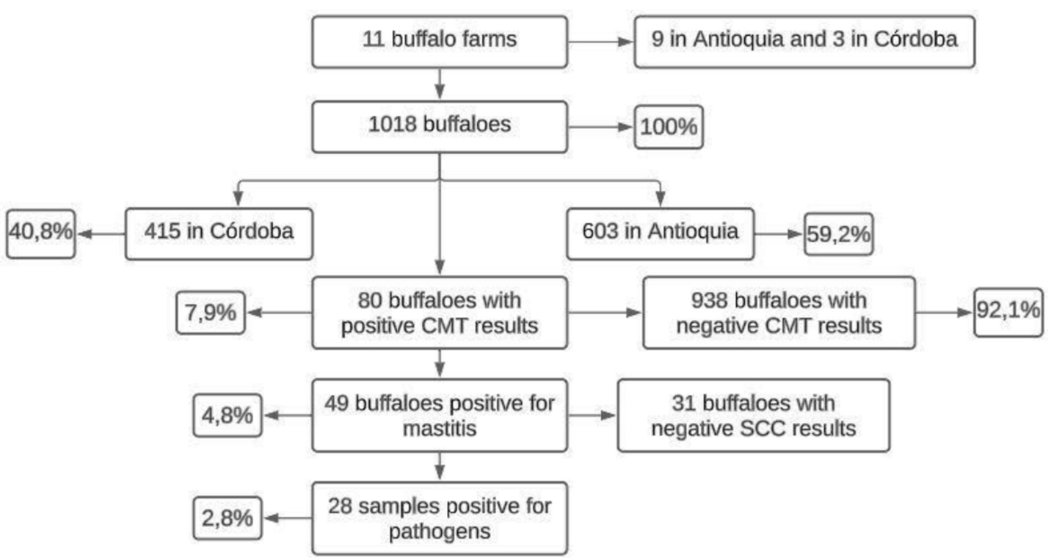

Figure 1: Buffalo selection algorithm for calculating risk factors.

Table 8: Frequency Distribution of Factors Related to the Milking Routine

Factors	n	%	95% CI
Milking type			
Manual	683	67.1	[64.1–70.0]
Mechanical	335	32.9	[30.0–35.9]
Milking site			
Room	320	31.4	[28.6–34.4]
Paddock	86	8.5	[6.8–10.3]
Stable	612	60.1	[57.0–63.1]
Do milkers wash their hands before milking?			
No	456	44.8	[41.7–48.0]
Yes	562	55.2	[52.2–58.0]
Do they wash their hands before milking each buffalo?			
No	848	83.3	[80.8–85.5]
Yes	170	16.7	[14.4–19.1]
Do they wash the udder?			
No	660	64.8	[61.8–67.8]
Yes	358	35.2	[32.2–38.1]
Do they wash the teats?			
No	669	65.7	[62.7–68.6]
Yes	349	34.3	[31.4–37.3]
Do they dry the udder or teats?			
No	810	79.6	[77.0–82.0]
Yes	208	20.4	[18.0–23.0]
Do they individually dry the teats?			
No	957	94.0	[92.4–95.4]
Yes	61	6.0	[4.6–7.6]
Type of paper with which they dry			
None	537	52.7	[49.6–55.8]
Newspaper	466	45.8	[42.7–48.9]
Towel	15	1.5	[0.8–2.4]
Is pre-milking teat dipping performed?			
No	588	57.8	[54.7–60.8]
Yes	430	42.2	[39.2–45.3]
Pre-milking teat dipping product			
Iodized	415	96.5	[94.3–98.0]
Chlorinated	15	3.5	[1.97–5.7]
Is the pre-milking teat dipping application container suitable?			
No	326	75.8	[71.5–79.8]
Yes	104	24.2	[20.2–28.5]
Do they dry each teat individually?			
No	326	75.8	[71. 5–79.8]
Yes	104	24.2	[20.2–28.5]
Type of paper used to dry the pre-milking teat dipping product			
Newspaper	415	96.5	[94.3–98.0]
Towels	15	3.5	[1.97– 5.7]

CI, confidence interval.

Table 9: Analysis of Milking Routine Factors Associated with Mastitis in Buffaloes

Factors	Mastitis					
	≥200,000	<200,000	%Yes	p	OR	95% CI
	n	n				
Type of milking						
Manual	38	16	70	0.016	3.24	[1.1–9.6]
Mechanical*	11	15	42.3			
Milking site						
Paddock	7	2	77.8	0.044**	6.10	[0.8–70.0]
Pen	34	15	69.4	0.009	3.96	[1.2–13.2]
Room*	8	14	36.4	1.0		
Do milkers wash their hands before milking?						
No	27	12	69.2	0.153	1.94	[0.7–5.3]
Yes*	22	19	53.7			
Do they wash their hands before milking each buffalo?						
No	42	21	66.7	0.056	2.86	[0.8–10.1]
Yes*	7	10	4.1			
Do they wash the udder?						
No	35	16	68.6	0.072	2.34	[0.8–6.6]
Yes*	14	15	48.3			
Do they wash the teats?						
No	31	23	57.4	0.309	0.6	[0.2–1.8]
Yes*	18	8	69.2			
Do they dry the udder or teats?						
No	39	16	70.9	0.009	3.66	[1.2–11.1]
Yes*	10	15	40			
Do they dry the teats individually?						
No	40	30	57.1	0.044**	0.15	[0.0–1.1]
Yes*	9	1	90			
Type of material with which it is dried						
None	30	14	68.2	0.035	1.35	[0.5–3.9]
Towel	0	5	0	0.016	0	[0.0–0.5]
Newspaper*	19	12	61.3	1.0		
Is pre-milking teat dipping performed?						
No	28	21	57.1	0.343	0.63	[0.2–1.8]
Yes *	21	10	67.7			
Pre-milking teat dipping product						
Iodized	21	5	80.8	0.001**	…	…
Chlorinated*	0	5	0			
Is the pre-milking teat dipping application container suitable?						
No	15	8	65.2	0.483**	0.63	[0.0–4.7]
Yes *	6	2	75			
Do they dry each teat individually?						
No	15	8	65.2	0.483**	0.63	[0.0–4.7]
Yes *	6	2	75			
Type of paper used to dry the pre-milking teat dipping product						
Towels	21	5	80.8	0.001**	…	…
Newspaper*	0	5	0			

*Reference category; **Fisher's exact test; ***Among the 49 buffaloes with mastitis, information was obtained from 21 that underwent pre-milking teat dipping. Among these, other questions about hygiene in pre-milking teat dipping were investigated.
CI, confidence interval.

Table 10: Frequency Distribution of Factors Related to Farm Facilities and Milkers' Actions

Factors	n	%	95% CI
Milking is performed in a covered area			
No	314	30.8	[28.0–33.8]
Yes	704	69.2	[66.2–72.0]
Milking is performed in areas that do not pose a risk of milk contamination			
No	87	8.6	[6.9–10.4]
Yes	931	91.4	[89.6–93.1]
Mud-free, dry, and trash-free area			
No	48	4.7	[3.4–6.2]
Yes	970	95.3	[93.7–96.5]
Washing is done after milking			
No	333	32.7	[29.8–35.7]
Yes	685	67.3	[64.3–70.2]
Milking area is free of other domestic animals			
No	325	31.9	[29.0–34.5]
Yes	693	68.1	[65.1–70.9]

Regarding the milking routine, the buffaloes that were milked manually exhibited a higher risk of mastitis (OR = 3.24) than those that were milked mechanically ($p < 0.05$). Furthermore, the place where milking was performed influenced the onset of mastitis ($p < 0.05$), as the buffaloes milked in the corral had a higher risk of developing mastitis (OR = 3.96) compared with those milked in a room. In addition, buffaloes whose udders or teats were not dried before milking had a higher risk of mastitis (OR = 3.66) compared with those whose udders or teats were dried before milking ($p < 0.05$).

Regarding the facilities and actions of the milkers, five possible risk factors were evaluated in the buffaloes. The distribution of frequencies with their respective 95% CIs is shown in Table **10**. None of the

Table 11: Analysis of Factors Related to the Facilities and the Actions of Milkers Associated with Mastitis in Buffaloes

Factors	Mastitis		% Yes	p	OR	95% CI
	≥200,000	<200,000				
	n	n				
Milking is performed in a covered area						
No	14	5	73.7	0.158**	2.08	[0.6–8.3]
Yes	35	26	57.4			
Milking is performed in areas that do not pose a risk of milk contamination						
No	3	1	75	0.495**	1.96	[0.1–106.0]
Yes	46	30	60.5			
Mud-free, dry, and trash-free area						
No	1	1	50	0.628**	0.63	[0.0–50.0]
Yes	48	30	61.5			
Washing is done after milking						
No	15	4	78.9	0.059**	2.98	[0.8–13.6]
Yes	34	27	55.7			
Milking area is free of other domestic animals						
No	17	16	51.5	0.134	0.5	[0.2–1.4]
Yes	32	15	68.1			

*Reference category; **Fisher's exact test.
CI, confidence interval.

Table 12: Frequency Distribution of Factors Related to Milking Hygiene

Factors	n	%	95% CI
Does the buffalo enter with clean flanks and tails at the time of milking?			
No	653	64.2	[61.1–67.0]
Yes	365	35.8	[32.9–38.9]
Buffaloes with dirty teats undergo pre-milking teat dipping, which does not affect the safety of the milk			
No	456	44.8	[41.7–47.9]
Yes	562	55.2	[52.1–58.3]
Hand milking is done with clean hands			
No	738	75.3	[72.5–78.0]
Yes	242	24.7	[22.0–27.5]
The drying of the teats is done with disposable material that does not affect the safety of the milk			
No	433	42.5	[39.5–45.6]
Yes	585	57.5	[54.4–60.5]
Milkers disinfect their hands before milking each buffalo			
No	871	85.6	[83.2–87.7]
Yes	147	14.4	[12.3–16.8]
Buffaloes secreting abnormal milk are milked last or with other equipment			
No	630	70.4	[67.3–73.3]
Yes	265	29.6	[26.6–32.7]
Abnormal milk is removed according to the inspection manual			
No	598	66.8	[63.6–69.9]
Yes	297	33.2	[30.1–36.3]
The equipment used to handle abnormal milk is treated with appropriate care			
No	100	22.9	[19.0–27.2]
Yes	336	77.1	[72.8–80.9]

CI, confidence interval.

factors related to the facilities' and milker's actions were associated with the presence of mastitis (Table **11**).

Table **12** shows the frequency distribution with 95% CIs for the eight factors assessed for hygiene during milking.

Nondisinfection of the hands by the milkers before milking was associated with an OR of 39.5 compared to the disinfection of hands before milking ($p < 0.05$). Moreover, compared with farms where abnormal milk was adequately disposed of, inadequate disposal of the abnormal milk was associated with an OR of 7.28 (Table **13**); in addition, it was associated with the presence of mastitis ($p < 0.05$).

No association was found between lactation trimesters in buffaloes and the presence of mastitis (Table **14**).

DISCUSSION

Buffaloes are long-lived animals with excellent maternal capacity, making them important milk producers [13]. In the present study, the animals had a mean age of 8.5 years, and they gave birth to four. This finding was consistent with the findings of the study by Cerón and Ramírez (2015), who reported production curves during lactation with data of buffaloes from the first to the twelfth calving. This result suggests a high longevity of buffaloes in Colombia [23]. Notably, overall milk production in Colombia reached an average of 3.1 L, and similar data were found on the Colombian Atlantic Coast throughout the lactation period (3.0–4.6 L) [24] and on the northern coast during the first lactation (3.9 L) [25]. This similarity in the findings can be explained by the fact that the data from these studies were collected in the same area where we found the productive systems to be relatively similar,

Table 13: Analysis of Milking Hygiene Factors Associated with Mastitis in Buffaloes

Factors	Mastitis					
	≥200,000	<200,000	% yes	P	OR	95% CI
	n	n				
Does the buffalo enter with clean flanks and tails at the time of milking?						
No	25	17	59.5	0.739	0.86	[0.3–2.3]
Yes *	24	14	63.2			
Buffaloes with dirty teats undergo pre-milking teat dipping, which does not affect the safety of the milk						
No	27	12	69.2	0.153	1.94	[0.7–5.3]
Yes *	22	19	53.7			
Hand milking is done with clean hands						
No	32	21	60.4	0.677	1.24	[0.4–4.0]
Yes *	11	9	55			
The drying of the teats is done with disposable material that does not affect the safety of the milk						
No	24	12	66.7	0.368	1.52	[0.6–4.2]
Yes	25	19	56.8			
Milkers disinfect their hands before milking each buffalo						
No	48	17	73.8	0.00**	39.52	[5.04–1704.6]
Yes	1	14	6.7			
Buffaloes secreting abnormal milk are milked last or with other equipment						
No	29	23	55.8	0.216	0.50	[0.1–1.7]
Yes	15	6	71.4			
Abnormal milk is removed according to the inspection manual						
No	39	15	72.2	0.000	7.28	[2.0–29.6]
Yes	5	14	26.3			
The equipment used to handle abnormal milk is treated with appropriate care						
No	9	8	52.9	0.224	2.25	[0.5–10.2]
Yes	7	14	33.3			

*Reference category; **Fisher's exact test.
CI, confidence interval.

Table 14: Analysis of Buffalo Factors in Milking Associated with Mastitis in Buffaloes

Factors	Mastitis					
	Yes	No	% Yes	p	OR	95% CI
	≥200,000	<200,000				
Days of milking						
First trimester (0–100 days)	4	6	40	0.437	0.3	[0.0–9.2]
Second trimester (101–200 days)	6	10	37.5	0.376	0.3	[0.0–7.4]
Third trimester (201–305 days) *	2	1	66.7	1.0		

*Reference category; **Fisher's exact test.
CI, confidence interval.

i.e., large dual-purpose farms with barn milking and predominantly Murrah breed. Compared to the buffaloes with the highest milk production in the study by Hurtado *et al*. [26], there was a difference in the average number of days in milk in the present study, which was 108 days (i.e., the time when production had already peaked); therefore, the data tended to be in the lower range [23]. Meanwhile, it is important to emphasize that the present study used only limited data on the inherent characteristics of the buffalo, such as age, days in milk, milk production, and number of lactations, owing to the lack of production records, a common feature of the Colombian countryside and notable finding in the buffalo herds included in this study. The milking routine used in buffalo farms on the Atlantic coast and in the Colombian mid-Magdalena area was described by Morales *et al*. [19], and it involved the arrival of the buffalo at the milking site, complete bathing of the buffalo, entry of the buffalo into the milking area; calf presentation; udder and/or teat washing, trimming, pre-milking teat dipping with an iodinated product, and drying the pre-milking teat dipping product with newspaper; hand washing by the milker; and predominantly manual milking (in 91.3% of the farms) [19]. Generally, this routine is consistent with the milking routine observed on the farms that participated in the present study. Notably, a significant percentage of the practices that contribute to the hygienic and sanitary quality of milk was performed on the buffalo farms in the present study, such as udder and/or teat washing (45.5%), hand washing by milkers (54.5%), and pre-milking teat dipping (45.5%). This trend may be attributed to the fact that in the study by Morales *et al*., these practices were described as risk or protective factors for high levels of colony-forming units, and in Colombia, this component may benefit or limit the payment of raw milk. Therefore, producers tend to strengthen these practices to achieve a better price for their products. In addition, the authors found a significant percentage (36%) of farms using on-site mechanical milking, where this type of practice is more common because of better access to technology.

No buffaloes with clinical mastitis were found in the present study in either of the two departments; this finding is consistent with the findings of a study from the Colombian municipalities of Puerto Boyacá and Granada, where only six lost quarters were found, possibly due to previous history of clinical mastitis [20]. These findings indicate that the clinical presentation of buffalo mastitis in our environment is rare. However, studies involving a larger number of farms with different conditions in the country are warranted to validate this hypothesis. The above results are not consistent with the result of 7.6% of buffaloes with clinical mastitis at a Jafarabadi buffalo farm in Chikhodra, Gujarat, India, found by Patel *et al*. [27] and that of 18.2% at Hisar University of Veterinary and Animal Sciences, Haryana, found by Sharma *et al*. [28] These differences could be attributed to the differences in methodology used by these studies. Furthermore, the prevalence of the clinical form is proportional to the subclinical form because the latter predisposes to the appearance of signs when not managed in a timely manner, and in our study, the prevalence of the latter was also low. Parity is another important factor because mastitis is more common during the first two lactations, and in the present study, the average number of births was 4.3 among buffaloes with ≤13 births [27-29].

In the present study, the prevalence of SM found using SCC was limited (4.8%). Further, we found no differences between the prevalence at Antioquia and Córdoba, which were 5.6% and 5.1%, respectively, possibly reflecting similar conditions of the farms in the two areas. It should also be noted that the farms in the present study were selected based on convenience and the producers' interest in participating, and one of the limitations of this study was the poor reception by the buffalo herds, which posed a difficulty in conducting this study. This can be attributed to the fact that the studies of mastitis in buffalo are new to the country, and little is known on the subject. Even during farm selection, the authors noted that some producers were unaware of the existence of the disease, and only a few producers were familiar with performing CMT in buffalo species.

The results of SM detected by SCC are consistent with those reported in Hisar (2.9%) [30] and Puducherry (6%), India, respectively [31]. Regarding mastitis detected with CMT, the results of the present study were similar to those observed in the dry (6.1%) and rainy (10%) seasons in a single farm in Sao Paulo, Brazil [32], in Iran (9.5%) [33], using the surf field test (similar to the CMT) at an experimental station in Punjab, Pakistan (9.3%) [34], and among 125 buffalo reported to the veterinary service of the NTR clinical complex, Krishna, India (13.6%) [10]. Both the above studies and the present study used small numbers of farms, indicating that the buffaloes had similar conditions and less variability in management factors. Regarding the findings of mastitis in the present study, the authors believe that the use of multiple practices that favor the hygienic and sanitary quality of the milk (e.g., hand washing by milkers, udder or teat washing, and pre-milking teat dipping) may decrease the frequency of the disease. Another factor that should be considered is the support given to the calf during milking on all farms in this study; although some researchers consider it a protective factor, others researchers consider it a risk factor for mastitis. The latter arises from the belief that the calves leave the teats clean after suckling, which can lead to failure to clean the teats, posing a risk for the presence of mastitis [35]. In bovines, this practice is controversial as some studies on dual-purpose cattle with characteristics similar to those of the present study have revealed a protective effect of suckling, and they have reported that under controlled conditions, stopping this practice is associated with an increased risk of mastitis. This could be explained by the presence of antimicrobial lysozymes in the saliva of calves in addition to the complete emptying of the udder, which eliminates the substrate for bacterial growth [36,37]. In dairy buffaloes, milking with a calf was associated with a shorter milk ejection time, shorter milking time, and lower residual milk volume than those with manual milking [38]. These conditions could explain the low prevalence of mastitis in the present study.

The results of this study are not consistent with those of the studies on SM in Bola, Bangladesh (20%) [39] and the Philippines (42.8%) [29]. According to the study by Biswas *et al*., this difference may be attributed to poor management of mammary gland health as milking hygiene is poor, and no disinfectant solutions are used to wash hands or udders before milking, even when the buffaloes remain in a muddy area after milking. In contrast, in the study by Salvador *et al*., [29] the differences were attributed to the fact that the latter was a retrospective study with data from a buffalo herd between 2006 and 2009.

The prevalence of SM in buffaloes has been extensively studied in India and Pakistan. In these countries, the hygienic and sanitary conditions in milking are poor, leading to a high prevalence of mastitis, which explains the differences between the results of their and our studies. For example, in India, a prevalence of 22.8% has been reported in Jafarabadi breed buffalo in Chikhodra, Gujarat, using CMT and SCC [27]. In Hisar, Sharma *et al*. reported a 33.8% prevalence in 2018 and 56.7% in 2014 using microbiological culture [40]. Moreover, in Hassan, Karnataka, the prevalence of 45.3% was determined using CMT and tests based on electrical conductivity [3]. Furthermore, studies in Pakistan have reported a prevalence of 15.2% in Faisalabad and Okara districts [35] and 44% in Punjab, Pakistan, using the white-side test [6]. In the district of Lahore, a prevalence of 43.6% was reported [41]. Similarly, Mustafa *et al*. [5] found 34 positive buffalo out of 272 examined buffalo; finally, a study in the districts of Bhimber and Lahore found a prevalence of 38.8% on 50 farms [42]. The discrepancies between the results of these studies can also be explained by the fact that most of them used many farms or buffaloes from different production lots. Conversely, in Colombia, Moscoso and Pinzón [20] reported a prevalence of 64% in two farms. Their study involved 30 lactating buffaloes, and particular conditions, such as the milkers' lack of hygiene, could have influenced these results. Previous studies have suggested a greater resistance of the buffalo species to mastitis; however, poor hygiene conditions in general and poor milking routines, in particular, imply that this species' immunity can be overcome by environmental conditions, increasing the risk of the disease. Therefore, good milking practices are considered important in the prevention of mastitis in buffaloes.

SCC is highly correlated with the presence of mastitis in buffaloes and is the main indicator of udder health; thus, it facilitates on-farm monitoring of mastitis prevalence as well as decision-making [43]. Although CMT is believed to be highly correlated with SCC [44], only 63.7% of the CMT-positive specimens in the present study had an SCC of ≥200,000 cells/mL, which is considered an indicator of mastitis in buffalo. A reason for this discrepancy could be that as CMT is a qualitative test, its results do not perfectly correlate with those of a quantitative test such as automated SCC, which has been proposed as a reference test for diagnosing this disease due to its high sensitivity (99.1%) and specificity (100%) [4,45]. Additionally, because CMT is a qualitative test, results may vary according to the observer. Although the researchers who performed the test were trained to reduce observer-induced variability, possible errors in interpreting the CMT results cannot be ruled out. Another reason for these variations could be the difficulty in taking the sample during milking in unruly animals, which sometimes made it difficult to discard the first milk streams, leading to a different composition of the milk sample for CMT in animals for which first jets could be excluded.

The geometric mean of positive quarters in the present study ranged from 412,948 to 462,126 cells/mL, which differs from the 625,640–892,110 cells/mL found in cattle in northern Antioquia [22]. This trend can be attributed to the fact that buffaloes have lower SCC than cows; however, these values were lower than those for bovines. For the buffalo species, a geometric mean of 400,001–1,000,000 cells/mL is considered to indicate high SCC [7].

The main cause of mastitis in the Antioquia and Córdoba departments was infectious mastitis, which is also the main cause of mastitis in dairy buffalo. The presence of samples from mastitis-positive quarters in which no microorganisms grew could be explained by insufficient amounts of microorganisms in the milk for growth in the laboratory (false negative), traumatic mastitis, or other conditions that increase SCC in milk (e.g., stage of lactation), season, and parturition number [9,46,47]. For economic reasons, in this study, it was not possible to process 100% of the milk samples with the automated SCC method. Therefore, the processing method of SCC was chosen only for samples positive for CMT; further, only for positive samples for SCC (≥200,000) culture was performed, which could have resulted in a lower apparent prevalence of pathogens causing moderate to low SCC. This is the case for CoNS and other mammary pathogens considered as minor.

Regarding the contamination of the samples, one possible explanation is that the help of the milkers was necessary at one of the farms to obtain the sample owing to the unruly nature of some buffaloes, which could have led to its contamination. In addition, it has been reported that there are various interactions between different microbes that cause SM in buffaloes, in which the microorganisms responsible for the infection predominate in conjunction with the normal udder flora. In the present study, various bacteria could be present in sufficient quantity to induce growth in the culture without necessarily being contaminants. However, standard laboratory methods consider a sample to be contaminated if three or more pathogens are growing [27]. The main microorganism isolated in this study was CoNS, which was also the main cause of mastitis among buffaloes in the studies by Moscoso & Pinzón in Colombia, Pizauro *et al*. in Brazil, Moroni *et al*. in Italy, and Chavoshi & Husaini in Iraq [4,20,32,33]. CoNS are a group of bacteria often considered to be minor pathogens of the mammary gland, which, if left untreated, can persist for several months during lactation; are mildly pathogenic [48]; are known to be pathogenic opportunists that adhere to metallic elements, persist in milking equipment and the hands of the milker, and colonize the skin of mistreated teats; and are generally associated with SM in buffalo, resulting in a slight increase in SCC [49]. They have also been isolated from the calf's mouth and nostrils [50]. The frequency of CoNS in the present study can be explained by calf-supported milking in all buffaloes, in addition to the lack of hand washing between milkings, leading to the transfer of bacteria from an infected buffalo to a healthy one by milkers.

Streptococcus dysgalactiae was the second most commonly isolated bacterium in the present study. In the study by Charaya *et al*. in 2014 [40], this pathogen was classified as environmental and considered capable of causing both clinical mastitis and SM. Further, it has been isolated in studies and implicated in mastitis in buffalo in Egypt [51], Brazil [9], Pakistan [6], Italy [4], and India [30]. Its frequency in the present study can be attributed to the concentration of the results from two farms where the hygiene conditions at the arrival of the buffalo and at milking, in general, were poor, favoring the colonization of the udder by this type of microorganism.

Regarding contagious mastitis caused by *S. aureus* and *S. agalactiae*, although these were the most important causative agents in buffalo mastitis, we found that contagious mastitis occurred to a lesser extent in the study herds [47].

Risk factors for mastitis among buffaloes have been previously explored, and it has been suggested that lactation stage, number of births, age of the buffalo, milking technique, suckling, and teat morphology are factors associated with the disease [10,29,35].

The present study found that buffalo milked by hand and in the corral had a higher risk of mastitis (OR = 3.24 and OR = 3.96, respectively). These two practices should be analyzed together as milking in the barn was always done by hand, and the staff on the farms with these conditions had fewer cleaning precautions than the operators on the indoor milking farms. In addition, the milking routine on these farms was more precarious, and the failure of staff to dress appropriately was often observed. De Oliveira *et al*. [9] reported that the waiting room is a critical point for the presentation of mastitis owing to the accumulation of feces, which could be extrapolated to the stalls where they were not cleaned by the end of milking due to conditions such as dirty floors, causing contamination and environmental-type mastitis. Accordingly, it is advisable to carry out the proper disinfection of implements associated with milking and to reinforce hygiene conditions for milkers, including the disinfection of teats, to mitigate bacterial exposure to the udder [52,53].

Incorrect elimination of abnormal milk, i.e., elimination of milk with mastitis or antibiotics, was associated with an OR of 7.28. It is known that the supply of this milk to calves in cattle favors the emergence of bacteria resistant to antibiotics [54,55]. A similar effect could occur in calves that become replacement buffalo on these farms, where the disease will be more complicated to treat. It is also important to highlight the role of CoNS, the main agent isolated in this study, as a reservoir favoring the development of antibiotic resistance in addition to resistance genes, such as *mecA*, that have been isolated in calves [56]. Discarded milk must, therefore, be disposed of correctly and should not be supplied to the calves.

Buffaloes whose udders or teats were not dried before milking exhibited a higher risk of mastitis (OR = 3.66) compared to those whose udders or teats were dried. This finding can be attributed to the fact that udders and teats become contaminated with manure and bedding material between milkings [57]. This further favors the accumulation of microorganisms at the tip of the teat, which can colonize the mammary gland. Therefore, it is recommended to dry the udder and teats thoroughly after washing, especially in the case of buffaloes prone to dirt accumulation due to their habits. Hence, individual paper or cotton towels have proven to be efficient in reducing SCCs [52].

Nondisinfection of hands by milkers before milking was associated with an OR of 39.5. It is known that milkers can transmit the bacteria observed in this study, such as CoNS and *S. aureus*, from sick to healthy animals. Therefore, washing hands between milkings may help reduce mastitis. For example, one study showed that washing hands with soap and water before milking, as well as drying with clean towels, decreased the bacterial load, specifically the count of *S. aureus*, as found in our study [53]. However, the high value of this OR needs careful analysis as non-disinfection was a rare practice (18.2%) in our study that may have affected this value. As previously mentioned, our study had a limitation related to the size of the sample collected from farms.

To the best of our knowledge, this is the first large-scale study to evaluate the prevalence and etiology of mastitis in buffalo in Colombia, in the departments of Antioquia and Córdoba, where the country's largest buffalo population is concentrated. However, further studies are needed to determine the prevalence and risk factors of this disease using a larger number of randomly selected farms to understand the reality of buffalo mastitis in these areas.

CONCLUSIONS

During the study period, the frequency of clinical and SM in the departments of Antioquia and Córdoba was low. The main microorganisms isolated in this study were CoNS and *S. dysgalactiae*. Hand milking, barn milking, incorrect removal of abnormal milk, failure to dry udders or teats, and failure to sanitize hands by milkers were identified as risk factors for mastitis during this study.

LIST OF ABBREVIATIONS

CFU = Colony-forming units

CMT = California mastitis test

CoNS = Coagulase-negative *Staphylococcus*

IDF = International Dairy Federation

OR = Odds ratio

SCC = Somatic cell count

SM = Subclinical mastitis

AUTHOR CONTRIBUTIONS

JAF-S and NFR designed the study; JFG, JDT, CFG, and CG collected the samples and the information through questionnaires; JFG drafted the manuscript, and CFG analyzed the data. All authors have read, reviewed, and approved the final manuscript.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

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