

ORIGINAL ARTICLE

Dynamics of somatic cell count and intramammary infection in lactating dairy cows

S. M. Mostafizur Rahaman Sumon^{1,2}, Mst. Sonia Parvin¹, Md. Amimul Ehsan¹, Md. Taohidul Islam¹

¹Population Medicine and AMR Laboratory, Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

²Department of Medicine, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

ABSTRACT

Objective: The influence of intramammary infection (IMI) and types of bacteria was assessed on somatic cell count (SCC) in dairy cows' milk with respect to breed, age, parity, stage of lactation, milk production, and mammary quarter location.

Materials and methods: After recording data in a structured questionnaire, 360 samples of quarter milk were collected. The samples were subjected to SCC and isolation and identification of bacteria. The data were analyzed to find out the significant influence of independent factors on SCC and IMI.

Results: The infected quarters had a significantly higher mean SCC (210.52×10^3 cells/ml) compared to uninfected ones (32.72×10^3 cells/ml). The mean SCC was the highest for IMI with *Enterobacter* spp. (338.00×10^3 cells/ml) followed by *Bacillus* spp. (319.20×10^3 cells/ml), coagulase-negative *Staphylococci* (CNS) (268.17×10^3 cells/ml), *Staphylococcus aureus* (218.31×10^3 cells/ml), and *Escherichia coli* (200.75×10^3 cells/ml) and the lowest for *Pseudomonas aeruginosa* (66.33×10^3 cells/ml). Milk of rear quarters had a significantly higher SCC than the front quarters. SCC increased with increasing age, parity, and lactation stage regardless of whether cows are infected or not. The IMI was more prevalent in rear quarters (42.2%) and cows at early (≤ 7 days) lactation (100.0%). Cows having a parity of ≥ 5 and crossbred and high yielding (> 5 l) cows had also a higher rate of IMI of 38.2%, 36.7%, and 38.2%, respectively.

Conclusion: The IMI and type of bacteria were the principal factors for SCC variation. Besides, mammary quarter location, age, and parity should be taken into consideration during the interpretation of SCC.

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Introduction

Somatic cell count (SCC) in individual cow's milk or bulk milk is routinely used to assess the status of subclinical mastitis (SCM) in dairy cow herds and recognized as the standard test for the quality of milk, worldwide. Usually, if the SCC is below 100,000 cells/ml of milk, then the quarter is considered healthy, i.e., free from SCM [1]. However, practically, to reduce the diagnostic error under field conditions, a quarter is defined as healthy when SCC is below 200,000 cells/ml of milk [2,3]. SCM is most often due to a bacterial intramammary infection (IMI), so in most of the cases, the words IMI and SCM are used indifferently

[4,5]. Thus, the term IMI explicitly refers to the presence of bacteria in the quarter [6,7]. The increased SCC in milk (including 75% of leukocytes and 25% of epithelial cells) is indicative of IMI, but also the types of bacteria could affect the SCC in milk [2,8]. The major pathogens typically cause a high increase in SCC, and an infection with minor pathogens normally leads to a slight increase in SCC and rarely to clinical mastitis [2,9]. The severity of udder inflammation is positively correlated with the amount of pathogens that shed from the infected quarter [10].

It has also been reported that diverse major or minor pathogens are responsible for the modest increase of

Correspondence Md. Taohidul Islam ✉ taohid@bau.edu.bd ☒ Population Medicine and AMR Laboratory, Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

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around 50,000 cells/ml of milk [11]. The degree of SCC elevation due to major pathogens varies among cows, but the distinction of the types of pathogens based on SCC alone seems impossible [11]. In certain cases, the elevated SCC is found with no growth of pathogens in milk samples, and it does not necessarily mean that the mammary quarter is healthy [12].

Therefore, many factors can affect SCC such as age, lactation, parity, seasons, stress, management practices, and daily variation [8,13]. The pathogens present in the quarter, however, is usually considered to be the main factor associated with the elevation of SCC in milk [14,15]. Thus, the culture of milk samples is important to diagnose mastitis accurately even though it was doubtful that SCC profiles indicated mastitis [16]. SCM should, therefore, be identified in a reliable and timely manner, based on the findings of SCC or culture; otherwise, SCM may become a clinical disease [17]. Despite a greater variation in SCC in dairy cows, scarce information is available on factors that may influence SCC in dairy cows submitted to a tropical environment such as Bangladesh, where the cows are mainly kept semi-intensive, as compared to the currently available data for cows maintained on temperate conditions. In light of these facts, the objective of this research was to identify the impact of IMI, types of bacterial species, and factors other than IMI on SCC in lactating cows.

Materials and Methods

Ethical statement

The milk samples were collected from the animals by following the Animal Welfare Guidelines without doing any harm to the animals. The data from the farms were collected with the prior written consent from the interviewers.

Study design and sampling

A cross-sectional study was employed to collect the data from 90 apparently healthy lactating cows of Bangladesh Agricultural University Dairy Farm and surrounding areas. The cows were randomly selected, and 360 quarter milk samples were collected aseptically into a separate sterile screw-capped sample tube and transported to the laboratory as described previously [15]. Before the collection of milk samples, the teat was cleaned and wiped with cotton soaked with 70% ethyl alcohol, and the first 2–3 streams of milk were discarded.

Data recording

The data were collected through face-to-face interviews of farm owners as well as from the farm records (if available) by using a pre-tested structured questionnaire. The data about breed, age, parity, lactation, average milk production, prior treatment with antibiotics, and management

system were recorded. The sampling did not include cows receiving treatment with antibiotics or having a history of prior treatment with an antibiotic. The sampled cows were indigenous (zebu) and/or crossbred. Cows were grouped into three categories based on age such as ≤ 4 , $>4-8$, and >8 years. Parity was grouped as 1–2, 3–4, and ≥ 5 . The lactation stage was categorized as ≤ 7 , $>7-60$, and >60 days. Milk yield was categorized as ≤ 3 , $>3-5$, and >5 l per cow. The informed consent was obtained from all the farm owners/managers before collecting data and milk samples.

Bacteriological examination of samples

All the collected samples were analyzed bacteriologically independent of SCC. About 100 μ l of milk sample was cultured into the nutrient broth (HiMedia, Mumbai, India) and incubated overnight aerobically at 37°C. The pure colony was obtained through culture and subculture onto various agar such as nutrient agar (HiMedia, Mumbai, India), blood agar (Merck, Darmstadt, Germany), mannitol salt agar (Merck, Darmstadt, Germany), MacConkey agar (Merck, Darmstadt, Germany), eosin-methylene blue agar (HiMedia, Mumbai, India), and triple sugar iron agar (HiMedia, Mumbai, India). The cultural and Gram stain properties along with the results of various biochemical tests such as catalase, coagulase, oxidase, indole, Methyl Red-Voges Proskauer, and basic sugar fermentation were interpreted to identify the bacteria as described earlier [18]. A quarter was identified as positive for IMI if the same pathogen was recovered from the duplicate culture of samples, and a cow was diagnosed as infected if at least one quarter was identified as positive for IMI [19]. Bacterial pathogens were grouped into four categories as described earlier: (i) major pathogens, comprising of *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter* spp., (ii) minor pathogens, comprising of *coagulase-negative Staphylococci*, (iii) uncommon pathogens, comprising of *Bacillus* spp., *Pseudomonas aeruginosa*, and unidentified, and (iv) mixed pathogens [15].

Somatic cell count

All the milk samples were subjected to SCC by using NucleoCounter® SCC-100™ (ChemoMetec). Milk samples were prediluted with the supplied reagent and then loaded into SCC-Cassette. Reading was recorded by placing the cassette into the instrument. All the procedures were followed as per the manufacturer's instructions.

Statistical analysis

The descriptive statistics, *Chi-square* test, Student's *t*-test, and one-way analysis of variance (wherever appropriate) were performed to find out the significant effect of independent factors on IMI and SCC. All the analyses were performed by using the Statistical Package for the Social

Sciences (SPSS 20.0), and the level of significance was set at $p \leq 0.05$.

Results and Discussion

Effect of IMI and types of bacterial species on SCC during the lactation period

The overall prevalence of IMI in the examined samples was 35.6% (Table 1). The presence of IMI and the type of microorganisms involved were the principal factors responsible for SCC variation. The infected quarters had a higher mean SCC ($p < 0.001$) compared with uninfected ones. Mean SCC was the highest for *Enterobacter* spp., followed by *Bacillus* spp., coagulase-negative *Staphylococci* (CNS), *S. aureus*, *E. coli*, unknown organisms, and *P. aeruginosa*. No significant variation in SCC was observed between major and minor pathogens causing IMI (Table 2). During infection, a number of somatic cells are increased as the body's and udder's

immune system activated to combat infection and play a role in repairing impaired udder tissues, and for this reason, the infected quarters might have a higher SCC. These findings support the report of a previous study [20]. The difference in SCC caused by various mastitic pathogens in this study may be due to the variation in cow's disposition of protective mechanism and the process of establishment of infection by the pathogen [15].

Factors influencing SCC other than IMI and on the pattern of IMI

The rear quarters had a higher SCC and IMI than the front quarters. The location of the mammary quarter (rear vs. front) significantly ($p = 0.008$) influences the occurrence of IMI in dairy cows (Table 3). Intramammary infection induces cow's immune response, and consequently, SCC increases; thereby a higher prevalence of infection occurred in hindquarters. The rear quarters are comparatively

Table 1. Mean SCC of mammary quarters according to the species of microorganisms as the cause of IMI in dairy cows.

Pathogen isolated	No. of quarters	% of total quarters	% of culture positive	SCC ($\times 10^3$ cells/ml) (Mean \pm SEM)	Level of significance
Quarters analyzed bacteriologically	360				
Culture negative	232	64.4		32.72 \pm 6.56	
Culture positive	128	35.6		210.52 \pm 27.75	$p < 0.001$
Single infections					
Major pathogens					
<i>S. aureus</i>	26	7.2	20.3	218.31 \pm 56.81	
<i>E. coli</i>	8	2.2	6.2	200.75 \pm 86.73	$p < 0.001$
<i>Enterobacter</i> spp.	8	2.2	6.2	338.00 \pm 96.80	
Minor pathogen					
Coagulase-negative <i>Staphylococci</i> (CNS)	22	6.1	17.2	267.73 \pm 121.42	$p < 0.001$
Others					
<i>Bacillus</i> spp.	10	2.8	7.8	319.20 \pm 126.37	
<i>P. aeruginosa</i>	6	1.7	4.7	66.33 \pm 19.81	$p < 0.001$
Unidentified	16	4.4	12.5	115.25 \pm 17.05	
Mixed infections					
<i>S. aureus</i> and <i>E. coli</i>	6	1.7	4.7	118.00 \pm 17.63	
<i>S. aureus</i> and <i>Enterobacter</i> spp.	4	1.1	3.1	176.00 \pm 72.17	
<i>S. aureus</i> and <i>P. aeruginosa</i>	2	0.6	1.6	202.00 \pm 0.00	
<i>S. aureus</i> and unidentified	2	0.6	1.6	61.00 \pm 0.00	
<i>E. coli</i> and CNS	2	0.6	1.6	273.000 \pm 0.00	
<i>E. coli</i> and <i>Bacillus</i> spp.	2	0.6	1.6	138.00 \pm 0.00	$p < 0.001$
<i>E. coli</i> and unidentified	2	0.6	1.6	206.00 \pm 0.00	
<i>Enterobacter</i> spp. and <i>Bacillus</i> spp.	2	0.6	1.6	84.00 \pm 0.00	
<i>Enterobacter</i> spp. and unidentified	2	0.6	1.6	302.00 \pm 0.00	
<i>Bacillus</i> spp. and <i>Pseudomonas</i> spp.	4	1.1	3.1	400.50 \pm 45.89	
<i>Bacillus</i> spp. and unidentified	4	1.1	3.1	22.50 \pm 2.59	

Table 2. Mean SCC of mammary quarters according to the pathogen groups of microorganisms causing IMI in dairy cows.

Intramammary infections	No. of IMI positive quarters (%)	Level of Significance	SCC ($\times 10^3$ cells/ml) (Mean \pm SEM)	Level of significance
Major pathogens	42 (32.8)	$p < 0.001$	237.76 \pm 42.737	$p > 0.05$
Minor pathogens	22 (17.2)		267.73 \pm 121.42	
Others	32 (25.0)		169.81 \pm 43.23	
Mixed pathogens	32 (25.0)		176.13 \pm 22.75	

Table 3. Mean values of SCC and IMI pattern by mammary quarter location, breed, age, parity, lactation stage, and milk yield of dairy cows.

Factors	No. of quarters	SCC ($\times 10^3$ cells/ml) (Mean \pm SEM)	Level of significance	No. of IMI positive quarters (%)	Level of significance
Front right	90	71.51 \pm 12.11	$p = 0.161$	26 (28.9)	$p = 0.072$
Front left	90	76.20 \pm 19.44		26 (28.9)	
Rear right	90	97.78 \pm 21.81		38 (42.2)	
Rear left	90	138.27 \pm 33.83		38 (42.2)	
Front quarters	180	73.86 \pm 11.42	$p = 0.057$	52 (28.9)	$p = 0.008$
Rear quarters	180	118.02 \pm 20.13		76 (42.2)	
Breed			$p = 0.533$		$p = 0.506$
Cross	240	97.51 \pm 11.20		88 (36.7)	
Indigenous	120	92.80 \pm 26.76		40 (33.3)	
Age (Years)			$p = 0.007$		$p = 0.699$
≤ 4	104	45.59 \pm 6.73		40 (38.5)	
$> 4-8$	176	102.44 \pm 13.64		62 (35.2)	
> 8	80	147.08 \pm 41.22		26 (32.5)	
Parity			$p = 0.004$		$p = 0.174$
1-2	200	68.76 \pm 9.65		64 (32.0)	
3-4	112	107.32 \pm 19.36		42 (37.5)	
≥ 5	48	182.63 \pm 61.54		22 (45.8)	
Lactation stage (days)			$p = 0.972$		$p = 0.001$
≤ 7	8	77.75 \pm 6.56		8 (100.0)	
$> 7-60$	128	95.64 \pm 23.73		46 (35.9)	
> 60	224	96.76 \pm 12.87		74 (33.0)	
Milk yield (L)			$p = 0.847$		$p = 0.644$
≤ 3	128	92.42 \pm 25.12		42 (32.8)	
$> 3-5$	80	87.25 \pm 21.29		28 (35.0)	
> 5	152	103.47 \pm 13.67		58 (38.2)	

more vulnerable to infections because of their conformation (usually larger), easy exposure to physical injuries, and other environmental factors. Furthermore, teats of hindquarters remain close to the floor, particularly in old-aged cows, and thus, they are most likely to be infected or injured [15,21,22]. Other earlier studies also observed a higher SCC in the milk of rear quarters than front quarters, but they did not find any marked difference between the

prevalence of IMI in the front and rear quarters [20,23]. However, the authors could not explain the quarter-wise prevalence of IMI.

Regarding breed, the crossbred cows had a higher SCC than indigenous ones. Crossbred cows were also more leaning to IMI than indigenous cows; however, the difference was not statistically significant (Table 3). This breed-wise difference in SCC may be due to the variation in protective

mechanism induced by the host to IMI, physiological factors, and stress to the high yielding crossbred cows as a result of the lack of adaptation to the environment. Similar to this finding, the earlier study also documented the higher SCC in the milk of high-yielding cows [11].

Mean SCC increased with increasing age, parity, and stage of lactation irrespective of IMI. Older (>8 years) cows and cows with many parities (5 or more parity) had significantly ($p = 0.007$ and 0.004) higher SCC than younger (≤ 4 years) cows with fewer parities (1–2 parity). An increased SCC with the advancement of age and parities also reported by various researchers [24–26]. Although Singh and Ludri [27] found no significant effect of parity on SCC between the first and sixth lactations, Kline et al. [13] reported that there was no significant correlation between an increased age of cows and higher SCC in milk. However, no significant variation of IMI with age and parity was observed in the findings though IMI was more prevalent (45.8%) in cows with many parities (five or more parity) than others. The stage of lactation had no significant effect on SCC, but early (≤ 7 days) lactating cows had significantly ($p = 0.001$) higher rate (100%) of IMI. An increased SCC in late lactation (>60 days) found in this study supports the previous observation [28,29]. They observed that SCC increases with the advancement of lactation. Somatic cell count elevation may be due to the cows' inherent response modulation to calving to boost up the udder's protective mechanism during the late pre-parturient transition period [11]. Similar to the findings, Fadlelmula et al. [30] reported the highest occurrence of mastitis (62.7%) during early lactation compared to late lactation (11.2%). Sharma et al. [11] reported that cows in late lactation may experience less oxidative stress and strong antioxidant defense compared to cows in the early lactation, and thereby, cows during the early lactation become more vulnerable to mastitis as well as other production diseases.

Considering milk production, high milk yielding (>5 l) cows had the highest SCC and were more vulnerable to IMI though they were not significant. The high yielding cows commonly experience more stress for the production of more milk, and they have a low level of immunity, which results in high SCC in milk [31]. In Bangladesh, 6.48 l/day average milk production is usually seen in crossbred dairy cows [32]. Crossbred dairy cows lean toward the production of more milk, but their milk normally has a high count of somatic cells. Upgrading dairy cows with high Holstein fraction make them disapproving with regard to resistance against mastitis and maintaining optimal health status [33]. For this region, farmers should select crossbred cows that are suitable for the prevailing environmental context. Alongside this, the utmost priority should be given in maintaining good farm practices to reduce the infection pressure and SCC in milk with the ultimate target to control mastitis for competitive profitable dairying. A limitation

of the study was the identification of organisms based on the cultural and biochemical properties only. It would be worthwhile if we could do molecular identification of the organisms. However, the identification was done very carefully based on the cultural and biochemical properties, and the culture of each sample was performed in duplicate to reduce the possibility of missing any organism.

Conclusions

The presence of IMI and the type of microorganisms involved were the principal factors responsible for SCC variation, and the infected quarters represented significantly higher mean SCC than uninfected ones. No significant change in SCC was observed between major and minor pathogens causing IMI. Mammary quarter location (rear vs. front), age, and parity were significantly associated with the SCC variation irrespective of intramammary infection. Thus, factors affecting SCC apart from IMI should be considered during the interpretation of SCC. The patterns of IMI of lactating cows were significantly influenced by mammary quarter location (rear vs. front) and lactation stage. Proper care and preventive measures should be taken to reduce IMI as well as SCM, especially for high yielding older dairy cows at early lactation and their mammary quarters.

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Conflict of interest

The authors declare that they have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's contributions

Sumon SMMR conceived the idea, designed the study, collected and analyzed data and sample, and drafted the manuscript. Islam MT contributed to conceptualize, design, and coordinate the study and interpreting the data and critical revision of the manuscript. Parvin MS greatly contributed to perform the study and analyze the data. Ehsan MA was involved in supervision, study design, and revision of the manuscript.

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