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# Morphometric Evaluation of Subclinical Mastitis in Holstein-Friesian Cows Using Ultrasonography

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**Abstract**

SUBCLINICAL mastitis poses significant economic and production challenges in the dairy industry. This study aimed to evaluate the efficacy of ultrasonography in diagnosing subclinical mastitis by assessing changes in teat parameters and supramammary lymph nodes in 48 Holstein-Friesian cows. Inclusion criteria included a positive California Mastitis Test (CMT) and somatic cell count (SCC) between $200\times10^3$/mL and $500\times10^3$/mL. There were significant difference between (SMLNL, $p<0.000$), (SMLNW, $p<0.000$) and other morphological parameters of teat such as, (TW, $p<0.008$), (TWrF, $p<0.001$), (TC,$p<0.010$), (TWT,$p<0.000$), and ratio's of (TC/TW, $p<0.000$) and (TWT/TW, $p<0.000$) whereas in case of teat canal length there was non-significant difference (TCL, $p<0.110$). Ultrasonographic findings revealed significant differences in teat width, teat wall thickness, and supramammary lymph node dimensions ($p<0.05$) in subclinically affected animals. Ultrasound proved to be an effective and novel tool for morphological assessment and diagnosis of subclinical mastitis, offering a non-invasive method for early detection, management and prognosis of mastitis.

**Keywords:** ultrasound, teat, lymph node, dairy cows, subclinical mastitis.

## Introduction

Livestock farming is the backbone of agriculture in many countries, including Pakistan, where it contributes significantly to the national economy and rural livelihoods. In fact, livestock accounts for 61.9% of the agricultural sector and 14% of Pakistan's GDP [1]. As the fourth-largest milk producer in the world, Pakistan relies heavily on its dairy industry, with over 8 million rural families depending on cattle farming for 35–40% of their income. However, this vital sector faces numerous challenges, one of the most pressing being mastitis—a common and costly disease affecting dairy herds worldwide [3,6,8].

Mastitis, an inflammation of the mammary gland, is broadly categorized into clinical and subclinical forms. While clinical mastitis is easily identifiable due to visible symptoms like swelling, redness, and abnormal milk, subclinical mastitis is more insidious. It often goes unnoticed because it lacks obvious signs of inflammation, yet it subtly reduces milk production and quality, leading to significant economic losses [17,24]. Chronic subclinical mastitis, which persists for months, exacerbates these issues, resulting in increased veterinary costs, reduced milk yields, and premature culling of affected animals [5].

Since the early 2000s, ultrasonography has emerged as a powerful tool in veterinary medicine, offering a non-invasive way to examine the morphological and pathological changes in the mammary gland [11,16,2]. Unlike traditional diagnostic methods, such as the California Mastitis Test (CMT) and somatic cell count (SCC), ultrasound provides detailed images of the udder and teat structures, enabling early detection of abnormalities [13,14,15]. The morphological parameters measured in this study are functionally

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relevant to udder health: teat width and wall thickness affect the teat's structural integrity and resistance to bacterial invasion, while teat cistern dimensions influence milk storage capacity and flow dynamics. Studies have shown that ultrasound can effectively differentiate between healthy and diseased tissues, making it a valuable asset for diagnosing and monitoring mastitis in dairy cows [10,11,18,24, 26,29,30].

Despite its growing use, there is a noticeable gap in research regarding the application of ultrasonography for evaluating supramammary lymph nodes (SMLNs) in cases of subclinical mastitis [20,27]. The SMLN, a key component of the immune response in the udder, undergoes significant changes during infection, yet its role in mastitis diagnosis remains underexplored. Similarly, while ultrasound has been used to assess teat parameters like teat width, teat wall thickness, and teat cistern dimensions [25,23,28], there is limited information on how these parameters correlate with subclinical mastitis.

This study aims to address these gaps by investigating the diagnostic potential of ultrasonography in Holstein-Friesian cows with subclinical mastitis. Specifically, we focus on identifying changes in teat morphology and supramammary lymph node dimensions, using digital image analysis to quantify echogenicity and heterogeneity. By doing so, we hope to provide a deeper understanding of the morphological alterations associated with subclinical mastitis and establish ultrasound as a reliable tool for its early detection and management. Ultimately, this research seeks to contribute to improved dairy herd health, enhanced milk production, and reduced economic losses for farmers.

## Material and Methods

### *Study Design and Animal Selection*

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan. This study was conducted on 48 adult Holstein-Friesian cows from various dairy farms in Lahore , Pakistan, and its surrounding areas. The cows were selected based on specific inclusion and exclusion criteria to ensure a focused investigation of subclinical mastitis. To be included in the study, cows had to meet the following criteria:

- A positive California Mastitis Test (CMT).
- A somatic cell count (SCC) between $200\times10^3$/mL and $500\times10^3$/mL.
- Only one quarter of the udder showing subclinical mastitis. Cows with a negative CMT and SCC below $150\times10^3$/mL were excluded. All selected animals were in their third lactation and not in the late stages of lactation. The cows were housed in loose-housing dairy barns and milked three times daily. The region’s climate is generally warm and dry, with temperatures ranging from 3°C to 45°C annually.

### *Laboratory Tests Milk*

Samples were collected during milking for two primary diagnostic tests: the California Mastitis Test (CMT) and somatic cell count (SCC). The CMT was used to identify subclinical mastitis, while the SCC was measured using the PortaSCC® Quick Test (PortaCheck, Inc., USA). Animals with an SCC below $500\times10^3$/mL were considered positive for subclinical mastitis.

### *Ultrasonographic Evaluation*

A portable ultrasound unit equipped with low-frequency convex (3.5–5 MHz) and high-frequency linear probes was used to examine the affected udder quarters and supramammary lymph nodes [11,14,28]. To ensure accurate imaging, a water bath and contact gel were applied to prevent deformation of the udder and teat during scanning [23]. The following parameters were measured:

**Teat Parameters:** Teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), and teat canal length (TCL).

**Ratios:** TC/TW and TWT/TW.

**Supramammary Lymph Node (SMLN) Dimensions:** Length (SMLNL) and width (SMLNW). Each measurement was taken three times using the ultrasound machine’s built-in software to ensure precision. The supramammary lymph nodes were evaluated based on echogenicity, with the capsule, cortical, and medullary areas clearly demarcated [9,11].

### *Sample Collection and Preparation*

Individual milk samples were collected from each quarter of the udder. Before sampling, the teat ends were cleaned with alcohol and allowed to dry. Foremilk was discarded, and samples were collected immediately afterward. The CMT and SCC tests were performed on each quarter to confirm subclinical mastitis. Ultrasonographic evaluations were conducted in the milking parlor without the use of anesthesia or sedatives.

### *Statistical Analysis*

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (version 23, USA). One-way ANOVA was used to compare parameters between subclinically affected and healthy quarters, while Pearson’s correlation test was applied to assess relationships between variables. A p-value of $<0.05$ was considered statistically significant.

## Results and Discussion

This study demonstrates the potential of ultrasonography as a non-invasive diagnostic tool for subclinical mastitis in Holstein-Friesian cows. By evaluating changes in teat parameters and supramammary lymph node (SMLN) dimensions, we identified significant morphological alterations associated with subclinical mastitis. These findings align with previous research and provide new insights into the diagnostic utility of ultrasound in dairy herd management [11,16,2].

*Teat Morphology and Subclinical Mastitis*

Ultrasonographic evaluation revealed significant changes in teat morphology in subclinically affected quarters. Specifically, teat width (TW), teat width at the rosette of Furstenberg (TWrF), and teat wall thickness (TWT) were all significantly higher in affected quarters compared to healthy ones. These findings are consistent with studies by [25] and [24], who reported similar increases in teat dimensions during subclinical mastitis. The thickening of the teat wall and narrowing of the teat cistern likely reflect inflammatory changes and tissue remodeling in response to infection.

Interestingly, the teat canal length (TCL) showed no significant difference between affected and healthy quarters. This contrasts with some earlier studies suggesting that longer teat canals may predispose cows to mastitis [22] (Martin, 2020). However, our findings support the idea that teat canal length alone may not be a reliable indicator of subclinical mastitis. Instead, other factors, such as teat end shape and sphincter function, may play a more critical role in udder health.

The ratios of teat cistern width to teat width (TC/TW) and teat wall thickness to teat width (TWT/TW) also showed significant differences, highlighting the value of ultrasound in quantifying subtle morphological changes. These ratios could serve as useful diagnostic markers for subclinical mastitis, providing a more comprehensive assessment than individual measurements alone.

*Teat Parameters in Subclinical Mastitis*

A total of 48 subclinically affected udder quarters from Holstein-Friesian cows were evaluated using ultrasonography (Fig. 1). Significant changes were observed in several teat parameters when compared to healthy quarters after treatment ($p<0.05$).

**Teat Width (TW):** The mean teat width in affected quarters was 2.21 ± 0.079 cm, significantly higher than the post-treatment measurement of 2.16 ± 0.077 cm Table 1.

**Teat Width at the Rosette of Furstenberg (TWrF):** This parameter also showed a significant increase in affected quarters (2.08 ± 0.074 cm) compared to post-treatment values (2.02 ± 0.073 cm) Table 1.

**Teat Wall Thickness (TWT):** The teat wall was thicker in affected quarters (1.42 ± 0.065 cm) than after treatment (1.35 ± 0.062 cm) Table 1.

**Teat Cistern Width (TC):** In contrast, the teat cistern width was narrower in affected quarters (0.79 ± 0.045 cm) compared to post-treatment measurements (0.81 ± 0.046 cm) Table 1. Ratios of teat parameters also revealed significant differences:

The **TC/TW ratio** was lower in affected quarters (0.56 ± 0.041) than after treatment (0.60 ± 0.044) Table 1.

The **TWT/TW ratio** was higher in affected quarters (0.643 ± 0.017) compared to post-treatment values (0.625 ± 0.017) Table 1. However, no significant difference was observed in **teat canal length (TCL)** between affected (1.69 ± 0.05 cm) and treated quarters (1.67 ± 0.05 cm) ($p>0.05$) Table 1.

*Supramammary Lymph Node (SMLN) Changes*

A total of 96 supramammary lymph nodes (48 left and 48 right) were evaluated (Fig. 2). The lymph nodes were located 1.5–2 cm below the udder surface, caudal and dorsal to each hind quarter. In healthy animals, the lymph nodes appeared as oval-shaped structures with a thin echogenic capsule, a hypoechoic parenchyma, and a central echogenic hilus. However, in subclinically affected cows, the lymph nodes were enlarged, and the echogenic hilus was less distinct.

**Left Lymph Node:** The mean length before treatment was 5.66 ± 1.15 cm, significantly larger than after treatment (4.63 ± 1.07 cm). Similarly, the mean width before treatment (3.65 ± 1.05 cm) was greater than after treatment (3.07 ± 0.88 cm) Table 1.

**Right Lymph Node:** The mean length before treatment was 4.78 ± 0.58 cm, significantly larger than after treatment (4.03 ± 0.48 cm). The mean width before treatment (2.99 ± 0.59 cm) was also greater than after treatment (2.52 ± 0.50 cm) Table 1. The supramammary lymph nodes (SMLNs) in subclinically affected cows exhibited significant enlargement and altered echogenicity. The loss of the distinct echogenic hilus and the overall hypoechoic appearance of the lymph nodes suggest an active immune response to infection. These findings are consistent with studies by [20] and [27], who reported similar changes in SMLNs during mastitis. The enlargement of lymph nodes likely reflects increased immune activity, as the body attempts to combat the infection.

The strong correlation between lymph node length and width further supports the idea that SMLN dimensions are a reliable indicator of subclinical mastitis. This finding underscores the potential of ultrasound not only for diagnosing mastitis but also for monitoring the immune response and progression of the disease.

These findings indicate that subclinical mastitis is associated with significant changes in both teat morphology and supramammary lymph node dimensions.

*Correlation Analysis*

Pearson's correlation analysis revealed several significant relationships between teat parameters and lymph node dimensions:

**Teat Width (TW):** Showed a strong positive correlation with teat width at the rosette of Furstenberg (TWrF, r=0.998) and teat wall thickness (TWT, r=0.822) Table 2.

**Teat Wall Thickness (TWT):** Positively correlated with the TWT/TW ratio (r=0.701) but negatively correlated with the TC/TWT ratio (r=-0.704) Table 2.

**Lymph Node Dimensions:** The length and width of the left and right lymph nodes showed strong positive correlations with each other (r=0.865 and r=0.837, respectively) Table 2. These correlations highlight the interconnected changes in teat and lymph node morphology during subclinical mastitis Table 2.

*Ultrasound as a Diagnostic Tool*

The results of this study highlight the value of ultrasonography in diagnosing and managing subclinical mastitis [13,2]. Unlike traditional methods like the California Mastitis Test (CMT) and somatic cell count (SCC), ultrasound provides detailed morphological information about the udder and associated structures [10,15,24]. This allows for early detection of subclinical mastitis, even before significant changes in milk composition or yield occur [18].

Moreover, the ability to quantify changes in teat and lymph node dimensions using ultrasound offers a more objective and reproducible approach to mastitis diagnosis [4,12]. This is particularly important in large dairy herds, where early and accurate detection of subclinical mastitis can significantly reduce economic losses.

*Limitations and Future Directions*

While this study provides valuable insights, it has some limitations. The sample size, though adequate, was limited to Holstein-Friesian cows from a specific region. Future studies should include a larger and more diverse population to validate these findings. Additionally, the relationship between teat canal dimensions and mastitis risk warrants further investigation, as this could provide additional diagnostic markers [22,23].

Future research could also explore the use of advanced imaging techniques, such as Doppler ultrasound, to assess blood flow changes in the udder during mastitis [21,27]. Combining ultrasonography with other diagnostic tools, such as bacterial culture and molecular testing, could further enhance the accuracy of mastitis diagnosis and management. The use of artificial intelligence and deep learning algorithms in analyzing ultrasound images could also provide more robust diagnostic capabilities [19,29].

The findings of this study have important practical implications for dairy farmers and veterinarians. By incorporating ultrasonography into routine herd health checks, farmers can detect subclinical mastitis earlier, reducing its impact on milk production and quality. Early intervention can also lower treatment costs and improve animal welfare by preventing the progression to clinical mastitis [4].

## Conclusion

In conclusion, ultrasonography offers a quick, non-invasive method for accurately diagnosing subclinical mastitis. Additionally, it was recommended to perform an ultrasound inspection of the udder and the superficial inguinal lymph node in order to reduce negative changes in milk production and quality, lower treatment costs, improve the animal culling program, and avoid major public health risks.

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*Conflict of Interest*

The authors have declared that there is no conflict of interests regarding the publication of this article.

**TABLE 1. The somatic cell count (SCC), teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), teat canal length (TCL), ratio of TC/TW, ratio of TWT/TW, lymph node length (LNL) and lymph node width (LNW) morphometric evaluation before and after treatment.**

| | Before Rx | After Rx | Sig. |
|---|---|---|---|
| **CMT** | Positive | Negative | |
| **SCC×10³** | 317.18±49.3 | 87.9±16 | 0.000 |
| **TW** | 2.21±0.08 | 2.17±0.08 | 0.008 |
| **TWrf** | 2.1±0.08 | 2.03±0.07 | 0.001 |
| **TWT** | 1.42±0.06 | 1.35±0.06 | 0.000 |
| **CD** | 0.79±0.04 | 0.81±0.05 | 0.010 |
| **CDTWT** | 0.56±0.04 | 0.60±0.04 | 0.000 |
| **TWTTW** | 0.64±0.02 | 0.62±0.02 | 0.000 |
| **TCL** | 1.69±0.05 | 1.68±0.05 | 0.110 |
| **LLNL** | 5.6±1.15 | 4.63±1.07 | 0.000 |
| **LLNW** | 3.66±1.05 | 3.1±0.9 | 0.004 |
| **RLNL** | 4.8±0.6 | 4.03±0.5 | 0.000 |
| **RLNW** | 3±0.6 | 2.52±0.5 | 0.000 |

**TABLE 2. Pearson correlation among, teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), teat canal length (TCL), ratio of TC/TW, ratio of TWT/TW, lymph node length (LNL) and lymph node width (LNW and somatic cell count (SCC).**

| | TW | TWrf | TWT | CD | CDTWT | TWTTW | TCL | LLNL | LLNW | RLNL | RLNW | SCC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TW** | 1 | | | | | | | | | | | |
| **TWrf** | .998** | 1 | | | | | | | | | | |
| **TWT** | .822** | .832** | 1 | | | | | | | | | |
| **CD** | .473** | .453** | -0.112 | 1 | | | | | | | | |
| **CDTWT** | -0.177 | -0.197 | -.704** | .782** | 1 | | | | | | | |
| **TWTTW** | 0.171 | 0.191 | .701** | -.787** | -.999** | 1 | | | | | | |
| **TCL** | -0.113 | -0.099 | -0.076 | -0.081 | -0.011 | 0.01 | 1 | | | | | |
| **LLNL** | 0.096 | 0.125 | 0.028 | 0.124 | 0.059 | -0.062 | .251* | 1 | | | | |
| **LLNW** | 0.184 | .202* | 0.121 | 0.134 | 0.007 | -0.012 | 0.149 | .865** | 1 | | | |
| **RLNL** | .248* | .283** | .329** | -0.078 | -.257* | .260* | -0.163 | 0.201 | 0.156 | 1 | | |
| **RLNW** | 0.041 | 0.066 | 0.188 | -.220* | -.274** | .275** | -.228* | 0.023 | -0.009 | .837** | 1 | |
| **SCC** | .225* | .285** | .417** | -.254* | -.439** | .439** | 0.136 | .395** | .288** | .560** | .397** | 1 |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

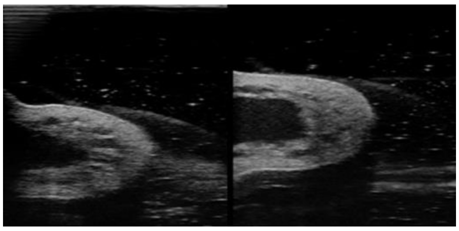

**Fig.1. ultrasonographic evaluation of teat cross-sections showing wall thickness differences.**

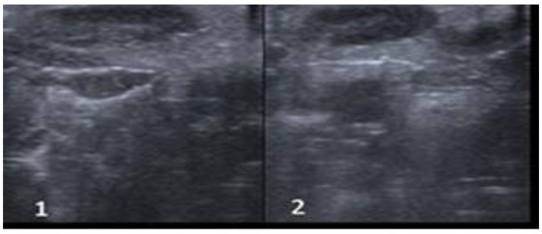


**Fig.2. ultrasonographic evaluation of Supramammary lymph node comparison.**


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