Haryana Vet. (March, 2022) **61(SI)**, 68-71

Research Article

ULTRASONOGRAPHIC CHANGES IN TEAT AND SUPRAMAMMARY LYMPH NODES IN DAIRY COWS AFFECTED WITH CLINICAL MASTITIS

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Received: 10.08.2021; Accepted: 22.11.2021

ABSTRACT

The aim of this study was to examine the ultrasonographic changes in the teat and supramammary lymph nodes in clinically mastitic cows. Thirty clinically mastitic cows presented to the Large Animal Clinics of GADVASU were examined ultrasonographically. Teat measurements such as overall teat diameter (OTD), teat wall thickness (TWT), diameter at level of Furstenberg (FTD), cistern diameter (CD) and teat canal length (TCL) were evaluated. The length and depth of both the supramammary lymph nodes were measured. Milk samples were collected aseptically to analyse bacteriological examination and the milk inflammatory parameters (CMT, pH, EC and SCC). OTD in clinically affected quarters were significantly larger ($p<0.05$) when compared to that of healthy ones. OTD was significantly correlated ($p<0.01$) with TWT and FTD. CMT had a significant correlation ($p<0.01$) with OTD and FTD. The supramammarylymph node had a mean length of 7.80 ± 0.20 cm (range 3.49 - 12.7 cm) and mean depth of 5.35 ± 0.19 cm (range 1.79- 13.4 cm). Significant positive correlation ($p<0.01$) was observed between the dimensions of the lymph node and CMT. The quarters with a higher CMT score had larger lymph nodes. On comparing the lymph node dimensions with the teat measurements, a significantly positive correlation ($p<0.01$) was observed between the length, depth and OTD of the ipsilateral side. The study concludes that there is an increased OTD and TWT with larger lymph node dimensions in mastitic cows.

Keywords: Cows, Mastitis, Supramammary lymph node, Ultrasonography

How to cite: Sunder, H.A., Gupta, D.K., Singh, R.S., Singh, S. and Randhawa, C.S. (2022). Ultrasonographic changes in teat and supramammary lymph nodes in dairy cows affected with clinical mastitis. *Haryana Vet.* **61(SI)**: 68-71.

India is the highest producer of milk in the world, contributing 22% to the world's milk production (Food and Agriculture Organization, 2018). Mastitis is one of the most important production diseases of dairy animals and has a major economic impact on the dairy industry of India. Impacts are not just due to loss of milk production, but also due to the losses through discarded milk and milk product, poor quality of products, veterinary services, treatment cost, extra labour cost and culling costs (Bansal and Gupta, 2009).

Ultrasonography is an excellent, non-invasive and economical technique which aids in the pathogenesis as well as diagnosis of mastitis (Kotb *et al.*, 2014). Ultrasonography helps to visualize the structures of the udder, teat and supramammary lymph node and to observe for the changes brought about by agents that cause mastitis.

Changes in the supramammary lymph node has been attributed to the proliferation of lymphocytes due to presence of a bacteriological organism. Khoramian *et al.* (2015) noted that quarters with a higher California Mastitis Test (CMT) score and SCC count had larger supramammary lymph nodes. This study attempted to observe the changes in teat tissue and supramammary lymph nodes and their relationship with milk inflammatory parameters and to assess the relevance of using ultrasonography as a diagnostic technique in mastitis.

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MATERIALS AND METHODS

The study was conducted from September 2020 to April 2021 at Large Animal Medicine OPD, GADVASU, Ludhiana. The study consisted of 30 clinically mastitic crossbred Holstein-Friesian (HF) cows, which were brought to the university clinic. The quarters were categorized into 3 grades, as healthy quarters which were CMT and bacteriologically negative, quarters without clinical signs (those which had no clinical abnormalities, but were CMT positive and had a presence of bacterial organism) and clinically affected quarters (visible changes in quarters and milk). Teat tissue measurements in relation to quarter health status of clinically mastitic cows was done.

Milk samples were examined for CMT as described by Pandit and Mehta (1969); pH was recorded using pH meter (Mettler-Toledo AG FEP20); EC was estimated with the Electrical Conductivity Meter (Mettler-Toledo AG) and the SCC was estimated using milk somatic cell counter (DELTA Instrument, The Netherland). SCC was estimated in healthy and non clinical quarters but not in clinical quarters as they had abnormal milk. Bacteriological isolation of the milk samples from respective quarters was done as per standard microbial procedures of National Mastitis Council (Brown *et al.*, 1969).

The ultrasound (US) was performed using a portable ultrasound system (Sonosite M-Turbo). The teat measurements were obtained using a 10-5 Mhz linear transducer (L38, Sonosite, serial number: 03RQ5k), using the water-bath technique as per standard protocols (Sendag and Dinc, 1999). The parameters recorded included the overall teat diameter (OTD), teat wall thickness (TWT), diameter at the level of rosette of Furstenberg (FTD), cistern diameter (CD) and teat canal length (TCL) (Fig. 1). Accurate measurements for TWT, CD and OTD, were obtained by measuring 1 cm above the Furstenberg rosette (FTD), the readings were taken prior to milking to ensure accurate readings. The supramammary lymph node was assessed using a convex probe (2-5 Mhz). The lymph node was located on the caudo-dorsal aspect, found between the udder and the thigh as per the description noted by Bradley *et al.* (2001) (Fig. 2 & 3). Isopropyl alcohol was applied at the location after which ultrasound contact gel was placed on the transducer and then the lymph node was scanned. The length and the depth of the lymph node was noted.

A total of 120 teats and 60 supramammary lymph nodes were ultrasonographically scanned to identify the changes brought about by intramammary infection. Independent sample student's t-test was used to compare the measured teat parameters with the milk inflammatory parameters - CMT, pH, EC and SCC. Pearson's correlation was used to observe the correlation between the teat measurements and the milk inflammatory parameters. Spearman's correlation was used to observe the relation between the lymph node dimensions and milk inflammatory parameters. The lymph node dimensions were also correlated with the teat measurements to observe for correlation between them. Data was analysed using SPSS package, version 26 (IBM).

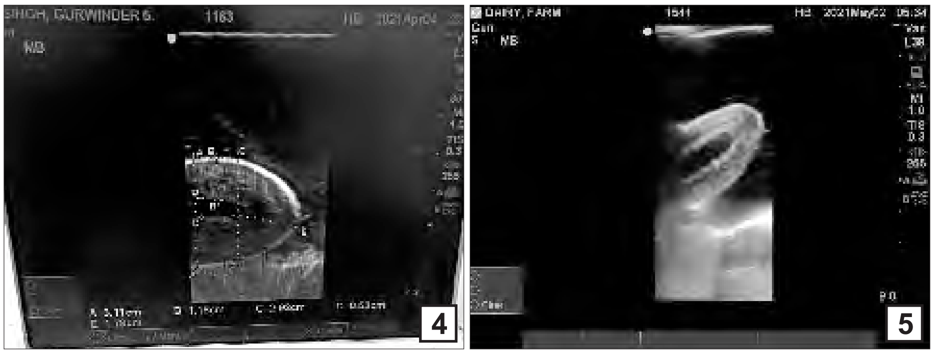
RESULTS AND DISCUSSION

Out of the 120 quarters from the 30 mastitic cows, 62 (51.67%) of the quarters were clinically affected, 18 (15%) quarters had subclinical affection and 40 (33.3%) quarters were healthy. The OTD in clinically affected quarters was found to be significantly higher ($p<0.05$) when compared to that of the healthy quarters (2.98 ± 0.07 vs 2.71 ± 0.08). The TWT and FTD non-significantly ($p>0.05$) increased in quarters without clinical signs and also in clinically affected quarters as compared to the normal ones as observed in Fig. 4. CD and TCL did not vary significantly ($p>0.05$) among the three grades (Table 1). Flock and Winter (2006) reported an increase in the teat wall thickness and changes in the teats of the mammary gland with intramammary infection; they further attributed these changes to the mild inflammation which led to proliferation of tissues and irregularities in the mucosal layer (Fig. 5). In addition, they also reported an increase in teat wall thickness and stenosis of the channel or teat cistern in mastitic quarters.Similar findings were observed in this study.The changes in the FTD could be explained by the findings of Kotb *et al.* (2014) who observed that buffaloes affected with subclinical mastitis had overlapping of the papillary duct leading to an eventual disappearance of the rosette of Furstenberg, possibly due to the damage to the udder parenchyma.

Major pathogens isolated from the milk samples were coagulase positive staphylococci (CS) 48.64% (36/74) and coagulase negative staphylococci (CNS) (40.05%). Other pathogens, isolated were *E. coli* (5.40%) and *Streptococcus* spp. (5.4%). The OTD and FTD significantly ($p<0.05$) increased in quarters affected with CS and CNS, when compared to quarters which had no growth. There was non-significant ($p>0.05$) increase in the TWT in quarters affected with CS and CNS. No significant ($p>0.05$) changes were observed in TCL.

On Pearson's correlation study, it was observed that the OTD had significant ($p<0.01$) positive correlation with TWT ($r=0.224$), FTD ($r=0.711$) and CD ($r=0.571$). TWT had significant ($p<0.01$) positive correlation with OTD ($r=0.224$) as well as FTD ($r=0.262$) along with a significant ($p<0.01$) negative correlation with CD ($r= -0.359$). A non-significant ($p>0.05$) positive correlation was observed between OTD and CMT and EC (Table 2). These findings are similar to the reports of Nak *et al.* (2005) who observed that in the case of the litis, the three-layered appearance of the teat wall was completely replaced, and that all layers of the teat wall had appeared as hyperechoic. The increase in the echogenicity of the teat wall could be associated with the severity of the condition. Venkatesan *et al.* (2017) reported that the teat canal length and teat wall thickness had comparable increase in the entire teat after milking, in both milk flow disorder (MFD) affected and unaffected teats. Nazire and Ismail (2015) reported a strong linear relationship between somatic cell count and teat wall thickness in clinical cases and indicated that teat tissue measurements such as OTD and TWT can be taken as good early indicators for early detection of subclinical mastitis.

A total of 60 supramammary lymph nodes were examined in 30 clinically mastitic cows. They were identified and located according to the descriptions by Bradley *et al.* (2001). The lymph nodes were identified as having a distinct hyperechoic capsule, a hypoechoic cortex with an inner hyperechoic medulla. The mean length of the left lymph node was 7.97±0.42 cm (3.49-12.7), and its



Figs. 1-5. **(1)** Appearance of non-infected teat; A: Overall teat diameter, B: Teat Wall Thickness, C: Diameter at the level of Furstenberg, D: Cistern Diameter, E: Teat Canal Length; **(2)** Appearance of healthy supramammary lymph node (kidney-shaped); **(3)** Appearance of a healthy ovoid supramammary lymph node; **(4)** Marked increase in the overall teat diameter as well as teat wall thickening in clinically affected cow; **(5)** Appearance of sub-clinically affected teat with an irregular inner mucosal layer along with presence of hyperechoic flakes in the teat cistern.

Table 1

Teat tissue measurements in relation to quarter health status of clinically mastitic cows

Type	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)	CMT	pH	EC (mS/cm)	SCC ($\times10^3$ cells /ml)
Healthy (n=40)	2.70±0.08^{a}	0.86±0.09^{a}	2.30±0.07^{a}	1.00±0.08^{a}	1.28±0.06^{a}	0.42±0.13^{a}	6.83±0.05^{a}	5.71±0.18^{a}	40.20±6.30^{a}
Quarters without clinical signs (n=18)	2.84±0.09^{a}	0.94±0.07^{a}	2.31±0.14^{a}	0.93±0.10^{a}	1.29±0.13^{a}	1.33±0.15^{b}	6.95±0.12^{a}	6.92±0.55^{b}	163.4±39.48^{b}
Clinical (n=62)	2.98± 0.07^{b}	0.92±0.03^{a}	2.48±0.05^{a}	1.10±0.08^{a}	1.40±1.10^{a}	2.36±0.39^{c}	6.88±0.03^{a}	7.55±0.22^{b}	-

Values in columns with different superscript differ significantly ($p<0.05$)

Table 2

Pearson correlations among various milk inflammatory parameters and ultrasonographic measurements of clinically mastitic cows

	OTD	TWT	FTD	CD	TCL	CMT	pH
OTD	1						
TWT	0.224*	1					
FTD	0.711**	0.262**	1				
CD	0.571**	-0.359**	0.176	1			
TCL	-0.121	-0.011	-0.084	-0.066	1		
CMT	0.086	0.074	0.126	-0.045	-0.036	1	
pH	-0.217*	-0.047	-0.203*	-0.012	-0.152	-0.039	1

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

mean depth was 5.5 ± 0.42 cm (2.51-9.66). The rightly mph node had mean length of 7.58 ±0.38 cm (3.63-12.2), and depth of 5.1 ±0.38 (1.71- 8.14). On Spearman's correlation, significant ($p<0.01$) positive correlation was found between the length and CMT (r= 0.265) (Table 3). Bradley *et al.* (2001) reported in their ultrasonographic study of fifty-four cattle that the lymph node dimensions ranged from 3.5-15 cm (mean 7.4 cm) for length and 1.2-5.7 cm (mean 2.5 cm) for depth; however those cows were free from any infection. In a herd infected with *Staphylococcus aureus*, Khoramian *et al.* (2015) observed that the mean node length was 9.2 cm (range 5.77-12.9)

Table 3

Correlation of the supramammary lymph node dimensions with milk inflammatory parameters

	Length	Depth	CMT	pH	EC
Length	1				
Depth	0.743**	1.000			
CMT	0.265*	0.217	1		
pH	-0.138	-0.136	0.300*	1	
EC	-0.022	-0.029	0.431**	0.140	1

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

Table 4

Correlation of the supramammary lymph node dimensions with teat measurements

	Length	Depth	OTD	TWT	FTD	CD	TCL
Length	1						
Depth	0.743**	1.000					
OTD	0.360**	0.320*	1				
TWT	0.056	0.175	0.249	1			
FTD	0.277*	0.319*	0.833**	0.222	1		
CD	0.291*	0.176	0.555**	-0.392**	0.380**	1	
TCL	0.152	0.295*	0.084	0.363**	0.316*	-0.162	1

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

and depth was 3.9 cm (range 2.07-6.41); however this study included both infected and uninfected cows. They reported a significant positive correlation between the cumulative scores of CMT in both quarters of each side with ipsilateral supramammary lymph node dimensions (length, $r=0.39$, $P<0.008$ and depth, $r=0.44$, $P<0.001$). In this study also, there was a significant ($p<0.01$) positive correlation between the lymph node dimensions and the CMT scores of the ipsilateral sides ($r= 0.265$). This indicated that the quarters with a higher CMT score had the tendency to be larger in dimension. There was a significant ($p<0.01$) correlation between the lymph node dimensions and the teat tissue measurements such as OTD ($r=0.360$) and FTD ($r=0.277$) (Table 4). This indicated that any insult to the mammary parenchyma was reflected both in the udder, as well the supramammary lymph node, as the same was visualized using ultrasonography.

While this study majorly documented the changes in clinically affected cows, future studies in cows with sub clinical mastitis could help reveal the potential utility of Udder and Teat Ultrasound as a possible early diagnostic/screening tool, even at subclinical stages and for appropriate early interventions.

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