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Study on Somatic Cell Count Using Tanuvas SCC Kit in Holstein Friesian Crossbred Cattle in Uttar Pradesh, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The incidence of subclinical mastitis among dairy cattle in Uttar Pradesh shows significant variation across different geographic zones, parities, lactation stages and seasons. Notably, the eastern plain zone experiences the highest prevalence, likely due to its humid climate conducive to bacterial proliferation. In contrast, the north-eastern and western plains report lower incidences. Higher parity cows are at an increased risk, attributed to prolonged exposure to pathogens over successive lactations, while cows in early lactation stages also exhibit heightened susceptibility due to lowered

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immune defences. Seasonal factors further impact mastitis rates, with rainy and summer months intensifying risks through elevated humidity and heat stress. These findings highlight the necessity for region-specific and adaptive mastitis management strategies. The TANUVAS SCC kit serves as a valuable tool for early detection of subclinical mastitis by measuring somatic cell count (SCC), an indicator of udder health and infection levels. Routine SCC monitoring with this user-friendly kit enables timely interventions, reducing the likelihood of severe mastitis outbreaks and subsequent milk production losses.

Keywords: Subclinical mastitis; zones; parity; lactation stage; TANUVAS SCC kit.

1. INTRODUCTION

India holds the number one position in global milk production, consistently producing more milk than any other country. As of recent data, India's milk production exceeds 200 million metric tons annually, contributing to approximately 22% of the world's total milk production (FAO, 2022). This remarkable achievement is driven by a large dairy sector, primarily composed of smallholder farmers, and the popularity of dairy products across the nation. The Indian dairy industry also employs millions of people, making it a critical sector in terms of livelihood, economic contribution, and nutritional value. However, the Indian dairy industry faces significant challenges, one of which is subclinical mastitis (SCM), a condition that affects a large proportion of dairy cows and buffaloes. Subclinical mastitis is an infection of the udder that does not show visible symptoms but leads to an increase in somatic cell count (SCC), which is an indicator of the immune response to infection. Despite its lack of obvious signs, SCM can result in substantial economic losses. Studies have shown that SCM leads to reduced milk yield, lower milk quality, and increased veterinary costs. In India, the economic loss due to SCM is estimated to be significant, with reports indicating that SCM can cause a reduction in milk production of up to 20-30% (Bansal et al., 2017). Additionally, the elevated SCC due to SCM can result in lower market prices for milk, as high SCC is considered an indicator of poor milk quality. Subclinical mastitis also increases the risk of the disease progressing to clinical mastitis, which is more severe and leads to visible symptoms such as swelling, heat, or discharge from the udder. This further exacerbates the economic losses, as affected animals require treatment, and their milk may need to be discarded. The loss from SCM not only affects farmers' income but also hampers the overall efficiency and productivity of the dairy sector in India. Efforts to reduce SCM through improved management practices, early detection, and preventive measures are essential to mitigate these losses and ensure the continued growth of India's dairy industry. Mastitis, particularly in India's dairy sector, leads to substantial economic losses due to reduced milk yield, poor milk quality and increased treatment costs. Visible or clinical mastitis directly impacts productivity by reducing lactation performance and often requires costly veterinary intervention. However, subclinical mastitis, which lacks overt symptoms, is especially detrimental because it goes undetected, silently impacting herd productivity and profitability. Subclinical mastitis primarily causes hidden losses by decreasing milk yield by 10-20% without visible signs. Subclinical mastitis poses a significant challenge for dairy farmers, especially those with Holstein Friesian crossbreeds, as it leads to hidden losses in milk yield and quality without visible symptoms (Kavitha et al., 2009). This condition often goes undetected, reducing productivity and overall herd health. Crossbreeds like Holstein Friesians are particularly vulnerable due to their high milk production demands and sensitivity to environmental and management conditions (Sharma et al., 2011). The TANUVAS SCC kit offers a practical solution, allowing farmers to monitor somatic cell counts (SCC) regularly. Early detection with this kit helps prevent severe infections, minimizing milk losses and maintaining herd profitability and health (Singh et al., 2014). V. Jayalalitha et al. (2020) used TANUCHECKS SCC kit on an on-farm test for quick determination of somatic cell counts which increases in milk samples upon infection or stress of the udder.

2. MATERIAL & METHOD

This study was designed to assess somatic cell counts (SCC) in Holstein Friesian crossbred cattle across diverse agroclimatic zones in Uttar Pradesh, India, utilizing the TANUVAS SCC kit for accurate and reliable SCC quantification. The research encompassed a total of 15 districts, strategically selected to represent the range of climatic and environmental conditions across five distinct agroclimatic zones as Central Plain Zone,Eastern Plain Zone,North Eastern Plain Zone, Tarai Zone, Western Plain Zone. Each zone reflects unique climatic conditions that could potentially influence somatic cell counts udder health and mastitis prevalence in dairy cattle. The study involved 1,401 dairy farmers across 253 villages, carefully selected to ensure representation of smallholder and large dairy operations within each zone. These farmers collectively managed a total of 2,755 Holstein Friesian crossbred cattle (Table 1). The sample size was determined to capture sufficient variability within each agroclimatic zone and account for factors like season, lactation stage and parity.Holstein Friesian crossbred cattle were chosen for their widespread use in Indian dairy farming, particularly due to their high milk yield. Inclusion criteria were the animals with clinically healthy udderat the start of sampling, and those with visible signs of clinical mastitis were excluded to specifically measure subclinical infections.

Data collection and analysis were structured around several key variables, namely:

1. Agroclimatic Zones: As noted, five zones were included to account for varying climatic influences on SCC.
2. Lactation Number: Cows were grouped according to lactation number, from the first lactation to the fifth and above, to evaluate parity effects on SCC. This stratification helps to isolate the impact of aging and cumulative environmental exposure on udder health.
3. Lactation Stage: Cows were categorized into three stages of lactation: Early Lactation (0–90 days post-calving), Mid-Lactation (91–180 days) andLate Lactation (181 days onward)
4. Seasons: Seasonal variation was captured by dividing the year into three seasons: Rainy, Summer and Winter

2.1 Data Collection and Testing Procedure

The study utilized somatic cell count (SCC) testing to assess udder health and subclinical mastitis prevalence in Holstein Friesian crossbred cattle. To ensure consistent and accurate measurements, a performance recorder conducted SCC tests every two months until the end of each cow's lactation period. This interval allowed the tracking of SCC trends over time, providing valuable data on variations associated with lactation stage, seasonal changes, and other factors. Milk sampling and SCC testing were conducted in the morning, following the milk recording and sample collection for milk components. Collecting milk samples in the morning standardized the timing, helping to reduce the impact of diurnal variations on SCC measurements. Proper sample handling was ensured by gently mixing the milk samples before testing to avoid any settling of cells or fat content, which could influence the SCC reading. The SCC measurement was conducted using the TANUVAS SCC kit, which is specifically designed for field-based testing and offers a reliable colorimetric method for estimating somatic cell counts in milk samples (include references using this kit) (Fig. 1). One drop of substrate solution was added to the test vial, followed by three drops of enhancer solution. These solutions are pre-mixed to react with somatic cells in the milk, leading to a color change that corresponds to SCC levels.

Fig. 1. Testing kit

Fig. 2. Microcentrifuge tube

Fig. 3. Substrate solutions

Fig. 4. Enhancer solutions

Fig. 5. Dropper

Fig. 6. Field use

Table 1. Details of farmers, villages, animals and somatic cell count (SSC) Kit

Zone	Farmers	Villages	Animals	SSC Test Count
Central Plain Zone	582	125	1087	3427
Eastern plain	368	56	803	2869
North eastern plain	125	17	319	785
Tarai	155	38	287	754
Western plain	170	16	258	749
Total	**1401**	**253**	**2755**	**8584**

After slowly shaking the milk container to ensure uniform distribution of cells, one drop of milk sample was carefully added to the prepared mixture of substrate and enhancer solutions. The vial was gently mixed and allowed to stand undisturbed for 20–30 minutes to permit full color development. This waiting period enabled the reagents to interact with the somatic cells present in the milk, producing a color intensity directly related to SCC concentration. Following the 20–30 minute incubation, the color was compared with a color grade card provided with the TANUVAS SCC kit. The color grades, available on both the card and the EGP (Enhancer Grade Powder) solution wrapper, offer a visual reference to match the color of the sample, indicating the SCC range. The kit's color grades allow for estimates of SCC levels between 1 lakh to 9 lakh cells per milliliter of milk. These ranges cover typical subclinical mastitis thresholds, allowing for an effective assessment of udder health status.

3. RESULT & DISCUSSION

Preliminary results indicate that SCC varies significantly across zones, lactation stages, and seasons, highlighting the influence of environmental and physiological factors. These findings provide insights into prevalence of subclinical mastitis across different agro-climatic conditions and informed region-specific management strategies to improve udder health in Holstein Friesian crossbreds.

Somatic cell count serves as an essential indicator of udder health, where elevated SCC is often correlated with subclinical mastitis, a key concern in dairy production due to its impact on milk quality and yield (Green et al., 1997).

3.1 Zone Wise Study

Zones are highly significant, indicating that there are significant differences in somatic cell count between the zones (Table 2). The factor zones explain a substantial portion of the variation in somatic cell count, suggesting that geographical or environmental factors may influence the health of the animals in different zones. Testing of milk samples by Somatic Cell Count kit show subclinical mastitis incidence varies across zones, with the eastern plain zone experiencing the highest rates, followed by the central plain-highest as per Table 3 and tarai zones. The lowest incidence rates are observed in the north-eastern and western plain zones. Several factors could explain these regional differences. The eastern plain zone has a hot and humid climate, which creates favourable conditions for bacterial growth, contributing to a higher risk of subclinical mastitis. Additionally, this region may have different management practices, with challenges related to hygiene, bedding quality, and heat management, which further increase susceptibility among crossbred cattle like Holsteins. In contrast, the north-eastern and western plain zones likely benefit from drier or cooler climates that naturally curb bacterial proliferation. Moreover, variations in herd management practices such as milking hygiene and nutrition may also contribute to the lower incidence rates in these areas (Table 2). The elevated somatic cell count (SCC) in Holstein crossbreed cattle in the eastern plain zone of Uttar Pradesh, including Varanasi, Faizabad, and Pratapgarh, is likely influenced by specific management practices, as well as environmental factors like the humid subtropical climate. Higher SCC is often associated with mastitis, a condition that arises from various management issues. Housing and Hygiene of households with Poor hygiene humid conditions, is a major contributor to high SCC. Literature indicates that unclean or muddy bedding fasten bacterial growth, increasing the risk of udder infections and SCC. Studies in tropical regions emphasize that clean, dry bedding and consistent hygiene practices reduce bacterial exposure and SCC (Kavitha et al., 2009). The method of milking and sanitation level during milking also play a crucial role in SCC. Manual milkingis commonly practiced in Uttar Pradesh, varies widely in cleanliness compared to mechanical milking. Research shows that inadequate hand hygiene and sanitation between animals can lead to cross-contamination and higher SCC. Crossbred cattle like Holsteins are often more sensitive to these variations compared to indigenous breeds (Barkema et al., 1999). Lack of essential nutrients or antioxidants weakens cattle immunity, increasing susceptibility to infections and SCC. Shook (1989) found that deficiencies in vitamins A, E and selenium correlate with higher SCC. Access to balanced feed remains challenging in many regions, where pasture management may not be optimal. The hot, humid climate of Uttar Pradesh can also elevate SCC, as heat stress weakens immune response. Studies show Holstein crossbreeds are more vulnerable to heat stress, and inadequate cooling measures can increase SCC levels (Collier et al., 1982). The stage of lactation is a critical factor, as early lactation cows typically experience higher SCC due to metabolic and immunological stress associated with the start of milk production (Mallard et al., 1998). Cows in mid- and late lactation stages often show varied SCC levels based on individual udder health and environmental exposures over time (Mehrzad et al., 2004). Lactation number ranged from first to fifth and above, allowing for analysis of how parity influences SCC. Research indicates that cows in their first and second lactations may experience different SCC dynamics compared to older cows due to their still-developing immune systems and varying milk production levels (Schukken et al., 1989). Higher parity cows often show increased SCC due to repeated exposure to pathogens over multiple lactations and a naturally declining immune response as age increases. Seasonal effects were also recorded, dividing the data into rainy, summer, and winter seasons. SCC levels are known to fluctuate seasonally, with higher counts frequently recorded in winter due to indoor housing and the resulting higher pathogen load in enclosed, damp environments (include reference). In contrast, the rainy season can introduce specific pathogens associated with wet and muddy conditions, which may also elevate SCC (Olde Riekerink et al., 2007).

3.2 Parity Wise Study

Parity number has a significant effect on somatic cell count. This suggests that somatic cell count may vary across different lactations, with higher lactation numbers potentially being associated with higher SCC due to age and potential udder health issues in later lactation (Table 2). The incidence of subclinical mastitis is often higher in second-parity dairy cows due to physiological, immunological and production-related factors that converge at this stage (Table 4). During the second lactation, cows experience an increase in milk production, which places considerable stress on the udder and immune system, potentially leading to a higher susceptibility to infections (Schukken et al., 1989). This increase in milk yield creates a greater demand on the mammary gland, which can lead to more wear and tear on teat tissues, making it easier for pathogens to enter the teat canal and establish infections (Smith et al., 2001). The high metabolic demands associated with increased milk production in second-parity cows can sometimes cause a transient immunosuppression, particularly during the periparturient period (Mallard et al., 1998). This immunosuppression can weaken the cow's ability to resist infections, increasing the likelihood of subclinical mastitis. Cows in their second parity are also at an age where they may not have yet developed full resistance to common mastitis pathogens encountered in the herd environment. Their exposure to these pathogens during the first lactation provides some immunity, but often not enough to prevent reinfection completely in subsequent lactations (Erskine et al., 1998). Monitoring and management practices, such as maintaining good udder hygiene and stress reduction strategies, are essential to help mitigate the risks of subclinical mastitis in second-parity cows, thereby supporting both their health and productivity (include reference).

3.3 Lactation Stage Wise Study

Lactation stage has significant effect over somatic cell count showing that somatic cell count differs depending on the lactation stage (Table 2). This implies that animals in different stages of lactation (early, mid, late) exhibit variations in somatic cell count, which could be due to changes in milk production, immune status, or udder health. The incidence of subclinical mastitis is notably higher during the early lactation period in dairy cows, primarily due to physiological and immunological challenges that cows face post-calving (Table 5). At the onset of lactation, cows undergo significant metabolic stress as their bodies transition from a dry period to milk production. This shift demands extensive energy resources, often leading to a negative energy balance, which can impair immune function and reduce the cow's ability to fight against infections (Goff & Horst, 1997). As a result, cows in early lactation are more susceptible to pathogens that cause subclinical mastitis, such as *Staphylococcus aureus* and *Escherichia coli*. The period around calving is also associated with periparturient immunosuppression. During this time, the cow's immune system is naturally downregulated to prevent rejection of fetal tissues, leading to a temporary reduction in immune function that persists after calving (Mallard et al., 1998). This immunosuppression increases the likelihood of infections in the mammary gland, especially in high-producing cows that are already under metabolic stress.Furthermore, increased milk production and the expansion of the mammary gland at this stage can create microtears in the teat sphincter, allowing pathogens easier access to the udder (Burvenich et al., 2007). The high frequency of milking early in lactation can also stress teat tissue, making it more vulnerable to infection.Effective udder hygiene, nutritional support, and stress management during this critical period can help reduce the incidence of subclinical mastitis in early lactation, promoting better health and productivity outcomes for the herd.

Table 2. Analysis of variance (ANOVA) for somatic cell count

Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Zone	5	6091792	1218358	101.686	< 2e-16	***
Lactation Number	4	205302	51325	4.284	0.00183	**
Lactation Stage	2	99095	49547	4.135	0.01603	*
Season	2	49246	24623	2.055	0.12814	
Residuals	8639	103508667	11982			

Table 3. Zone wise details of somatic cell count

Zone	Somatic Cell Count (1000 X cells/ml)					Total
	0	100	300	500	700	
Central Plain Zone	440(283)	2088(902)	855(376)	43(40)	1(1)	3427(1087)
Eastern plain	12(9)	1584(617)	1152(526)	116(107)	5(5)	2869(803)
North eastern plain	2(2)	664(301)	118(91)	1(1)	()	785(319)
Tarai	27(21)	576(197)	120(113)	30(29)	1(1)	754(287)
Western plain	72(64)	420(159)	232(166)	20(18)	5(4)	749(258)
Total	**553(379)**	**5332(2176)**	**2477(1272)**	**210(195)**	**12(11)**	**8584(2754)**

Table 4. Parity wise details of somatic cell count

Lactation Number	Somatic Cell Count (1000 X cells/ml)					Total
	0	100	300	500	700	
1	68(66)	1177(388)	535(213)	62(19)	0	1844(557)
2	111(61)	1317(655)	556(355)	47(59)	2(2)	2035(937)
3	95(96)	958(695)	431(360)	40(43)	4(3)	1527(951)
4	184(78)	1082(493)	608(287)	41(39)	3(3)	1918(695)
5 & above	553(117)	5332(533)	2477(315)	210(40)	3(3)	8584(711)
Total	**553(379)**	**5332(2176)**	**2477(1272)**	**210(195)**	**12(11)**	**8584(2754)**

Table 5. Lactation stage wise details of somatic cell count

Lactation stage	Somatic Cell Count (1000 X cells/ml)					Total
	0	100	300	500	700	
Early	166(149)	1865(1259)	851(634)	98(97)	3(3)	2983(1782)
Mid	272(208)	1696(1170)	795(571)	46(43)	6(5)	2815(1662)
Late	115(103)	1771(1203)	831(649)	66(64)	3(3)	2786(1633)
Total	**553(379)**	**5332(2176)**	**2477(1272)**	**210(195)**	**12(11)**	**8584(2754)**

3.4 Season Wise Study

season (e.g., summer, winter, rainy) does not have a statistically significant effect on somatic cell count in this dataset (Table 2). However, seasonal effects might still exist but are not strong enough to be detected in this analysis. Subclinical mastitis often shows an increased incidence during the rainy as per the Table 6 season due to several interacting factors that stress the animal's immune system and create an environment conducive to bacterial growth (Table 6). Dairy cows are typically kept indoors for extended periods during winter to protect them from cold temperatures, leading to overcrowded and less sanitary conditions that can promote the spread of mastitis-causing pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Schukken et al., 2003).Cold stress itself can compromise the immune response, as the animal diverts energy toward maintaining body heat, thus reducing its capacity to fight off infections effectively (Green et al., 1997). Furthermore, damp and poorly ventilated winter housing, combined with infrequent bedding changes, can lead to a buildup of moisture, manure and urine, providing an ideal habitat for bacteria that cause subclinical mastitis (Olde Riekerink et al., 2007). The cold also makes maintaining udder hygiene more challenging. With cold water and slow drying times, teats can remain damp, increasing

Table 6. Season wise details of somatic cell count

Season	Somatic Cell Count (1000 X cells/ml)					Total
	0	100	300	500	700	
Rainy	264(176)	2159(1413)	910(720)	81(78)	7(6)	3421(1980)
Summer	140(131)	1827(1128)	894(605)	57(53)	0	2918(1526)
Winter	149(130)	1346(1010)	673(502)	72(71)	5(5)	2245(1458)
Total	**553(379)**	**5332(2176)**	**2477(1272)**	**210(195)**	**12(11)**	**8584(2754)**

Note – numbers in bracket represent number of animals while outside bracket represent somatic cell test count

susceptibility to bacterial invasion through the teat canal (Slettbakk et al., 1990). Additionally, changes in feed quality and intake during winter can impact the cow's nutritional status, which in turn can affect immune strength and make cows more vulnerable to infections (Giesecke et al., 1994). Preventive measures, including maintainence of clean, dry bedding, enhancing ventilation and ensuring proper nutrition, are essential to managing winter mastitis risks. Regular monitoring of somatic cell counts (SCC) can aid in early detection and intervention, reducing the impact of subclinical mastitis on herd health and milk production.

4. CONCLUSION

In summary, zone, lactation number, and lactation stage are significant factors affecting somatic cell count, while the season does not show a statistically significant impact in this model. The incidence of subclinical mastitis in Uttar Pradesh varies significantly across zones, parity, lactation stages, and seasons. The Central as per Table 3 plain zone shows the highest incidence, possibly due to humid conditions that favor bacterial growth, whereas the north-eastern and western plains exhibit lower rates. Higher parity cows are more prone due to prolonged exposure to pathogens, while early lactation stages often see increased susceptibility as immune defenses are lower. Seasonally, the rainy and summer months further exacerbate mastitis risk through increased humidity and heat stress. These factors underscore the importance of tailored strategies for managing subclinical mastitis across diverse conditions. Dairy farmers can use the TANUVAS SCC kit to detect subclinical mastitis early, helping prevent further milk production losses. This user-friendly kit measures somatic cell count (SCC), indicating udder health and infection levels. Regular monitoring with this kit allows timely intervention and reduces the risk of severe mastitis outbreaks.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. **ChatGPT-4**, the latest version of OpenAI's conversational AI model as of 2024.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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