Relationship of Udder, Flank and Leg Hygiene Scores with Elevated Somatic Cell count and Prevalence ofSubclinical Mastitis in Dairy cow

Abstract

Aim: To examine the association of udder, flank and leg hygiene scores with elevated somatic cell count (SCC) and risk of subclinical mastitis (SCM) in dairy cows in Nepal.

Study design: Longitudinal

Place and duration of the study: National Cow Research Program, Chitwan, Nepal; from September, 2019 to March, 2020

Methodology: Eighty (Holstein Frisian, Jersey and Lulu) SCM-free cows were enrolled in the research and a single observer obtained the duplicate hygiene scores of said body parts on one (clean) to four (extremely dirty) scale. The individual SCC was analysed fortnightly and the data were transformed at log10 base. The effect of animal cleanliness on SCC and risks of SCM were statistically analysed using ANOVA by GLM (univariate); SPSS (version 25). Likewise, the association of hygiene score with SCC and risks of SCM were performed by Pearson's correlation analysis and prevalence of SCM was assessed by Chi- square test.

Results: Mean hygiene scores were 2.39, 2.21 and 2.25 for udder, flank and lower legs, respectively. Majority of animals (UHS- 41.3%, FHS- 36.3% and LHS- 38.7%) had score 2 or 3 on all three body parts. The results showed a significant association ($P<.001$) between poor hygiene scores and increased SCC, with dirty udders, flanks, and lower legs linked ($P<.001$) with higher SCC and a greater incidence of SCM. Further, cleanliness in these areas significantly influences udder health, as evidenced by increased bacterial counts and SCM prevalence in cows with poor hygiene scores. These findings highlighted the importance of maintaining dairy animal hygiene to reduce the risk of SCM and to improve milk quality. Cleanliness of the udder and associated areas was vital for minimizing pathogen exposure. These findings also proved a strong correlation among poor hygiene scores and elevated SCC, which was associated with an increasing load of major bacteria (both contagious and environmental) and a higher prevalence of SCM.

Conclusion: Keeping dairy cows clean, especially around the udder, can significantly reduce the risk of SCM and ensure sound UH and milk quality. Further research is needed to optimize hygienic management for enhancing udder health in dairy cows.

Keywords: Cleanliness, udder health, log10 SCC, intramammary infection

INTRODUCTION

Sound udder health is critical for ensuring the production, productivity and well-being of dairy cows[1–3]. Mastitis, both clinical and subclinical, poses a significant concern in dairying due to its adverse effects on udder health (UH) and milk quality; the laterpositions a bigger challenge, often creating problem onearly detection of mastitis and prolonged losses[4–6].

The environment, precisely that of in-and-around shed, is one of the major causes of any form of intramammary infection (IMI) in either stall-fed [7,8] or in pasture-based dairy management[9,10]. The two major determinants, contact to pathogens and the competency of the immunity status of focus animals determines the risk of UH[11–13]. Contact to pathogens may arise from various sources, such as the cow's surroundings, pre-existing IMI, and the microbial flora present on the teat surface [14,15]. Udder cleanliness is the determinant of bacterial load and diversity on teat surfaces;so dirty udders and teats are the primary source of environmental contaminants in milk [16–18]. Therefore, hygiene scores are taken as practical tools for assessing cleanlinessof dairy animals and identifying potential risk factors for IMI[19]. In this regard, udder, flank and lower leg cleanlinessmight play a crucial role in reducing SCC, as poor hygiene can facilitate the entry and proliferation of pathogens, leading to mammary gland infections, specifically by environmental contaminants including the pathogenic micro-organisms[20,21].

Exposure to contagious and environmental pathogens often occurs during milking by direct or indirect contact with infected quarters and when teats are exposed to contaminated housing facilities, especially the barn floor and gutter regions. Therefore, potential sources of exposure to IMIs were identified as bedding materials, manure, shed accessories and mud [20–22]. In addition, dairy cows spent about 40 to 65% of their time lying down, during which the udder and teat surfacescome in contact with bacteria present in the bedding [22–24], thus, lessening bacterial contact at the teat end is imperative for preventing mastitis. [25,26] observed that the bacterial populations on teat ends are the major determinant of the incidence of IMI and these bacterial populations in turn had been found to be correlated with those found in barn floor and bedding [27–29].Similarly, the mastitis wasin high odds in the early dry period, mainly due to the discontinuation of pre-milking teat washing [30], and cessation of pre- and post-milking teat dipping [3,31]. The saidroutine deedswererecognized to reduce bacterial load at the teat end and consequently cut-off the chances of IMI[32].

Furthermore, the udder preparation before milking is an important factorinfluencingSCC and the milk quality. [14,15]describedthat a higher incidence of mastitis was associatedto the increased load of bacteria on the teat end. Theyalso reported a positive association between poor pre-milking udder preparation, such as cleaning and drying of the uddersand increase in bacterial counts in the milk.More specifically, the hygiene score of animals, especially at the hind quarter regions and udders had significant effect on the SCC than any type of dry cow therapy[33,34]. In the same line,[35]reported the use of environmental sanitation index for estimating the incidence of coliform mastitis in dairy cows.Consequently, hygiene scoring system had been developed and used to examine the cleanliness of dairy cows and the farm environment [33,36–38]. Therefore, dairy herdskept in fresh environments with cleaner udders and associated regions showed lower SCCs than those with dirtier udders and legs [38,39].

In spite of the recognized importance of udder, flank and leg hygiene scores in preventing mastitis, limited research has explored their specific relationship with elevated SCC and SCM in dairy cows. Elevated SCC is a hallmark of mammary gland inflammation and serves as a key indicator of SCM, even in the absence of visible symptoms. Therefore, this research aimed to explore the relationship between udder, flank and leg hygiene scores and their association with elevated SCC and SCM in dairy cows, thereby informing targeted management strategies for improving dairy cow welfare and milk quality.

MATERIALS AND METHODS

Experimental animals and location

This research was conducted at Cattle Research Program (NCRP), Rampur, Chitwan, Nepal during September, 2019 to March, 2020. From the herd of 224, a total of 80 apparently healthy and SCM- free lactating crossbreds (with iSCC, 84–109 × 1000 cells/ml), HF (40) and Jersey (32) and indigenous Lulu cows (8) were selected, after screening for SCM using Somaticell Kit (Intervet Schering Plough, Whitehouse, NJ). The screening test was conducted according to the manufacturer's note as detailed by [40]. Throughout the research period, the cows, milked twice daily at 6.00 AM and 17.00 PM, were maintained in a tail-to-tail tie- stall management under conventional housing system with assess to free grazing for 3- 4 hours daily at day time and standard housing space was provided according to national guidelines. All the daily farm management procedure, feeding management as well as the feed ingredients were kept similar for the herd. Commercial concentrates on the basis of production level and *ad libitum* green fodders were offered to address the nutritional requirements of the study cows.

Collection of milk samples and SCC analysis

Approximately 30 ml composite milk samples during morning milking were collected aseptically in sterilized sampling bottles from enrolled dairy cows at 15 days interval for six months. Udders and teats were washed and wiped off with towels soaked in betadine solution (1:1)and allowed to dry. The few streaks of foremilk (~5 ml)were discarded before collecting samples to avoid the initial milks collected in teat canal. The collected samples were dispatched to the laboratory immediately in a cool box maintaining temperature of <4°C and Lactoscan SCC (Milkotronic Ltd., Bulgaria; http://www.milkotronic.com) based on fluorescent microscopic techniques with dedicated Lactochipswere used for analyzing SCC following the standard procedures; both the absolute (×1000 cells/ml) and $\log_{10}$SCC values were determined.

Microbiological procedures and analysis

Microbiological procedures were run as per the [41]. Milk samples were immediately frozen upon receiving at the laboratory. For bacterial culture and further assessment, the samples were inoculated on blood agar plates and incubated at 37°C for 48 h. The morphology and haemolysis patterns were noted, and major organisms were identifiedby standard microbiologic protocols.*Pseudomonas spp*. was noted using catalase reaction, motility test, presence of pigments (blue/green) and triple sugar iron agar;*Staphylococcus aureus*by mannitol and coagulase test and*Streptococcus agalactiae*by the esculin reactions, agglutination and CAMP test. Gm-ve bacteria were grown on MacConkey agar, and identified by motility, indole, and ornithine reactions, and triple sugar iron slants. Samples were marked as negative (no growth), contagious bacteria (*Pseudomonas spp*.,*Staphylococcus aureus, Streptococcus agalactiae*), environmental bacteria (*Escherichia coli, Klebsiella spp., Streptococcus spp., Enterococcus spp.*), minor bacteria (coagulase-negative *Staphylococcus spp., Actinomyces spp., and Corynebacteria spp.*) or contaminated (any culture with more than two bacterial species per sample except*Strep. agalactiae*). Environmental and contagious pathogens were combined and categorized as major bacteria for some analyses.

Hygiene scoring

Udder, flank and leg hygiene scores were determined before milk sample collection in each animal [21,42]. Respective body parts of focus animals were compared to films and photos and scoredas 1) little, or hadnomanure, 2) minor bands of manure, 3) visible distinct flakes of manure, or dominantly covered in manure, or 4) completely covered with flakes of caked manure.Hygiene scorings were set out by singleobserverfor the entire enrolled animals. Immediately after completion of first scoring, the second hygiene assessment was done by the same observer without having access to the initial scores to reduce the influence on second scoring, for examining the reliability and agreement between the scores.

Statistical analysis

The reliability and repeatability of udder, flank and lower leg hygiene scores and the agreement between the duplicated scores were examined by using Kappa statistics. The absolute SCC were first transformed at log10 base to achieve the normal distribution and minimize the heterogeneity of variance. Then, the effects of hygiene scores of dairy animals on SCC were worked out for analysis of variance by using General Linear Model (univariate); SPSS statistical packages (version 25) and expressed as mean and standard error of the mean. Mean separation was done by Bonferronitest for stratification of effect of hygiene scoreson SCC. Further, the relationship of udder, flank and lower leg cleanliness with udder SCC were examined by Pearson'scorrelation analysis, while association of prevalence of subclinical mastitis with different hygiene scores were analysed by Chi- square test.Mean differences were maintained at $p \leq 0.05$.

RESULTS

Animals

The descriptive characters of hygiene score and somatic cell count of focus dairy cows are presented in Table 1 and 2. The mean parity, lactation stageand initial SCC (iSCC) of animals (n = 80) were 3.13 (0.16),5.04 (0.08) months and 0.95×10^5 cells/ml,respectively. The mean udder, flank and lower leg hygiene at enrolment were 2.39 (0.10), 2.21 (0.10) and 2.25 (0.10) respectively. Similarly, the mean log10 and absolute SCC were 5.43 (0.03) and 3.18 (0.03) $\times 10^5$ cells/ml (Table 1).Additionally, the flank region of the animal was observed to have highest cleanliness (65%), based on hygiene score, followed by the lower leg region (60%) and the udders (57.5%). For all three hygiene scores covered in this research, majority of animals on each group earned score 2 (moderately clean) or 3 (dirty)(Table 2).

Table 1. Descriptive statistics of hygiene score and somatic cell count of dairy cows (n= 80), Chitwan, Nepal

	iSCC ($\times 10^5$ cells/ml)	Log10 SCC	Udder SCC ($\times 10^5$ cells/ml)	UHS	FHS	LHS
Mean	0.95	5.43	3.18	2.39	2.21	2.25
Median	0.95	5.43	2.66	2.0	2.0	2.0
SEM	0.05	0.03	0.024	0.10	0.10	0.10
Range	0.84–1.09	4.81–6.05	0.64–11.35	1–4	1–4	1–4
Variance	0.01	0.60	2.16	0.82	0.90	0.82
Skewness	0.27	0.40	2.10	0.19	0.28	0.21

iSCC- initial somatic cell count, Log10 SCC- log 10 transformed somatic cell count, UHS- udder hygiene score, FHS- flank hygiene score, LHS- lower leg hygiene score

Table 2. Distribution of Udder, Flank and lower leg hygiene score in dairy cows (n= 80), Chitwan, Nepal

	Hygiene score[#]			Udder	Flank	Lower leg
	Udder	Flank	Lower leg	Clean vs. soiled		
Clean	13 (16.2)	21 (26.2)	18 (22.5)	□	□	□
Moderately clean	33 (41.3)	29 (36.3)	31 (38.7)	□	□	□
Dirty	24 (30.0)	22 (27.5)	24 (30.0)	□	□	□
Extremely dirty	10 (12.5)	8 (10.0)	7 (8.8)	□	□	□
Clean	□	□	□	46 (57.5)	52 (65.0)	48 (60.0)
Soiled	□	□	□	34 (42.5)	28 (35.0)	32 (40.0)

[#]Figure in parenthesis indicated the percentage, udder,flank and leg hygiene scores were categorized as “clean” (score of 1 and 2) or “soiled” (score of 3 or 4).

Hygiene Scores

Repeated scoring of dairy cows by a single observer showed a high degree of repeatability and agreement between hygiene scoring. The finest agreement (*P*<.001) between observations (UHS- 93.79%, FHS- 89.34% and LHS- 93.70%)were evident when scores were combined and worked- out into clean or soiled categories (Table 3). Comparatively lowerbut substantial agreements (*P*<.001) were found between duplicated observations of UHS(Table 3)

Table 3. Agreement within the observation first and second for repeated hygiene score in dairy cows, Chitwan, Nepal

Comparison	Observed agreement	Kappa value	Approx. t	Sig.
UHS-1 vs. UHS- 2	81.28%	0.726	10.36	<0.001
FHS- 1 vs. FHS- 2	87.50%	0.822	12.08	<0.001
LHS- 1 vs. LHS- 2	90.10%	0.855	12.20	<0.001
Clean vs. Soiled[a]				
Udder scores	93.79%	0.870	7.85	<0.001
Flank scores	89.34%	0.835	7.47	<0.001
Lower leg scores	93.70%	0.869	7.78	<0.001

[a]*clean*means dairy cows with hygiene score of 1 or 2 and *soiled*means that with hygiene score of 3 or 4, UHS- udder hygiene score, FHS- flank hygiene score, LHS- leg hygiene score

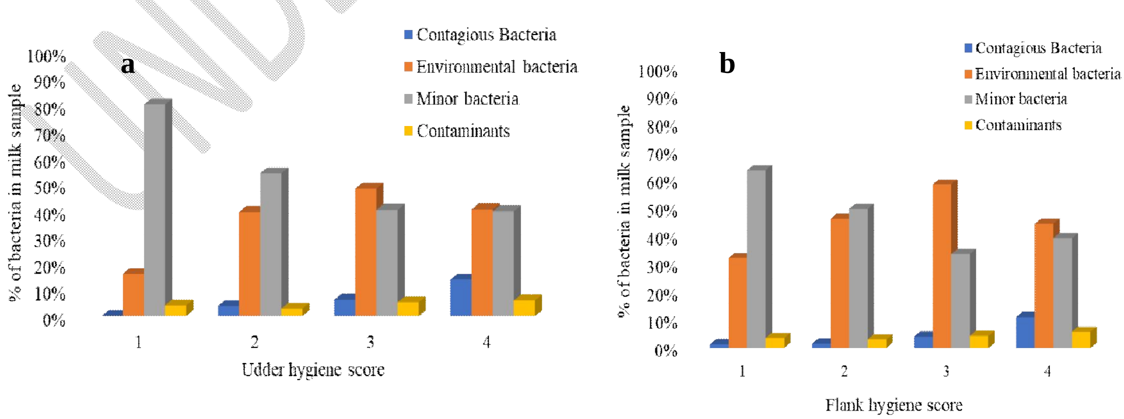
Mean log10 SCC and absolute SCC according to the degree of cleanliness of udder, flank and lower leg are presented in Table 4. The overall log10 SCC of UHS, FHS and LHS ranged from 5.42–5.49, which correspond with 3.69–3.89 $\times 10^5$ cells/ml absolute SCC of each score. The log10 SCC differed significantly (P<.001) among the hygiene scores for each body parts in question and was observed lowest on the clean score, which increased along the score and were recorded highest in extremely dirty score(Table 4). The absolute SCC too follow the trend of log10 SCC with lowest value in clean score and the highest in extremely dirty score for UHS, FHS and LHS but difference between clean and moderately clean score were non- significant.

Table 4. Milk log10 Somatic Cell Count (SCC) and absolute SCC (mean and SEM) according to hygiene score in dairy cows (n= 80), Chitwan, Nepal

	log10 SCC		95% CI	SCC ($\times 10^5$ cells/ml)		95% CI
	Mean	SEM		Mean	SEM	
UHS						
Clean	5.12[a]	0.04	5.05–5.20	1.39[a]	0.34	0.71–2.07
Moderately clean	5.35[b]	0.02	5.31–5.40	2.33[a]	0.21	1.90–2.75
Dirty	5.52[c]	0.03	5.47–5.58	3.52[b]	0.25	3.02–4.02
Extremely dirty	5.85[d]	0.04	5.77–5.92	7.51[c]	0.38	6.74–8.28

Overall	5.46	0.02	5.43–5.50	3.69	0.15	3.38–3.99
F- value	62.26			56.67		
Significance	<0.001			<0.001		
FHS						
Clean	5.17[a]	0.03	5.11–5.24	1.57[a]	0.30	0.96–2.17
Moderately clean	5.41[b]	0.03	5.35–5.46	2.61[a]	0.26	2.10–3.13
Dirty	5.57[c]	0.03	5.50–5.63	3.91[b]	0.30	3.32–4.50
Extremely dirty	5.81[d]	0.06	5.70–5.92	7.48[c]	0.49	6.50–8.46
Overall	5.49	0.02	5.45–5.53	3.89	0.17	3.54–4.24
F- value	41.14			38.50		
Significance	<0.001			<0.001		
LHS						
Clean	5.18[a]	0.04	5.10–5.26	1.63[a]	0.38	0.87–2.39
Moderately clean	5.38[b]	0.03	5.32–5.44	2.47[a]	0.29	1.89–3.05
Dirty	5.58[c]	0.04	5.51–5.65	4.25[b]	0.33	3.59–4.91
Extremely dirty	5.78[d]	0.06	5.65–5.90	6.65[c]	0.61	5.43–7.87
Overall	5.48	0.02	5.43–5.52	3.75	0.21	3.32–4.17
F- value	29.57			21.67		
Significance	<0.001			<0.001		

Log10 SCC- log 10 transformed somatic cell count, UHS- udder hygiene score, FHS- flank hygiene score, LHS- lower leg hygiene score, CI- confidence interval



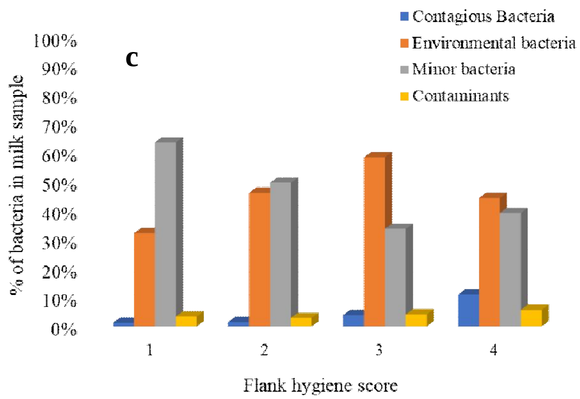


Figure 1. Distribution of contagious bacteria (*Staphylococcus aureus, Pseudomonas spp.* and *Streptococcusagalactiae*), environmental bacteria (*Escherichiacoli, Klebsiella spp., Streptococcus spp.*and *Enterobacter spp.*), minor bacteria (coagulase negative *Streptococcus spp., Actinomyces spp.*and *Corynebacteria spp.*) and contaminant bacteria (when more than two microorganisms are present excluding *Streptococcusagalactiae*) in milk sample according to the udder (**a**), flank (**b**) and lower leg (**c**) hygiene score

Relationship of prevalence of Subclinical Mastitis and hygiene score

The prevalence of subclinical mastitis was 46.2% and was associated with all three hygiene scores, i. e. UHS (χ^2=39.70; df= 3; *P*<.001), FHS (χ^2=38.60; df= 3; *P*<.001)and LHS (χ^2=32.09; df= 3; *P*<.001). Hygiene scores'clean' and 'moderately clean' did not show any issue of intramammary infection while 'dirty' score experienced the highest odds of SCM (Table 5). When scores were combined as 'clean' and 'soiled', more than three- fourth issues of SCM were significantly associated (χ^2=33.66; df= 3; *P*<.001) with score 'soiled'. The contagious bacteria increased with the hygiene score and were observed in the highest level at score 'extremely dirty' in all three scorings, while environmental bacterial number increased till score 'dirty' and then experienced the decreasing tendency (Figure 1a, b and c). The prevalence of major bacteria (contagious and environmental combined) increased with the hygiene score until it reached score 3 (dirty) and then it either remained constant or reduced (Figure 1). In addition, the absolute and log10 SCC were positively and significantly associated with SCM and hygiene scores (*P*<.001). There was significant association (*P*<.001) between all categories of hygiene scores too (Table 6).

Table 5. Association of prevalence of subclinical mastitis with different hygiene scores in dairy cows, Chitwan, Nepal

Variables	Hygiene score n (%)					Significance of results
	1	2	3	4	Overall	
Prevalence of SCM						
UHS	0	8 (21.6)	19 (51.4)	10 (27.0)		χ^2=39.70; df= 3; *P*<.001
FHS	0	11 (29.7)	19 (51.4)	7 (18.9)	37 (46.2)	χ^2=38.60; df= 3; *P*<.001
LHS	1 (2.7)	10 (27.0)	20 (54.1)	6 (16.2)		χ^2=32.09; df= 3; *P*<.001
Clean vs. soiled (Combined)			9 (24.3)	28 (75.7)		χ^2=33.66; df= 3; *P*<.001

Figures in parenthesis indicates percentage; *clean* means dairy cows with hygiene score of 1 or 2 and *soiled* means that with hygiene score of 3 or 4, UHS- udder hygiene score, FHS- flank hygiene score, LHS- leg hygiene score, SCM- subclinical mastitis

Table 6. Correlation of absolute and log10 SCC with udder, flank and lower leg hygiene score in dairy cows(n= 80), Chitwan, Nepal

	Absolute SCC	Log10 SCC	SCM	UHS	FHS	LHS
Absolute SCC	1	0.928**	0.644**	0.761**	0.730**	0.659**
Log10 SCC		1	0.756**	0.833**	0.783**	0.734**
SCM			1	0.686**	0.667**	0.605**
UHS				1	0.637**	0.727**
FHS					1	0.716**

**Significant at the 0.01 level (2-tailed) and *at the 0.05 level (2-tailed); SCC- somatic cell count, Log10 SCC- log 10 transformed somatic cell count, UHS- udder hygiene score, FHS- flank hygiene score, LGS- lower leg hygiene score, SCM- subclinical mastitis

DISCUSSION

The wet dirt and manure in the shed are the main sources of exposure to mastitis bacteria, and hygiene status of cows, whichvisuallydescribe the degree of exposure of animals to these sources. The presence of contagious and environmental bacteria on teat ends [43] and udder surfaces [21] is associated to the incidence of different form of mastitis. Therefore, all the approaches for monitoring and preventing udder infection in dairy animals revolve around the effective measures for controlling new infections and checking the new ones. Findings of this research lighted the relationship between the hygiene scores and milk somatic cell count in dairy cows for managing the risks of subclinical mastitis.

The animals at the start of research had 0.95×10^5 cells/ml SCC (iSCC), but the mean log10 and absolute SCC reached 5.43 (0.03) and 3.18 (0.03) $\times10^5$ cells/ml, respectively, at the end, ending up with overall SCM prevalence of 46.2%.The average iSCC in this research herd is recently reported to range from 84–109×105 cells/ml [44].This might reflect that most of the study cows enrolled started the study period with good udder health. The prevalence of SCM in the herds were found almost comparable to the figure (28- 55%) reported by [45] in mid- western region and [46]in Chitwan (42.8%), but was higher than that reported by [47]as the authors proclaimed 28.6% at animal level and 24.2% at quarter level in Lamjung. The prevalence of SCM in this study might have been found higher because we did not segregate the causative bacteria for working out the overall prevalence.In addition, the study area is the dairying pocket of Nepal, feeding capital city by around 50% of its demand but prevalence of SCM were reported critically higher in this region of the country and are the ever-long challenge for quality milk production. Moreover, the results could have been due to individual animal characteristics such as age, health status, stress level, parity, milk yield, lying behaviour, hygiene score and the routinehusbandry at farm.

The highest cleanliness was found in flank region of the animal (65%) and the lowest in udders (57.5%); the mean UHS, FHS and LHS at enrolment were 2.39 (0.10), 2.21 (0.10) and 2.25 (0.10), respectively. Themajority of animals on each group (UHS- 41.3%, FHS- 36.3% and LHS- 38.7%) earned score 2 or 3 in this research, which highlighted that the hygiene status to skew toward poorer side.Similar to these results, [48] observed 2 and 3 hygiene scores most frequently for all three body parts in free- stall cows. [49] reported the similar hygiene scores in automatic machine milked cows and described mean scores of upper thighs (2.5), legs (2.5) and udder (2.8). However, [20] found higher frequencies of score 3 and 4 in free- stall lactating cows. The surface type of the barn and the type bedding material used are the important determinants of hygiene score. Furthermore, the shed floor cleanliness, degree of animal movement, overcrowding, milking system adopted, schedule of barn scrapping, use of water, feeding systems, consistency of faeces, bedding material, stages of lactation and a lot of factors contribute to the barn hygiene status and thereby to that of animals. The shed cleaning and scrapping routine might had contributed in obtained hygiene score in this study as the cow mats used were observed poorly aligned and barn management is compromised in comparison to subsistence framers shed in periphery.

Kappa statistic showed a high degree of consistency and agreement between double hygiene scoring of study cows by a single observer and observed to be practically repeatable. The finest consistency between observations were observed when scores were combined into clean or soiled class while comparatively weaker but substantial agreements were documented between duplicated observations of UHS and FHS. These finding are in close agreement with[21] who observed the greatest consistency within combined observations marked as clean or dirty but weaker agreement between udder and leg hygiene scores.The finest consistency between clean and dirty category in this research might be associated with immediate second scoring after completing the initial one as well as the higher differences in observable traits during scoring, while comparatively lower agreement for udder and lower leg hygiene were hard to defend on this basis.

The log10 SCC significantly increased along the hygiene scores of udders, flank and lower legs and reached its peak at score 4 (extremely dirty). The absolute SCC too followed the trend of log10 SCC for all body scores but difference between score 1 and 2 were non-significant. The elevated SCC and increased odds of SCM had been consistently related with poor hygiene in dairy cows [21,37,38,49]. Additionally, [50] reportedthat the lower bulk milk SCC has been correlated with higher level of cleanliness in farm. However, [20] observed the different fact on free- stall dairy herds and reported lack of associations between cleanliness status and risk of increased SCC. The increasing log10 SCC along the hygiene scores in all three body parts in this study also agree with the loads of contagious (*Staphylococcus aureus, Pseudomonas spp*. and *Streptococcusagalactiae*) which increased with compromised cleanliness till score 4 and environmental bacteria (*Escherichiacoli, Klebsiella spp*., *Streptococcus spp.* and *Enterobacter spp*.) that amplified till score 3 and then remain static. Thus, the number of different pathogens, especially the contagious and environmental bacteria in teats and udder surface might have contributed the larger SCC in animals with poor hygiene status. As expected, the absolute and log10 SCC were positively and significantly associated with SCM and hygiene scores of all three body parts. There was significant association between all categories of hygiene scores too, signifying the risks of contamination of other body parts once either udder, flank or lower leg regions are covered with filths.The association of environment, in-and-around shed, is described as the key reason for any form of udder infection in stall-fed [7,8] and in pasture-based management [9,10]. In the same line, [16–18] reported that the load and diversity of bacteria on teat surfaces are influenced by the level of cleanliness of udders and associated structures. Furthermore, [33,34]observed significant contribution ofthe hind quarter and udder hygiene to the milk SCC in dairy animals than any other factors.

Thus, theresearch identifieddampfilths and manure as an important source of mastitis-pathogens, with poor hygiene proving a determinant to higher SCC and increased prevalence of SCM. Further, various shed conditions and management factors, such as barn surface, bedding material, and management routines, along with different cow factorswere evident to influence the hygiene scores. Consistency in hygiene scoring was encouraging and a significant relationship was found between animal cleanliness scores and SCC, emphasizing its importance to manage udder health and reduce the odds ofSCM.

CONCLUSION

The results of this research highlighted the imperative share of udder, flank, and lower leg cleanliness on UH in dairy cows, emphasizing the essential role of hygiene in preventing SCM and enhancing milk quality. Maintaining the udder and its surroundings clean was vital to lowering the SCC and limiting pathogen exposure. Theseresultsexpressed a strong associationbetween poor hygienicconditions and elevated SCC, which waslinked with a risein major bacterial numbers (both contagious and environmental) and a higher odd of SCM. Additionally, theresults alsodemonstrated that keeping dairy cows clean, especially around the udder, can significantly reduce the risk of SCM. These insights thus emphasizedhow importance hygieneis to ensure UH and milk quality.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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