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Research article

Intramammary bacterial profile and its impact on the number and differential distribution of somatic cells in the context of drying off in dairy cows

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Abstract: Mastitis remains one of the most significant conditions in dairy cattle farms, with major consequences for animal health and milk quality. The aim of this study was to determine the prevalence of intramammary infections (IMI) and to analyze the relationship between the pathogens involved, the total somatic cell count (SCC), and their differential distribution (DSCC) in two family-owned farms of Romanian Spotted cows, evaluated before and after drying-off. A total of 140 milk samples were collected and analyzed using standard microbiological examinations, as well as SCC and DSCC determinations by flow cytometry. The results revealed an overall IMI prevalence of 32.9%, with differences between the two farms: in Farm 1, major pathogens predominated (*Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*), whereas in Farm 2, minor pathogens were more frequently isolated (coagulase-negative staphylococci, *Corynebacterium* spp.). The mean SCC value was significantly higher in positive samples (807,557 cells/mL) compared to negative samples (98,243 cells/mL), with the highest values associated with *E. coli* infections. Similarly, DSCC showed marked differences between uninfected (26.2%) and positive samples (68.4%), with a tentative threshold of 65% suggested as indicative of intramammary infection. The results confirm the utility of combining SCC and DSCC for more accurate mastitis detection and highlight the importance of pathogen characterization at the farm level to optimize management strategies and apply selective therapy at drying-off.

Keywords: mastitis; intramammary infection; dairy cows; somatic cell count; differential somatic cell count;

1. Introduction

In the current context of dairy cattle farms, mastitis, defined as inflammation of the mammary gland, remains one of the most significant conditions, with direct effects on both animal health and milk quantity and quality [1]. Considering that the International Dairy Federation (IDF) indicates that the etiology of mastitis is most frequently associated with infectious agents [2], knowledge of the epidemiological situation on farms is essential for rapid diagnosis and informed decision-making regarding udder health management at both herd and individual levels [1]. In the scientific literature, pathogens involved in mastitis are commonly grouped into two major categories: contagious and environmental pathogens, each playing a distinct role in the disease dynamics and its management [3].

The main contagious pathogens include *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma bovis*, recognized for their ability to cause persistent and difficult-to-eradicate infections. Regarding environmental mastitis, the etiological spectrum is more diverse, with coliforms such as *Escherichia coli, Klebsiella spp*, and *Enterobacter spp*, environmental streptococci such as *Streptococcus uberis* and *Streptococcus dysgalactiae*, and non-aureus staphylococci (NAS) most frequently identified [4]. Most infections caused by these pathogens manifest as clinical mastitis, which is readily detectable on the farm through visible changes in the mammary gland or milk. However, a substantial proportion of cases remain undetected clinically, presenting as subclinical mastitis (SM) [5], which is primarily identified through elevated somatic cell counts (SCC) [2]. Milk SCC reflects the cellular population of the mammary gland and consists mainly of leukocytes,

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including macrophages, neutrophils, and lymphocytes, along with a small proportion of mammary epithelial cells, representing less than 7% of the total [6]. Under normal physiological conditions, the mammary gland maintains a low cell count, internationally considered healthy up to approximately 200,000 cells/mL [2]. Thus, the SCC in an individual cow's milk or bulk milk represents a reliable quantitative indicator of mammary gland inflammation [7,8] and is used worldwide for assessing udder health in lactating animals [9], as well as for guiding selective dry cow therapy (SDCT) [10]. Although several factors can influence SCC variation in lactating cows, such as age, parity, lactation stage, and season, the presence of infection remains the primary determinant of these fluctuations [11].

An essential aspect highlighted by previous research is the correlation between SCC variations and the type of pathogen involved in mastitis. Major pathogens, including *S. aureus*, *S. agalactiae*, coliforms, and other *Streptococcus species* (excluding *S. agalactiae*), are associated with the highest SCC elevations, whereas minor pathogens, such as *Corynebacterium bovis* and coagulase-negative staphylococci (CNS), generally produce only moderate increases in SCC [12–14]. Understanding this dynamic is crucial for developing realistic models to support decision-making regarding treatment and culling, ensuring economic efficiency and optimal udder health management. In recent years, to enhance the sensitivity and accuracy of SCC in identifying mammary inflammation, researchers have introduced an additional parameter for evaluating udder health: the differential somatic cell count (DSCC) [15]. Unlike SCC, which indicates the total cell count in milk, DSCC highlights the proportions of lymphocytes, macrophages, and polymorphonuclear cells directly involved in the defense mechanisms and inflammatory response of the mammary gland [16]. Studies have proposed DSCC thresholds between 56% and 72% [15,17], but have also shown that this parameter varies depending on the pathogen involved [18–20], the stage of mastitis, and parity [21]. Higher DSCC values have been observed in the early phases of intramammary infections (IMI) caused by major pathogens such as environmental streptococci, *S. aureus*, and coliforms [22].

However, the literature still provides limited information on the impact of mastitis on the dynamics of this indicator. Therefore, DSCC is recommended to be used in conjunction with SCC and other farm-level data, with thresholds adapted to specific farm conditions and management strategies [17]. The objectives of this study were to determine the prevalence of IMI caused by different microorganisms in two family-owned Romanian Spotted dairy farms during the drying-off period and immediately thereafter, and to analyze the relationships between microbial agents, total somatic cell counts, and their differential distribution. Obtaining this information aims to provide an epidemiological overview at the farm level, supporting informed decisions regarding udder treatments and management strategies during the drying-off period.

2. Materials and Methods

2.1. Description of the farms and animals included in the study

The study was conducted between November 2024 and June 2025 in two family-owned Romanian Spotted dairy farms located in Cluj County, Romania. Farm 1 included a herd of 63 lactating cows with an average milk production of 26.4 L/cow, while Farm 2 comprised 51 lactating cows with an average milk production of 20.1 L/cow. Both farms operated under similar management conditions. Cows were housed in a free-stall system, with individual resting areas and access to common feeding spaces. The feed ration was comparable between the two farms, consisting of corn silage, alfalfa hay, straw, and protein concentrates with mineral-vitamin supplements, administered as a Total Mixed Ration (TMR). Milking was performed twice daily, in the morning and evening, in parlors equipped with milk hygiene and monitoring systems. In both farms, cows were dried off gradually by progressively reducing the number of milkings, following standard dairy farm practices. Drying off was performed using Blanket Dry Cow Therapy (BDCT), whereby antibiotics were administered to all quarters of all cows, irrespective of their infection status. This standardized approach to housing, feeding, and milking ensured the comparability of data obtained from the herds.

2.2. Sampling protocols before and after drying-off

For this study, 68 cows approaching the dry period were selected, 34 from each farm. Milk samples were collected in the morning, before milking, and included milk from all four udder quarters. Sampling was performed at two distinct time points: 5–10 days before drying-off and 4–7 days after drying-off, except for four cases (two from each farm) that required a third sampling due to the development of clinical mastitis, resulting in a total of 140 milk samples. For each animal, two samples were collected at each time point: one for SCC and DSCC determination, and the other for microbiological analysis. The SCC/DSCC sample was collected using a milkometer, a standardized device that measures milk quantity and composition and

allows a representative sample by proportionally collecting milk from all udder quarters. These samples were placed in sterile tubes without preservatives and transported directly to the laboratory immediately after collection. Microbiological samples were collected under aseptic conditions following the protocol recommended by the National Mastitis Council [23]. Teat preparation included removing dirt and debris by brushing, performing forestripping, and disinfecting each teat with an iodine-based solution (KerbaWasch 2%, Kerbl, Germany) left for 30 seconds. Teats were then dried with individual paper towels. Immediately before sampling, the teat tip and barrel were wiped with sterile gauze soaked in 70% isopropyl alcohol. Samples were collected under hygienic conditions using disposable nitrile gloves. A few streams of milk were manually discarded from each quarter, after which 10–20 mL of milk were collected in sterile plastic containers for standard aerobic bacterial culture. All samples were properly labeled, and container numbers were correlated with each cow's identity. Samples were kept on ice and transported promptly to the laboratory for processing.

2.3. Determination of SCC and DSCC

SCC and DSCC were determined at the Milk Quality Control Foundation laboratory in Cluj-Napoca using the automated Fossomatic™ system (FOSS, Hillerød, Denmark), based on flow cytometry and in accordance with IDF standards. DSCC was determined using the method described by Damm et al. [15], which allows the identification, in a milk sample, of macrophages (MAC) and the combined population of polymorphonuclear leukocytes (PMN) and lymphocytes (LYM). DSCC is expressed as the combined proportion (%) of PMN and LYM relative to the total somatic cells in milk.

2.4. Bacterial Identification

Mastitis pathogens were identified at the Microbiology Laboratory of the Milk Quality Control Foundation, Cluj-Napoca. Milk samples were initially inoculated on chromogenic, selective, and differential media to isolate the main bacteria involved in mastitis: CPS (ChromID® CPS® Elite agar) for detection and differentiation of coliforms and enterococci, and Baird-Parker agar for selective isolation of *S. aureus*. Colonies with low growth or small size on CPS were subsequently transferred to Columbia Blood Agar, a nutrient-rich medium, to allow full bacterial development and observation of morphological characteristics and hemolysis. Plates were incubated aerobically at 37 °C for 18–24 h to obtain isolated colonies. Culture purity was verified through morphological inspection and Gram staining, and representative colonies were selected for identification. Growth of three or more bacterial types was considered a contaminated culture and excluded from analysis. From compliant cultures, bacterial suspensions were prepared in sterile saline (0.45–0.50% NaCl), adjusted to McFarland 0.5 standard (~1.5×10^8 CFU/mL) using the DensiCHEK system (bioMérieux, France). Standardized suspensions were inoculated into VITEK® 2 cards appropriate for Gram-positive or Gram-negative microorganisms and processed automatically in the VITEK® 2 Compact system (bioMérieux, France) according to manufacturer instructions and IDF bacterial identification standards.

2.5. Statistical Analyses

Statistical analyses were performed using Microsoft Excel**.** Descriptive statistics were applied to characterize SCC and DSCC in animals according to mastitis pathogens and infection status. Arithmetic mean, geometric mean, standard deviation, and median were calculated to describe data distribution and highlight variations between different groups.

3. Results

3.1. Microbiological examinations and infection prevalence

A total of 140 samples were analyzed through microbiological examinations, SCC, and DSCC determination. Of these, 94 samples (67.1%) showed no bacterial growth, while 46 samples (32.9%) were positive for at least one mastitis pathogen. Among all samples from animals, 4 (2.9%) were classified as contaminated during microbiological examination, according to the criteria of Harmon et al. [24]. From the milk samples with bacterial growth, the following bacteria were isolated: 21 samples (50%) with CNS, with the most frequent species being *S. chromogenes* (28.81%), *S. epidermidis* (19.05%), *S. simulans* (19.05%), and S. *xylosus* (14.29%), and other species including *S. haemolyticus, S. warneri*, and *S. sciuri.* Additionally, 7 samples (16.67%) were positive for *Streptococcus uberis*, 5 samples (11.90%) for *S. agalactiae*, 3 samples (7.14%) for *S. aureus*, 2 samples (4.76%) for *S. dysgalactiae*, 2 samples (4.76%) for *E. coli*, and 2 samples (4.76%) for *Corynebacterium spp*.

In Farm 1, out of 70 samples, 28 were positive, of which two were excluded due to contamination. From the remaining 26 positive samples (37.14%), 12 samples (46.15%) were infected with minor pathogens, represented by CNS, while 14 samples (53.85%) contained major pathogens, including *S. agalactiae, S. dysgalactiae, S. uberis*, and *E. coli*. Infection prevalence before drying off was 38.24% (13 positive samples out of 34 collected) and after calving was 36.11% (13 positive samples out of 36 collected). The mean SCC before drying off was 482,735 cells/mL, and the mean DSCC was 47.8%. After calving, the mean SCC was 384,176 cells/mL, while the mean DSCC was 42.5%.

In Farm 2, out of 70 samples, 18 were positive, of which two were excluded due to contamination. From the remaining 16 positive samples (22.86%), 11 samples (68.75%) were infected with minor pathogens, represented by CNS *and Corynebacterium spp*., and 5 samples (31.25%) with major pathogens, namely *S. aureus* and *S. uberis*. Infection prevalence before drying off was 29.41% (10 positive samples out of 34 collected), and after calving was 16.67% (6 positive samples out of 36 collected). The mean SCC before drying off was 268,485 cells/mL, and the mean DSCC was 33.88%. After calving, the mean SCC was 270,250 cells/mL, and the mean DSCC was 29.7%.

The results regarding the prevalence of pathogens identified in the two farms are summarized in Figure 1. Overall, total infection prevalence was higher in Farm 1 both before drying off and after calving, as reflected by the greater number of positive samples. In Farm 1, post-calving prevalence remained relatively constant, whereas in Farm 2 it decreased significantly. Moreover, the prevalence of major pathogens was higher in Farm 1 compared to Farm 2, while infections with minor pathogens predominated in Farm 2. CNS and *S. uberis* were commonly isolated from both herds. Additionally, both before and after drying off, the mean SCC and DSCC were higher in Farm 1 compared to Farm 2.

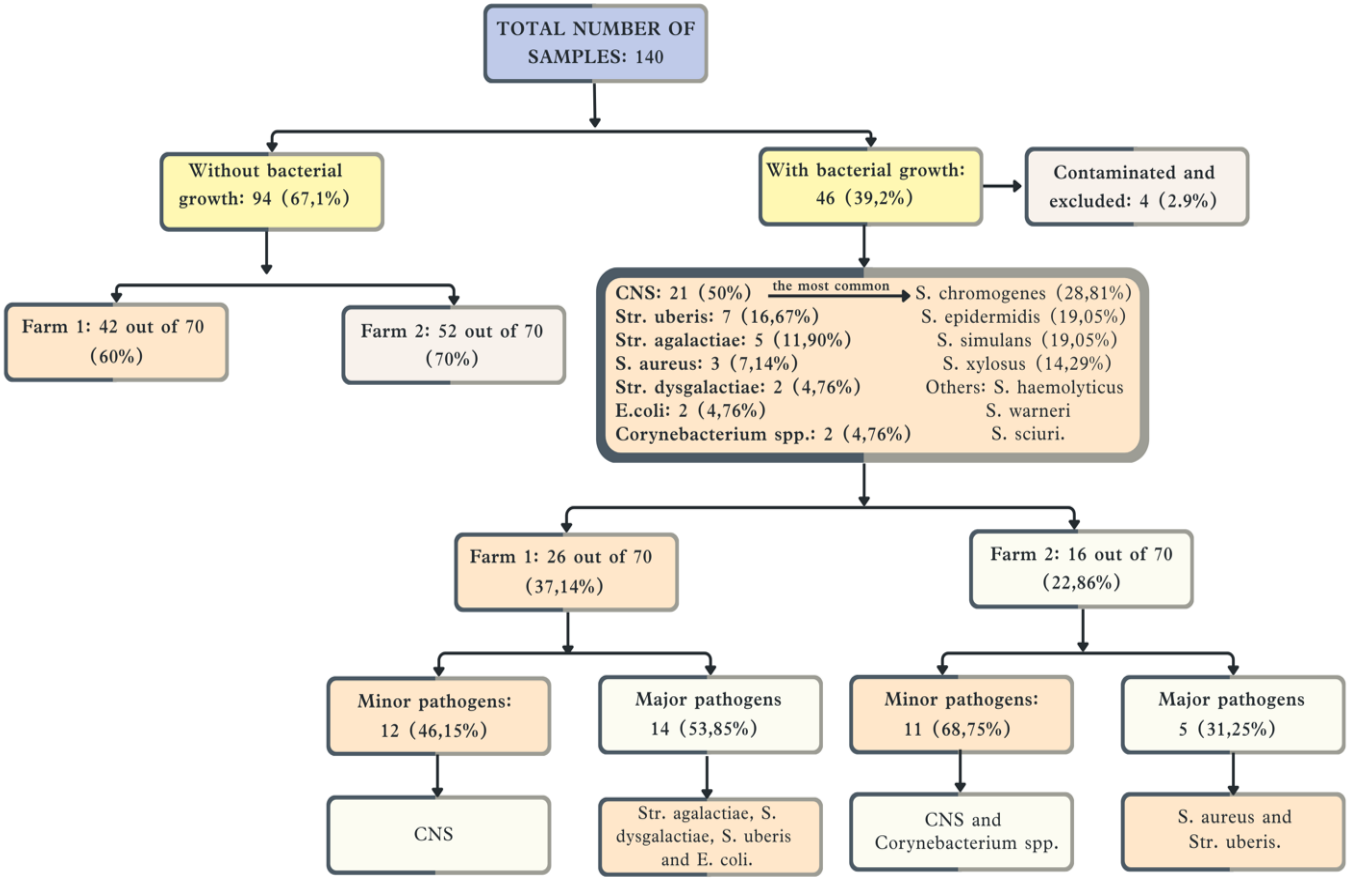

Figure 1. Prevalence of Mastitis Pathogens Identified in the Two Dairy Farms

3.2. Correlation between Bacterial Type, SCC, and DSCC

The analysis of SCC revealed significant differences between milk samples without and with bacterial infection. Samples without bacterial growth had a geometric mean SCC of 98,243 cells/mL, with a median value of 120,000 cells/mL. In samples with pathogen isolation, the mean SCC increased to 807,557 cells/mL,

with a median of 832,000 cells/mL. Notably, 23% of samples with SCC > 200,000 cells/mL showed no bacterial growth. Infections with minor pathogens were associated with a geometric mean of 423,179 cells/mL (median: 424,000), whereas infections with major pathogens exhibited much higher values (mean: 1,390,369 cells/mL; median: 1,154,000), indicating a more intense inflammatory response.

Analysis of the differential somatic cell count (DSCC) also confirmed the association with infection. Samples without bacterial growth had a mean DSCC of 26.2%, while samples with pathogens showed substantially higher values, with a mean of 68.4%. Infections with minor pathogens had a mean DSCC of 65.6%, and infections caused by major pathogens had mean values of 74.4%, highlighting the intensity of the inflammatory response according to the type of etiological agent. Considering these differences, a tentative threshold of 65% for DSCC can be suggested to indicate the presence of intramammary infection, with values above this threshold reflecting a significant inflammatory response.

At the level of specific pathogens, infections with coagulase-negative *Staphylococcus spp.* (STACN) had lower mean values (SCC – 392,520 cells/mL, median: 372,000; DSCC – 65.4%), while infections with *Corynebacterium spp*. (CORIN) were slightly higher (SCC – 815,000 cells/mL, median: 814,823; DSCC – 67.1%). Infections with *Streptococcus spp.* other than *S. agalactiae* (STREP), *S. agalactiae* (STRAG), and *S. aureus* (STPHA) generated intermediate mean values, with SCC ranging from 1,041,552 to 1,490,496 cells/mL and DSCC between 73.2% and 74.6%. The highest values were observed in infections with *E. coli* (SCC – 2,560,163 cells/mL; DSCC – 80.7%), reflecting the particularly intense inflammatory response to this pathogen. Arithmetic and geometric means, standard deviations and medians of SCC and DSCC according to bacteriological results are presented in Table 1 and Table 2.

Table 1. Variation of SCC (cells/mL) according to the presence of intramammary infection and the type of etiological agent.

SV	Category	N	AM	SD	GM	Median
Presence of infection	NO	94	129.989	92.569	98.243	120.000
	YES	42	1.066.116	769.087	807.557	832.000
Etiological agent type	Min. p	23	463.435	198.440	423.179	424.000
	Maj. p	19	1.511.737	675.311	1.390.369	1.154.000
Etiological agent	STACN	21	425.190	174.029	392.520	372.000
	STPHA	3	1.687.000	1.079.417	1.490.496	1.132.000
	STRAG	5	1.678.800	559.469	1.608.703	1.374.000
	STREP	9	1.063.111	239.626	1.041.552	972.000
	CORIN	2	815.000	24.042	814.823	815.000
	ECOLI	2	2.562.000	137.179	2.560.163	2.562.000

SV – source of variation; N – number of samples; AM – arithmetic mean; SD – standard deviation; GM – geometric mean; Min. p – minor pathogens; Maj. p – major pathogens; STACN – coagulase-negative Staphylococcus spp.; STPHA – *S. aureus*; STRAG – *S. agalactiae*; STREP – *Streptococcus* spp. other than *S. agalactiae*; CORIN – *Corynebacterium* spp.; ECOLI – *E. coli*.

Table 2. Variation of DSCC (%) according to the presence of intramammary infection and the type of etiological agent.

SV	Category	N	AM	SD	GM
Presence of infection	NO	94	26,2	19,5	30,1
	YES	42	68,4	9,7	67,9
Etiological agent type	Min. p	23	65,6	6,4	65,4
	Maj. p	19	74,4	4,6	73,4
Etiological agent	STACN	21	65,4	6,8	65,3
	STPHA	3	74,6	7,4	78
	STRAG	5	73,4	3,0	73,4
	STREP	9	73,2	3,9	71,3
	CORIN	2	67,1	1,1	67,1

ECOLI	2	80,7	3,2	80,7

SV – source of variation; N – number of samples; AM – arithmetic mean; SD – standard deviation; Min. p – minor pathogens; Maj. p – major pathogens; STACN – coagulase-negative *Staphylococcus* spp.; STPHA – *S. aureus*; STRAG – *S. agalactiae*; STREP – *Streptococcus* spp. other than *S. agalactiae*; CORIN – *Corynebacterium* spp.; ECOLI – *E. coli*.

4. Discussion

The results obtained in our study indicate that the overall prevalence of IMI was 32.9%. The isolated bacteria were identified as *S. agalactiae*, *S. uberis*, *S. dysgalactiae*, *E. coli*, *S. aureus*, CNS, and *Corynebacterium* spp., with findings similar to those reported by other authors [14,25]. Over 80% of IMI in this study were caused by *Streptococcus spp.*, *S. aureus*, and CNS, which contrasts with more recent studies showing a decrease in IMI caused by *S. aureus* and an increase in the prevalence of Gram-negative pathogens [26]. In Farm 1, prevalence was higher both before drying off (38.24%) and after calving (36.11%) compared to Farm 2, where values were 29.41% and 16.67%, respectively. These data suggest a possible influence of dry period management and hygiene conditions on mastitis incidence. Similar to observations reported by Haw et al. [27] and Emidio et al. [28], where "no significant growth" was the most frequent result of milk cultures (75.2% and 66.0%, respectively), in our study a considerable proportion of samples (67.1%) showed no bacterial growth. These finding highlights that many inflammatory changes in the mammary gland may be associated with non-bacterial factors or transient infections, which are difficult to detect using conventional bacteriological culture.

Regarding the distribution of etiological agents, CNS were the most frequently isolated microorganisms (50% of positive samples), followed by *S. uberis* and other major pathogens such as *S. aureus*, *S. agalactiae*, and *S. dysgalactiae*. These results are consistent with recent literature reporting non-aureus staphylococci as the most commonly identified agents involved in subclinical mastitis [27,29,30]. The predominant species in our study, *S. chromogenes*, *S. epidermidis*, and *S. simulans*, are also among the most frequently reported internationally [27,31,32]. Moreover, *S. chromogenes* and *S. simulans* have been cited in multiple studies as being associated with persistent infections and chronic subclinical mastitis [32,33], underlining the clinical relevance of their frequent isolation in the samples analyzed in our study.

Certain differences between the two farms were observed regarding pathogen distribution. In Farm 1, major pathogens predominated (53.85%), whereas in Farm 2, the largest proportion corresponded to minor pathogens (68.75%). These results suggest that environmental contamination control may be insufficient in Farm 2, while in Farm 1, cow-to-cow transmission appears to be facilitated by improper milking parlor management. Similar situations were described by Nunes De Souza et al. [14], where the distribution of infections with *S. agalactiae*, *S. aureus*, and environmental streptococci varied depending on the farm and local management practices.

Our study results highlight significant differences in SCC depending on the presence and type of pathogens. Uninfected samples showed a geometric mean of 98,243 cells/mL (median: 120,000), whereas positive samples had much higher values (mean: 807,557 cells/mL; median: 832,000), confirming the role of mammary infections in increasing SCC. SCC values in uninfected quarters generally fall within the ranges reported by Dohoo et al. [34] (113,000–251,000 cells/mL) and are comparable to Emídio et al. [28], who reported a geometric mean of 52,000 cells/mL and a median of 70,000 cells/mL, as well as the meta-analysis by Djabri et al. [35], which indicated a mean of 68,000 cells/mL, suggesting consistency and practical relevance. Regarding positive samples, the mean SCC in our study exceeds the ranges reported by Dohoo et al. [34] (190,000–519,000 cells/mL) and Nunes de Souza et al. [14] (mean 228,000 cells/mL; median 342,000), which may be explained by methodological variability, herd management, or factors related to physiological status and stress. All studies, however, confirm a clear increase in SCC in the presence of pathogens, highlighting the consistency of the mammary gland's immune response. It should be noted that in both our study and Petzer et al. [36], over 20% and 30% of samples with elevated SCC were culture-negative, emphasizing the importance of using multiple diagnostic methods for IMI.

Beyond general differences between positive and negative samples, our analysis revealed a clear separation of SCC values according to pathogen type. Infections with minor pathogens were associated with a geometric mean of 423,179 cells/mL (median: 424,000), whereas infections with major pathogens resulted in significantly higher values (mean: 1,390,369 cells/mL; median: 1,154,000), indicating a stronger inflammatory

response. Previous studies [11,34] have shown that when bacteria are classified into major pathogens (streptococci, *S. aureus*, and coliforms such as *E. coli* and *Klebsiella spp*.) and minor pathogens (coryneforms and CNS), quarters infected with major pathogens had SCC > 600,000 cells/mL on average, while minor pathogens yielded SCC between 100,000 and 300,000 cells/mL. Our results indicate even higher levels, suggesting either greater infection severity in the studied herd or differences in herd management or detection sensitivity. Similarly, Eberhart et al. [37] reported SCC increases from 190,000 to 320,000 cells/mL for minor pathogens and from 614,000 to 986,000 cells/mL for major pathogens. A relevant methodological difference between studies lies in sample type: composite milk versus milk from individual quarters. In both our study and Eberhart et al. [37], evaluations were performed on composite samples, whereas Harmon [11,34] and Dohoo et al. [34] used individual quarter samples.

Analysis of SCC by pathogen showed clear species differences: the highest geometric mean and median SCC values were recorded for *E. coli* (2,560,163 and 2,562,000 cells/mL), followed by *S. agalactiae* (1,608,703 and 1,374,000 cells/mL), *S. aureus* (1,490,496 and 1,132,000 cells/mL), and other streptococci (1,041,552 and 972,000 cells/mL). The lowest values were recorded for *Corynebacterium spp.* (815,000 and 814,823 cells/mL) and CNS (392,520 and 372,000 cells/mL). Overall, the arithmetic mean of SCC increased progressively in the order: no bacterial growth, CNS, *Corynebacterium spp*., other streptococci, *S. aureus*, *S. agalactiae*, and *E. coli.* CNS infections showed the lowest SCC, slightly above Emídio et al. [28] values (mean 138,000 cells/mL) but close to Nunes de Souza et al. [14] (mean 400,000 cells/mL; median ≤ 205,000). For *Corynebacterium* spp., the values were significantly higher compared to literature data. Nunes de Souza et al. [14] reported a mean of approximately 400,000 cells/mL (median ≤ 166,000), and Wilson et al. [25] confirmed similar values. These differences highlight the variability of the inflammatory response, likely influenced by management factors or specific characteristics of the strains involved. In the case of *Streptococcus spp.,* infections with *S. agalactiae* in our study generated a mean very close to the values reported by Djabri et al. [35] (1,572,000 cells/mL). The results are also consistent with Nunes de Souza et al. [14], who showed that *S. agalactiae* consistently produces the highest SCC values, with means of 662,000 cells/mL and medians ≥ 923,000. Wilson et al. [25] confirmed this trend, highlighting the same pathogen as responsible for the most significant increases in SCC.

For *Streptococcus spp*. other than *S. agalactiae* (e.g., *S. uberis*, *S. dysgalactiae*), our values are comparable to those reported by Emídio et al. [28], who found 1,024,000 and 547,000 cells/mL, respectively, but higher than those mentioned by Nunes de Souza et al. [14], where means were around 800,000 cells/mL, with medians ≥ 641,000. Regarding *S. aureus*, the SCC values observed in our study significantly exceed the means reported by Emídio et al. [28] (357,000 cells/mL) and Djabri et al. [35] (587,000 cells/mL) but are close to those in the study by Nunes de Souza et al. [14] (966,000 cells/mL, median ≥ 509,000). This variation between studies may be partly explained by the intermittent shedding of the bacterium in milk, a characteristic of *S. aureus*, which influences SCC levels [38] and can lead to differences in reported means. The results also confirm *S. aureus* as one of the pathogens most frequently associated with high SCC values. The highest values in our study were associated with *E. coli* (2,560,163 cells/mL), confirming the intensity of the inflammatory response to Gram-negative pathogens. Djabri et al. [35] and Harmon [11] also reported that *E. coli* causes the highest SCC levels, typically in the context of clinical mastitis, which aligns with our observations. Overall, comparison of our results with the literature reveals the same general trends: CNS and *Corynebacterium* spp. result in low to moderate SCC values, *S. aureus* and streptococci are associated with intermediate to high values, and Gram-negative pathogens, especially *E. coli*, induce the highest SCC levels.

Additionally, the DSCC analysis conducted in our study confirmed its correlation with the presence of IMI. Samples with no bacterial growth recorded a mean DSCC of 26.2%, while positive samples showed significantly higher values, with a mean of 68.4%. Infections caused by minor pathogens were associated with a mean DSCC of 65.6%, whereas those caused by major pathogens showed a mean of 74.4%, highlighting the differentiated intensity of the inflammatory response depending on the type of etiological agent. To the best of our knowledge, the dynamics of DSCC in relation to pathogen type are very poorly described in the literature, which limits the interpretation of results at a longitudinal level. Nevertheless, our findings are consistent with the observations of Schwarz et al. [20], who reported that most cases of IMI caused by major pathogens showed high DSCC values (>60%). In contrast, in IMI caused by minor pathogens, DSCC varied considerably (14–89%), a variation attributed mainly to the different levels of pathogenicity of NAS [39]. The significantly higher DSCC values in cows with IMI caused by major pathogens can be explained by the fact that this parameter primarily reflects the proportion of PMN [15], immune cells known to be dominant in the presence of major pathogens [16]. Conversely, Kirkeby et al. [21] demonstrated that DSCC appears to be

a useful indicator for the presence of any pathogen, but less specific for differentiating between pathogen groups.

Based on the differences observed in this study, our results suggest a preliminary threshold of 65% for DSCC, with values above this level indicating a significant inflammatory response and, consequently, the presence of intramammary infection. This threshold aligns with values proposed in the scientific literature, where DSCC between 65% and 72% has been suggested as an indicative cut-off for the detection of subclinical mastitis [15]. Furthermore, Schwarz et al. [20] reported that at an SCC of 200,000 cells/mL, a DSCC > 60% may signal mammary gland infection (sensitivity 67–87%), while Zecconi et al. [40] identified DSCC thresholds differentiated by days in milk (66.3% for <100 DIM, 69.2% for 101–200 DIM, and 69.3% for >200 DIM), with an accuracy of 81% and sensitivity of 67%. Our tentative threshold of 65% falls within the range proposed by these studies, confirming overall agreement while also emphasizing the need to adjust reference values according to the specific context of each herd and local management conditions. Recent studies highlight that DSCC, in combination with SCC, can identify more IMI cases compared to SCC alone, suggesting its high potential for widespread use in udder health monitoring [20,40,41]. This approach can be particularly useful in selective dry cow therapy (SDCT), for the accurate identification of animals requiring treatment. It should be noted that, given the relatively small number of samples analyzed in our study, the 65% threshold cannot be considered definitive and applies strictly within the context of this research. In practice, DSCC values should be interpreted alongside other clinical and laboratory criteria to accurately assess the health status of dairy cows.

The main limitation of this study is the relatively small number of samples and farms included, which may affect the generalizability of the results. Additionally, sampling was conducted at relatively infrequent intervals, which may not fully capture the rapid dynamics of DSCC and SCC changes during the course of infection. The lack of detailed differentiation by lactation stage and parity represents another limitation, given the known impact of these factors on the inflammatory response. For future research, studies involving larger herds and more frequent sampling are needed to better capture the variability and progression of DSCC over time. Integrating DSCC with other parameters (SCC, milk yield, lactation stage) into complex predictive models could improve the diagnosis and management of mastitis**.** Furthermore, validating DSCC thresholds according to each farm's context and the characteristics of the pathogens involved will be essential for practical applicability.

5. Conclusions

The main bacteria identified in this study included both major pathogens (*E. coli*, *S. agalactiae*, *S. uberis*, *S. dysgalactiae*, and *S. aureus*) and minor pathogens (CNS, *Corynebacterium* spp.), each exerting a different influence on the inflammatory response (as reflected by SCC and DSCC) and milk quality. Their distribution varied between farms, with higher prevalence observed on Farm 1, both before dry-off and after calving. This highlights the importance of proper dry period management and minimizing cow-to-cow transmission, along with careful monitoring of udder health through SCC and DSCC, key tools for the prevention and control of mastitis.

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