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Prevalence Of Subclinical Staphylococcal Mastitis And Milk Hygienic Quality In Relation To Individual Risk Factors In Dairy Cattle Farming At Mechroha Region (Northeastern Algeria)

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Abstract
This study aimed to evaluate the prevalence of subclinical mastitis induced by Staphylococci and the hygienic quality of milk for particular individual risk variables in semi-intensive dairy cattle farms in the Mechroha region (Wilaya of Souk Ahras, Algeria). The study involved 100 lactating cows from three farms between March and September 2023. Milk samples were collected and tested for subclinical mastitis using the California Mastitis Test (CMT). Positive samples were then analyzed for the presence of Staphylococcus spp. In terms of mastitis prevalence, 75.4% of the 61 cows tested by CMT were positive. Bacteriological analysis of positive samples revealed that 63.04% were contaminated with Staphylococcus spp. The most prevalent species was Staphylococcus xylosus (31%), followed by Staphylococcus aureus (13.8%). Additionally, 13.8% of the samples contained either Staphylococcus aureus alone or in combination with Staphylococcus micrococcus. Combinations of Staphylococcus aureus with Staphylococcus intermedius or Staphylococcus xylosus, and samples containing only Staphylococcus micrococcus accounted for (10.3%) each. Less frequent combinations, including Staphylococcus aureus, Staphylococcus intermedius, and Staphylococcus xylosus, or Staphylococcus aureus, Staphylococcus sciuri, and Staphylococcus micrococcus, were identified in (3.4%) of samples. The results also indicated that cows on Farm 3 had a significantly higher probability of being CMT positive compared to those on Farm 2. Daily milk production exceeding 30 liters was associated with a higher probability of CMT positive results, as well as higher levels of total and fecal coliforms. In contrast, breed, lactation rank, stage of lactation, and stage of gestation showed no significant associations with CMT results. No samples showed the presence of Salmonella. This study reveals a high prevalence of subclinical Staphylococcus mastitis on farms in the Mechroha region. High milk production emerges as an important risk factor for subclinical mastitis and microbiological contamination of milk. These findings highlight the urgent need to improve hygiene and management practices to reduce the incidence of mastitis and enhance milk quality.

Keywords: Test (CMT), risk factors, subclinical mastitis, staphylococcus, hygiene quality

Introduction

Milk production is expanding, with cattle being the primary producers. However, due to its susceptibility to microbial infections, milk poses significant risks to food safety (FAO, 2020). In Algeria, milk production has progressed, reaching 2,414,552 tons of raw cow's milk per year, accounting for 75.8% of the total milk supply per capita (FAO, 2020). Despite these advances, self-sufficiency has not yet been achieved due to challenges such as climatic conditions and forage supply (Haou et al., 2021; Eulmi et al., 2023). The udder health of cows is crucial for milk yield, as mammary infections can reduce production by up to 20% and affect milk quality (Seegers et al., 2003; Sharun et al., 2021; Hogeveen et al., 2011).

It leads to a significant reduction in milk production, exacerbating production deficits in a context where the local supply struggles to meet internal demand and increases dependence on imports (Islam et al., 2011; Viguier et al., 2009). Furthermore, mastitis deteriorates milk quality by increasing somatic cell counts and affecting its microbiological properties, which can lead to financial penalties and damage the reputation of the dairy industry (Blowey & Edmondson, 2010; Ndahetuye et al., 2019). Treatment costs, including veterinary care and medications, increase farmers' expenses and reduce their profit margins (Smith & Hogan, 1993; Langer et al., 2014). Moreover, these economic challenges may hinder the development and modernization of the dairy sector by limiting investments in farm expansion (Erskine, 2015). Mastitis also increases the risk of milk contamination, raising food safety concerns and generating additional costs for quality control.

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The Mechroha region, located in the northeast of Algeria, is considered one of the country's leading milk-producing areas, providing a significant portion of the national milk supply. To better understand this dynamic, this study aims, for the first time in this region, to assess the hygienic quality of milk and analyze staphylococcal infections in dairy cattle farms. The objective is to identify the main risk factors associated with bovine mastitis, detected by the California Mastitis Test (CMT), and their impact on raw milk quality. Additionally, the study seeks to determine the prevalence of Staphylococcus species in CMT-positive milk samples.

Materials and Methods

Study Area

The study was conducted between March and September 2023 across three dairy herds (E1 located in Ain-Nafra, E2 in Gueleaa, and E3 in Meris), in the Mechroha region, Souk-Ahras province, in northeastern Algeria (Figure 1). The northern part of the region is influenced by Mediterranean climatic conditions, while the southern part is characterized by a semi-arid climate. The province experiences hot, dry summers with an average temperature of 33°C, peaking at 42°C, and cold, wet winters with an average temperature of 12°C. The Souk-Ahras region (approximately 553 km from Algiers) is considered the main dairy basin in the eastern part of the country, with an annual milk production of 50 million liters (DSA, 2022).

Animals

The study was conducted on a dairy farm with 100 cows, distributed among the Montbeliarde, Prim'Holstein, and Cross-breed. Among these, 61 lactating cows were selected. Cows with clinical mastitis, those in the dry period, and heifers were excluded from the study. The farms were chosen based on accessibility and the availability of the farmers. An epidemiological survey was conducted using a data sheet focusing on milking practices, hygiene, and dry-cow treatment. All three farms followed a semi-intensive management system. Milking was done twice a day, morning and evening, using a milking trolley at all three farms.

Farm 1: 43 cows (37 Montbeliarde, 6 Prim'Holstein)
Farm 2: 25 cows (13 Montbeliarde, 12 Cross-breed)
Farm 3: 32 Montbeliarde cows

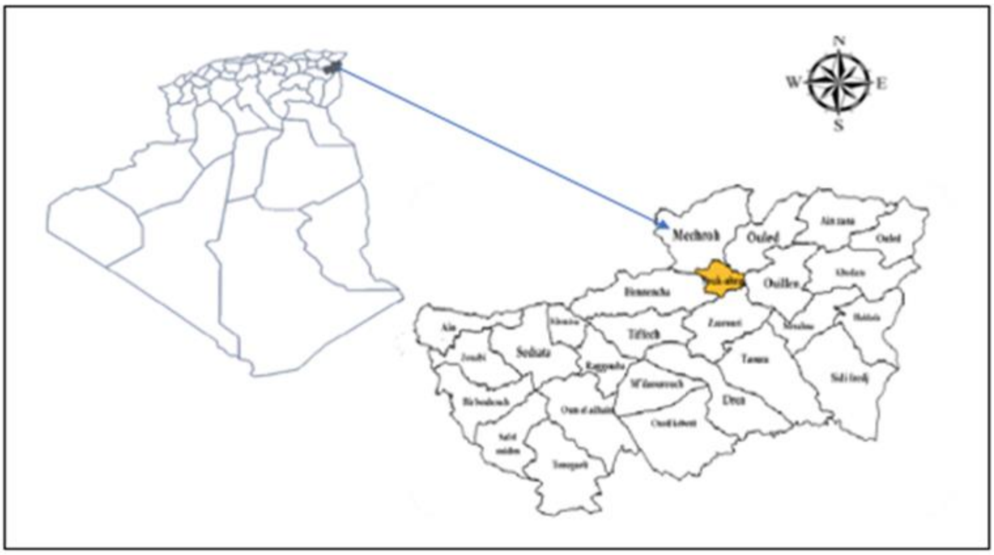

Figure 1. Location of the study area

Sampling

A total of 244 milk samples were collected from 61 clinically healthy lactating cows. The samples were taken directly from the udder before the morning milking. The first sample was used for the California Mastitis Test (CMT), and the second sample was collected in sterile bottles (125 ml) for bacteriological analysis.
Only samples that tested positive for CMT were selected for further analysis.

Preparation

Teats were washed with water and disinfected with 70% alcohol, and the first milk jets were discarded (National Mastitis Council, 1999). All samples were identified and transported to the Microbiology and Physicochemistry Laboratory of Edough Dairy (GIPLAIT), Annaba, under strict refrigeration conditions (4°C) on the same day.

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CMT (California Mastitis Test)
The California Mastitis Test (CMT) was used to screen for subclinical mastitis in the cows' udders across 244 quarters, following the procedure described by Quinn et al. (1999). After discarding the first milk jets, approximately 2 ml of milk was collected from each quarter into a black dish, to which an approximately equal amount (about 3 ml) of reagent was added. The mixture was shaken for 15 seconds to ensure proper mixing of the reagent and milk. The reaction was observed by transparency to assess the appearance of the mixture. According to Saidi et al. (2010), any change in the phase towards milk flocculation was considered a positive reaction. CMT scores of 0 and trace were considered negative, while scores of 1, 2, and 3 were considered positive. A cow with at least one quarter showing a CMT score of 1+ was considered a positive cow (Abebe et al., 2016).

Bacteriological Milk Analysis
Isolation and counting of Total Mesophilic Aerobic Flora (TMAF) were performed on PCA (Plate Count Agar) after incubation at 30°C for 72 hours. Total coliforms (TC) and thermotolerant coliforms (TTC) were cultured on VRBL (violet red bile lactose) agar, with incubation at 30°C for TC and at 40°C for TTC for 24 to 48 hours. Salmonella detection followed international standards (ISO 6888).

Staphylococcus spp. were cultured on selective Chapman agar and incubated at 37°C for 48 hours. A volume of 100 µl of milk was plated by surface spreading, then incubated at 37°C for 24 to 48 hours. Suspected S. aureus isolates were purified and subjected to a series of biochemical and physiological identification tests, including catalase test, Gram staining, and coagulase test (Society for General Microbiology, 2006).

Statistical analysis
Data were collected and organized using Excel spreadsheets. Independent variables comprised farm characteristics and individual cow parameters (breed, lactation rank, lactation stage, pregnancy stage, and daily milk production), while dependent variables included CMT results and bacterial counts (total coliforms, fecal coliforms, and Staphylococcus sp.) in CMT-positive samples. Bacterial count thresholds and Staphylococcus species prevalence were assessed through frequency distributions. Associations between CMT results and categorical variables were examined using Chi-square tests. After confirming normality (Kolmogorov-Smirnov and Shapiro-Wilk tests), Kruskal-Wallis analysis of variance compared bacterial counts across individual risk factors. Binary logistic regression analyzed associations between risk factors and four dependent variables: CMT results (N=61), and within CMT-positive samples (N=46), counts of total coliforms, fecal coliforms, and Staphylococcus sp. The model used was: $\text{Logit}(Y)=\alpha+\beta_1X_1+\beta_2X_2+\cdots+\beta_nX_n$, where Y represents the binary outcome, α the intercept, and $\beta_1, \beta_2, \ldots, \beta_n$ the regression coefficients for risk factors $X_1, X_2, \ldots, X_n$. Odds ratios (OR) with 95% confidence intervals quantified association strengths. Statistical significance was set at $P<0.05$, and all analyses were conducted using SPSS 26 software.

Results
CMT test
Figure 2, displays the results of the CMT tests per udder quarter of the dairy cows. The highest rate of positive CMT tests was recorded for quarter B (49.2%). However, quarters C and D showed the highest rates of CMT level 3. The percentage of dairy cows with positive CMT tests was significantly higher (75.4%) compared to those with negative results (24.6%) (Figure 3).

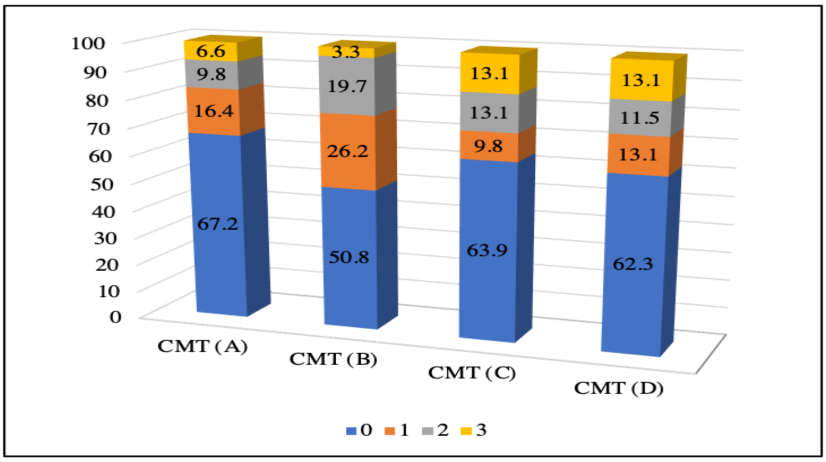

Figure 2. Percentage of different CMT levels for the udder quarters of the studied dairy cows (N=61).

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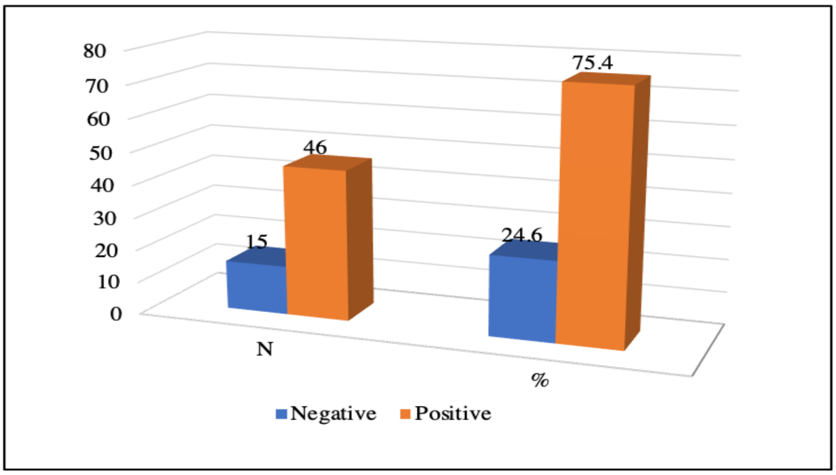
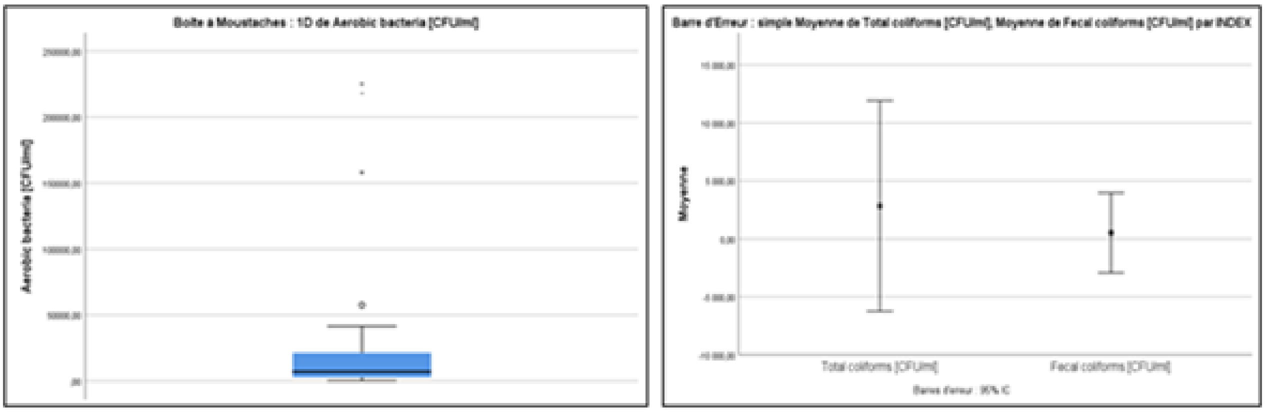


Figure 3. Percentage of dairy cows with positive CMT test results (N=61).

Aerobic, total coliform and fecal coliform bacteria count

Among the 46 cows with positive CMT results, the mean counts of Total Aerobic Bacteria (AB), Total Coliform Bacteria (TCB), and Fecal Coliform Bacteria (FCB) were 39859.21 ± 68649.24 CFU/ml, 2826.24 ± 4545.72 CFU/ml, and 511.45 ± 1720.91 CFU/ml, respectively (Figure 4 a, b). For these cows, 80.43% and 89.31% showed satisfactory counts of Total Coliform (TC) and Fecal Coliform (FC), respectively (Figure 4). Additionally, 15.21% and 10.86% of these dairy cows exhibited acceptable TC and FC counts (Figure 4). However, the percentage of milk samples from dairy cows with positive CMT results showing unsatisfactory TC and FC counts were 4.34% and 0%, respectively (Figure 5).



(a) **(b)**

Figure 4. Mean and standard deviation (SD) of Total Aerobic Bacteria (TAB), Total Coliform (TC), and Fecal Coliform (FC) counts in CMT test-positive samples (N=46).

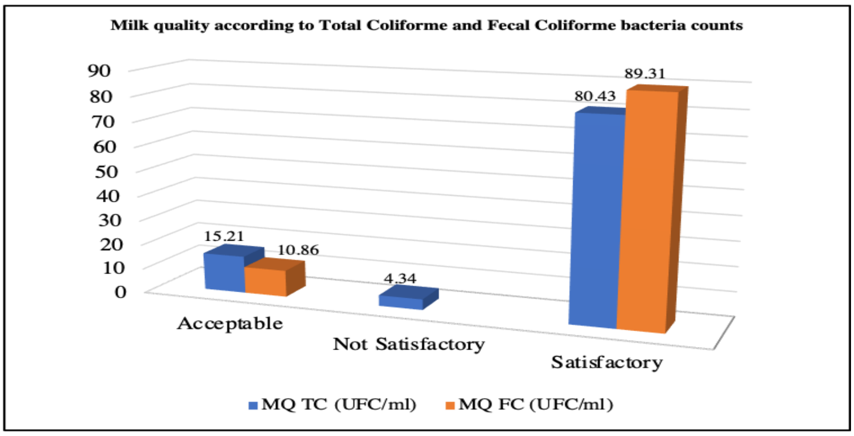


Figure 5. Percentage of milk samples with satisfactory TC and FC counts from dairy cows with positive CMT (N=46).

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Staphylococcus and Salmonella

Of the 46 positive CMT test samples, 63.04% (N=29) tested positive for *Staphylococcus spp.* (Figure. 6). The distribution of different Staphylococcus species among the positive samples is shown in (Figure.7). The most prevalent species was *Staphylococcus xylus*, present in 31% of the samples. This was followed by samples containing *Staphylococcus aureus* or combination of *Staphylococcus aureus* with *Staphylococcus micrococcus*, each accounting for 13.8% of the samples. In the third rank, samples with a combination of *Staphylococcus aureus* and *Staphylococcus intermedius*, *Staphylococcus aureus* with *Staphylococcus xylosus*, or only *Staphylococcus micrococcus* each had a prevalence of 10.3%. Finaly, samples containing *Staphylococcus aureus*, *Staphylococcus intermedius*, and *Staphylococcus xylosus; Staphylococcus aureus, Staphylococcus sciuri,* and *Staphylococcus micrococcus;* or only *Staphylococcus intermedius* each had a prevalence of 3.4%. All positive CMT samples were tested negative for Salmonella (Figure. 7).

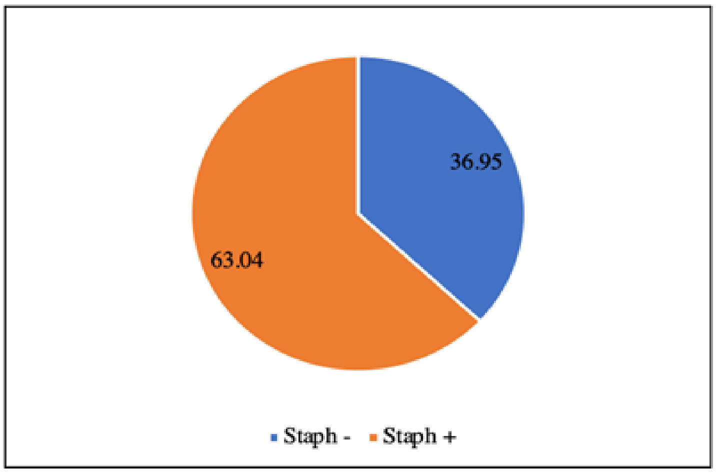

Figure 6. Percentage positive (staph+) and negative (staph-) tested samples for Staphylococcus sp. from dairy cows with positive CMT (N=46).

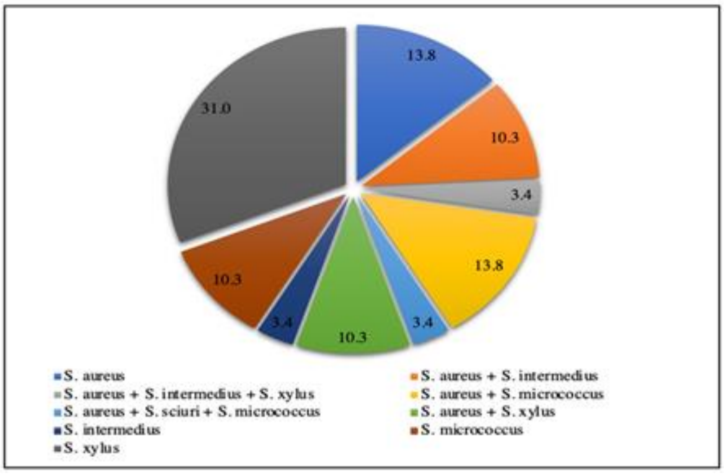

Figure 7 . Percentage of milk samples tested for Staphylococcus species from dairy cows with positive CMT (N=46).

CMT

Logistic regression analysis revealed significant associations between certain risk factors and the results of the California Mastitis Test (CMT) in cows. In particular, cows belonging to Herd 3 had a much higher probability of obtaining a positive CMT test (OR=22.43, 95% CI: 4.25-73.56) compared to those from Herd 2, with Herd 1 being the reference group. Regarding daily milk production, cows producing more than 30 liters per day had a significantly higher probability of having a positive CMT test (OR=4.50, 95% CI: 3.77-35.81). However, other risk factors studied, such as breed, lactation rank, lactation stage, and pregnancy stage, did not show significant association with CMT test results. The Montbéliarde, Prim Holstein, and Cross-breed cows, as well as different lactation ranks, lactation stages, and pregnancy stages, did not present significant differences in CMT test positivity rates.

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Table 1 Analysis of risk factors for CMT and microbiological quality. Logistic regression analysis result for association of potential risk factors with CMT test results (N=61)

Risk factors	Risk factors category	f_i	f_n	CMT-	CMT+	P	OR	95% CI for OR
Herd	Herd 1	25	41,0	3(4,9)	22(36,1)	0,01	Reference	
	Herd 2	15	24,6	8(13,1)	7(11,5)		0.44	0.10-1.88
	Herd 3	21	34,4	4(6,6)	17(27,9)		22.43	4.25-73.56
Breed	Montbeliarde	47	77,0	12(19,7)	35(57,4)	0,893	Reference	
	Prim Holstein	6	9,8	1(1,6	5(8,2)		0.37	0.06-3.60
	Cross-breed	8	13,1	2(3,3)	6(9,8)		0.63	0.12-3.35
Lactation rank	1	17	27,9	7(11,5)	10(16,4)	0,598	Reference	
	2	15	24,6	3(4,9)	12(19,7)		1.25	0.53-5.22
	3	13	21,3	3(4,9)	10(16,4)		1.67	0.55-5.06
	4	11	18,0	2(3,3)	9(14,8)		1.87	0.51-6.89
	5	3	4,9	0(0.0)	3(4,9)		3.33	0.44-25.33
	6	1	1,6	0(0.0)	1(1,6)		2.50	0.15-40.72
	7	1	1,6	0(0.0)	1(1,6)		2.50	0.15-40.72
Lactation stage	Beginning	16	16,0	3(4,9)	13(21,3)	0,617	Reference	
	Middle	34	34,0	2(3,3)	9(14,8)		0.58	0.11-3.04
	End	11	11,0	10(16,4)	24(39,3)		3.88	0.12-6.66
Pregnancy stage	Empty	16	26,2	3(4,9)	13(21,3)	0,359	Reference	
	1st Third	4	6,6	1(1,6)	3(4,9)		0.56	0.05-6.18
	2nd Third	30	49,2	6(9,8)	24(39,3)		1.69	0.33-9.74
	3rd Third	11	18,0	5(8,2)	6(9,8)		1.69	0.21-13.56
Daily Milk Production	<20	17	27,9	0(0.0)	17(27,9)	0,008	Reference	
	20-30	3	4,9	0(0.0)	3(4,9)		2.44	0.23-25.56
	>30	41	67,2	15(24,6)	26(42,6)		4.5	3.77-35.81

CMT-: Negative CMT Test, CMT +: Positive CMT Test, f_i: frequency, f_n: relative frequency, OR: odds ratio, 95% CI: 95% Confidence Intervals.

Total coliform and fecal coliform bacteria

The analysis of factors influencing total coliform counts in cow milk revealed compelling insights. Daily milk production emerged as the most significant determinant, with cows producing over 30 liters per day showing a substantial likelihood (OR = 4.50, 95% CI: 3.77-35.81, P = 0.046) of higher coliform counts. Conversely, factors such as herd origin, breed type, lactation rank, lactation stage, and pregnancy stage did not demonstrate significant associations with coliform levels.

Table 2. Logistic regression analysis result for association of potential risk factors with total coliform (TC) bacteria count in milk CMT-positive samples (N=46)

Risk factors	Risk factors category	f_i	f_n	Acceptable	Satisfactory	Not Satisfactory	P	OR	95% CI for OR
Herd	Herd 1	22	47,8	2(4.3)	18(39.1)	2(4.3)	0,1 37	Reference	
	Herd 2	7	15,2	3(6.5)	4(8.7)	0(0.0)		0.66	0.28-3,94
	Herd 3	17	37,0	2(4.3)	15(32.6)	0(0.0)		0.85	0.32-3.58
Breed	Monbeliarde	35	76,1	4(8.7)	29(63.0)	2(4.3)	0,1 14	Reference	
	Prim Holstein	5	10,9	0(0.0)	5(10.9)	0(0.0)		1,34	0.05-6,22
	Cross-breed	6	13,0	3(6.5)	4(6.5)	0(0.0)		0.78	0.16-4.50
Lactation rank	1	10	21,7	2(4.3)	8(17.4)	0(0.0)	0,1 26	Reference	
	2	12	26,1	0(0.0)	12(26.1)	0(0.0)		0,72	0.073-2.37
	3	10	21,7	2(4.3)	8(17.4)	0(0.0)		1.25	0.38-4.15
	4	9	19,6	1(2.2)	6(13.0)	2(4.3)		1.87	0.51-6.89
	5	3	6,5	1(2.2)	2(4.3)	0(0.0)		3.33	0.44-25.33
	6	1	2,2	0(0.0)	1(2.2)	0(0.0)		2.50	0.15-40.72
	7	1	2,2	1(2.2)	0(0.0)	0(0.0)		2.50	0.15-40.72
Lactation stage	Beginning	13	28,3	4(8.7)	9(19.6)	0(0.0)	0,3 1	Reference	
	Middle	9	19,6	1(2.2)	7(15.2)	1(2.2)		8.11	0.98-13.91
	End	24	52,2	2(4.3)	21(45.7)	1(2.2)		3.88	0.12-6.66
Pregnancy	Empty	13	28,3	4(8.7)	9(19.6)	0(0.0)	0,4	Reference	

stage	1st Third	3	6,5	0(0.0)	3(6.5)	0(0.0)	21	0.56	0.05-6.18
	2nd Third	24	52,2	3(6.5)	19(43.3)	2(4.3)		1.69	0.33-9.74
	3rd Third	6	13,0	0(0.0)	6(13.0)	0(0.0)		1.69	0.21-13.56
Daily Milk Production	<20	17	37,0	1(2.2)	16(34.8)	0(0.0)	0,0 46	Reference	
	20-30	3	6,5	1(2.2)	1(2.2)	1(2.2)		1.67	0.20-12.45
	>30	26	56,5	5(10.9)	20(43.5)	1(2.2)		4.50	3.77-35.81

Acceptable or Satisfactory or Not Satisfactory: Quality of milk, fi: frequency, f_n: relative frequency, OR: odds ratio, 95% CI: 95% Confidence Intervals

Fecal coliform count

The Table 3 showed the results of logistic regression analysis examining several key risk factors influencing the presence of acceptable fecal coliform counts in cow milk samples (N=46). Among the factors investigated, herd composition showed no significant differences between Herd 2 (OR = 0.75, 95% CI 0.29 - 1.95) and Herd 3 (OR = 0.87, 95% CI 0.37 - 2.05) compared to Herd 1, the reference category. Breed analysis revealed no occurrences of acceptable counts for Prim Holstein, precluding meaningful odds ratio calculations. Cross-breeds exhibited lower odds of acceptable counts (OR = 0.46, 95% CI 0.05 - 4.25) compared to Montbeliarde. Lactation rank demonstrated varying impacts; Lactation Rank 4 (OR = 0.48, 95% CI 0.13 - 1.83) and Lactation Rank 5 (OR = 0.57, 95% CI 0.08 - 4.03) had lower odds compared to Rank 1, though not statistically significant. Regarding lactation and pregnancy stages, no statistically significant differences were found in mid to end lactation stages compared to the beginning stage, nor in different pregnancy thirds compared to an empty stage. Daily milk production showed a significant association, with cows producing over 30 liters per day having significantly higher odds of acceptable counts (OR = 5.54, 95% CI 1.39 - 22.13) compared to those producing less than 20 liters per day.

Table 3. Logistic regression analysis result for association of potential risk factors with fecal Coliform (FC) bacteria count in milk CMT-positive samples (N=46)

Risk factors	Risk factors category	f_i	f_n	Acceptable	Satisfactory	P	OR	95% CI for OR
Herd	Herd 1	22	47,8	2(4.3)	20(43.5)	0,918	Reference	
	Herd 2	7	15,2	1(2.2)	6(13.0)		0.75	0.29 - 1.95
	Herd 3	17	37,0	2(4.3)	15(32.6)		0.87	0.37 - 2.05
Breed	Montbeliarde	35	76,1	4(8.7)	31(67.4)	0,661	Reference	
	Prim Holstein	5	10,9	0(0.0)	5(10.9)			
	Cross-breed	6	13,0	1(2.2)	5(10.9)		0.46	0.05 - 4.25
Lactation rank	1	10	21,7	1(2.2)	9(19.6)	0,163	Reference	
	2	12	26,1	0(0.0)	12(26.1)		0,33	0.03-1.29
	3	10	21,7	0(0.0)	10(21.7)			
	4	9	19,6	3(6.5)	6(13.0)		0.48	0.13 - 1.83
	5	3	6,5	1(2.2)	2(4.3)		0.57	0.08 - 4.03
	6	1	2,2	0(0.0)	1(2.2)			
	7	1	2,2	0(0.0)	1(2.2)			
Lactation stage	Beginning	13	28,3	2(4.3)	11(23.9)	0,805	Reference	
	Middle	9	19,6	1(2.2)	8(17.4)		0.74	0.22 - 2.46
	End	24	52,2	2(4.3)	22(47.8)		1.25	0.47 - 3.36
Pregnancy stage	Empty	13	28,3	2(4.3)	11(23.9)	0,697	Reference	
	1st Third	3	6,5	0(0.0)	3(6.5)		1,86	0,52-6,44
	2nd Third	24	52,2	3(6.5)	21(45.7)		1.89	0.52 - 6.88
	3rd Third	6	13,0	0(0.0)	6(13.0)			
Daily Milk Production	<20	17	37,0	0(0.0)	17(37.0)	0,124	Reference	
	20-30	3	6,5	1(2.2)	2(4.3)		0.40	0.08 - 1.97
	>30	26	56,5	4(8.7)	22(47.8)		5.54	1.39 - 22.13

Acceptable or Satisfactory: Quality of milk, f_i: frequency, f_n: relative frequency, OR: odds ratio, 95% CI: 95% Confidence Intervals

***Staphylococcus sp.* Count**

The logistic regression analysis investigating risk factors affecting Staphylococcus sp. counts in cow milk yielded several notable insights. Among the factors examined, herd differences were observed, with Herd 2 showing a slight

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increase in odds (OR = 1.33, 95% CI: 0.19 - 9.24) in bacterial counts compared to the reference Herd 1, although this association was not statistically significant. Herd 3 did not demonstrate a significant relationship with bacterial counts (OR = 1.00, 95% CI: 0.15 - 6.78). Breed-wise, the Prim Holstein category lacked negative counts, making OR calculation impractical. Similarly, the Cross-breed category showed no significant association with bacterial counts (OR = 1.00, 95% CI: 0.11 - 9.00). Lactation rank analysis indicated that higher ranks did not significantly affect bacterial counts, with Lactation Rank 2 showing a non-significant decrease in odds compared to Rank 1 (OR = 0.65, 95% CI: 0.04 - 10.72, P = 0.17). Conversely, cows in the later stages of lactation (Lactation Stage End) showed a significantly higher odds ratio (OR = 3.16, 95% CI: 1.60 - 16.75, P = 0.014). Additionally, while the 3rd Pregnancy Stage showed an elevated odds ratio (OR = 2.60, 95% CI: 0.34 - 19.67). Daily Milk Production >30 liters also exhibited a higher odds ratio (OR = 1.30, 95% CI: 0.23 - 7.34), indicating a potential trend towards increased bacterial counts with higher milk production levels, though not reaching statistical significance.

Table 4. Logistic regression analysis result for association of potential risk factors Staphylococcus sp. count in milk CMT- positive samples (N=46)

Risk factors	Risk factors category	f_i	f_n	Negative	Positive	P	OR	95% CI for OR
Herd	Herd 1	22	47,8	8(17.4)	14(30.4)	0,938	Reference	
	Herd 2	7	15,2	3(6.5)	4(8.7)		1.33	0.19 - 9.24
	Herd 3	17	37,0	6(13.0)	11(23.9)		1.00	0.15 - 6.78
Breed	Montberiarde	35	76,1	18(39.1)	21(45.7)	0,173	Reference	
	Prim Holstein	5	10,9	0(0.0)	5(10.9)			
	Cross-breed	6	13,0	3(6.5)	3(6.5)		1.00	0.11 - 9.00
Lactation rank	1	10	21,7	9(19.6)	4(8.7)	0,17	Reference	
	2	12	26,1	1(2.2)	11(23.9)		0.65	0.04 - 10.72
	3	10	21,7	5(10.9)	6(13.0)		0.46	0.04 - 5.75
	4	9	19,6	4(8.7)	5(10.9)		1.20	0.12 - 11.65
	5	3	6,5	2(4.3)	1(2.2)		0.50	0.03 - 7.64
	6	1	2,2	0(0.0)	1(2.2)		0.50	0.03 - 7.64
	7	1	2,2	0(0.0)	1(2.2)		0.50	0.03 - 7.64
Lactation stage	Beginning	13	28,3	10(21.7)	4(8.7)	0,014	Reference	
	Middle	9	19,6	4(8.7)	6(13.0)		0.67	0.03 - 14.16
	End	24	52,2	7(15.2)	19(41.3)		3.16	1.60 - 16.75
Pregnancy stage	Empty	13	28,3	10(21.7)	4(8.7)	0,039	Reference	
	1st Third	3	6,5	2(4.3)	2(4.3)		0.50	0.04 - 8.11
	2nd Third	24	52,2	7(15.2)	18(9.1)		1.00	0.10 - 10.02
	3rd Third	6	13,0	2(4.3)	5(10.9)		2.60	0.34 - 19.67
Daily Milk Production	<20	17	37,0	5(10.9)	12(26.1)	0,454	Reference	
	20-30	3	6,5	2(4.3)	1(2.2)		0.57	0.07 - 4.40
	>30	26	56,5	14(30.4)	16(34.8)		1.30	0.23 - 7.34

Negative: samples negative staphylococcus sp, Positive: samples positive staphylococcus sp, fi: frequency, f_n: relative frequency, OR: odds ratio, 95% CI: 95% Confidence Intervals

Discussion

Subclinical mastitis represents a major challenge for the dairy industry, as it does not present obvious clinical signs or visible changes in milk (Ndahetuye et al., 2019). Its prevalence is significantly higher than that of clinical mastitis, with an estimated frequency 10 to 40 times greater according to some studies (Islam et al., 2011). This disparity underscores the importance of early and effective detection. In this context, we conducted a screening in three different dairy farms using the CMT test. The latter proves to be a valuable tool. Although it is an indirect method, the CMT is recognized for its practicality and sensitivity in detecting subclinical mastitis (Langer et al., 2014). Its effectiveness is primarily due to its ability to detect the increase in somatic cell count in milk, a reliable indicator of mammary inflammation (Viguier et al., 2009).

The present study highlights the high prevalence of subclinical mastitis, with 75.4% of the cows testing positive for the CMT. This rate is particularly alarming, especially when compared to the results reported in several studies, which range from 31.09% (Palma et al., 2020) to 31.80% in the study by Sarba and Tola (2017) in Ethiopia, 31.79% in northeastern Algeria (Abderrazak et al., 2021), 31.09% in Nepal (Aavash et al., 2023), 35.5% (Saidani et al., 2024), 33.13% in South Africa (Khasapane, et al., 2023), and only 28% in Bangladesh (Sohidullah et al., 2023). In contrast, Akkou et al. (2024) reported a rate of 45.94% in northern Algeria, which is still significantly lower than our findings. Moreover, other studies have reported prevalence rates similar to ours. In Ethiopia, Mekibib et al. (2010) observed a rate of 71.0%, Belay et al. (2022) found a rate of 64.3%, and Zeryehun et al. (2013) reported a rate of 74.7% in Addis

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Ababa. Abrahmsén et al. (2014) recorded a rate of 86.2% in Uganda, which is higher than in our study. Abebe et al. (2016) noted a prevalence of 62.6% in southern Ethiopia. Gitau (2014) found a rate of 61.21% in Kenya. Medrano-Galarza et al. (2021) reported a rate of 50% in Colombia. Al Harbi et al. (2021) recorded a prevalence of 72% in various regions of Australia. Meskini et al. (2021) observed a rate of 61.21% in dairy cattle farming in northwestern Algeria, while F. Seddar-Yagoub et al. (2024) reported a rate of 62.8% in northwestern Algeria. Prevalence rates can vary between and within regions due to differences in livestock management and climatic conditions (abed et al., 2021).

The significant disparity of subclinical mastitis rates can be explained by several factors. First, milking hygiene practices play a crucial role. A study by Abebe et al. (2016) demonstrated that inadequate hygiene practices during milking significantly increased the risk of subclinical mastitis. The use of the same drying towel spreads mastitis pathogens, as confirmed by Mekonnen et al. (2017). Second, the maintenance of milking equipment is also a key factor. According to Breen et al. (2009), poor maintenance of milking equipment can lead to teat injuries, thereby increasing susceptibility to mammary infections. The absence of green forage, an essential source of vitamins and trace elements, negatively impacts udder health and reduces resistance to intramammary infections (Mbindyo et al., 2021). These factors are primarily associated with subclinical mastitis caused by pathogens with a mammary reservoir. Microorganisms such as Staphylococcus aureus and Streptococcus agalactiae can easily spread from one cow to another if hygiene rules are not followed during milking (Ruegg, 2017). This spread may explain the high prevalence observed in our study. In fact, the proportion of milk samples from positive CMT quarters that were carriers of one or more Staphylococcus species was considerable, reaching 63.04%.

Our study highlights moderately low levels of total aerobic bacteria, total coliform bacteria, and fecal coliform bacteria, reflecting only mildly concerning levels of unsatisfactory total coliform (TC) and fecal coliform (FC) counts, which were 4.34% and 0%, respectively. These bacteria are primarily of environmental origin, related to herd management practices. A study by Kathambi al. (2019) showed that overcrowding, inadequate bedding, and poor drainage in cow resting areas were associated with an increase in cases of subclinical mastitis. Similarly, farming conditions are closely linked to milk hygiene quality, as well as to the levels of coliform and aerobic bacteria (Radostits et al., 2007).

The left posterior quarter (B) was most frequently affected by mastitis, with a prevalence of 49.2%. This rate is similar to that found by Abderrazak et al. (2021) in northeastern Algeria, which was 33.33%, but higher than the 13.25% reported by Saidani et al. (2024). Although relatively high, this figure was still surpassed by the more evident CMT scores observed in the right and left anterior quarters (C and D), which showed higher readings.

Mastitis transmission between quarters can occur due to malfunctioning milking machines. Incorrect machine settings or inadequate management conditions can increase the risk of new infections. Mein (2012) emphasizes that air leaks and vacuum fluctuations in milking systems can facilitate the transfer of pathogenic bacteria between quarters, thereby exacerbating infections. To prevent such issues, it is crucial to regularly maintain and check milking equipment, as well as to adopt good hygiene practices.

The higher frequency of subclinical mastitis, and thus positive CMT results, in Farm 3 can be explained by milking hygiene practices. In this farm, hygiene measures during milking are not followed, and additionally, the milking order is not respected in all three farms. The systematic application of the CMT test is also not in place, meaning that farmers lack a clear understanding of the subclinical infection levels in their cows. This complicates the control of pathogen spread during milking, which is further confirmed by the presence of Staphylococcus, particularly Staphylococcus aureus.

Cows with higher milk production are more likely to develop subclinical mastitis (OR=4.50, 95% CI: 3.77–35.81, P=0.008). The high prevalence of subclinical mastitis in high-producing dairy cows is due to multiple factors. Metabolic stress and production demands impair the immune system (Ingvartsen & Moyes, 2013). Genetic selection for high yield reduces resistance to mastitis (Heringstad et al., 2003). Physical characteristics and intensive milking practices increase the risk of infections (Sørensen et al., 2000; Neijenhuis et al., 2001). Nutritional imbalances and intensive farming systems exacerbate exposure to pathogens (Bhardwaj et al.,2024; Barkema et al., 2006). This combination underscores the need for a holistic approach to prevent and control the condition.

Additionally, the absence of significant differences in the prevalence of subclinical mastitis related to breed, lactation rank, and stage of gestation in our study can be explained by several factors. Regarding breed, uniform management practices across animals, regardless of breed, can minimize the variations observed in other studies. For example, Sharma et al. (2012) found increased susceptibility in Holstein-Friesians compared to Indian native breeds, but such variation may be reduced in a standardized management environment. Sharun et al. (2021) also reported significant variations between breeds in Ethiopia, highlighting the importance of local conditions.

Lactation rank, often associated with increased risk in multiparous cows due to the wear and tear of mammary defense mechanisms (Vergara et al., 2014; Stanek et al., 2024), may not show significant differences in our study due to the rigorous and uniform management of cows, whether they are primiparous or multiparous. Regarding gestational stage, hormonal and metabolic changes at the end of gestation typically increase the risk of subclinical mastitis (Green et al., 2007). However, uniform management across different stages of lactation and gestation may reduce these variations. Ashan et al. (2018) identified higher-risk periods at the beginning and end of lactation, but consistent management can moderate these effects. Finally, the complex interaction between these factors, as highlighted by Ruegg (2003) and

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Fauteux et al. (2014), could be mitigated by effective and uniform management. Standardized management minimizes the observed variations and contributes to the absence of significant differences in our study.

The total mesophilic aerobic flora, considered an indicator of general hygiene, provides an assessment of the level of microbial contamination and the quality of the product. In the present study, this flora meets the standards established by the Algerian Official Journal in 2017 (JORA, 2017). Royster & Wagner (2015) in the Tissemsilt region (western Algeria), found values ranging from 1.1×10^5 to 5.6×10^6 CFU/ml, while Tanjaoui et al. (2023) in Morocco reported an average bacterial load in TAMF in milk of 3.8×10^8 CFU/ml. These relatively high values reflect poor hygiene practices in those farms. In contrast, the milk in this study can be considered to have good hygienic quality overall.

The notable difference between our results and those of other studies, which suggest poorer hygienic quality, can be explained by several factors. Geographical and environmental variations between the regions studied, as highlighted by Elmoslemany et al. (2010), can significantly influence the microbiological quality of milk. The improvement of hygiene practices over time, as observed by Elmoslemany et al. (2009), could also have contributed to the better results seen in our study. Differences in farming practices, particularly milking methods and general hygiene, have a direct impact on the total mesophilic aerobic flora, as demonstrated by Pantoja et al. (2009). Seasonal variations, highlighted by O'Connell et al. (2015), and differences in herd size and management, studied by Zucali et al. (2011), may also contribute to these discrepancies. Furthermore, sampling and analysis methods, along with the implementation of mastitis control and prevention programs, can influence the results, as pointed out by Zajác et al. (2015) and Ruegg (2017). This positive trend may reflect a general improvement in hygiene practices within the Algerian dairy industry, emphasizing the ongoing importance of research and the application of good dairy hygiene practices.

The evaluation of total coliform counts in our study reveals generally satisfactory results. The majority of samples meet the standards set by the Algerian Official Journal in 2017 (JORA, 2017), with only 4.34% of samples exceeding these limits. These results are encouraging compared to those reported by Afif et al. (2008), who observed higher concentrations reaching 3.2×10^5 CFU/ml. This improvement could be attributed to better implementation of hygiene practices in dairy farms over time. Regarding fecal coliform counts, our study shows that all samples comply with the standards, which contrasts with more concerning results reported by Royster & Wagner (2015) and Aggad et al. (2009), who highlighted alarmingly high levels, indicating poor overall farm hygiene, particularly during milking (Farougou et al., 2011). This positive difference could be explained by several factors. The evolution of regulations and their stricter enforcement may have encouraged farmers to improve their practices. Additionally, increased awareness of the public health risks associated with poor milk hygiene likely played a role. As noted by Elmoslemany et al. (2009), ongoing training for farmers and the adoption of good hygiene practices are essential for improving milk microbiological quality. Furthermore, technological advances in milking and milk storage equipment may have contributed to this improvement. Pantoja et al. (2011) demonstrated that the use of modern, well-maintained equipment can significantly reduce microbial contamination in milk. Geographic and seasonal variations also need to be considered. O'Connell et al. (2015) highlighted that these factors can influence milk microbiological quality, so the differences observed between our study and previous ones could partly be attributed to these variations.

The positivity rate for Staphylococcus spp. in animals with positive CMT results is significant, reaching 63.04% of samples. This is similar to the 64% reported by Bentayeb et al. (2023) in Tizi-Ouzou, but much higher compared to the 16.96% observed in Bangladesh by Sohidullah et al. (2023). Among these cases, Staphylococcus aureus, either alone or in association with other species, accounted for 13.8% of samples, which is close to the 18% reported by Bentayeb et al. (2023). This high prevalence of S. aureus is particularly concerning, as this pathogen is recognized as a major cause of contagious mastitis, with a mammary reservoir (Ruegg, 2017). Transmission of S. aureus during milking can occur through several routes, including poorly maintained milking machines, inadequate hygiene practices during milking, or contaminated hands of the milkers (Donkor et al., 2007). In our study, the lack of a fixed milking order likely exacerbates the risk of pathogen spread, as noted by Stanek et al. (2024) in their review of risk factors for intramammary infections caused by S. aureus.

In addition to S. aureus, other Staphylococcus species were identified, which could contribute to the observed subclinical mastitis cases. While traditionally considered minor pathogens, recent studies, such as that by Jenkins et al. (2019), suggest that certain coagulase-negative Staphylococcus species can have a significant impact on udder health and milk production.

Pathogen associations, although less common than single-agent infections in subclinical mastitis, were observed in our study. These associations included S. aureus with S. micrococcus, S. intermedius, and S. xylosus. Such co-infections can complicate diagnosis and treatment, as demonstrated by Bonsaglia et al. (2017) in their study on the microbial diversity of intramammary infections. The complexity of these microbial associations emphasizes the need for a holistic approach to managing subclinical mastitis. As Ruegg (2018) pointed out, an effective control strategy must not only address milking hygiene practices but also the overall herd management, including cow environment and nutrition.

Regarding salmonella, the absence of detection in our microbiological encouraging, as Salmonella can pose a serious public health risk when present in raw milk (Mureithi et al., 2017). However, caution should be maintained, as Salmonella shedding in milk can be intermittent, as shown by Ait Kaki et al. (2019) in their study on the prevalence of pathogens in raw milk.

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Conclusion

This study offers a comprehensive analysis of the factors affecting bacterial contamination levels in cow milk, highlighting critical aspects of management and farming practices. The findings emphasize the importance of daily milk production as a major determinant of total coliform bacteria levels, with cows producing more than 30 liters per day exhibiting a significantly higher risk of contamination. This observation underscores the need to adjust management practices for high-production herds in order to minimize associated risks.

The lack of Salmonella in the samples is advantageous; nonetheless, the elevated incidence of subclinical mastitis is very alarming in specific farms. These often silent but problematic infections are strongly associated with increased levels of Staphylococcus sp. in the milk. The results show a notable prevalence of subclinical mastitis, suggesting an urgent need for improved milking and hygiene practices in these farms. Enhanced attention to the management of milking equipment, hygiene of facilities, and regular monitoring of cows is crucial to reduce these infections and improve milk quality.

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