Indian J. Vet. Med. Vol. 42, No. 1, 2022 pp. 33-39

Ultrasonographic evaluation of udder and teats in bovine clinical mastitis

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Abstract

The experiment involved ultrasonographic evaluation of teats and udder in natural cases of mastitis in dairy cows. A total of 35 clinical mastitis cows with 125 functional quarters were scanned at the clinic. The measurements of teat were calculated later on using Image J software. Out of 125 functional quarters from mastitic cows, 28 (22.4%) quarters were healthy, 49 (39.2%) were non-clinical (with no visible signs but positive for tests) and 48 (22.4%) quarters were clinically affected (with visible signs in udder and milk). A total of 15 quarters were fibrosed, 10 with udder edema, one each with abscess and teat obstruction, and remaining with milk abnormality only. The Log_{10} SCC and California Mastitis Test (CMT) score were found to be significantly increased in clinical and non-clinical quarters in comparison to healthy ones. When correlation among milk inflammatory parameters and echogenic changes in teats was evaluated, Log_{10} SCC was significantly ($p<0.05$) correlated with teat wall thickness (TWT) ($p<0.01$) and negatively with cistern diameter (CD) ($p<0.05$). However, CMT score was significantly correlated with TWT ($p<0.01$) and teat diameter at the level of the Furstenberg rosette (FTD)($p<0.05$). The teat wall thickness (TWT) and cistern diameter (CD) differed significantly ($p<0.05$) in clinical quarters as compared to healthy ones.

Keywords: Ultrasonography, Cow, Clinical Mastitis, CMT, SCC

Dairy industry in India has become an important sector in the agricultural economy and milk is the largest agricultural commodity, which valued more than the total value of paddy and wheat together contributing 17.4 per cent to the GDP according to economic survey 2017-18 (http://mofapp.nic.in:8080/economicsurvey/). India, the world's largest milk producing country with 22 per cent of global production, yielded about 165.4 million tonnes of milk per annum (2016-17). The major threat to dairy industry is mastitis, which causes huge economic losses to the industry and serious hazards for public health (Lee *et al*., 2005). In India, the annual economic loss due to mastitis has been calculated to be Rs. 7165.51 crores. At Punjab level it was calculated to be Rs 561 crores (Bansal and Gupta, 2009). Therefore, speedy and confirmatory diagnosis and prognosis are very important. Here lies the significance of the usage of modern, accurate and quick methods for mammary gland examination.

At field level mainly symptoms of mastitis are observed and CMT is performed to diagnose mastitis. This can further be supplemented with ultrasonography. Ultrasonography, a quick, non-invasive method for the diagnosis, can visualize udder and teat abnormalities such as abscess, hematoma, udder edema, fibrosis etc. which may go unnoticed or may be difficult to diagnose on physical examination. Udder and teat scanning is generally performed for diagnosis of milk flow disturbances but also is increasingly used for examination and measurement of different anatomical structures (Bobic *et al.,* 2014 and Strapak *et al.,* 2017). Ultrasonographic scanning of the teat is used primarily in cows and sheep for the diagnosis of obstructions, stenoses, and the rosette of Furstenberg, fibrous changes in the area of the teat canal, or the boundary between the teat and gland cisterns (Stadnik *et al.,* 2010, Szencziova and Strapak 2012 and Bobic *et al.,* 2014). Also, ultrasonography is more objective than glandular palpation and can be used in diagnosing both clinical and subclinical mastitis (Franz *et al.,* 2009). Practically, analysis of real time ultrasound images is an arduous task, so software (Image J) was used for the first time to analyze the stored images. Keeping the above facts in view, the present study was envisaged to evaluate the ultrasonographic changes in udder and teats of dairy cows affected with clinical mastitis.

Materials and Methods

A total of 35 lactating cows with clinical mastitis (140 quarters including apparently normal and clinically affected) presented to the University clinic from June 2018 to May, 2019 were scanned. The ultrasonographic changes in udder and teats of these cows were noted by scanning of all the four teats and udder parenchyma. The

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quarters were categorized as clinical (with visible signs in udder and milk), non-clinical (with no visible signs but positive for tests) and healthy (neither visible signs nor positive for tests).

The scanning was performed with a portable ultrasound scanning system (Sonosite M-Turbo) using a 10-5 MHz linear transducer (L38, Sonosite, serial number: 03RQ5K). The water bath technique (Franz *et al*., 2009) or cup method (Fig. 1) was used for sonography of the teat structures. It was done by immersion of the teat in a plastic container filled with warm water (30–35°C), with the transducer placed in contact with the container. A layer of gel was applied on the probe head to enhance contact with the plastic glass. The probe was manipulated vertically until a clear image of teat appeared on the ultrasound screen. When the picture was obtained, the image was frozen on screen. A vertical cross-section of teat was scanned and stored in scanning device (Singh 2012). All four teats were scanned and teat dimensions such as teat canal length (TCL), teat cistern diameter (CD), teat wall thickness (TWT), overall teat diameter (OTD), the teat diameter at the level of the Furstenberg rosette (FTD) and changes associated with teats were observed. Each quarter was also scanned placing the probe directly on the skin surface using gel as medium (Fig. 2)

Quarter foremilk samples were collected aseptically in sterilized labeled test tubes, kept in ice box and immediately transferred to the laboratory. The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council (1990). Milk tests such as BTB card test, CMT (Pandit and Mehta 1969), pH and EC (Mettler Toledo), SCC of non-affected quarters (Delta instruments, Netherlands) and manually for clinically affected quarters by direct microscopic method were also performed.

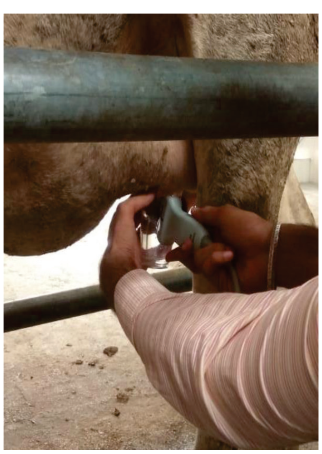
Fig 1. USG of teat (cup method)

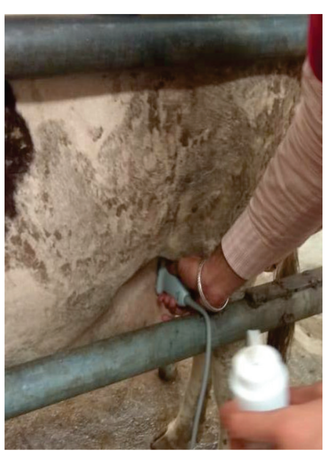
Fig 2. USG of udder

Definition of quarter health status: The health status of non-affected quarters was assessed and defined on the basis of bacteriology and SCC of quarter foremilk samples using IDF criteria as described below:

Milk SCC (cells/ml)	Microbial pathogen	
	Not detected	Detected
≤ 400 000	Healthy	Latent infection
> 400 000	Nonspecific mastitis	Specific mastitis

Use of software for evaluation of USG images: All teat scans were stored on the integrated flash drive in JPEG format and transferred to a computer with a USB for image processing. Images were evaluated and measurements were performed by using an open source software program (Image J, National Institutes of Health, Bethesda, MD). Wieland *et al.* (2018) used the same software for measuring teat canal dimensions and found no significant difference between measurement methods. We also standardized this technique and found similar findings.

Statistical analysis: Data was analyzed using SPSS package (IBM, SPSS version 25, 2017). Descriptive statistics were performed using frequency as well as mean and standard error (SE) to describe numeric data. Independent samples t test was used to compare the mean of normally distributed numeric variables. Correlation of milk inflammatory parameters and ultrasonographic udder and teat parameters were analyzed using Pearson's correlation co-efficient for numeric variables. The SCC values were converted to Log_{10} to achieve normality.

Results and Discussion

A total of 35 animals with 125 functional teats presented to Large Animal Clinical complex were ultrasonographically scanned. Out of these, 28 (22.4%) quarters were healthy, 49 (39.2%) were non-clinical (no visible signs but affected with specific subclinical mastitis) and 48 (22.4%) quarters were clinically affected (Table 1).

Staphylococci have been reported as a predominant organism for causing mastitis by several workers Saidi *et al.* (2013), Marimuthu *et al.* (2014) and Alemu *et al.* (2014), Biressaw and Tesfaye (2015), Seid *et*

Table 1: Distribution of quarters in clinically mastitic cows

Health status	N	Percentage	LF	LH	RF	RH	Fore quarters	Hind quarters
Healthy	28	22.40	7	6	6	9	13	15
Non-clinical	49	39.20	8	16	14	11	22	27
Clinical	48	38.40	13	10	12	13	25	25
Blind	15	-	7	3	3	2	10	5
Total	140	-	-	-	-	-	-	-

Table 2: Overall distribution of organisms in clinical mastitis cases

Organism	Frequency	Quarters affected			
		LF	LH	RF	RH
No growth (NG)	81	18	20	18	25
Coagulase positive staphylococci (CS)	16 (16/44; 36.36%)	4	4	6	2
Coagulase negative staphylococci (CNS)	21 (21/44; 47.72%)	5	6	5	5
Streptococcus spp.	3 (3/44; 6.81%)	0	1	2	0
Corynebacterium spp.	3 (3/44; 6.81%)	0	1	1	1
Pseudomonas spp.	1 (1/44; 2.27%)	1	0	0	0

al. (2015), Mekonnin *et al.* (2016) which is similar to our results where *Staphylococcus* is major pathogen causing the disease. Out of all the organisms isolated, coagulase negative *Staphylococci* (CNS) alone comprised 47.72 per cent (Table 2). This is in agreement with findings of Prasad (1997) where out of 133 bacteriologically positive milk samples, CNS were the predominating isolates, followed by *S. aureus* and then *Streptococci* spp.

In the study conducted by Lalrintluanga *et al.* (2003), the organisms isolated included CNS (55.10%), Streptococci (22.45%), *S. aureus* (7.14%), *Corynebacterium* spp. (6.12%), *E. coli* (3.06%), *Proteus* spp. (2.04%), *Klebsiella aerogenes* (2.04%) and *Citrobacter* spp. (2.04%) isolated from the milk samples, which favours our results however *E.coli*, *Klebsiella aerogenes* and *Citrobacter* were not found as causative agents in our case.

Corneybacterium spp. in our cases contribute only 6.81 per cent (Table 2), which is in close agreement with results found by Sumathi *et al.* (2008) which showed lower prevalence while absence of *Corneybaterium* spp. was revealed by Amin *et al.* (2011) and Saidi *et al.* (2013).

The quarters with non-clinical (1.225±0.124) and clinical (2.270±0.129) mastitis were found to have significantly ($p < 0.05$) more CMT score as compared to healthy (0.178±0.046). The difference between clinical and non-clinical was found to be significant. Similarly, the BTB card score increased significantly in clinical (2.125±0.125) and non-clinical (0.714±0.123) quarters when compared to healthy ones (0.142±0.111). The BTB score was also significantly more in case of clinical mastitis as compared to non-clinical (Table 3). The EC also showed the similar trend.The mean pH (7.049±0.047) for clinically affected quarters was recorded to be significantly more than both non-clinical (6.732±0.035) and healthy (6.634±0.049) quarters. The difference between healthy and non-clinical quarters was found to be non-significant. The Log_{10}SCC was found

Table 3: Milk parameters in relation to udder health status of clinical mastitic cows

	BTB	CMT	EC (mS/cm)	pH	Log_{10} SCC
Healthy	0.14 ± 0.11^{a}	0.18 ± 0.05^{a}	5.25 ± 0.21^{a}	6.63 ± 0.05^{a}	5.00 ± 0.08^{a}
Non-clinical	0.71 ± 0.12^{ab}	1.225 ± 0.12^{ab}	6.58 ± 0.30^{ab}	6.73 ± 0.04^{b}	5.83 ± 0.10^{ab}
Clinical	2.13 ± 0.13^{ab}	2.27 ± 0.13^{ab}	7.98 ± 0.34^{ab}	7.05 ± 0.05^{ab}	6.30 ± 0.12^{ab} (n = 34)

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

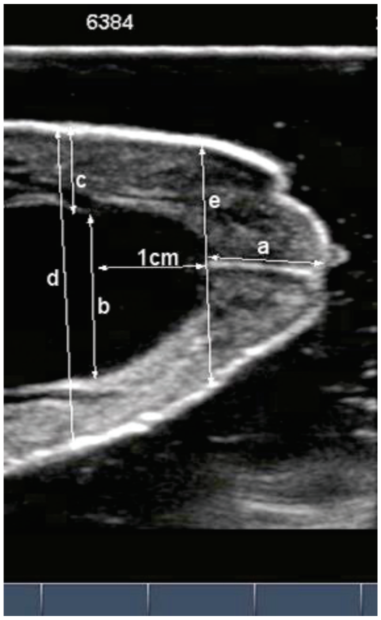

Fig. 3. Ultrasound image of a normal teat
a - Teat canal length (TCL), b - Cistern Diameter (CD), c - Teat wall thickness (TWT), d - Overall Teat diameter (OTD) and e - Teat diameter at the level of Furstenberg's rosette (FTD)

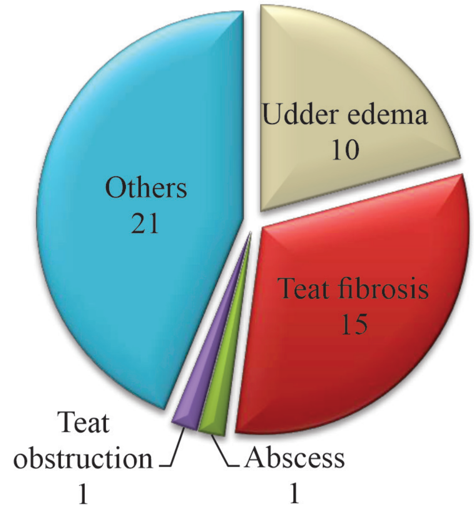

Fig. 4. Affections observed in clinically infected quarters

to be significantly increased in clinical and non-clinical quarters in comparison to healthy ones. The Log_{10} SCC values were also observed to be significantly ($p < 0.05$) different between clinical and non-clinical quarters.

In our study, there was significant increase in EC in clinical and non-clinical cases which is in agreement with Pyorala (2003) and Raj (2010). The increased EC value could be due to increased sodium and chloride concentration due to infection. Also, in our study, it was found that there was significant increase in CMT score and Log_{10}SCC in clinical and non-clinical quarters as compared to healthy ones which were in accordance with various researchers (Mahendra and Dang 2001, Raj 2010).

There were various clinical affections which could be detected ultrasonographically. During the study, 15 quarters were fibrosed and ten quarters were presented with udder edema. Abscess and teat obstruction was observed in one case each and the remaining clinical cases included teats or udder tissue which appeared normal externally and did not have any teat or udder swelling but the secreted milk was not normal (Fig.4).

On comparing the teat tissue changes in quarters of clinical mastitis cases, it was noted that there was a significant increase in TWT for both clinical (0.813±0.036) and non-clinical (0.818±0.044) as compared to healthy quarters (0.674±0.041). The remaining teat measurements TCL, CD, OTD and FTD had no significant difference between the groups (Table 5). However, CD was observed to be reduced in clinical mastitis quarters. The variation in CD and TWT indicate the role of infection to ensue the tissue changes. Singh and Singh (1994) and Uppal *et al.* (1994) stated that the longer teat canals and a stronger teat sphincter were the important reasons for a lower incidence of mastitis in buffaloes than in cows.

In this study, TCL was found to be more in clinical and non-clinical teats as compared to healthy ones but the difference was non-significant (Table 4). Raj (2010) found that the mean values of teat canal length of cows with diseased teat condition showed a statistically significant difference from the mean values of infected teats. Similar

Table 4: Teat tissue measurements in relation to quarter health status of clinical mastitis cases

	TCL (cm)	CD (cm)	TWT (cm)	OTD (cm)	FTD (cm)
Healthy(n=28)	0.98±0.06	1.29±0.12	0.67±0.04[a]	2.65±0.10	1.90±0.06
Non-clinical(n=49)	1.06±0.04	1.15±0.10	0.82±0.04[a]	2.75±0.07	2.03±0.04
Clinical (n=48)	1.08±0.04	1.13±0.10	0.81±0.04[a]	2.75±0.07	2.03±0.05

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

Table 5: Pearson correlations among various milk inflammatory parameters and ultrasonographic measurements

	BTB	CMT	EC	pH	Log_{10} SCC	TCL	CD	TWT	OTD	FTD
TCL	.126	.115	.099	-.055	.115	1				
CD	.033	-.158	-.122	.033	-.209[b]	-.167	1			
TWT	.101	.282[a]	.170	.053	.311[a]	.376[a]	-.726[a]	1		
OTD	.031	.018	-.055	-.053	-.031	.208[b]	.444[a]	.012	1	
FTD	.096	.219[b]	-.019	-.067	.143	.441[a]	.163	.309[a]	.721[a]	1

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

observations were recorded by Querengasser *et al.*(2002). Also, teat measurement data were in accordance with the findings of Cartee *et al.*(1986), Nudda *et al.*(2000), Franz *et al.* (2001), Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006).

It was observed in our study that there was significant increase in TWT of clinical and non-clinical mastitic teats as compared to healthy teats. This is in accordance with Zecconi *et al.,*(1992 and 1996) who showed an increase of teat thickness of over five per cent after milking is associated with an increased incidence of intramammary infection. The findings were in accordance to the findings of Nudda *et al*., (2000), Querengasser *et al.,* (2002) and Flock and Winter (2006).There was non-significant reduction in CD. These findings were contrary to the findings of Nudda *et al.*(2000), Querengasser *et al.* (2002) where the change was significant.

The correlation among milk inflammatory parameters and sonographic changes in teats was evaluated (Table 3). CMT score was significantly

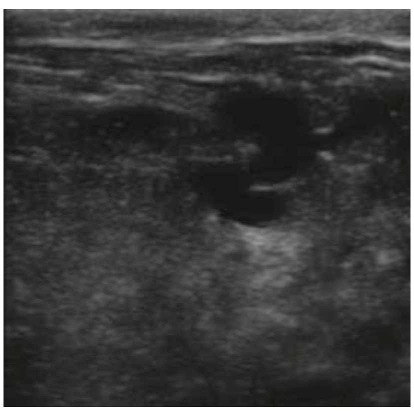

Fig. 5. Normal udder sonogram

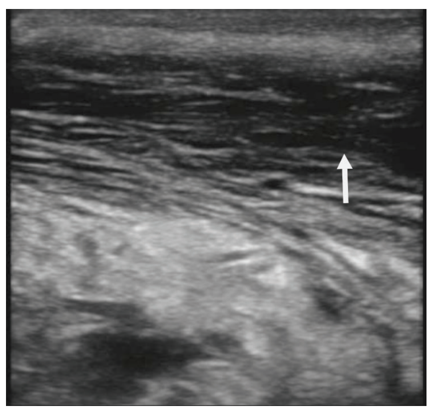

Fig 6. Ultrasonographic changes In udder edema

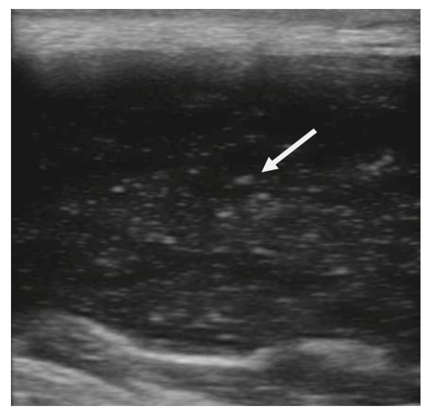

Fig 7. Ultrasonographic changes in abscess

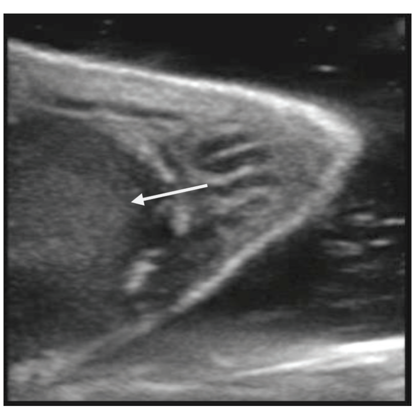

Fig. 10. USG of teat obstruction

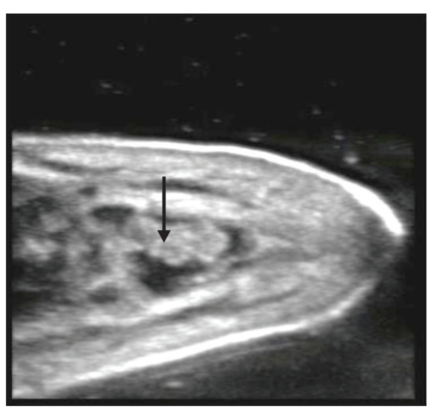

Fig. 11. USG of teat fibrosis

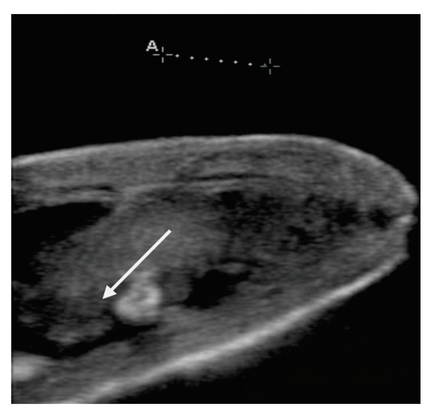

Fig. 12. USG of teat with abnormal milk

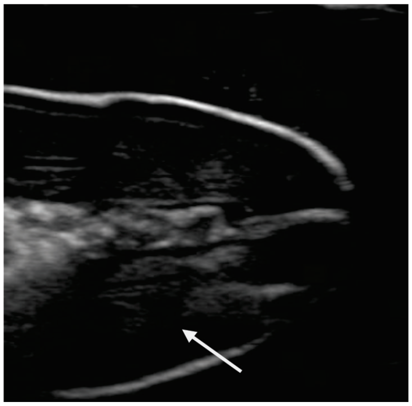

Fig. 13. USG of inflamed teat

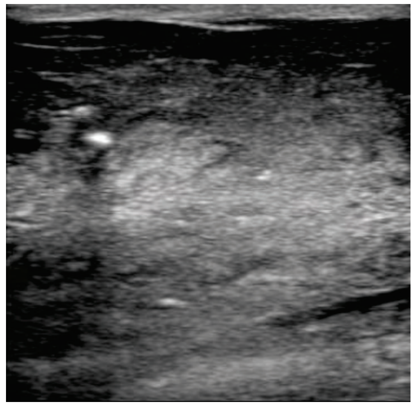

Fig 8. Ultrasonographic view of fibrosed udder

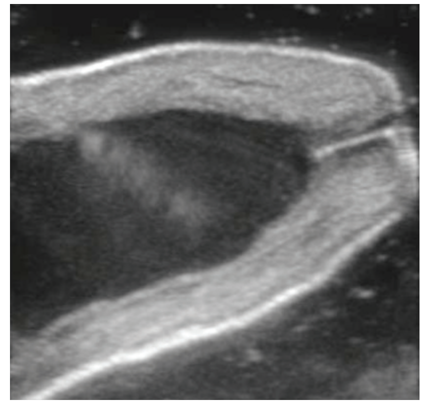

Fig 9. Normal teat ultrasonogram

correlated with TWT ($p<0.01$) and FTD ($p<0.05$). CD was negatively correlated with CMT score but statistically non-significant. Log_{10} SCC was significantly negatively correlated with CD ($p<0.05$) and TWT ($p<0.01$). This indicates a smaller CD and higher TWT in quarters with CMT score and SCC values (Gulyas and Ivancsics 2001). The echographic measurements did not correlate significantly with BTB, EC and pH.

Various udder and teat affections can be diagnosed in clinical mastitis cases ultrasonographically immediately at field level. Echographic measurements of teat (TWT and CD) were well correlated with milk inflammatory parameters so, they can be taken into consideration while assessing for infection.

Acknowledgement

The authors thank the Director of Research, GADVASU and Head, Department of Veterinary Medicine, for providing the necessary facilities.Thanks are also due to the farmers for providing the test subjects and assistances during this study.

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Received : 27.03.2022
Accepted : 29.05.2022