

# UDDER AND TEAT BIOMETRY IN RELATION TO ELEVATED SOMATIC CELL COUNT AND RISK OF SUBCLINICAL MASTITIS IN DAIRY COWS

# DIPESH K. CHETRI 1\*, NABA R. DEVKOTA 2, HOM B. BASNET 3 and

#### DAINIK B. KARKI<sup>4</sup>

<sup>1,3,4</sup> Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal.

<sup>2</sup> Gandaki University, Pokhara, Nepal.

ORCID ID: <sup>1</sup>https://orcid.org/0000-0002-8669-3818, <sup>2</sup>https://orcid.org/0009-0006-6258-6838, <sup>3</sup>https://orcid.org/0000-0002-3077-8660, <sup>4</sup>https://orcid.org/0009-0005-0631-7994 \*Corresponding Author Email: dipeshvet@gmail.com

#### Abstract

The association between udder and teat biometrics with occurrence of subclinical mastitis (SCM) in dairy cows was investigated in this study. The relationship of udder and teat biometrics with udder health status were analyzed by using Chi- square test, one-way ANOVA, and a binomial logistic regression model. Funnel teats and round udder were the most common, and their distribution among the breeds differed. Teat shape (TS) was associated with SCM, and the cows with bottle shaped teats experienced the highest odds of SCM, while cylindrical teats were least affected. The udder SCC was higher in Jersey while quarter SCC was larger in fore teats in both Jersey and HF. Similarly, quarter and udder SCC were higher in SCM cows compared to the healthy ones. Teat length (TL), teat diameter (TD), and teat-to-floor distance (TFD) were associated with increased risk of SCM. Teats were longer and wider in HF and placed farther from floor, while Lulu had the shortest and thinnest teats placed nearer to the floor. The SCM animals had low set udders, longer and thicker teats with smaller udder length, width, TFD and distance between teats than in healthy animals. The increase in TL and TD and decrease in TFD showed an intensification of the risk of SCM by 11.55 folds in fore, 3.88 folds in hind, 9.58 times in right teats and 8.52 folds in thicker teats. Each unit increase in TFD cuts off probability of SCM by 43% in fore, 11% in hind and 10% in left teats. Thus, biometric traits are important characteristics for SCM management and need to be duly considered in the selection and breeding program of dairy cows to cut off the incidence of SCM.

Keywords: Udder Health, SCC, Teat Morphometry, Udder Shape.

#### **INTRODUCTION**

Subclinical mastitis (SCM), a latent udder infection, is one of the biggest challenges in Nepalese dairy herds with 4–18 % production loss per infected quarter (Nielsen *et al.*, 2009). This results in a loss of approximately 63\$ per animal per lactation (Ng *et al.*, 2010) and undermines the motivation of developing dairy industry (Bhattarai *et al.*, 2020). Thus, SCM in dairy cows is a consistent hurdle for enhancing quality milk production in Nepal and around the globe. Somatic cell count (SCC) serves as a proxy indicator of udder infection and is an important component of milk for monitoring its hygiene and quality. It also functions as an early diagnostic aid for SCM control (Vieira *et al.*, 2021). The prevalence of SCM is 42.8% (Bhandari *et al.*, 2021) in cattle in Chitwan, 28.6% and 24.2% at animal and quarter level in Lamjung (Khanal & Pandit, 2013) whereas it ranges 28- 55% in cattle in mid-western Nepal





(Sah *et al.*, 2020). The higher prevalence of SCM is associated with hitches in animal husbandry, milking practices, and the cow itself (Mein *et al.*, 2004). Seasonal variations, ambient temperature, associated diseases, and production status pose risks for SCM at the farm level (Neculai-Valeanu & Ariton, 2022; Singha *et al.*, 2021). However, the risk associated with udder and teat biometrics, though few works are reported, are often under looked aspects and shedding light on these factors can help identifying high-risk animals for SCM management (Miles *et al.*, 2019). Subjective annotation of relationships among udder confirmation, teat biometrics and odds of SCM is quite common.

Early research in these areas has defined the association among the above-mentioned traits in dairy animals (Gavan & Riza, 2021; Guarin *et al.*, 2017; Kaur *et al.*, 2018; Singh *et al.*, 2017; Sinha *et al.*, 2022). However, significant disparities exist in past works regarding the relationships of SCM and associated SCC to various udder and teat traits. For example, Sharma *et al.* (2016) reported the higher prevalence of SCM in pendulous udder, thick or wider barreled teats (Zwertvaegher *et al.*, 2013) and inverted and flat teat end shapes in cows. In contrast, other researchers observed so in narrower teat (Guarin *et al.*, 2017) and described that SCM and teat end shape are unrelated traits (Chrystal *et al.*, 2001; Guarin *et al.*, 2017). Singh *et al.* (2017) observed the least association of pencil-shaped teats with occurrence of SCM in Frieswal cows, but misaligned teats might end up with larger cases of SCM.

Bhutto *et al.* (2010) reported non-significant association of udder shape with teat- SCC and SCM, in contrast to Bharti *et al.* (2015) and Sharma *et al.* (2016) who elucidated that the pendulous udder was associated with higher incidence of SCM. Similarly, higher cases of mastitis in cows were reported with larger udder depth or udder reaching up to hock joint, with longer and thicker teats and smaller teat end-to-floor distance, i.e., teat ends nearer to the floor (Gavan & Riza, 2021; Sinha *et al.*, 2022). In the same vein, longer and wider teats that reach closer to the floor were found to experience more cases of SCM in buffaloes (Kaur *et al.*, 2018). The udder and teat traits contribute about 40% to a standard scorecard of ideal dairy animal confirmation, show medium to high heritability and can be recorded relatively early in the life of animals. Under these contexts, this study was conducted with a focus on elucidating the magnitude of the association of various traits of udder and teat biometrics and the odds of elevated SCC and risk of SCM in crossbreds (Holstein Friesian and Jersey) and indigenous Lulu cows.

# MATERIALS AND METHODS

#### **Selection of Animals**

A total of 80 lactating crossbred HF, Jersey and Lulu cows that were free from SCM were randomly selected from the lactating herd of National Cattle Research Program (NCRP), Rampur, Chitwan, Nepal. SCM-free cows were screened from the milking herd with Somaticell Kit (Intervet Schering Plough, Whitehouse, NJ), an on-farm rapid test and was conducted in accordance with the manufacturer's recommendations. The experiment was carried out from September, 2019 to March, 2020, spanning a period of 180 days. The cows were maintained in tail-to-tail tie- stall management with mat on barn floor and were both hand and machine





milked twice daily (6.00 AM and 17.00 PM).

# **Measurement of Udder and Teat Biometrics**

Udder shape (US), teat shape (TS) and teat-end shape (TES) were categorized through visual examination half an hour before the first sampling of milk. US was evaluated and classified into bowl, round, goaty and stepped type; TS as funnel, bottle, cylindrical and pear; whereas TES was categorized as flat, concave, inverted, and pointed. Udder length (UL), udder depth (UD), udder width (UW), teat length (TL), teat diameter (TD), teat-to-floor distance (TFD), distance between fore and hind teats and right and left teats were measured using a measuring tape and vernier caliper.

# Milk SCC Analysis

Approximately 30 ml morning milk samples were collected aseptically in sterilized sampling bottles from each quarter at fortnightly interval for 180 days. Udders and teats were washed with clean water and wiped off with towels soaked in antiseptic solution. The first 2-3 streaks of foremilk were discarded before collecting milk samples. SCC was performed using Lactoscan SCC (Milkotronic Ltd., Bulgaria; www.milkotronic.com) following the standard procedures; both the absolute (×1000 cells/ml) and log<sub>10</sub>SCC values were determined. The udder health was defined based on the teat SCC as a SCM group or a healthy one. If a single quarter showed higher SCC ( $\leq$ 275,000) at least once during 12 events of count, the udder was assigned as SCM group, and if it remained consistently fresh throughout the study period, it was categorized as the healthy one.

# **Statistical Analysis**

The statistical analysis was conducted using SPSS statistical packages (version 25). The relationship between udder and teat shapes with different breeds and udder health was analyzed by Chi- square test of independent factors. Data on teat and udder biometrics and SCC were analyzed using one way analysis of variance and expressed as mean and standard error of the mean. Mean differences were maintained at p $\leq$ 0.05. To examine the association of teat and udder biometrics with the risk of SCM, the cows in the study with SCC  $\geq$ 275,000/ml at least once during the study period were designated as the SCM group and scored 0, while cows consistently showing lower SCC (<275,000/ml) were grouped as the healthy one and scored 1. Consequently, the association was analyzed for odds ratio (OR) at a 95% confidence interval and assessed using dichotomous logistic regression as follows:

logit (*p*)= 
$$\beta 0 + \beta_1 X_1 + \beta_2 X_2 \dots + \beta_{17} X_{17}$$

Where,

Scopus

logit (p)	Log [p/1-p] = odds of occurring SCM in study animals
$eta_0$	Interception at y- axis
β117	Partial regression coefficient
X117	Predictor variables, here different teat and udder traits



#### **RESULTS AND DISCUSSION**

#### Udder and teat morphometry

A total of 320 teats (128 in Jersey, 160 in HF and 32 in Lulu) were analysed for distribution of TS, TES and US in different breeds of cow (Table 1). Overall, funnel-shaped teats were predominant (51.2%) followed by cylindrical (27.5%), and bottle (11.2%) shaped teats. Funnel shaped teats were the most common in all breeds and the distribution of teat shapes among the breeds differed ( $\chi^2$ =14.07; df= 6; *p*=0.03). These results are similar to the findings of Poudel *et al.* (2022) as the authors described the dominant distribution of funnel shaped teats in Murrah buffaloes but are different than that of Singh *et al.* (2017) and Kaur *et al.* (2018) where a higher frequency of cylindrical teats in all breeds may indicate selection preferences in this region, linked with better production and a lower risk of mastitis.

The most prevalent US was round (about one third of data) followed by goaty whereas bowl and stepped udders were much uncommon. Jersey cows were found to have round udders in most of the cases, but goaty udders were more frequent in HF; thus, the distribution of US among breeds differed significantly ( $\chi^2=32.07$ ; df= 6;  $p\leq0.001$ ). These results are consistent with the findings of 50% round udders in Bushuev cows (Nosirov *et al.*, 2021) and 19.12% in Sahiwal and 24.68% in Karan Fries cows (Danish *et al.*, 2018). However, Basavaraj *et al.* (2019) reported only 3.12% of round udders in Deoni cows. The high prevalence of round udders in Jersey might be due to the intended selection focus linked with higher production along with appealing visual characteristics.

Variables	В	reed n (%	)	Overall	Gi i 6i 6 14		
	Jersey	HF Lulu		Overall	Significance of results		
Teat shape							
Funnel	56 (34.2)	84 (51.2)	24 (14.6)	164 (51.2)	$\chi^2$ =14.07; df= 6; <i>p</i> =0.03		
Bottle	16 (44.4)	20 (56.6)	0	36 (11.2)			
Cylindrical	40 (45.5)	40 (45.5)	8 (9.0)	88 (27.5)			
Pear	16 (50.0)	16 (50.0)	0	32 (10.0)			
Teat end shape							
Pointed	16 (28.6)	36 (64.3)	4 (7.1)	56 (17.5)	$\chi^2$ =8.35; df= 6; <i>p</i> =0.21		
Round	44 (47.8)	40 (43.5)	8 (8.7)	92 (28.8)			
Flat	52 (40.6)	60 (46.9)	16 (12.5)	128 (40.0)			
Concave	16 (36.4)	24 (54.5)	4 (9.1)	44 (13.8)			
Udder shape							
Bowl	4 (25.0)	8 (50.0)	4 (25.0)	16 (20.0)	$\chi^2$ =32.07; df= 6; <i>p</i> =0.00		
Goaty	4 (16.7)	20 (83.3)	0	24 (30.0)			
Round	20 (71.4)	4 (14.3)	4 (14.3)	28 (35.0)			
Stepped	4 (33.3)	8 (66.7)	0	12 (15.0)			

Table 1: Association of teat and udder shape with different breeds of dairy cow at NCRP
farm, Chitwan, 2019-2020

Figures in parenthesis indicates percentage





The overall TL and TD were measured 5.44 cm and 2.39 cm respectively, with an average TFD being 47.18 cm (Table S1). All these traits were associated with increased risk of SCM (p<0.001). While, teat location did not affect TL and TFD, right-side teats were observed to be longer and wider. Hind teats were shorter and thicker than fore teats and were positioned farther away from the floor. Teats were longer and wider in HF and they were placed farther from floor compared to other breeds (p<0.001); Lulu had the shortest and thinnest teats which were positioned nearer to the floor (p<0.001). On the basis of quarter health, TL, TD and TFD differed (p<0.001) among the breeds; the longer and wider teats with shorter TFD were found in SCM animals compared to healthy one.

Rupp and Boichard (1999) described the positive genetic correlation between SCC and TL suggesting that cows having longer teats are at higher risk of udder infection. Longer and thicker teats are more prone to injuries and are placed closer to the floor (Bharti *et al.*, 2015; Bhutto *et al.*, 2010). Siagian and Amidjaya (2022) documented positive association of TL and SCM with evidence of higher risk at TL >5.48 cm while negligible at TL 5.11 cm in cows. Similar findings were shared by Shinde *et al.* (2020) with higher incidence of SCM in Gir crossbreds having TL >7 cm. However, Hickman (1964) reported that TL was independent of the incidence of any form of mastitis in cows, and a different perspective was presented by Sharma Buragohain *et al.* (2016) in cows and by Hussain *et al.* (2013) in buffaloes indicating a significant association between smaller teats and mastitis.

Teats with broader diameter have the larger barrel and wider orifices, which remain open for a longer duration post-milking compared to thinner teats, increasing the risk of pathogens entering the udder. A positive association between TD and SCC in cows was described by Jerstad *et al.* (1989) and Bharti *et al.* (2015). Shinde *et al.* (2020) reported that TD >2.5 cm and TFD <42 cm in Gir crossbreds predisposed them to a higher odd of SCM.

The udder biometrics in cows are presented in Table S2. UL was longer in Jersey (p<0.05) compared to Lulu cows. All udder biometrics were smaller in Lulu cows compared to Jersey and HF cows. Nemcova *et al.* (2007) reported the highest SCS and risk of SCM in cows with deep udders, weak central ligaments and pendulous udder. Disparities in the findings of udder measurements might be linked to the variation in production potential, and genetic differences among the breeds, and to some extent, the milking method followed.

# Milk Somatic Cell Count

The overall SCC and teat SCC (mean and SEM) in different breeds of cow are presented in Table 2. Udder SCC (cells/ml), both log10 and absolute (×10,000), were higher (p<0.001) in Jersey cows compared to HF and Lulu. At the teat level, the SCC in forequarters was higher (p<0.01) in Jersey and HF than in Lulu, indicating the potential risk of forequarter infection. These findings may be attributed to the differences in biometric characteristics of teats and udder in these cows, as discussed earlier, and the level of milk production. Jersey and HF cows recorded longer and thicker teats that reached closer to the floor compared to Lulu, making them more prone to SCM with a higher score of SCC. Zwertvaegher *et al.* (2013) reported longer and wider fore teats and higher SCC compared to rear teats in crossbred cows, with





these teat biometrics showing an increasing trend with parity. The trend of SCC variations has been described for various breeds of cows (Alhussien *et al.*, 2016; Alhussien & Dang, 2017, 2018a, 2018b). The shape/size of teats and udder, along with their attachment, vary according to the breed type; thus, well-balanced udders pose less odds of mastitis than the pendulous one (Dang *et al.*, 2007). High-producing breeds like Jersey, Brown Swiss and HF have a higher SCC (241,000 cells/ml) in milk (Alhussien & Dang, 2018b) than low-producing indigenous cows like Tharparkar, Lulu, etc. In the same vein, lower SCC was reported in Tharparkar compared to Karan Fries by Alhussien *et al.* (2016a) and Alhussien *et al.* (2016b).

T	Table 2: Overall and teat-level SCC (mean and SEM) in different breeds of dairy cattlein tie-stall management at NCRP farm, Chitwan, 2019-2020							
		LF	LH	RF	RH	SEM	Overall	

	LF	LH	RF	RH	SEM	Overall				
Factors		#Log10	SCC Ce	lls/ml		Log10SCC Cells/ml	SEM	SCC (×10,000)	SEM	
Jersey	5.12 <sup>a</sup>	5.05	5.15 <sup>a</sup>	5.04	0.20	5.34 <sup>a</sup>	0.03	27.78ª	2.07	
HF	5.03 <sup>a</sup>	5.01	5.13 <sup>a</sup>	5.02	0.06	5.22 <sup>b</sup>	0.03	21.68 <sup>b</sup>	1.43	
Lulu	4.66 <sup>b</sup>	5.04	4.67 <sup>b</sup>	5.05	0.15	5.11 <sup>b</sup>	0.08	18.05 <sup>b</sup>	2.56	
F- value	5.09		5.09			5.56		4.71		
Sig.	0.007	ns	0.007	ns		0.004		0.01		

<sup>#</sup> Log base 10 transformation of absolute SCC; Mean within the same column with different superscripts differ significantly ( $p \le 0.01$ ) and ns- non significant (p > 0.05)

The teat and udder SCC were higher (p < 0.001) in cows with SCM (3,78,799 cells/ml) compared to the healthy ones (1,26,769 cells/ml) (Table S3). This finding is due to the fact that a basal picture of SCC (250,000 to 275, 000 cells/ml), is maintained by a healthy udder in cows (IDF, 2013; Middleton *et al.*, 2017). However, when pathogens enter the gland, somatic cells play a key role as the first line of defense against pathogens, leading to an increase in their numbers along with epithelial cells and signal the inflammatory status (Mukherjee *et al.*, 2015; Sharma Buragohain *et al.*, 2016).

The prevalence rate of SCM at udder level was 36.2% (Table 3), which is in line with Sah *et al.* (2020) [28-55%] in mid-western Nepal but is lower than the rates reported by Bhandari *et al.* (2021) [42.8%], Tiwari *et al.* (2022) [42.8%]. However, lower prevalence of SCM was reported by Khanal and Pandit (2013) [28.6%], and Chen *et al.* (2023) [10%] in cows and by Kaur *et al.* (2018) [21%] in buffaloes. The differences in prevalence of SCM might be due to the diverse range of breeds covered with variations in production, gaps in basis of defining the udder health, variations in husbandry and milking practices, diagnostic methods employed, environmental conditions, etc.

TS demonstrated a significant association with SCM ( $\chi 2=10.78$ ; df=3; p=0.01; Table 3). Animals with bottle-shaped teats exhibited the highest odds of SCM (100%), followed by funnel-shaped (42.9%) and pear-shaped teats (41.7%), while cylindrical teats (22.2%) had the lowest risk. The increased risk associated with bottle and pear-shaped teats may be due to their broader teat orifice and narrower base, making them more susceptible to teat end traumatization from higher milking pressure during hand milking (Hickman, 1964). This is so due to occlusion







of the orifice between the gland cistern and teat cistern by the teat cups resulting in a decrease and complete stoppage of milk flow which ends up in teat-end lesions in machine milking. These teat end lesions are in general the first click for mastitis in cows (Guarin *et al.*, 2017; Sharma *et al.*, 2016; Singh, 2022). However, Bhutto *et al.* (2010) found non-significant association between teat end lesions and quarter SCC. Modh *et al.* (2017) was in line with these results who described higher risk of SCM in bottle and funnel shaped teats in Gir cows. With respect to TES and US, the effect on udder health was non-significant, though odds of SCM was higher in round udder with pointed teat end. However, Tesfay *et al.* (2023) found 2.84- and 11.85-times higher risks of mastitis in cows with round and flat teat ends, while Bharti *et al.* (2015) reported higher incidence of SCM in pendulous udders.

Variables	Udder hea	alth n (%)	Significance of results			
	SCM	Healthy				
Teat shape						
Funnel	48 (42.9)	64 (57.1)				
Bottle	16 (100.0)	0				
Cylindrical	32 (22.2)	112 (77.8)	$\chi^2 = 10.78$ ; df= 3; <i>p</i> =0.01			
Pear	20 (41.7)	28 (58.3)				
Overall	116 (36.2)					
Teat end shape						
Pointed	28 (50.0)	28 (50.0)				
Round	40 (43.5)	52 (56.5)				
Flat	40 (31.2)	88 (68.8)	$\chi^2$ =3.57; df= 3; <i>p</i> =0.31			
Concave	8 (18.2)	36 (81.8)				
Overall	116 (36.2)					
Udder shape						
Bowl	4 (25.0)	12 (75.0)				
Goaty	8 (33.3)	16 (66.7)				
Round	13 (46.4)	15 (53.3)	$\chi^2$ =2.26; df= 3; <i>p</i> =0.52			
Stepped	4 (33.3)	8 (66.7)				
Overall	29 (36.2)					

# Table 3: Association between teat morphometric traits and the odds of sub clinicalmastitis in dairy cattle at NCRP farm, Chitwan, 2019-2020

Figures in parenthesis indicates percentage

The results of dichotomous logistic regression analysis describing the association between teat and udder biometrics with the odds of SCM in cows are depicted in Table 4. Among different 17 traits in question, FTL, HTL, RTL, FTD, FTFD, HTFD, LTFD and UD were observed to be the most important traits associated with the risk of SCM. The animals with SCM had dipper udder, longer and thicker teats with smaller UL, UD, TFD and distance between teats than in healthy animals. However, the risk of SCM were significant in fore (p<0.01), hind (p<0.05), right TL (p<0.05), fore TD (p<0.05) and in TFD (p<0.05) except the left TFD. The results indicated that an increase in TL correlates with a higher risk of SCM in cows. The odds of SCM were 11.55 times higher in fore teats, 3.88 times higher in hind teats, and 9.58 times higher in right teats.





Consistent with these findings, previous literature has also reported higher odds of SCM in cattle and buffaloes with longer teats (Bharti *et al.*, 2015; Kaur *et al.*, 2018; Sharma *et al.*, 2016; Siagian & Amidjaya, 2022; Singh *et al.*, 2013; Sinha *et al.*, 2022). The teat canal, along with its associated structures, serves as the primary defense line against invading microorganisms and a source of antimicrobial substances against potential intruders (Zecconi *et al.*, 2002). However, it also represents a crucial pathway for the initial interactions between infectious microorganisms and the structures of the mammary gland. Furthermore, Sharma Buragohain *et al.* (2017) reported an inverse relationship of phagocytic index of milk neutrophils and macrophages with the TL as an increased susceptibility to SCM. Similarly, Lund *et al.* (1994) described a genetic correlation between TL and SCC which increases the risks of mastitis with increasing TL. In contrast, Slettbakk *et al.* (1990) reported that TL and risks of mastitis were merely related while significant association between smaller teats and risks of mastitis was described in Nili-Ravi buffaloes (Hussain *et al.*, 2013).

Biometric traits (cm)	<b>B</b> <sup>1</sup>	SE <sup>2</sup>	OR <sup>3</sup>	95% CI <sup>4</sup>	<i>p</i> value
Fore teat length (FTL)	2.447	0.94	11.55	1.84-72.61	0.01
Hind teat length (HTL)	1.356	0.63	3.88	1.13-13.35	0.03
Left teat length (LTL)	0.420	0.65	1.52	0.43-5.46	0.52
Right teat length (RTL)	2.259	1.10	9.58	1.11-82.20	0.04
Fore teat diameter (FTD)	2.151	0.93	8.59	1.39- 53.12	0.02
Hind teat diameter (HTD)	0.970	0.68	2.63	0.70-9.93	0.15
Left teat diameter (LTD)	0.496	1.58	1.64	0.08-35.97	0.75
Right teat diameter (RTD)	2.113	1.48	8.28	0.45-51.15	0.15
Fore teat to floor distance (FTFD)	-0.567	0.23	0.57	0.36-0.90	0.02
Hind teat to floor distance (HTFD)	-0.118	0.05	0.89	0.81-0.98	0.02
Left teat to floor distance (LTFD)	-0.110	0.05	0.90	0.82-0.98	0.02
Right teat to floor distance (RTFD)	-0.075	0.21	0.93	0.61-1.41	0.72
Udder length (UL)	-0.079	0.09	0.92	0.77-1.10	0.38
Udder width (UW)	-0.263	0.25	0.77	0.47-1.26	0.30
Udder depth (UD)	0.269	0.11	1.31	1.05-1.16	0.02
Distance of fore to hind teats (DFH)	-0.480	0.30	0.62	0.34-1.11	0.11
Distance of left to right teats (DLR)	-0.016	0.17	0.98	0.71-1.36	0.92

 Table 4: Logistic regression model showing association of teat and udder biometric traits with the risks of SCM in dairy cattle at NCRP farm, Chitwan, 2019-2020

<sup>1</sup>regression coefficient, <sup>2</sup>standard error of regression coefficient, <sup>3</sup>odds ratio, <sup>4</sup>95% confidence interval

Cows with thicker teats demonstrated a higher risk of SCM, 8.59 times greater compared to those with normal teats. This heightened risk is attributed to the larger orifice of thick-barreled teats, which takes a longer time to close post-milking, increasing the probability of microbial entry and consequently, the risks of mastitis. Sharma Buragohain *et al.* (2017) highlighted a negative correlation between the phagocytic index of milk neutrophils and macrophages and TD, signifying a predisposition to higher susceptibility to SCM in cows with thicker teats. This finding aligns with earlier studies where teats with larger diameters were identified as key factors in elevating SCC and the risks of mastitis in cows (Bharti *et al.*, 2015; Guarin *et al.*,





2017; Sharma *et al.*, 2016; Singh *et al.*, 2013; Sinha *et al.*, 2022) and in buffaloes (Hussain *et al.*, 2013; Kaur *et al.*, 2018). Similarly, Sharma Buragohain *et al.* (2017) reported the strong positive correlation of mid teat and teat base diameter with SCC in cows, elucidating the higher susceptibility to mastitis with larger TD. Besides, the cows with deeper or pendulous udder were at 1.31 times risk of experiencing SCM than those with normal udder depth. This finding is in close agreement with the past works (Bhutto *et al.*, 2010; Miles *et al.*, 2019; Singh *et al.*, 2013; Sinha *et al.*, 2022) who described the deeper udder in cows as a risk multiplier of odds of mastitis because of their relative closeness to the floor and increasing risks of teat lesions during lying down. Bhutto *et al.* (2010) reported that the cows with udder frame reaching at lower level of hock joint experienced the higher incidence of mastitis because of increased possibility to have poor udder and flank hygiene score. The loose and weak central suspensory ligament of udder had been described as the main reason that increases the susceptibility for lesions and diseases (Nemcova *et al.*, 2007).

The higher TFD was associated with the decreasing risk of SCM in cows; each unit increase in TFD cuts off chance of SCM by 43% in fore teats, 11% in hind teats and 10% in left teats. This falls in line with Singh *et al.* (2013), Sharma Buragohain *et al.* (2017), Kaur *et al.* (2018) and Gavan and Riza (2021) who described that cows with teats placed nearer to the floor were more susceptible to mastitis. However, Sinha *et al.* (2022) found such association in distance between the rear teats only, not on TFD and odds of mastitis in Karan Fries and Sahiwal. This may be due to the fact that shorter TFD increases the possibility of teat soiling, thereby the entry of microorganisms. Moreover, when teat ends were at the level of the hock joint or below, the risks of teat injury elevated during standing and lying of cows, which leads to different form of intramammary infections, mostly seeded up in subclinical form.

# CONCLUSION

This study showed the significant association between certain udder and teat morphometric traits with elevated SCC and SCM in dairy cows. Bottle and funnel-shaped teats experienced the higher likelihood of SCM. Animals with a deeper udder, characterized by longer and wider teats that reach closer to the floor, demonstrated a notable susceptibility to SCM. Biometric characteristics such as udder height, shape, length, and teat thickness, along with the shortest distance from teat end to floor, emerged as crucial traits in the context of SCM management. Therefore, these factors should be carefully considered in the selection and breeding programs of dairy cows to mitigate the incidence of SCM and enhance milk production.

#### Ethical approval

We, authors, hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The work did not include approaches that challenge well-being and welfare of animals.

#### Acknowledgements

We thank National Cattle Research Program, Chitwan, Nepal for providing access to work on dairy cows and the Faculty of Animal Science, Veterinary Science and Fisheries, Chitwan, Nepal for equipment and consumables support.





#### **Disclosure statement**

Authors have declared that no competing interests exist.

#### Data availability statement

The data and associated information will be shared upon request

#### References

- Alhussien, M., Manjari, P., Mohammed, S., Sheikh, A. A., Reddi, S., Dixit, S., & Dang, A. K. (2016). Incidence of mastitis and activity of milk neutrophils in Tharparkar cows reared under semi-arid conditions. Tropical Animal Health and Production, 48(6), 1291–1295. https://doi.org/10.1007/s11250-016-1068-8
- Alhussien, M., Manjari, P., Sheikh, A. A., Seman, S. M., Reddi, S., Mohanty, A. K., Mukherjee, J., & Dang, A. K. (2016). Immunological attributes of blood and milk neutrophils isolated from crossbred cows during different physiological conditions. Czech Journal of Animal Science, 61(5), 223–231. https://doi.org/10.17221/63/2015-CJAS
- Alhussien, M. N., & Dang, A. K. (2017). Integrated effect of seasons and lactation stages on the plasma inflammatory cytokines, function and receptor expression of milk neutrophils in Sahiwal (Bos indicus) cows. Veterinary Immunology and Immunopathology, 191, 14–21. https://doi.org/10.1016/j.vetimm.2017.07.010
- 4) Alhussien, M. N., & Dang, A. K. (2018a). Diurnal rhythm in the counts and types of milk somatic cells, neutrophil phagocytosis and plasma cortisol levels in Karan Fries cows during different seasons and parity. Biological Rhythm Research, 49(2), 187–199. https://doi.org/10.1080/09291016.2017.1350442
- Alhussien, M. N., & Dang, A. K. (2018b). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. In Veterinary World (Vol. 11, Issue 5, pp. 562– 577). Veterinary World. https://doi.org/10.14202/vetworld.2018.562-577
- 6) Basavaraj, H., Waghmare, P., M. Patil, V., Suranagi, M. D., Biradar, U. S., Chandra, S., Desai, A. R., Mallikarjun, H., & Prasad, M. (2019). Study the Morphological Characteristics of Udder and Teat and Its Relation with Lactation Milk Yield in Deoni Cattle. International Journal of Current Microbiology and Applied Sciences, 8(10), 2369–2376. https://doi.org/10.20546/ijcmas.2019.810.274
- 7) Bhandari, S., Subedi, D., Tiwari, B. B., Shrestha, P., Shah, S., & Al-Mustapha, A. I. (2021). Prevalence and risk factors for multidrug-resistant Escherichia coli isolated from subclinical mastitis in the western Chitwan region of Nepal. Journal of Dairy Science, 104(12), 12765–12772. https://doi.org/10.3168/jds.2020-19480
- 8) Bharti, P., Bhakat, C., Pankaj, P. K., Bhat, S. A., Arul Prakash, M., Thul, M. R., & Puhle Japheth, K. (2015). Relationship of udder and teat conformation with intra-mammary infection in crossbred cows under hothumid climate. Veterinary World, 8(7), 898–901. https://doi.org/10.14202/vetworld.2015.898-901
- 9) Bhattarai, A., Kaphle, K., & Adhikari, P. (2020). A Review on "Bovine Sub-Clinical Mastitis in Nepal: Sustainable Management Strategy." International Journal of Food Science and Agriculture, 4(1), 80–89. https://doi.org/10.26855/ijfsa.2020.03.012
- Bhutto, A. L., Murray, R. D., & Woldehiwet, Z. (2010). Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. Veterinary Journal, 183(1), 63–67. https://doi.org/10.1016/j.tvjl.2008.08.024
- 11) Chen, S., Zhang, H., Zhai, J., Wang, H., Chen, X., & Qi, Y. (2023). Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982–2022: a systematic review and meta-analysis. Frontiers in Veterinary Science, 10, 1185995. https://doi.org/10.3389/fvets.2023.1185995





- 12) Chrystal, M. A., Seykora, A. J., Hansen, L. B., Freeman, A. E., Kelley, D. H., & Healey, M. H. (2001). Heritability of Teat-End Shape and the Relationship of Teat-End Shape with Somatic Cell Score for an Experimental Herd of Cows. Journal of Dairy Science, 84(11), 2549–2554. https://doi.org/10.3168/JDS.S0022-0302(01)74707-8
- 13) Dang, A. K., Kapila, S., Tomar, P., & Singh, C. (2007). Relationship of blood and milk cell counts with mastitic pathogens in Murrah buffaloes. Italian Journal of Animal Science, 6(SUPPL. 2), 821–824. https://doi.org/10.4081/ijas.2007.s2.821
- 14) Danish, Z., Bhakat, M., Rasool Paray, A., Ahmad Lone, S., Rahim, A., Sinha, R., Ziaullah Danish, C., & Mohanty, T. (2018). Udder and teat morphology and their relation with incidence of sub-clinical and clinical mastitis in Sahiwal, Karan Fries cows and Murrah buffaloes. Journal of Entomology and Zoology Studies, 6(5), 2138–2141.
- 15) Gavan, C., & Riza, M. (2021). Somatic Cell Count in Relation to Udder and Morphometry in Holstein Friesian Dairy Cows. Journal of Agricultural Science and Technology A, 11(1), 47–52. https://doi.org/10.17265/2161-6256/2021.01.003
- 16) Guarin, J. F. i, Paixao, M. G., & Ruegg, P. L. (2017). Association of anatomical characteristics of teats with quarter-level somatic cell count. Journal of Dairy Science, 100(1), 643–652. https://doi.org/10.3168/jds.2016-11459
- 17) Hickman, C. G. (1964). Teat Shape and Size in Relation to Production Characteristics and Mastitis in Dairy Cattle. Journal of Dairy Science, 47(7), 777–782. https://doi.org/10.3168/jds.S0022-0302(64)88763-4
- 18) Hussain, R., Javed, M. T., Khan, A., & Muhammad, G. (2013). Risks factors associated with subclinical mastitis in water buffaloes in Pakistan. Tropical Animal Health and Production, 45(8), 1723–1729. https://doi.org/10.1007/s11250-013-0421-4
- 19) IDF. (2013). Guidelines for the use and interpretation of bovine milk somatic cell counts (scc) in the dairy industry. www.fil-idf.org
- 20) Jerstad, A., Farver, T. B., & Riemann, H. (1989). Teat canal diameter and other cow factors with possible influence on Somatic Cell Counts in cow milk. Acta Veterinaria Scandinavica, 30(3), 239–245.
- 21) Kaur, G., Bansal, B. K., Singh, R. S., Kashyap, N., & Sharma, S. (2018). Associations of teat morphometric parameters and subclinical mastitis in riverine buffaloes. Journal of Dairy Research, 85(3), 303–308. https://doi.org/10.1017/S0022029918000444
- 22) Khanal, T., & Pandit, A. (2013). Assessment of sub-clinical mastitis and its associated risk factors in dairy livestock of Lamjung, Nepal. International Journal of Infection and Microbiology, 2(2), 49–54. https://doi.org/10.3126/ijim.v2i2.8322
- Lund, T., Miglior, F., Dekkers, J. C. M., & Burnside, E. B. (1994). Genetic relationships between clinical mastitis, somatic cell count, and udder conformation in Danish Holsteins. Livestock Production Science, 39, 243–268.
- 24) Mein, G., Reinemann, D., Schuring, N., & Ohnstad, I. (2004). Milking Machines and Mastitis Risk-A Storm in a Teatcup. Proceeding of the National Mastitis Council Annual Meeting, 176–188. https://pdfs.semanticscholar.org/b8a2/6d9e7229ed01984dfd235b5138b3532e34f8.pdf
- 25) Middleton, J. R., Fox, L. K., Pghetti, G., & Petersson-Wolfe, C. (2017). Laboratory Handbook on Bovine Mastitis (J. R. Middleton, L. Fox, G. Pghetti, & C. Petersson-Wolfe, Eds.; 3rd ed.). National Mastitis Council.
- 26) Miles, A. M., McArt, J. A. A., Leal Yepes, F. A., Stambuk, C. R., Virkler, P. D., & Huson, H. J. (2019). Udder and teat conformational risk factors for elevated somatic cell count and clinical mastitis in New York Holsteins. Preventive Veterinary Medicine, 163, 7–13. https://doi.org/10.1016/j.prevetmed.2018.12.010



- 27) Modh, R. H., Nauriyal, D. S., Islam, M. M., Modi, R. J., & Wadhwani, K. N. (2017). Morphological study on types of udder and teats in association with subclinical mastitis in Gir cows. International Journal of Science, 6(4), 2688–2693. https://www.researchgate.net/publication/340583322
- 28) Mukherjee, J., De, K., Chaudhury, M., & Dang, A. K. (2015). Seasonal variation in in vitro immune activity of milk leukocytes in elite and non-elite crossbred cows of Indian sub-tropical semi-arid climate. Biological Rhythm Research, 46(3), 425–433. https://doi.org/10.1080/09291016.2015.1020200
- 29) Neculai-Valeanu, A.-S., & Ariton, A.-M. (2022). Udder Health Monitoring for Prevention of Bovine Mastitis and Improvement of Milk Quality. Bioengineering, 9(11), 608. https://doi.org/10.3390/bioengineering9110608
- 30) Nemcova, E., Stipkova, M., Zavadilova, L., Bouska, J., & Vacek, M. (2007). The relationship between somatic cell count, milk production and six linearly scored type traits in Holstein cows. Czech Journal of Animal Science, 52(12), 437–446.
- 31) Ng, L., Jost, C., Robyn, M., Dhakal, I. P., Bett, B., Dhakal, P., & Khadka, R. (2010). Impact of livestock hygiene education programs on mastitis in smallholder water buffalo (Bubalus bubalis) in Chitwan, Nepal. Preventive Veterinary Medicine, 96(3–4), 179–185. https://doi.org/10.1016/j.prevetmed.2010.06.012
- 32) Nielsen, C. H., Emanuelson, U., Berglund, B., & Strandberg, E. (2009). Relationship between somatic cell count and milk yield in different stages of lactation. Journal of Dairy Science, 92(7), 3124–3133. https://doi.org/10.3168/jds.2008-1719
- 33) Nosirov, B. J., Safarov, M. M., Gaipov, M., Askarkhodjayeva, S. S., Soatov, U., & Javkarashev, K. H. T. (2021). Correlation of udder shape, size and udder size of Bushuev breed of cows with milk yield. E3S Web of Conferences, 244, 1–7. https://doi.org/10.1051/e3sconf/202124402048
- 34) Poudel, S. P., Chetri, D. K., Sah, R., & Jamarkatel, M. (2022). Relationship Between Udder and Teat Conformations and Morphometrics. Journal of Agriculture and Forestry University, 5, 209–217.
- 35) Rupp, R., & Boichard, D. (1999). Genetic parameters for clinical mastitis, somatic cell score, production, udder type traits, and milking ease in first lactation Holsteins. Journal of Dairy Science, 82(10), 2198–2204. https://doi.org/10.3168/jds.S0022-0302(99)75465-2
- 36) Sah, K., Karki, P., Shrestha, R. D., Sigdel, A., Adesogan, A. T., & Dahl, G. E. (2020). MILK Symposium review: Improving control of mastitis in dairy animals in Nepal. Journal of Dairy Science, 103(11), 9740– 9747. https://doi.org/10.3168/JDS.2020-18314
- 37) Sharma, A., Sharma, S., Singh, N., Sharma, V., & Pal, R. S. (2016). Impact of udder and teat morphometry on udder health in Tharparkar cows under climatic condition of hot arid region of Thar Desert. Tropical Animal Health and Production, 48(8), 1647–1652. https://doi.org/10.1007/s11250-016-1138-y
- 38) Sharma Buragohain, T., Das, P. K., Ghosh, P. R., Banerjee, D., & Mukherjee, J. (2017). Association between udder morphology and in vitro activity of milk leukocytes in high yielding crossbred cows. Veterinary World, 10(3), 342–347. https://doi.org/10.14202/vetworld.2017.342-347
- 39) Sharma Buragohain, T., Kumar Das, P., Ghosh, P. R., Banerjee, D., Chandra Das, B., & Mukherjee, J. (2016). Alteration in the in vitro activity of milk leukocytes during different parity in high yielding cross-bred cows. Biological Rhythm Research, 47(4), 519–527. https://doi.org/10.1080/09291016.2016.1153713
- Shinde, A. S., Mote, M. G., Bhoite, S. U., & Bhoite, U. Y. (2020). Relationship between teat biometry and subclinical Mastitis in crossbred cows. Indian Journal of Animal Production and Management, 36(1–2), 84– 90.
- Siagian, T. B., & Amidjaya, S. H. (2022). Correlation between teat length and lactation periods on the level of subclinical mastitis occurrence in Sappy Valley Farm. E3S Web of Conferences, 348, 1–5. https://doi.org/10.1051/e3sconf/202234800031





- 42) Singh, A. K. (2022). A comprehensive review on subclinical mastitis in dairy animals: Pathogenesis, factors associated, prevalence, economic losses and management strategies. CABI Reviews, 17, 057. https://doi.org/10.1079/cabireviews202217057
- 43) Singh, R. S., Bansal, B. K., & Gupta, D. K. (2013). Udder health in relation to udder and teat morphometry in Holstein Friesian × Sahiwal crossbred dairy cows. Tropical Animal Health and Production, 46(1), 93–98. https://doi.org/10.1007/s11250-013-0454-8
- 44) Singh, R. S., Bansal, B. K., & Gupta, D. K. (2017). Relationship between teat morphological traits and subclinical mastitis in Frieswal dairy cows. Tropical Animal Health and Production, 49(8), 1623–1629. https://doi.org/10.1007/s11250-017-1368-7
- 45) Singha, S., Koop, G., Persson, Y., Hossain, D., Scanlon, L., Derks, M., Hoque, M. A., & Rahman, M. M. (2021). Incidence, etiology, and risk factors of clinical mastitis in dairy cows under semi-tropical circumstances in Chattogram, Bangladesh. Animals, 11(8), 2255. https://doi.org/10.3390/ani11082255
- 46) Sinha, R., Sinha, B., Kumari, R., Vineeth, M. R., Shrivastava, K., Verma, A., & Gupta, I. D. (2022). Udder and teat morphometry in relation to clinical mastitis in dairy cows. Tropical Animal Health and Production, 54(2), 1–7. https://doi.org/10.1007/s11250-022-03077-y
- 47) Slettbakk, T., Jorstad, A., Farver, T. B., & Hird, D. W. (1990). Impact of Milking Characteristics and Teat Morphology on Somatic Cell Counts in First-lactation Norwegian Cattle. Preventive Veterinary Medicine, 8, 253–267.
- 48) Tesfay, Y., Abda, S., & Sheferaw, D. (2023). Bovine mastitis: Prevalence, causes and associated risk factors in Silte Zone, Ethiopia. Ethiopian Veterinary Journal, 27(2), 88–103. https://doi.org/10.4314/evj.v27i2.5
- 49) Tiwari, B. B., Subedi, D., Bhandari, S., Shrestha, P., Pathak, C. R., Chandran, D., Pandey, G., Mohankumar, P., & Dhama, K. (2022). Prevalence and Risk Factors of staphylococcal Subclinical Mastitis in Dairy Animals of Chitwan, Nepal. Journal of Pure and Applied Microbiology, 16(2), 1392–1403. https://doi.org/10.22207/JPAM.16.2.67
- 50) Vieira, R. K. R., Rodrigues, M., Santos, P. K. S., Medeiros, N. B. C., Cândido, E. P., & Nunes-Rodrigues, M. D. (2021). The effects of implementing management practices on somatic cell count levels in bovine milk. Animal, 15(4), 100177. https://doi.org/10.1016/j.animal.2021.100177
- 51) Zecconi, A., Hamanno, J., Bronzo, V., Moroni, P., Giovannini, G., & Piccinini, R. (2002). Relationship Between Teat Tissue Immune Defences and Intramammary Infections. In Biology of the mammary gland (pp. 287–293). Springer.
- 52) Zwertvaegher, I., De Vliegher, S., Verbist, B., Van Nuffel, A., Baert, J., & Van Weyenberg, S. (2013). Short communication: Associations between teat dimensions and milking-induced changes in teat dimensions and quarter milk somatic cell counts in dairy cows. Journal of Dairy Science, 96(2), 1075–1080. https://doi.org/10.3168/jds.2012-5636

