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Pioneering Precision In Dairy Health: A Novel Approach To Bovine Mastitis Assessment Through B-Mode And Doppler Ultrasonography

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Abstract
This study aimed to evaluate the effectiveness of ultrasound in detecting changes in teat and supra mammary lymph node parameters in dairy cows with mastitis. The study focused on teat width, teat wall thickness, teat canal length, and supra mammary lymph node size, using ultrasound to scan 48 udder quarters and 28 lymph nodes of sub-clinical, and clinical groups respectively. The findings revealed that ultrasonography can identify clinical mastitis and provide valuable additional information on the udder's condition. Mastitis caused a considerable thickening of the teat wall and a loss of anechoic zones in the cistern. In contrast, the lymph nodes on the mammary gland's bacteriologically positive side were significantly larger and more hypoechogenic than those on the opposing side. The study concludes that ultrasound can be a useful tool in diagnosing mastitis in dairy cows and in evaluating the health of the mammary gland.

Keywords: Mastitis; Ultrasonography; Teat parameters; Lymph nodes; Dairy cows

Introduction:

Livestock is the primary component of our agriculture sector (61.9%) and Pakistan's GDP (14%). The fourth-largest producer of milk in the world is Pakistan. More than 8 million rural families depend on raising cattle for 35 to 40% of their income (Ahmad et al., 2020) (Ahmad and Ma, 2020). Mastitis is frequently regarded as one of the most common and economically significant production illnesses globally, particularly in dairy herds. Clinical and subclinical mastitis are standard terms used to describe intramammary infections (Cobirka et al., 2020)

Mastitis, or mammary gland inflammation, is mainly caused by bacterial infections. Mastitis is more common in animals that produce milk for dairy products. Climatic circumstances, seasonal change and farming techniques all impact the disease's incidence and aetiology. Mastitis in cattle is a contagious and inflammatory disease of the mammary gland. It is the most common and expensive disease of dairy cattle. Early diagnosis and timely treatment of the disease are essential parts of its treatment. Mastitis impacts labour and replacement expenses, lower lactation persistence, premature culling, milk quantity and quality (Cheng and Han, 2020). To produce milk of the highest standard, contemporary animal husbandry prioritizes maintaining ruminant mammary gland health. At dairy farms, disturbances that reduce milk output are a big issue. Various mastitis forms result in losses and poor modifications to the milk's quality. The adverse economic impact also includes higher healthcare costs and the early euthanasia of animals (Marchant-Forde and Boyle, 2020).

The text discusses intramammary infections (IMIs) categorized as subclinical or clinical mastitis. Subclinical mastitis denotes hidden infections lacking visible inflammation, while chronic infections last over two months. Clinical mastitis involves inflammation, leading to abnormal milk and potential udder changes. Severity ranges from local symptoms to systemic effects. Subclinical mastitis, despite lacking apparent signs, decreases milk quality and output, leading to economic losses due to lowered production, higher medical costs, and premature culling. Chronic infections persisting for two months are also highlighted (Argaw, 2016)(Argaw, 2016).

Due to its growing significance in veterinary medicine, ultrasound is one of the non-invasive methods for the morphological and pathological examination of the mammary gland. (Fasulkov et al., 2018b); (Stan et al., 2020). Imaging techniques of ultrasound scans provide a precise measurement of the udder and teats to assess the various changes occurring within the mammary gland. In dairy animals, mammary gland ultrasound has been used to distinguish between distinct structures found in the udder parenchyma, describe normal and pathological changes in the morphology of the udder and teats, to diagnose and monitor pathological mammary abnormalities. (Themistokleous et al., 2022); (EZ Kotb et al., 2020). Conventional ultrasonographic imaging enables the repetitive and safe assessment of diverse interior organ areas. The use of this approach to assess the mammary gland has been tested in a variety of animal species of veterinary interest. The Doppler method estimates vascular parameters of blood flow, which can help in the differential diagnosis of primary mammary gland diseases (Samir et al., 2021).

Doppler ultrasonography determines the speed and direction of a moving body. This is most typically used in veterinary medicine to describe blood flow. The word "doppler effect" was invented by Christian Doppler, who defined the phenomenon in 1842(Kaveh et al., 2022). The effect is the shift in frequency of a wave caused by its contact with a moving object. If an ultrasonic wave reflected off a stationary object returns to the transducer at the same frequency it delivered, the wave is said to be reflected.

The extent of mastitis and modifications in supra mammary lymph node (SMLN) size was linked by ultrasound, and other morphological parameters were established. These parameters included teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), and teat canal length (TCL). Additionally, if the wave is reflected from a moving object, the frequency of the returning echoes changes. The ultrasound equipment may detect this change in across-the-board quantity and show it as colour pixels or in graphical representation (Khoramian et al., 2015). Areas of aberrant or turbulent blood flow related to cardiac abnormalities can be visualized by mapping the velocity and direction of blood flow. Flow pressure variations can be predicted by measuring blood velocity inside heart chambers. Doppler Shift is a shift in frequency that may be detected by ultrasound equipment and presented as colour pixels or in graphical representation. When doing abdominal ultrasonography or echocardiography, the Doppler Effect might offer extra information (Garcia et al., 2019). Doppler ultrasonography may be used to examine blood flow to the udder, with the discovery that ultrasonographic parameters related to blood flow to the udder increased with milk supply. Pulsed wave Doppler ultrasonography is used to measure Time-Averaged Maximum Velocity (TAMV), blood flow volume, Systolic Peak Velocity (SPV), end-diastolic velocity (EDV) and Resistant Index (RI) in udder vasculature (Oglat et al., 2018).

As a result, the goal of this study was to see if B-mode and Doppler ultrasonography could help with the diagnosis and prognosis of mastitis and proves themselves as practical imaging and morphological assessment tool for healthy and mastitic animal. Future research should look at the effect of udder blood flow on lactation in cows.

Material and Methods:

Ethical approval (DAS/440, 03-02-2022) was sought from the Advanced Studies and Research Board (ASRB) at the University of Veterinary and Animal Sciences in Lahore, Pakistan. The ASRB waived approval due to procedures being routine and carried out by qualified veterinarians.

Forty-eight udder quarters and ninety-six supra mammary lymph nodes of adult dairy cows were subjected to each group among two groups of sub-clinical and clinical, respectively. The Holstein-Friesian was chosen due to their highest milk production than other breeds and the availability of Holstein-Friesian around the globe (Strączek et al., 2021). They were kept under identical conditions, fed TMR twice a day, and milked twice daily by an automatic milking system. Inclusion criteria: All animals grouped based on (1) CMT and SCC, (2) adult dairy cows (3rd lactation), (3) the animal must be in between lactation (60 days after parturition and before drying off), and (4) animal included in a sub-clinical and clinical study must have only one affected quarter.

This study was conducted in private livestock farms focusing on milk production in the Lahore district (31.5204° N, 74.3587° E), Punjab, comparing outcomes of three stages of Holstein-Friesian dairy cows. The research encompassed 48 cows in their third lactation stage, subjecting udder quarters and lymph nodes to healthy, sub-clinical, and clinical groups. Criteria included CMT and SCC classifications, third lactation stage, specific lactation period, and single affected quarters. Diagnosis was conducted by a Veterinary Physician using CMT and SCC tests. The study aimed to analyze mastitis impacts and was performed under controlled conditions with standardized feeding and milking practices. The experiments were conducted in private livestock farms committed to milk production under a semi-intensive production system.

Ultrasonographic scanning of the teats was carried out in a water-filled plastic cup for 30 min before milking with a 7.5 MHz linear array transducer. The same clinician performed each examination and measurement. The water in the cup was renewed after each use. The teats were carefully and slowly submerged in the water. To acquire clear images, contact gel (Konix®, Turkuaz medikal kozmetik, Istanbul) was applied to both the transducer and cup. Ultra-sonographic morphometry was done in forty-eight clinically affected teats, and forty-eight supra mammary lymph nodes from forty-eight dairy cows must be in their third lactation. Teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), and teat canal length (TCL), as well as supra mammary lymph node size (SMLNL, SMLNW), were scanned.

The study involved ultrasound measurements on the udder's mammary artery using different Doppler techniques. The probe was positioned near the udder's entry into the abdomen to locate the artery. Color mapping aided in identifying the artery's position. The spectral Doppler utilized an angle below 60° between the sound beam and artery. Power Doppler enhanced flow sensitivity and changed angle independence. A 2-3mm gate was placed on the vessel for waveform analysis. Parameters like Time-Averaged Maximum Velocity (TAMV), blood flow volume, Systolic Peak Velocity (SPV), end-diastolic velocity (EDV), and indices such as vascular resistance (RI= [SPV-EDV]/SPV), and pulsatility (PI= [SPV-EDV]/mean velocity) were measured using pulsed wave Doppler ultrasonography, with values generated automatically by software.

Any abnormalities in secretions, size, consistency, or temperature of all milking cows in the selected farms were thoroughly evaluated. Mastitis was characterized by palpatory pain, milk alterations (blood-mixed milk, watery discharges, flakes, pus), and udder integrity variation. Udder health was assessed by examining the udder; California Mastitis Test and Somatic cell count were performed. Milk samples were collected before the ultra-sonographic examination. Clinically affected quarters (mastitis) of the udder were subjected to the present study. They showed a minimum level of 150000 and 500000 cell count per mL which would then be characterized as healthy, subclinical and clinically affected cattle. The farms were surveyed twice a year, once during the dry and once during the wet seasons. Farmers were visited to gather information about their farming system. To detect clinical mastitis, udders and milk were analyzed. The udder and CMT scores were recorded following the National Mastitis Council method (1999). Udder palpation findings were rated as 1, 2, 3, and 4 for no swelling or discomfort in the udder, swollen ventral quarter, generalized swollen quarter, and swollen and painful udder, respectively.

Statistical analysis was performed using the programs of SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The data were analyzed by using analysis of variance (ANOVA). The significance level was set at 5 %. Pearson correlations were performed using the same software to investigate the relationship between different parameters. The data were shown as mean ± standard deviation (SD).

Results:

A total of 144 healthy, subclinical and clinically affected animals were grouped and evaluated (n=48) in each group, respectively, from seven different dairy farms in the Lahore district and its surroundings. Teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), and teat canal length (TCL), as well as supra mammary lymph node size (SMLNL, SMLNW), were scanned (Table I) (Fig. I & II).

Table 1: Ultrasonographic measurements of the teat's parameter and lymph node were compared between groups (healthy, subclinical and clinical). The data is presented as mean ± standard deviation.

		Mean±SD (cm)	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**	**Sig.**
		(BRx-ARx)	Lower Bound	Upper Bound			
Teat width	Subclinical mastitis	0.04±0.05b	0.043	0.045	0.03	0.05	0.000
	Clinical mastitis	0.31±0.03a	0.297	0.32	0.25	0.36	
Teat width(rF)	Subclinical mastitis	0.05±0.003b	0.05	0.052	0.04	0.06	0.000
	Clinical mastitis	0.21±0.02a	0.2	0.212	0.17	0.24	
Teat wall thickness	Subclinical mastitis	0.07±0.05b	0.07	0.07	0.06	0.08	0.000
	Clinical mastitis	0.33±0.03a	0.32	0.34	0.27	0.39	
Teat cistern diameter	Subclinical mastitis	-0.02±0.005a	-0.026	-0.02	-0.03	-0.02	0.416
	Clinical mastitis	-0.02±0.005a	-0.025	-0.023	-0.03	-0.02	
Teat canal length	Subclinical mastitis	0.016±0.005b	0.015	0.018	0.01	0.02	0.000
	Clinical mastitis	0.24±0.005a	0.24	0.25	0.2	0.28	
Lymph node length	Subclinical mastitis	0.83±0.18b	0.78	0.88	0.61	1.35	0.000
	Clinical mastitis	1.33±0.28a	1.25	1.41	0.91	1.76	
Lymph node width	Subclinical mastitis	0.53±0.14b	0.49	0.57	0.33	0.87	0.000

	Clinical mastitis	0.74±0.16a	0.69	0.79	0.43	1.02

The data were statistically evaluated for the differences in the values between subclinical and clinical mastitic animals before and after treatment. The difference between the teat width (TW) in subclinically (before and after treatment) affected quarters was 0.04±0.01 cm, significantly lower than the difference of recovered clinical (before and after treatment) mastitic cattle 0.31±0.03 cm ($p<0.05$) (Table 1). The teat width at the Furstenberg rosette (TWrF) the difference in subclinically (before and after treatment) affected quarters was 0.05±0.003 cm, significantly lower than the difference of recovered clinical (before and after treatment) mastitic cattle 0.21±0.02 cm ($p<0.05$) (Table 1). The difference in mean teat wall thickness (TWT) measurement in subclinically (before and after treatment) affected quarters was 0.07±0.01 cm, showing a significant difference from the recovered clinical (before and after treatment) mastitic cattle 0.33±0.05 cm ($p<0.05$) (Table I). The differences in mean teat cistern diameter in subclinically (before and after treatment) affected quarters was -0.025±0.01 cm, showing a nonsignificant difference from the recovered clinical (before and after treatment) mastitic cattle -0.024±0.01 cm ($p = 0.416$). The difference in mean teat canal length (TCL) measurement in subclinically (before and after treatment) affected quarters was 0.016±0.01 cm, showing a significant difference from the recovered clinical (before and after treatment) mastitic cattle 0.25±0.02 cm ($p<0.05$)

A total of 48 supramammary lymph nodes from each group (healthy, subclinical and clinical mastits) were evaluated on both sides. The supra mammary lymph node was situated 1.5–2 cm below the udder surface, caudal and dorsal to each hind quarter. It was possible to recognize the typical lymph node as an oval-shaped object with a thin echogenic capsule. The parenchyma of the node was hypoechoic, and a central linear echogenic structure represented the hilar region with its arteries. This finding is consistent with what was found in earlier investigations on sheep (Hussein et al., 2015) and cattle (Amin et al., 2017). A portable ultrasound machine with a (3.5-5 MHz) convex transducer was used to identify the supra mammary lymph node size (Kaixin 5600, VET Portable Ultrasound). The length (dorsoventral dimension) and depth (caudocranial measurement) were measured (Khoramian et al., 2015).

The differences in the lymph node length of subclinically (before and after treatment) affected quarters was shown as 0.83±0.18 cm, significantly lower than the difference of recovered clinical (before and after treatment) mastitic cattle's lymph node 1.33±0.28 cm ($p<0.05$). The differences in the lymph node width of subclinically (before and after treatment) affected quarters were shown as 0.53±0.14 cm, significantly lower from the difference of recovered clinical (before and after treatment) mastitic cattle's lymph node 0.74±0.16 cm ($p<0.05$) (Table 1).

On Pearson's correlation, it was statistically observed (Table II) that TW has a significant ($p<0.01$) difference and, at the same time, shows a positive correlation with TWrF ($r=0.996$), TWT ($r=0.999$), LNL ($r=0.713$), LNW ($r=0.551$) and between groups (subclinical and clinical mastitic) ($r=0.984$), whereas shows negative correlation TCL ($r=-0.615$) and nonsignificant ($p>0.05$) correlation with teat cistern width (CD) ($r=0.084$). TWrF has a significant ($p<0.01$) difference and, at the same time, shows a positive correlation with TWT ($r=0.997$), LNL ($r=0.707$), LNW ($r=0.567$) and between groups (subclinical and clinical mastitic) ($r=0.985$) and negative correlation shown by TCL ($r=-0.613$), whereas shows nonsignificant ($p>0.05$) correlation with teat cistern width (CD) ($r=0.065$). TWT has a significant ($p<0.01$) difference and, at the same time, shows a positive correlation with TCL (0.980), LNL ($r=0.713$), LNW ($r=0.556$) and between groups (subclinical and clinical mastitic) ($r=0.983$), whereas shows nonsignificant ($p>0.05$) correlation with teat cistern width (CD) ($r=0.077$). CD has a nonsignificant ($p>0.05$) difference with all except with TCL and shows an ipsilateral ($p<0.05$) positive correlation ($r=0.222$). TCL has a significant ($p<0.01$) difference but at the same time shows a negative correlation with TW, TWrF, TWT, LNL ($r=-0.465$), LNW ($r=-0.336$) and between groups (subclinical and clinical mastitic) ($r=-0.621$), whereas shows significant ($p<0.05$) positive correlation with teat cistern width (CD) ($r=0.222$). LNL has a significant ($p<0.01$) difference and, at the same time, shows a positive correlation with LNW ($r=0.600$) and between groups (subclinical and clinical mastitic) ($r=0.729$). In contrast, it shows a nonsignificant ($p>0.05$) negative correlation with teat cistern width (CD) ($r=-0.023$) and shows an ipsilateral negative but significant correlation ($p<0.05$) with TCL. LNW has a significant ($p<0.01$) difference and, at the same time, shows a positive correlation between the groups (subclinical and clinical mastitic) ($r=0.566$). In contrast, it shows a nonsignificant ($p>0.05$) negative correlation with teat cistern width (CD) ($r=-0.016$) (Table 2).

Table 2: Correlation of the ultrasonographic morphometry of teat's parameters and lymph node

	TW	**TWrf**	**TWT**	**CD**	**TCL**	**LNL**	**LNW**	**Groups**
TW		.996**	.999**	0.084	-.615**	.713**	.551**	.984**
TWrf	.996**		.997**	0.065	-.631**	.707**	.567**	.985**
TWT	.999**	.997**		0.077	-.613**	.713**	.556**	.983**
CD	0.084	0.065	0.077		.222*	-0.023	-0.016	0.084
TCL	-.615**	-.631**	-.613**	.222*		-.465**	-.336**	-.621**
LNL	.713**	.707**	.713**	-0.023	-.465**		.600**	.729**
LNW	.551**	.567**	.556**	-0.016	-.336**	.600**		.566**
Groups	.984**	.985**	.983**	0.084	-.621**	.729**	.566**	

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

Doppler ultrasound of the mammary artery indicated statistically significant variations between groups. The artery's systolic peak velocity (SPV), end-diastolic velocity (EDV), vascular resistance index (RI= [SPV-EDV]/SPV), and pulsatility index

(PI= [SPV-EDV]/mean velocity) were measured using pulsed Doppler. The indices mentioned above were generated automatically. PI showed a significant difference (p <0.05) and an increase in the severity of inflammation as (4.02±1.74 vs 5.86±2.82 vs 9.44±3.12). RI showed a significant difference between healthy and clinically affected animals (p <0.05) and increased with the severity of inflammation as (1.51±0.26 vs 1.47±0.37 vs 1.36±0.32). Still, there isn't a significant difference between healthy and subclinical mastitic cows. SPV showed a significant difference between healthy and clinically affected animals (p <0.05) and increased with the severity of inflammation (40.63±12.53 vs 52.58±15.23 vs 55.36±13.44). Still, there is no significant difference between clinical and subclinical mastitic cows. EDV showed a significant difference between healthy and clinically affected animals (p <0.05) and increased with the severity of inflammation (22.65±10.53 vs 27.69±10.35 vs 29.27±11.69). Still, there isn't any significant difference between clinical and subclinical mastitic cows. EDV showed a significant difference between healthy and clinically affected animals (p <0.05) and increased with the severity of inflammation (8.27±2.49 vs 10.26±3.36 vs 18.72±5.86) (Table 3).

Table 3: The pulsed-wave Doppler ultrasonographic measurements of the pudendoepigastric trunk were compared between groups (healthy, subclinical and clinical). The data is presented as mean ± standard deviation.

	Healthy(Control)	Subclinical mastitis	Clinical mastitis	Sig.
SPV(cm/s)	40.63 ± 12.53^{b}	52.58 ± 15.23^{a}	55.36 ± 13.44^{a}	0.000
EDV(cm/s)	22.65 ± 10.53^{b}	27.69 ± 10.35^{a}	29.27 ± 11.69^{a}	0.007
PI	4.02 ± 1.74^{c}	5.86 ± 2.82^{b}	9.44 ± 3.12^{a}	0.000
RI	1.51 ± 0.26^{a}	1.47 ± 0.37^{ab}	1.36 ± 0.32^{b}	0.066
TAMV(cm/s)	8.27 ± 2.49^{c}	10.26 ± 3.36^{b}	18.72 ± 5.86^{a}	0.000

On Pearson's correlation, it was statistically observed (Table II) that SPV has a significant (p<0.01) difference and, at the same time, shows a positive correlation with TAMV (r=0.253) and a negative correlation with PI (r=-0.335) and with groups (H, SCM and CM) (r=-0.399), whereas has significant (p<0.05) difference and at the same time shows a positive correlation with EDV (r=0.192). EDV has a significant (p<0.01) difference and, at the same time, shows a negative correlation with groups (H, SCM and CM) (r=-0.247) and a nonsignificant difference between PI and RI. Whereas significant (p<0.05) difference and at the same time shows a positive correlation with SPV (r=0.192) and TAMV (r=0.211). PI has a significant (p<0.01) difference and, at the same time, shows a positive correlation with groups (H, SCM and CM) (r=-0.643) and a nonsignificant difference between EDV and RI. In contrast, it has a significant (p<0.01) difference and, at the same time, shows a negative correlation with SPV (r=0.335) and TAMV (r=0.416). RI has a significant (p<0.05) difference and, at the same time, shows a positive correlation with groups (H, SCM and CM) (r=-0.189) and a nonsignificant difference with SPV, EDV and PI. TAMV has a significant (p<0.01) difference and, at the same time, shows a negative correlation with PI (r=-0.416) and with groups (H, SCM and CM) (r=-0.697) and a nonsignificant difference with RI. In comparison, it has a significant (p<0.01) difference and, at the same time, shows a positive correlation with SPV (r=0.335) and a significant (p<0.05) difference and positive correlation with EDV (r=0.211). Groups (H, SCM and CM) has significant (p<0.01) difference and, at the same time, shows a positive correlation with PI (r=0.643) and a negative correlation with SPV (r=-0.399), EDV (r=-0.247) and TAMV (r=-0.697) and ipsilateral significant and negative correlation with RI (r=-0.189) (Table 4).

Table 4: Correlation of the Doppler ultrasonographic measurements of the pudendoepigastric trunk was compared between groups.

	SPV	EDV	PI	RI	TAMV	Groups
SPV		.192*	-.335**	0.156	.253**	-.399**
EDV	.192*		-0.107	-0.056	.211*	-.247**
PI	-.335**	-0.107		-0.148	-.416**	.643**
RI	0.156	-0.056	-0.148		0.103	-.189*
TAMV	.253**	.211*	-.416**	0.103		-.697**
Groups	-.399**	-.247**	.643**	-.189*	-.697**	

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

Discussion:

Teat parameters such as teat width (TW), teat width at the rosette of Furstenberg (TWrF), teat cistern width (TC), teat wall thickness (TWT), and teat canal length (TCL) as well as supra mammary lymph node size (SMLNL, SMLNW), were scanned through ultrasound. These measurements have been demonstrated to be a valuable method for evaluating changes in the features of the teat and supra mammary lymph node induced by mastitis.

According to (Paulrud and Rasmussen, 2004) and (Smolenski, 2018), the longer the teat canal, the more noticeable the keratin cap, which functions as a natural barrier, avoiding teat contamination against mastitis-causing microorganisms. Furstenberg's rosette was seen as parallel, short hyperechoic lines running from the teat cistern into the papillary duct. Teat ultrasonography typically shows that the teat wall has three distinct layers, namely an outside hyperechoic layer, a middle thicker hypoechoic layer, and an interior hyperechoic layer. However, in the case of an infection, the ultrasonography revealed a considerable thickening of the teat wall and hypoechoic cisternal content. Nonetheless, after the mastitis therapy, the teat cistern returned to its normal state, which is anechoic.

Conserving a healthy mammary gland in ruminants is critical in current animal husbandry to ensure high milk output and quality (Fasulkov, 2012). Subclinical mastitis is a significant cause of worry in cattle worldwide; consequently, early and precise identification of this kind of mastitis is critical to avoiding severe economic losses.

In a clinical situation, ultrasonography cannot replace bacteriological investigation, but it may give vital additional information on the state of the udder, which may assist in prognosis. Ultrasonography alone was capable of identifying clinical mastitis in our investigation. Although (Fasulkov et al., 2018a) found thicker teat walls in goats with mastitis, we did not find this in our study.

In mastitis, the gland cistern loses its anechogenicity and appears with mixed hypoechogenic contents. The teat cistern had a homogeneous hypoechogenic lining, an irregular contour lining, a smaller lumen, a somewhat thicker wall, and a lack of the distinctive three-layered appearances(Santos et al., 2014). The Furstenberg rosette, papillary ducts, and papillary orifice were all overlapping and difficult to distinguish. Before infection, the teat canal length was substantially shorter. There were no statistically significant variations in teat canal diameter before and after infection between subclinical and clinical mastitic measures(Smolenski, 2018).

During the infection, teat ultrasonography revealed hypoechoic material in the teat cistern. The scans of the parenchyma revealed just a few hyperechoic spots, with the majority of its structure being hypoechoic. The picture of the teat was identical to that seen on the initial vertical scan after infection. Again, the cistern's milk secretion was hypoechoic, with no usual anechoic zones. Hyperechoic structures were more common during this period, although hypoechoic zones dominated (Abshenas et al., 2014).

The outcomes of the ultrasonographic tests were consistent with the SCC results in all three study groups. Similarly, (Amin et al., 2017) reported that ultrasonographic measurements of the lymph node varied across animals. Bradley et al. employed an ultrasound machine with a 7.5 MHz linear transducer and a depth of field of 8 cm in a prior investigation, which was not a limit for determining the depth of the nodes. The breadth of the field was just 5 cm, which was less than the length of the nodes. In order to address this constraint, the researchers used a 2-5 MHz convex ultrasound machine.

They are proportional to the quantity of somatic cells and are helpful in diagnosing mastitis. The healthy lymph node was an oval-shaped structure with a thin echogenic capsule, hypoechoic parenchyma, and a linear echogenic centre region representing the node hilus. (Khoramian et al., 2015).

Bradley et al. (2001) discovered that ipsilateral supra mammary lymph nodes on bacteriologically positive sides of the mammary gland were substantially more significant than those on the negative sides. Furthermore, our findings demonstrated a positive link between lymph node length and mean log SCC on both sides and a substantial positive correlation between node depth and mean log SCC on both sides. Although the mammary gland is divided into quarters, each side's quarters are linked to the same supra mammary lymph node; therefore, mastitis in one-quarter of each side has a more substantial influence on expanding supra mammary lymph node growth than mastitis in both quarters.

Mastitis lymph nodes were easily identifiable, swollen, and essentially lost their highly echogenic hilus core region, rendering the entire structure hypoechogenic. Lymph nodes on the mammary gland's bacteriologically positive side are significantly larger than those on the opposing side, this is because the quarters on each side are connected to the same supra mammary lymph node. Mastitis in one-quarter of each side influences this growth more than mastitis in both quarters. Based on the findings of this study, which revealed that the dimensions of the supra mammary lymph node change in mastitis cases, ultrasonography of the supra mammary lymph node is likely an advantageous method for confirming mastitis cases. Future research should be done to apply this method for mastitis detection in some groups of cows, such as heifers and dry cows, where milk testing is impossible and can be used for selective dry cow therapy or additional treatment in the This strategy should be tested on heifers and dry cows in the future.

Blood-flow volumes rose parallel to somatic cell count in a study of Escherichia coli mastitis in cows. Udder blood flow is expected to rise because of inflammatory alterations and metabolic activity. In the early stages of acute mastitis, transrectal color-Doppler ultrasonography might be utilized to identify changes in blood flow. TAMV values, in particular (Potapow et al., 2010).

Researchers have explored various innovative methods, including aetiology, diagnosis, therapy, and ultrasonography, to understand mastitis and its impact on udder blood flow using Doppler techniques. Studies indicate that cows with mastitis exhibit increased systolic peak velocity (SPV) in udder arteries and decreased resistance index (RI) in milk veins. These changes are attributed to inflammation-induced increased blood flow and decreased vascular resistance. Additionally, research involving healthy Swiss Brown cows (Braun and Hoegger, 2008) showed mean blood-flow velocities before and after delivery (Ntemka et al., 2022).

a study on mastitis in goats and its effects on Doppler ultrasonographic measurements of blood flow. The research observes differences in blood flow parameters between goats with clinical mastitis and those without. Increased blood flow is noted in infected goats, while specific measurements like TAMV are lower but not significantly so (Nielsen et al., 1990). The study suggests that subclinical mastitis might influence Doppler data. However, the complexity of Doppler measurements, influenced by factors like breed, age, and environmental conditions, is acknowledged. It recommends broader studies involving multiple goat breeds to better understand these effects and their underlying factors (Abdelnaby, 2020).

the influence of udder inflammation on blood flow dynamics in goats. In response to infection, blood vessels in the udder dilate, leading to increased blood flow to combat the infection. (RIŞVANLI et al., 2018) supports this connection between infection and increased local blood flow. Schwarz et al.'s 2020 study, focusing on cows, found that only one parameter, TAMV, was lower in cows with udder inflammation compared to other measured parameters. This suggests inflammation might alter blood flow dynamics in cows. However, Santos et al.'s research contradicted this, reporting no significant differences in hemodynamic measures between healthy goats and those with subclinical mastitis (Schwarz et al., 2020). These discrepancies highlight the complexity of the relationship between inflammation and blood flow, possibly due to factors like infection stage or individual variability. Further research is needed to clarify this intricate interaction for a comprehensive understanding of its implications for animal health.

the influence of inflammation on hemodynamic measurements and its practical implications. The discussion proposes that the observed differences in parameters like SPV and PI could be attributed to the organism's strategy of increasing blood flow to transport defense cells to the site of inflammation, particularly in response to bacterial invasion. (Santos et al., 2015)research supports this concept by associating increased SPV values with heightened blood flow in diseased organs. The article suggests that these findings have potential applications in standardizing computerized image analysis for diagnosing and monitoring conditions like mastitis. The understanding of how inflammation affects blood flow dynamics could aid in improving diagnostic accuracy and tracking disease progression through the analysis of ultrasonograms and Doppler values in the mammary gland.

The focus is on diagnosing subclinical mastitis in ruminants, emphasizing the widely used approach of microbiological culture of milk samples. The method's significance is supported by extensive literature on ruminant mastitis. (Fragkou et al., 2014)study further validates this approach and highlights the prevalence of macrophages in milk samples from healthy ewe udders, shedding light on their role in udder health and defense. The discussion underscores the importance of quantifying somatic cell count (SCC) as a reliable indicator of udder inflammation. Overall, the article contributes to the understanding of subclinical mastitis diagnosis, encompassing microbial and cellular aspects for a comprehensive assessment of udder health.

The article discusses advancements in early detection tools for mastitis in ruminants. Researchers are focusing on molecular techniques that identify microorganism nucleic acids, ensuring accurate diagnoses. Modern methods like infrared thermography are being explored for subclinical mastitis detection in cows, with varying success (Neculai-Valeanu and Ariton, 2022).. The study notes the lower pulsatility index (PI) in sheep with subclinical mastitis, indicating increased blood flow during intramammary inflammation. Integration of artificial intelligence (AI) could automate diagnostics, providing insights into udder health, milk output, and more. The article highlights a pioneering study using ultrasonography for subclinical mastitis diagnosis in sheep, contributing to innovative diagnostic approaches (Underwood et al., 2015). In conclusion, the article emphasizes ongoing efforts to improve early mastitis detection using diverse techniques, offering potential benefits for animal health and dairy production.

Conclusion:

In conclusion, the study's findings hold significant potential for establishing reference points and ranges for computerized image analysis of mammary gland ultrasonograms and Doppler values in the mammary artery. This application can greatly benefit the diagnosis and monitoring of various pathological conditions, including mastitis. The study highlights a clear relationship between ultrasonographic findings and the severity of the disease, indicating that the imaging results accurately reflect the extent of the condition. Both conventional and Doppler ultrasound techniques offer valuable supplementary insights into udder health, aiding in the precision of diagnosis and prognosis. Ultimately, the research underscores the practical utility of these imaging techniques in enhancing the understanding and management of udder-related health issues.

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