ULTRASONOGRAPHIC EVALUATION FOR EARLY DETECTION OF MASTITIS AND USE OF INFRARED THERMOGRAPHY IN BOVINE MASTITIS

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University in partial fulfilment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY MEDICINE
(Minor Subject: Veterinary Microbiology)

By

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(L-2019-V-50-M)

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2021

CERTIFICATE – I

This is to certify that the thesis entitled, **"ULTRASONOGRAPHIC EVALUATION FOR EARLY DETECTION OF MASTITIS AND USE OF INFRARED THERMOGRAPHY IN BOVINE MASTITIS"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Microbiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Habbu Aishwarya Sunder (L-2019-V-50-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that the thesis entitled, **"ULTRASONOGRAPHIC EVALUATION FOR EARLY DETECTION OF MASTITIS AND USE OF INFRARED THERMOGRAPHY IN BOVINE MASTITIS"** submitted by **Habbu Aishwarya Sunder (L-2019-V-50-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in the partial fulfilment of the requirements for the degree of **M.V.Sc.** in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Microbiology**) has been approved by Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ACKNOWLEDGEMENT

*I would like to begin, by acknowledging the love, support, constant encouragement and guidance I received from my family, without which I would not have achieved the dreams and goals that I aimed for. They have been a source of happiness, humor and fierce love which helped me on darker, harder days. The blessings of **GOD ALMIGHTY** helped me sail smoothly through this endeavor.*

*I am eternally indebted to my esteemed Major Advisor **Dr. D.K. Gupta**, Senior Scientist, Department of Veterinary Medicine for teaching me some of the most important lessons in the slice of these two years of my life here. He has been a source of constant support, motivation and extremely dedicated towards the working and completion of my research studies. I am forever thankful for the guidance, keen supervision, constructive criticism, generous help, and support throughout the pursuance of my research studies. His dedication and genuine passion to the field of veterinary medicine, has made me a better student, clinician and researcher, and for that I am filled with gratitude.*

*Simply words are inadequate to express my earnest and whole hearted thanks to my advisory committee members **Dr. Shukriti Sharma**, Professor Department of Veterinary Medicine, **Dr Mudit Chandra**, Senior scientist Department of Veterinary Microbiology, and Dean PGS nominee **Dr Swaran Singh Randhawa**, Professor Department of Veterinary Medicine and Director of Clinics for their inspiring guidance, invaluable time, motivation, suggestions, encouragement and generous help throughout my M.V.Sc programme.*

*It is my privilege to express my gratitude towards **Dr. Charanjit Singh Randhawa**, Professor cum Head, Department of Veterinary Medicine, for providing necessary input during my research work.*

*I express my sincere thanks to **Dr. S.K. Uppal**, Dean, Postgraduate Studies, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, for his valuable suggestions and constructive guidance. I would like to acknowledge **Dr. Ajeet Singh,** Professor, Veterinary Gynecology and Obstetrics for providing facility of infrared thermography camera and technical knowledge of the instrument.*

*I would like to extend my special acknowledgement to **Dr. Raj Sukhbir Singh,** Professor for giving his valuable suggestions on data analysis, **Dr. S.T. Singh and Dr B.K. Bansal**, Principal scientist for providing help at University Dairy Farm. I will forever be grateful for the support, encouragement and motivation to faculty members of my department **Dr. K. Dua, Dr. S. S. Randhawa, Dr. Ashwani Kumar, Dr. Sushma, Dr. Neetu Saini, Dr. Sujata Turkar, Dr. Randhir Singh, Dr. Asmita Narang and Dr Shabnam Sidhu** for rendering help and moral support.*

The fruits of this research would not be rendered without the love, support and inspiration given to me by my beautiful family. I am eternally thankful to my *parents, **Dr Sunder Habbu and Mrs Shaila Habbu** who raised me to believe to aim for the stars. The constant belief in me and support through all my decisions and choices have made me who I am, and for that I am eternally thankful. The love and support given to me by my brothers **Kamaksha Pradhan and Aditya Habbu** cannot be expressed into words.*

*Special thanks to my friends who became like family over time **Drs. Tejini M Kumar, Anugraha Mercy Easaw, Deepak Sharma, Aparna Mariyam Thomas and Shilpa B.** for their constant presence, love, encouragement and for all of the beautiful memories we made together.*

*A sense of heartful gratitude and deep love is felt towards my incredible seniors **Drs. Iqra Khan, Smriti Kapoor, Sheetal Dogra, Lakhan Sachdeva and Amrinder,** my wonderful batchmates **Drs. Bava, Rashmi, Sameer, Jasnit, Avtar, Anish, Jaspreet, Reema, Sahil, Ravnit, Himanshu and Kuljeet and my juniors Drs. Kanika, Sanjeev, Sarthak, Ragini and Shivani** for their ever-willing co-operation, motivation, moral support, rendering ungrudging assistance whenever and wherever need arose.*

*The help rendered by **Mrs. Mini, Mr. Ganga, Mr. Kewal, Mr. Randhir, Mr. Baljinder, Mrs. Anita, Mr. Satinder** and all the other non-teaching staff of the department of veterinary medicine is duly acknowledged.*

Last but not the least I would like to express my sincere thanks to all those who led me to accomplish this task with solemn effort directly or indirectly. All may not be mentioned but none is forgotten.

Place: Ludhiana

Date: ***(Habbu Aishwarya Sunder)***

Title of the Thesis	:	**ULTRASONOGRAPHIC EVALUATION FOR EARLY DETECTION OF MASTITIS AND USE OF INFRARED THERMOGRAPHY IN BOVINE MASTITIS**
Name of the Student	:	Habbu Aishwarya Sunder
Admission No.	:	L-2019-V-50-M
Major Subject	:	Veterinary Medicine
Minor Subject	:	Veterinary Microbiology
Name and Designation of Major Advisor	:	Dr. D.K. Gupta Senior Scientist
Degree to be awarded	:	M.V.Sc.
Year of award the Degree	:	2021
Total pages of Thesis	:	100 + VITA
Name of University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004 (Punjab), India

ABSTRACT

The first objective involved ultrasonographic evaluation of teat and supramammary lymph node in apparently healthy and clinically mastitic dairy cows. A total of 52 apparently healthy dairy cows having 208 functional teats were ultrasonographically scanned at university dairy farm. Besides, 30 clinically mastitic cows with 120 functional quarters were also scanned at the large animal clinic. The second objective was to standardize the use of infrared thermography (IRT) in healthy and mastitis cases. This included 68 images of all the quarters of 17 apparently healthy cows and 48 images of all the quarters of 12 clinically mastitic cows. Upon comparison of teat tissue measurements in relation to quarter health status, the OTD was found to be significantly ($p<0.05$) increased with a non-significant ($p<0.05$) increase in TWT in subclinically affected mastitic quarters compared to that of healthy ones. All milk inflammatory parameters were found to be significantly ($p<0.05$) elevated in subclinically affected quarters. TCL was found to be significantly ($p<0.05$) increased in quarters with SCC of 2-4 lakh cells/ml as compared to quarters with SCC <2 lakh cells/ml. A significant ($p<0.05$) increase in the OTD and TWT in CMT positive quarters when compared to CMT negative quarters. A significant ($p<0.05$) increase in the OTD was observed in quarters with a CMT score of 0.5 and those with a score of 2-3 when compared to those with a CMT score of 0. pH, EC, SCC and CMT were significantly ($p<0.01$) positively correlated with OTD and TWT. A total of 104 supramammary lymph node of apparently healthy cows were scanned. Log_{10}SCC and CMT had significant ($p<0.01$) positive correlation with the length and depth of the lymph node. The length and depth had a significant ($p<0.05$) positive correlation with the OTD and TWT of the ipsilateral sides. Out of 120 functional quarters from clinically mastitic cows, the OTD differed significantly ($p<0.05$) in clinical quarters as compared to healthy ones. Total of 60 supramammary lymph nodes of 30 clinically mastitic cows were also scanned. CMT was significantly ($p<0.05$) correlated with length of the lymph node. The length of the lymph node was significantly ($p<0.05$) correlated with OTD and FTD. USST and TSST in clinical quarters was significantly ($p<0.05$) higher as compared to healthy ones. A significant ($p<0.05$) positive correlation was obtained between USST and the CMT, EC and Log_{10}SCC. The USST in quarters with SCC of >4 lakh cells/ml increased non-significantly ($p>0.05$). Quarters with CMT score >1 had a significantly ($p<0.05$) higher USST when compared to those quarters with a CMT score of <1.

Keywords: Bovine, infrared thermography, mastitis, ultrasonography

Signature of Major Advisor **Signature of the student**

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LIST OF ABBREVIATIONS

%	:	Percentage
<	:	Less than
>	:	Greater than
µl	:	micro litre
CD	:	Cistern diameter
Cfu	:	colony forming units
CM	:	clinical mastitis
CMT	:	California Mastitis Test
E. coli	:	*Escherichia coli*
E.C.	:	Electrical Conductivity
e.g	:	For example
EMB	:	Eosin Methylene Blue
et al	:	and Co-workers
Fig.	:	Figure
FTD	:	Diameter at the level of furstenberg rosette
g	:	gram
h	:	hour
i.e.	:	that is
IRT	:	Infrared thermography
ml	:	millilitre
mm	:	millimeter
mS	:	Millisiemens
MSA	:	Mannitol Salt Agar
^{o}C	:	Degree Celsius
OTD	:	Overall teat diameter
QFM	:	Quarter Fore Milk
S.aureus	:	*Staphylococcus aureus*
S.E	:	Standard Error
SCC	:	Somatic Cell Count
SCM	:	Sub Clinical Mastitis
sec	:	second
SLN	:	Supramammary Lymph Node
TCL	:	Teat canal length
TCS	:	Teat Condition Score
TES	:	Teat End Score
TSST	:	Teat skin surface temperature
TWT	:	Teat wall thickness
USG	:	Ultrasonography
USST	:	Udder skin surface temperature
vs	:	Versus
w.r.t	:	With respect to

CHAPTER I

INTRODUCTION

India produces over 180 million metric tonnes (MMT) of milk, accounting for about 20% of global production, and it is expanding at a rate of 4.5 percent. Milk is India's most valuable crop, valued Rs. 6.5 lakh crore far more than paddy and wheat combined. Mastitis is a heavy burden for the dairy sector, not only in India but world-wide; loss of milk production, discarded milk and milk product quality, veterinary services and treatment cost, labour cost and culling cost (Bansal & Gupta, 2009). Mastitis, both clinical and subclinical forms are a major concern for dairy farmers, veterinary specialists, and policy makers.

Mastitis is an outcome of the interaction between three vital factors: infectious agents, the resistance of the host and environmental factors (Gera & Guha, 2011). Mastitis is the body's response to an injury to udder tissue caused mostly by intramammary bacterial infection, but it can also be caused by mycoplasma, fungal, or algal infections. Mechanical or thermal stress to the udder may predispose it to the infections (Radostits et al., 2007). Mastitis is therefore, characterized by a set of pathological changes in the mammary parenchyma which eventually leads to physical and chemical changes in the milk. The most important changes in the milk include discolouration, the presence of clots and a significant rise in the somatic cell count due to the presence of a large number of leukocytes. In clinical cases, there will be swelling, heat, pain, and oedema. By manual palpation or visual examination of the milk with a strip cup, a considerable fraction of mastitic glands are not immediately apparent; these quarters reflect subclinical infections, and because of the vast number of subclinical cases, the diagnosis of mastitis largely depends on indirect tests which in turn depend on the somatic cell concentration or electrolyte concentration of milk. The total economic losses due to mastitis at national level is assessed to be Rs. 7615 crores and state (Punjab) level, losses were found to be about Rs 561 crore per annum (Bansal & Gupta, 2009). This further emphasizes the need for a quick, non-invasive, easily performed and economic way of diagnosing mastitis at the field level.

For the diagnosis of mastitis, milk culture and bacterial isolation is considered as the gold standard technique. At the field level, CMT, BTB card tests and SCC are performed to diagnose mastitis. This is further supplemented with ultrasonography, a quick non-invasive method for the early diagnosis and visualization of changes in teat tissue and supramammary lymph nodes which otherwise, are difficult to diagnose on physical examination (Kotb et al., 2014).

For the timely and more economical detection of subclinical mastitis, ultrasonography is used to observe the changes in the udder and teat tissues (Dinc et al., 2000). It is observed that in cases of mastitis there are changes in teat and glandular tissue and supramammary lymph nodes which are easily visualized using ultrasonography and is used as a supplement along with field techniques such as the California Mastitis Test or the Sodium Lauryl Sulphate test.

Several methods are attempted before concluding that for the ultrasonographic examination of the udder, the direct contact method using a 7.5 or 10 MHz convex transducer is the ideal technique to visualize changes in the udder and to detect any pathological changes in the udder such as inflammation or fibrosis. The changes in clinical mastitis are very well perceivable by ultrasonography, however, in the case of subclinical mastitis the ultrasound findings are not as distinct and clear but may be visualized as a slight increase in echogenicity in the affected quarters and teats.

The teat is best visualized using the water-bath technique which would provide excellent contrast and detail of the teat structures using a linear probe with a frequency of 7.5 – 10 MHz which enables us to visualize the contour of the teat and the different structures quite well (Szencziov and Strapak, 2012). The teat wall outer skin is visualized as distinct hyperechoic line, followed by a middle less echogenic area which would again be followed by a thin hyperechoic line. The teat cistern will be anechoic, while the Furstenberg rosette will appear as several hypoechoic cords directed towards the teat sinus arising from the teat canal. The teat canal was visualized as the area between the two hyperechoic lines of the teat wall and forms an anechoic lumen (Ragab et al., 2017). The changes in the teat must also be noted as they are specific according to the duration or type of mastitis whether clinical, subclinical, or even in cases of fibrosis or obstructions found in the teat canal or teat cistern. In subclinical mastitis it is observed that the teat canal and sinus appeared irregular, and the teat wall loses the three-layered appearance significantly with the presence of an overlapped appearance of the rosette of Furstenberg. On ultrasonography, the teat wall appears thickened in clinical mastitis, with a total loss of the usual three-layered appearance of the teat wall and potentially complete occlusion of the teat canal with the loss of the Furstenberg rosette. These changes are incredibly significant and distinct and are used to easily identify the changes brought about by subclinical as well as clinical mastitis which further aid as a diagnostic tool in field as well as in clinical conditions.

Studies done by Seker et al. (2009), evaluated ultrasonography to assess the changes in the different teat parameters brought about by mastitis to find a correlation between the California Mastitis Test and the different teat measurements. It is found that the number of lactations, lactation period and pregnancy did not affect any of the teat measurements however the cistern diameter was found to be significantly smaller in CMT positive quarters. There are also differences in the teat parameters according to the breed such as the Brown Swiss and the Holstein- Friesian having the shortest teat canals whereas breeds such as the Simmental with a longer teat canal. These conditions must be kept in mind as several studies find a correlation between mastitis with a higher somatic cell count and a shorter teat canal length and one of the findings has been that found that healthy animals usually possess a longer teat canal however, no particular correlation has been made to mastitis and an increase in teat wall thickness.

Lymphadenopathies are indicators of any form of disease whether neoplastic, inflammatory or traumatic. In human studies done by Bruneton et al. (1994), lymph nodes were examined to study the etiological agent and pathogenesis of a disease and they found that a 7-10 MHz convex transducer is excellent to view the lymph node and the changes occurring within it. The lymph node on ultrasonography has a distinct appearance in which it has a hyperechogenic capsule due to the presence of smooth muscle and elastic fibres followed by the cortex which appear as a hypoechoic structure corresponding to the accumulation of lymphocytes in the form of multiple follicles followed by an inner hyperechoic medulla. The medulla appears to be hyperechoic due to the presence of lymphatic sinus where the lymph drains into it, it is uncertain whether the hyperechogenicty in the medulla is due to the lymphatic sinus or the presence of fat or it could be due to both these factors (Gilks & Gordon, 1988). The appearance of the lymph node help to differentiate them between neoplastic, inflammatory, and non-neoplastic causes. It is observed that in conditions that are benign, a middle central hyperechogenic line is usually present representing the medulla and the absence of the medulla might indicate more of malignant conditions

(Sambhaji, 2009). Similarly, enlargement of the supramammary lymph node is also as an indicator of infections of the udder.

The location of supramammary lymph node in the udder is described by Bradley et al. (2001) and stated that there are two supramammary lymph nodes present caudo-dorsal to the udder, between the udder and the thigh; however, the exact location of these lymph nodes is variable and may differ from animal to animal. One of the lymph nodes is present laterally and is slightly larger ranging from 7-8 cm and is usually kidney shaped and the superficial vessels drain into this lymph node. The other one being slightly smaller in size, has a more ovoid shape and is present more medially and receives the vessels from the deeper part of the udder.

Each of the quarter drains into the respective lymph node, therefore an infection in any of the quarters would leads to an increase in the size of the lymph node due to the proliferation of the lymphocytes which is correlated to an increase in the somatic cell count. Studies done by Bradley et al. (2001) reveal that CMT positive quarters have a larger lymph node size. Similarly, studies done by Khoramian et al. (2015) revealed a positive correlation between the lymph node dimensions and CMT score as well as mean log $_{10}$SCC of the ipsilateral sides, this indicated that this technique is excellent in detecting mastitis in heifers, where obtaining milk samples is not possible. Hence, the increase in length and width of the lymph node could be indicatory of mastitis in the animal. However, there are certain factors that need to be kept in mind such as the fact that size of the lymph node is said to increase with the age and number of lactations. Bradley et al. (2001) observed that older cows had larger lymph nodes. They also found a positive correlation between the number of lactations and the dimensions of the lymph node, however, they did not find a correlation in the lymph node size with number of days calved and stage of pregnancy.

Besides ultrasonography, infrared thermography (IRT) is also gaining momentum as a technique of future for early diagnosis of mastitis with ease of applicability. IRT is a revolutionary, advanced technique that was originally developed for military and industrial applications (Eugeniusz-Herbut & Mazur, 2006). All objects emit infrared radiation proportional to their temperature in accordance with Stefan-law. Using this law, a thermal camera is utilized which detects the infrared radiation and creates a pictorial representation based on the heat generated without exposing users to harmful radiation (Kunc et al., 2007).

IRT is used in both the medicine and veterinary field for a long period; it may not be a confirmatory diagnostic tool although it very well can be used to detect the location of a lesion so long as it is on the surface of the animal. IRT involves not only studying the temperature of the location of interest but in the patterns of temperatures in the location of interest and the vicinity of the location would help in accurately diagnosing the disease in an easy, non-invasive technique that can be then supplemented with the required specific diagnostic tool.

IRT identifies changes in skin surface temperature that are influenced by internal states of tissues and organs, as well as fluctuations in capillary blood flow volume and rate. Thermography in the veterinary field has been primarily used to detect lesions in the digit for the early detection of hoof lesions such as digital dermatitis. Purohit and McCoy (1998) found that using infrared thermography they could detect an increase in temperature in the hoof indicating early inflammatory signs of the hoof atleast two weeks before a clinical diagnosis could be made. Alsaaod et al. (2014) detected that the highest temperature in the digit was found at the coronary band and the front and rear of the hooves.

Poikalainen et al. (2012) have attempted in using infrared thermography as a method to set as a health index for livestock. There are essentially three pain spots, the udder, the feet and around skin injuries and in these pain spots, the thermographic patterns are not uniform. It has also been found that the temperature of the udder increases to a certain extent while using machine milking. The presence of any infection in a particular quarter, leads to increased blood supply to the affected site increasing the temperature of that region and hence straying from the normal thermal pattern. This deflection from the normal thermal pattern is used as a quick and non-invasive technique to presence of any antigenic challenges that may be present in the udder. Hovinen et al. (2008) recorded an elevation of about 1 to 1.5 °C in the udder skin surface temperature in mastitic cows.

Studies were conducted in which the udder skin surface temperature was taken at standard distances ranging from 60 cm to 1 m away from the animal (Berry et al., 2003; Sathiyabharathi et al., 2018). It has been found that an increase in udder skin temperature can be correlated to a significant increase in the somatic cell count of the respective quarter, along with which a positive correlation can also be made with an increase in the pH, electrical conductivity and the CMT status of the milk. Infrared thermographic thermal patterns are present throughout the body and they do not tend to vary from animal to animal but there are most certainly species differences. These thermal patterns can be used to detect diseases easily in field conditions as well.

The increase in temperature of the udder can be further correlated to the increase in quarter cell count and CMT to detect the presence of any infection in the udder. It has been observed in studies (Juozaitiene, 2019. Da Silva, 2016) that there could be upto an 8.5 °C increase in temperature in cases of clinical mastitis and an increase in 2.5 °C in cases of subclinical mastitis which was primarily due to the dilation of the blood vessels present in the udder.

There are certain precautions and limitations, however to this technique as it is sensitive, the infrared thermographic image must be taken at a set distance from the animal which ranges from about 60 cm to 1m, and this image must be taken ensuring that there is no direct sunlight. The images must be also preferably taken prior to milking, as milking would increase the temperature of the udder (Yang et al., 2015). IRT needs to be performed in ambient environmental conditions as a higher environmental temperature could lead to an increase in the udder skin temperature even in healthy animals.

IRT has been demonstrated to be sensitive enough to identify thermal changes in udder skin produced by inflammation; on addition to being a non-invasive and quick tool, it may aid veterinarians and farmers in the early detection of udder inflammation and, as a result, the administration of prompt and successful therapy.

In field conditions we would need diagnostic techniques that would help in a quick and preferably non-invasive technique to detect subclinical mastitis. Ultrasonography is an economical, non-invasive method which could help us to visualize the changes that may occur in the udder, teat tissue and supramammary lymph node in case of subclinical and clinical mastitis. Ultrasonography of the supramammary lymph node would help to detect and isolate early cases of mastitis in lactating animals as well as heifers. There has been no study in local conditions and very few studies have been done on this aspect in countries abroad.

Infrared thermography is a newer technique, whose standards and use has not been established yet. It is a more advanced, non-invasive technique which would easily detect an increase in the udder temperature indicating signs of inflammation. Even the slightest increase in temperature could indicate signs of infection as this instrument is extremely sensitive. IRT is a technique that has been frequently used in the field of equine medicine, however not many studies have been done in the use of IRT in detecting mastitis. Only one study on IRT has been done in local conditions and very few abroad. Therefore, thorough standardization would be required to establish the use of IRT in field conditions. Keeping in view the above facts the present study was designed with the following objectives:

1. To evaluate the ultrasonographic changes due to mammary infection in udder, teats and supramammary lymph nodes of cross bred dairy cows
2. To standardize the use of infrared thermography (IRT) in healthy and mastitis cases

CHAPTER II

REVIEW OF LITERATURE

2.1 Ultrasonography of udder and teats of dairy cow

2.1.1. Ultrasonography of udder and teats.

Cartee et al. (1986) used brightness mode (B-mode) ultrasonography to evaluate the udders and teats of cows. Scanning the teat sinus, gland sinus, and lactiferous ducts in lactating animals was revealed to be simple. With 5-MHz and 10-MHz mechanical sector probes, they observed that the distinct layers of the teat wall were easily identifiable and a distinct ring of mucosal tissue arising from the teat sinus into the papillary duct.

Takeda (1989) examined the udders of 51 lactating cows ultrasonographically and found that the udder in healthy animals, the glandular parenchymal region appeared as a large non-structural homogenous hypoechoic area with fine diffuse echo spots. Udder abscesses could be visualized as localized regions which had a clear boundary and even those that could not be palpated were easily visualized ultrasonographically. He found that in cases of acute purulent mastitis the echogenicity was not homogenous and the boundaries were indistinct. In cases of chronic diffuse mastitis, however, the border-line echo of the gland sinus increased significantly, and diffuse cords of high echogenicity were seen in cases of fibrosis. In the cases with subclinical mastitis, milder findings like those of chronic mastitis were obtained. There were no associations between the echo findings and the histological findings or the ability to produce milk in the case of diffuse mastitis.

Weiss et al. (2004) analysed the milking characteristics of 148 quarters of 38 cows and measured the overall teat diameter, the teat wall thickness and the teat canal length ultrasonographically. It was found that the hindquarter teats were found to be much shorter and thicker as compared to the forequarter teats. However, no such difference was found in the teat canal length and the teat wall thickness. They also revealed that the rear teats yielded more milk and had a higher peak flow rate. They found no between traits involved in milking and external dimensions of the teat such as teat length or overall teat diameter.

Baer & Bilkei (2005) examined the udders of 1125 post parturient sows dividing them into two groups, one which had previously been diagnosed with Mastitis metritis agalactia (MMA) and one group which was found to be normal and healthy animals. They were examined ultrasonographically and then culled to observe the gross pathological findings of the udder. It was found that the mammary glands of the sows having suffered MMA had highly ($p<0.001$) echogenicity, when compared to those sows which did not have a history of MMA. They also noted that the abdominal glands were more ($p<0.01$) prone to pathological changes as compared to the pectoral glands. Sows of high parity had more hyperechogenic images and gross pathological changes in their mammary glands compared with the sows of low parity.

Nak et al. (2005) used ultrasonography to diagnose various teat lesions in dairy cows. The teats collected from the abattoir were scanned by placing the probe linear to the teat within the water bath, and in live animals, the teat was dipped into a clear plastic glass of water, followed by placing a linear probe directly on the glass, to visualize the teat. They discovered that ultrasonography was more accurate and effective than standard clinical examination in detecting issues like stenosis, blockages of the teat canal, and any deformities in the teat.

Flock and Winter (2006) evaluated the udder of fifty-two cattle employing the use of ultrasonography between 2000 and 2004. They used convex transducers (3.5 – 5 Mhz) and linear transducers, along with which bacterial isolation was done as well. The udder health status was examined using ultrasonography and they noted that certain causative agents showed specific changes in the udder parenchyma. They also explained in detail how there would be changes in the teat according to the breed such as Brown Swiss was found to have the shortest cistern length (1.57 cm) followed by Holstein-Friesian (1.72 cm) and Simmental (1.83 cm) and the same study also spoke about how healthy udders had a longer cistern length (1.78 cm) as compared to the one that had mastitis (1.65 cm).

Celik et al. (2008) examined the use of the linear probe to visualize the appearance of the teat canal in four hundred teats. They found that most teat canals had a straight course, however about twenty percent of the teat canals were found to be angled and bent at some locations, however the functionality appeared to be unaffected by this. The mean length of the teat canal was found to be 1.51 cm. It was demonstrated by them that the length of the teat canal as well as the milk yield could be correlated to the age of the animal. It was revealed that younger animals with a short teat canal had a higher milk yield and vice versa. They concluded that ultrasonographic examination was effective in teat canal imaging and morphological assessment.

Rambabu et al. (2008) used direct contact, gel application, water bath, and standoff methods to visualize the udders and teats of buffaloes using ultrasonography. The gel application and water bath methods were deemed the most effective methods for viewing the udder and teats of buffaloes.

Seker et al. (2009) examined the ultrasonographic teat measurements in dairy cows to observe for any correlation between the teat dimensions and the CMT score. The most significant findings where changes observed as increase in diameter at the level of the rosette of Furstenberg (FTD) and reduction of teat cistern diameter (CD). They found that the number of lactations, lactation period and pregnancy did not affect any of the teat measurements however the cistern diameter was found to be significantly smaller in CMT positive quarters ($P < 0.01$).

Rambabu et al. (2009) conducted ultrasonography of the udder and teats. They observed that the different structures of the udder and teat could be identified and visualized based on the different echogenicities. Any afflictions of the udder could be differentiated from the normal parenchyma due to the difference in echogenicty as in conditions such as oedema, abscess and fibrosis. Other lesions such as mammitis, abscess and hemtoma which could not be easily diagnosed by manual palpation were diagnosed easily using ultrasonography. Ultrasonographic diagnosis of lesions such stenosis of the teat, intraluminal foreign bodies, teat fistula, atresia, thelitis and fibrosis could also be diagnosed with this.

Porcionato et al. (2009) examined eighty Gir cows in which the external teat dimensions were examined using ultrasonography of the right anterior teat and measuring of the teat externally. Bacteriological culture and somatic cells were checked in the milk samples, then the milk flow was observed and evaluated with parameters such as the milk yield, somatic cell count, external teat dimensions and measurements. It was found that milk flow had a strong negative relationship with the milk production i.e. those with a slow and steady milk flow had the tendency to have higher milk production. It was also found that the somatic cell count of Gir cows was influenced by the teat- end to floor distance.

Condino et al. (2011) evaluated at the teats of five cows with toxic mastitis and divided them into three categories based on milk flow, imaging, and histological features. In five cows with toxic mastitis, study examined at the milk flow, imaging, and histopathological characteristics of the teat. Those in Group I had no gross abnormalities, those in Group II had minor histological findings, and those in Group III had both (gross and histopathological findings). The irregular mucosal surfaces found in Group II and the absence of a hyperechoic line along the teat canal in Group III were two of the most notable ultrasonography findings.

Javadi & Accorda. (2011) used a complete histogram analysis of ultrasonograms of the udder and teat in Holstein Friesan Sahiwal crossbred cows to evaluate ultrasonographic alterations and echo mean values. Based on the CMT, they divided them into three groups: healthy, subclinical, and clinical. They discovered that ultrasonography of the teats and udders revealed that cows with mastitis (CMT grades 2 and 3) had more echogenic sinuses and cisterns than healthy animals. The echo value was significantly higher in the subclinical and clinical groups, according to the histogram analysis. They came to the conclusion that ultrasonography would be a beneficial tool in the early detection of mastitis.

Szencziová & Strapak. (2012) evaluated the use of ultrasonography as an excellent technique to visualize the internal and external structures of the teat. The udder and teats were ultrasonographically examined to diagnose milk flow disturbances and to observe the different inner structures of the teat like overall diameter and length of the teat canal, the appearance of the teat wall along with the thickness (TWT). The diameter of the teat cistern (CD) was also measured. The ultrasonography evaluation of the udder parenchyma done using the direct-contact method with the help of a linear probe that provided a depth of 28–29 cm. The water-bath approach using a high frequency linear probe was shown to be excellent to visualize the various structures of the teat.

Diaz et al. (2013) used ultrasonography to observe the changes in the teat wall thickness in Murciano-Granadina and found that frequencies of 5 and 7.5 MHz were the most ideal to visualize the changes in the teat wall caused by mechanical milking.

They also determined that this method could be used to achieve similar results and findings in Hostein Friesan cows as well.

Kotb et al. (2014) examined 55 lactating buffaloes for subclinical mastitis by clinical examination, CMT, SCC, microbiological tests and ultrasonography. In buffaloes with subclinical mastitis, the inner mucosal lining of the teat canal and sinus was observed to be irregular, combined with a significant loss of the three-layered look of the afflicted wall and an overlapping appearance of the papillary duct and the Furstenberg rosette. In clinical mastitis, the ultrasonographic examination of a thickened teat wall with complete loss of the three-layered appearance, complete obstruction of the teat canal, and disappearance of the Furstenberg rosette was observed. They concluded that ultrasonography and laboratory diagnosis complement each other in correct diagnosis of mastitis in buffaloes.

Fasulkov et al. (2015) examined and studied the teat structures and features of 12 clinically healthy cattle using a multifrequency linear transducer. Teat structures and features such as teat canal length and diameter were investigated. The scans were performed at intervals of 1 hour, beginning with one at pre-milking, 1 hour post-milking, and a few hours after milking. The TCL was found to be significantly ($p<0.001$) lower even 2 hours after milking, however the diameter of the teat canal length was only found to be significantly different immediately after milking. They observed that post-milking the CD decreased ($p<0.001$) significantly, along with an increase in the overall increase in Overall teat diameter (OTD) and teat wall thickness (TWT). They determined that ultrasonography is a reliable, non-invasive method for visualizing the teat and its architecture in order to observe changes in the teat before and after milking.

Fasulkov et al. (2015) detected a presence of several hyperechoic specks in the teat cistern, after milking which they attributed to be milk coagula. They discovered clear evidence of inflammation in experimentally generated *Staphylococcus aureus* infection in goats. On post-infection hour 72, ultrasonography of the udder parenchyma revealed extensive hyperechoic zones, statistically significant narrowing of lactiferous ducts, and an inability to visualize anechoic blood vessels. The results showed that ultrasonography combined with histological exams might be utilized to successfully detect and track the progression of goat udder changes caused by experimental *S. aureus* mastitis.

Hussein et al. (2015) evaluated ultrasonography to test the use of ultrasonography in diagnosing subclinical mastitis in 56 sheep udder halves along with their accompanying superficial inguinal lymph nodes. The ultrasonographic alterations were compared to the milk samples' bacteriological analysis, somatic cell count (SCC), and California mastitis test (CMT). They discovered that the parenchyma of diseased udders was homogeneous and hypoechoic, that the gland cisterns were anechoic, and that the teat canal appeared as a longitudinal echogenic line. The superficial inguinal lymph nodes were easily identified as an oval-shaped structure with a thin echogenic capsule. They also discovered that the ultrasonographic length, depth, and area of the superficial inguinal lymph nodes were significantly increased in the udder infected groups ($p<0.05$), and that there was a positive correlation between the SCC values and the ultrasonographic length ($r = 0.5$).

Ismail & Nazire (2016) performed ultrasonography on a total of seventeen Awassi sheep to determine the the changes caused by mastitis in the udder and teat specifically by *Staphylococcus aureus.* They found a significant difference in the gland cistern size, width and the wall of the gland cistern in the case of clinical mastitis. They also detected a significant positive correlation between SCC and TWT in clinical quarters. They therefore determined that ultrasonography could be used to determine the degree of damage caused by mastitis as well as a diagnostic tool.

Santos et al. (2015) evaluated nine Jersey cattle using a 6 Mhz convex sector transducer to observe the internal structures and ultrasonographic appearance as well as evaluation of the udder parenchyma. It was found that ultrasonography helped in visualizing the changes occurring in the mammary gland and some cases could also be used to point out causative agents such as in the case of *E.coli* where there is greater tissue damage along with abscess formation.

Venkatesan et al. (2016) assessed teat morphology of 32 hand-milked cattle and it was observed that ultrasonography was found to be an efficient tool in the detection of teat obstruction which may cause disorders in the flow of milk, when compared to clinical detection or manual palpation. Ultrasonography was also helpful in detecting the exact etiology and location of the lesion.

Amin et al. (2018) examined thirty-eight bovines (cow-26 and buffalo-12). They examined the udder and teats by ultrasonography using the gel direct-contact method and water-bath 7 technique, respectively. It was found that the direct contact method provided an accurate visualization of the teat cistern and the water bath technique helped in the visualization of the teat as it provided a contrast of the distal portion of the teat. The mammary gland on ultrasonography appeared as uniformly distributed with a hypoechoic appearance with the alveoli being visualized as anechoic structures. The mammary vessels and veins of the glandular parenchyma were identified with their typical anechoic appearance. The three layers of the teat wall were distinctly seen as an outer hyperechoic, middle hypoechoic and inner hyperechoic. The teat cistern appeared as an anechoic area surrounded by the inner mucosal layer of the teat wall which appeared as a hyperechoic line.

Barbagianni et al. (2017) evaluated ultrasonography as an ancillary technique for a detailed study of disorders of the udder and found that this technique had many uses and could support decisions during diagnostic or health management procedures. They visualized the different structures of the udder such as the gland cister, the teats, the mammary veins and the two supramammary lymph nodes. But they found that ultrasonography should be used in combination with established diagnostic methods, including clinical, bacteriological and cytological techniques.

Galfi et al. (2017) examined the udders of 56 apparently healthy animals by ultrasonography. In these 56 animals, they found that 32 of these cows were found to be sub clinically affected with the help of CMT. On further ultrasonographic evaluation using the direct contact method they found that 27 of the cows with subclinical infections were found to have non-homogenous parenchyma with an increased anechogenicity of lactiferous ducts and rest 5 had parenchyma which appeared homogenous and hyperchogenic structure with anechoic areas that corresponded to blood vessels and lactiferous ducts. They concluded that in subclinical mastitis, the udder parenchyma appeared non-homogeneous, with a lack of clear visibility of mild alveoli and lactiferous ducts, and that this change in the udder could be caused by an increased somatic cell count, which is due to the inflammatory processes in the parenchyma.

Miranda et al. (2017) observed the udder of a Girolando breed. They used techniques such as ultrasonography and theloscopy, along with methods such as histopathology. Using imaging methods such as theloscopy and ultrasonography as diagnostic techniques combined with histopathological examination and helped confirm the clinical suspicion of obstruction of milk between the gland cistern (GC) and teat canal as a result of fibrosis at the level of rosette of Fürstenberg.

Ragab et al. (2017) examined the udder and teats of 30 cows, 20 ewes and 45 does. It was found that in the normal udder, mammary parenchyma on ultrasonographic examination appeared as homogenously hyperechoic with regions of anechoic alveoli. The outer teat skin was found to be highly echogenic, the central layer was found to be less echogenic than the skin in a uniform pattern, with the teat canal present with two distinct hyperechoic lines with an anechoic lumen. The glandular parenchyma of the udder exhibited as several hyperechoic regions surrounded by a hypoechoic border in many abscesses. The presence of hypoechoic to anechoic zones separated by hyperechoic septa due to each teat h ad independent milk cisternae in supernumerary teats. The presence of a hyperechoic obstructive mass within the teat canal was present in case of full teat obstruction.

Singh et al. (2017a) performed ultrasonography using the water-bath technique on the teats of 18 adult buffaloes to determine the different characteristics of the teat and classified them into - dry, lactating and unhealthy group each containing six buffaloes. They measured the teat length and OTD by using scale and vernier calliper, respectively. The teat canal length, teat end width, teat wall thickness and teat cistern width were measured sonographically of the fore and hind teats of dry, lactating and unhealthy buffaloes, respectively and dimensions were established for future references.

Singh et al. (2017b) examined 1040 quarters from 261 lactating cows to examine the different shapes of the teat, the shape of the teat-end, orientation of the teat and position of teat. They were screened for mastitis using the California mastitis test. Subclinical mastitis was prevalent at the rate of 30.6. The majority of the teats were normal or cylindrical in shape (48%), dished teat-ends were found in 40.7% of the cases and those that were aligned (central or squared) in orientation were found to be 65 %. They discovered a strong relationship between subclinical mastitis and teat shape, teat location, teat orientation, number of lactations, and lactation stage (P 0.05 to P 0.001). They also found significant ($p<0.07$) correlation between the teat-end shape and occurrence of subclinical mastitis. It was observed that teats with a particular pencil shape had the least chances of mastitis. They found that the prevalence of subclinical infection was the highest in the hind teats, in those teats where the placement of the teat was abnormal, late lactation and in multiparous cows.

Amin et al. (2018) used the direct contact with gel method and the water bath technique, to visualize the udder and teats of 38 bovine (cattle and buffaloes). The glandular parenchyma was found to be homogeneously hypoechoic with interspersed alveoli which appeared anechoic. The milk ducts and mammary vessels of the bovine mammary gland were anechoic. The three distinct layers of the teat wall were visualized.

Singh et al. (2019) utilized ultrasonography to examine the internal post-milking teat tissue changes to evaluate the changes caused by machine milking and the time required for the recovery after milking. They examined the left sided teats in twenty Holstein Friesian x Sahiwal crossbred dairy cows and scanned them sixfold – before milking– prior to milking, immediately after hour 1, 2, 3,4 hours after milking. They found that machine milking caused a significant lengthening of the teat canal, an increase in the overall teat diameter and an increase in the teat wall thickness and narrowing of the cistern diameter. They concluded that it took at least 4 hours for the recovery of these parameters back to their physiological status and the time taken for the recovery of the parameters was affected by the position of the teat.

Bonelli et al. (2020) evaluated the udder cistern size, ultrasonographically during dry period. They evaluated the size of forty healthy udder quarters to observe the changes of the udder cistern in the dry period and found that the size of the udder cistern decreased throughout the dry period and only increased in size at the beginning of the next lactation and found that this technique was useful to monitor the udder during the dry period.

Mourya et al. (2020) examined the udders and teats of 55 lactating cattle of livestock farm using ultrasonography MCMT, pH and SCC. All of the cows were screened by contact gel and water bath technique, in which 23 cows were subclinically infected. They found normal udder parenchyma to have homogenous hypoechogenic parenchyma with interspersed anechoic blood vessels, the teat wall to have a three-fold layer structure, the gland cistern is observed as a large region of homogenous anechogenicity with a few specks of hypoechoic dots representing the milk present within it. The rosette of Furstenberg appeared as hyperchoic cords, emerging from the teat cistern into the papillary duct. Udder parenchyma of cows found positive for subclinical mastitis by CMT and somatic cell count appeared non-homogenously hypoechoic, however the inner structures of the duct and alveoli lost its normal appearance. The inner layer of the teat cistern and canal were irregular. The three-layered appearance of the teat wall was absent in quarters affected with subclinical mastitis

Senthilkumar (2020) examined the udder parenchyma and teats of both, lactating and non-lactating Madras Red Ewes with the help of a linear transducer (7.5 – 12.5 MHz) in a transverse and longitudinal plane by the direct method. It was found that in lactating animals, the parenchyma showed increased anechoic fields within it, due to the accumulation of milk and increased vascularity. The water-bath technique was used to visualize the different structures of the teat. The three different layers of the teat wall- including the hyperechoic teat skin. The teat cistern was anechoic, and there were hypoechoic specks in it, indicating that milk was present. The rosette of Furstenberg was reported to be a circular, homogeneous hypoechoic or anechoic structure. The teat orifice was hyperechoic, a teat canal observed as a thin hyperechoic cord. They also found no significant changes in other teat tissue measurements such as Teat canal width (TCW), Teat canal length (TCL), Cistern diameter (CD) and diameter at level of Furstenberg rosette (FTD) between lactating and non-lactating Madras Red ewes.

Suzuki et al. (2020) performed ultrasonographic examination of mastitic quarters of Holstein Friesian Cows during physical and clinical examination. They observed that clinically mastitic cows had three major abnormalities: the loss of the homogenous appearance of the parenchyma, increased regions of hyperechoic spots throughout the udder and structural changes throughout the milk duct. The ultrasonographic finding of the loss of the homogeneous hypoechoic structure of the glandular parenchyma in the initial examination could detect the outcome of clinical mastitis. They concluded that ultrasonography evaluations of the mammary glands during the primary clinical examination could be a valuable and cost-effective way to predict the outcome and prognosis of clinical mastitis.

2.1.2 Ultrasonography of Supramammary lymph node

Bradley et al. (2001) examined the supramammary lymph node of fifty-four cows, determined the structure, location and architecture of the lymph node. They measure the various sizes of the supramammary lymph node and found that the lymph nodes were significantly larger in cows with more lactations although no correlation could be found between their size and either the time calved or the somatic cell count of the milk. They concluded that lymph nodes on sides that were CMT positive had significantly larger lymph nodes when compared to CMT negative sides.

Gürbulak et al. (2009) collected the milk from 1184 quarters of 296 Holstein cows between the age of 3-9 years old in Saray farms, Turkey. They examined the supramammary lymph nodes of these cows using a 7.5 MHz convex probe and measured the length and width of these lymph nodes. They found that the increase in size of the supramammary lymph node could be correlated with an increase in the somatic cell count, electrical conductivity, and bacteriological examination of the milk. It was also determined that *Staphylococcus aureus* was the most common etiological agent for mastitis. However, they concluded that the increase in the size of the lymph node could be used as an adjunct in the diagnosis of mastitis but not the sole tool for it.

Ghaemmaghami et al. (2014) examined the supramammary lymph node of twenty Sannen goats. The milk was collected, and the culture and somatic cell count was checked. After determining that these animals were healthy by a low somatic cell count and no growth in culture, the supramammary lymph nodes were examined ultrasonographically. They located the presence of two supramammary lymph nodes, one found to be larger comparatively. The lymph node had a distinct hyperechogenic capsule, hypoechogenic cortex and a hyperechogenic medulla. The length was found to be approximately 1.2cm and the width was 2.25 cm. They concluded that these lymph nodes could be visualized to determine the health status of the udder.

Khoramian et al. (2015) examined thirty-five cattle affected with Staphylococcus aureus infection and noted a significant correlation between CMT as well as mean log SCC of each side and size of supramammary lymph node in the same side. There was a strong positive correlation between lymph node dimensions and the bacteriological results on the same quarters, indicating that lymph node ultrasonography is a helpful tool for mastitis screening, especially when milk testing is not accessible.

Al-Galil & Ahmed (2016) evaluated the use of ultrasonography for the purpose of diagnosis of subclinical mastitis on fifty-four buffaloes. They were first classified into three groups based on CMT and the somatic cell count. They performed ultrasonography of both the udder and the supramammary lymph node using the 7.5 MHz linear transducer. They found that those with subclinical mastitis might be characterized ultrasonographically by presence of a uniform characterized a heterogenous hypoechoic parenchyma, increase in echogenicity of the contents of the gland cistern, the non-uniform mucosal lining of teat canal, thickening of teat wall with the loss of the distinctive three-layered appearance of the teat wall and disappearance of the rosette of Furstenberg. The superficial supramammary lymph node which was located and found to be large and distinct appearing completely hypoechoic. The lymph node had a significant increase in dimensions such as the length and depth. The area, volume and teat wall thickness, was also significantly ($P \leq 0.05$) increased along with which there was a significant ($P \leq 0.05$) increase in echogenicity of the udder parenchyma leading to an increase in gray scale analysis.

Risvanli et al. (2019) examined 102 lactating cows to test the hypothesis that B-Mode, colour doppler ultrasonographic measurements could be used to identify clinical mastitis. They were divided into three groups and the CMT was performed. It was found that colour doppler ultrasonography measurements of the supramammary lymph node revealed distortion type vascular morphology and a type 4 vascular density and the highest mixed type vascular distribution in the clinical mastitis group. Concluding that the use of B-mode and colour Doppler ultrasonographic measurements of the supramammary lymph nodes can provide useful information about the current condition of mastitis in cows.

2.2 Infrared Thermography in the detection of subclinical and clinical mastitis

Barth (2000) captured 93 infrared thermographic images of six cows over a period of 8 days to detect temperature differences within the different parts of the udder and teat and therefore the changes observed during mastitis. They found that the teat temperature increased from the teat tip to the udder base from 30 °C to 35.1 °C. They also noted that there was least variability within the udder cistern temperature comparatively. They captured images of the teat from the medial, lateral, and caudal side to work out presence of any changes and located that in cases of clinical mastitis the temperature of the teat when taken laterally was much above when taken medially. Quarter foremilk samples were taken to analyze the somatic cell count during which they found a direct correlation between a rise in cell count and a rise in cistern temperature.

Berry et al. (2003) examined the daily and variation of the udder temperature in ten dairy cow using infrared thermography (IRT). it had been found that the udder temperature rose significantly after an exercise period. They performed regression amalysis to predict udder skin surface temperature supported the environmental temperature, and that they could successfully predict the daily USST supported the equation derived from it. They concluded that infrared thermography had excellent potential in its application, for the aim of detection of subclinical mastitis.

Paulrud et al. (2005) used both ultrasonography and infrared thermography to detect the changes caused by overmilking eight Danish cows with machine milking using 1 mm liners. The cows were overmilked for a period of 4 minutes and on day 1 the ultrasonography was performed and on day 2 infrared thermography. On examination of the teat it had been observed that in milking the teat canal increased by 30-40% and after a period of over milking the teat thickness increased also. They observed that the teat canals remained elongated even twenty minutes after milking. within the case of infrared thermography they observed a drop by temperature when the pre-milking dipping followed by a spike within the temperature during milking, the temperature of the teat kept increasing until the post-milking dipping was through with ethanol solution. They concluded that ultrasonography and infrared thermography were excellent tools to review the teat tissue integrity.

Hovinen et al. (2008) experimentally induced mastitis in six lactating cows to watch the changes observed within the rectal and udder temperatures observed within the infrared thermography and concluded that the udder skin temperature rose simultaneously with the rectal temperature. Yet, local inflammatory changes of the udder, appearing before the TR increase, were not detected with IRT in experimental conditions as in field conditions.

Polat et al. (2010) assessed the relation among milk inflammatory parameters to evaluate the efficiency of Infrared thermography as a diagnostic tool for mastitis in comparison to conventional field techniques such as SCC and CMT. They observed significant relationship between udder surface skin temperature (USST), somatic cell count and CMT.

Poikalainen et al. (2012) used an infrared thermographic camera to review the various thermal patterns during a cow, to use these thermal patterns as a health index for livestock. It had been found that pain sense spots were mostly considered at udders, feet and around skin injuries and it had been therefore found that using machine milking affected the udder surface skin temperature to some extent and therefore the minimum temperature was observed at hooves, but the utmost temperature belonged to the coronary band of hooves.

Alejandro et al. (2013) observed that thermal imaging could be utilized to identify temperature variations in the udder of dairy goats as a result of mechanical milking. They discovered that utilizing thermal imaging, machine milking could raise the udder's surface temperature by 1.5 to 6.6 degrees Celsius.

Martinsa et al. (2013) evaluated the use of infrared thermography in ewes to detect and differentiate subclinical and clinical mastitis. They concluded that IRT was an excellent apparatus which not only detected subclinical quarters, but a significant difference between subclinical and clinical USST quarters was present, indicating that IRT had great potential for the diagnosis of mastitis.

Castro-Costa et al. (2014) examined eighty-three lactating dairy ewes to evaluate the use of IRT, for the purpose of detection of mastitis by observing for changes in the udder skin surface temperature. It was found that the initial signs of inflammation and intramammary infection were visualized as a significant elevation in udder skin temperature, at 4 hours after experimental infusion. At 6 hours, the highest temperature was recorded. They concluded that IRT was beneficial tool in detecting the changes brought about by intramammary infection.

Samara et al. (2014) examined sixty-five lactating dairy camel with an infrared thermal camera to observe the correlation between IRT, CMT and SCC. It was found that subclinical mastitic udders elevation of upto 1.45 °C when compared to healthy quarters. USST could be successfully correlated with CMT. It had been noted that the USST linearly increased with the increase in the CMT score. Additionally, high correlations were obtained between USST and SCC score.

Bortolami et al. (2015) evaluated the udder health status in subclinical mastitis affected dairy cows by using conventional techniques such as bacteriological isolation, detection of an increase in somatic cell count and comparing it to an increase in USST using IRT. It was noted that SCC was the most efficient technique in detecting subclinical quarters, as it detected those quarters with non-specific infection as well. Infrared thermography was correlated to SCC ($p<0.05$). but Thermographic imaging was found to be promising in evaluating the inflammation status of cows affected by subclinical mastitis but was found to be of diagnostic value.

Metzner et al. (2015) captured infrared images of the hindquarters of five healthy cows. This was done to observe the changes in the glandular parenchyma after the experimental infusion of *E.coli* into the right hindquarter. They found that in those animals which had a fever due to the intramammary infusion, there was a significant increase in udder skin temperature as well as somatic cell count. They found that one of the first apparent changes attributed to inflammation was a shift in udder skin temperature, which may be seen as early as 6 hours after inoculation.

Yang et al. (2015) compared the variation of hindquarter skin surface temperature pre- and post- milking in dairy cows to determine the optimal time to capture infrared thermographic images to improve the sensitivity and specificity of mastitis detection in dairy cattle. It was concluded that the sensitivity and specificity of IRT in mastitis detection was influenced by milk yield and the optimum time to capture thermographic images was pre-milking to avoid changes in the udder skin surface temperature due to milking.

Hanusova et al. (2016) used infrared thermography to analyse the quality of milking by checking the temperature changes in the teats of six Holstein Friesian dairy cows. They evaluated the milking procedure by comparing two partial vacuum pressures 40 and 45 kPa respectively. They checked the temperature during, immediately after milking, two minutes post-milking and four minutes post- milking and found that the highest temperature of the teat skin was found immediately after milking and most decrease in temperature was found two minutes post-milking. An average increase of about +2.44K was found using 45 kPa whereas an increase of about +1.93 K was observed at 40 kPa.

Pampariene et al. (2016) examined cows in the negative and at positive ambient temperature. Infrared thermographic images were taken of the teats at the level of the area of Furstenberg rosette and then analyzed and correlated with the findings found in CMT and it was found that there was a correlation to that of subclinical mastitis. It was observed that Infrared thermography was successful in detecting subclinical mastitis at an early stage. They noted that along with the udder skin surface temperature, the teat skin surface temperature could also be correlated to the CMT score. IRT was found to be a non-invasive, sensitive, rapid and portable method for subclinical various origins of inflammatory processes in the udder.

Sathiyabarathi et al. (2016) used infrared thermography as a novel diagnostic technique to diagnose early cases of subclinical mastitis. They found infrared thermography to be a simple, effective and non-invasive type of diagnostic method that is based on the comparative thermal difference of skin or udder surface which could be visualized in the form of images and are helpful in the diagnosis of inflammation of udder. They determined that clinical quarters had a much higher temperature as compared to subclinical quarters, in both sheep as well as cattle. Thus, IRT was found to be a sensitive, non-invasive technique which could be used in field conditions for the early detection of mastitis.

Da Silva (2016) examined a total of twenty-four lactating Girlandow cattle (healthy, subclinical and clinical mastitis). The Udder surface temperature (USST), Eyeball temperature (ET) and rectal temperature were recorded. There was an increase of upto 8.5 ° C and 2.46 ° C in clinical and subclinical quarters respectively. A difference of 7.9 and 8.0% was found between the ocular temperatures of subclinical and clinical mastitis when compared with healthy animals. When the rectal temperatures were taken a difference of 2.9% and 5.5% was found in subclinical and clinical cases as compared to healthy animals. This indicated that IRT was a sensitive technique which detected minor thermal changes occurring in the udder.

Golzarian et al. (2017) studied the correlation between an increased udder skin temperature using Infrared Thermography and the somatic cell count. It was observed that in cases of bovine mastitis the thermal images showed that there was average elevation of upto 0.45 ˚C in subclinically infected quarters when compared to healthy ones. They found that the accuracy of this method was 57.3% hence concluding that

Infrared Thermography could be used as a non-invasive, effective technique of diagnosis of mastitis.

Zaninelli et al. (2018) tested the use of infrared thermography (IRT) as a possible tool for evaluating cow udder health in field conditions. Each udder's thermographic indices were calculated automatically and in a standard way using images collected from different farms and evaluated using software. They a significant correlation between udder surface skin temperature (USST) and somatic cell count in the milk samples. They further, concluding that infrared thermography could be used as an effective tool to detect mastitis in field conditions.

Byrne et al. (2018) quantified the relationship between udder skin temperature (USST) and somatic cell count (SCC) in lactating dairy cows. Infrared thermography alone, it was concluded, could not be used to detect high SCC in dairy cattle in a pasture-based system in real time.

Sathiyabarathi et al. (2018a) evaluated the efficiency of IRT for the early diagnosis of intramammary infection in 200 quarters of crossbred cows by comparing the body temperature and udder skin surface temperature (USST) of the animals. The images were taken prior to milking, using a FLIR (Forward Looking Infrared) i5 camera. A positive correlation between the udder skin surface temperature and somatic cell count was reported concluding that the IRT technique could be utilized as a potential non-invasive, quick field test for the purpose of screening and early detection of mastitis in crossbred cows.

Sathiyabarathi et al. (2018b) used a FLIR i5 (forward-looking infrared) camera to measure ocular surface temperature (OST) and compare this with the udder skin surface temperature (USST). Thermal images of fifty-six quarters of lactating draught (Dongari) cattle before morning and evening milking were taken. They discovered a positive linear relationship between the USST of subclinical mastitis quarters and the SCC as well as EC. They concluded that IRT could be utilized as an effective non-invasive diagnostic technique for mastitis identification.

Zaninelli et al. (2018) tested the use of infrared thermography as a health index in the field of veterinary. Thermographic photos from several farms were collected and analyzed using a customized software application to calculate the thermographic indices of each udder automatically and in a uniform manner. They discovered that an increase in somatic cell count led to a simultaneous increase in udder skin temperature (USST), indicating that infrared thermography might be utilized to identify mastitis in the

Juozaitiene (2019) examined the teats of 230 Lithuanian Black and White cows and captured thermal images to assess teat-end conditions and the thermographic characteristics were determined before evening milking. On examination and classification into three groups, the images at the teat sinus level showed that one group had a lower teat temperature (0.93-1.32 ° C) than group 2 and group 3 ($p<0.01$) indicating a significant positive correlation between milk SCC and temperature of the teats evaluated by hyperkeratosis scores N, S, and R. The results of the studies clearly showed that there was a significant connection between different levels of hyperkeratosis and teat temperature in all groups which were good indicators of mastitis.

Wollowski et al. (2019) examined the use of a handheld dynamometer and an infrared thermometer to differentiate 426 cattle into clinical mastitis, subclinical and healthy animals. They found that the dynamometer was an excellent tool in the diagnosis of mastitis whereas the use of the infrared thermometer was limited as it was heavily influenced by the ambient temperature as the udder surface temperature increased by 0.15 to 0.18°C for each degree of ambient temperature.

Marcela et al. (2020) examined the quarters of hundred cattle with subclinical mastitis in which infrared images were taken from a short- milking tube of each experimental quarter at two minutes of each experimental quarter at two after milking- unit attachment in thirty-second intervals during the milking process it was found that the use of infrared thermography in dairy cows could be a valuable method for distinguishing between subclinically mastitic quarters with and without active infection.

Sarubhi et al. (2020) examined the udders of 192 buffaloes using a FLIR I7 thermal camera to evaluate the use of infrared thermography to detect subclinical mastitis in buffaloes. They collected the milk samples on the same day as well. They found that a significant positive correlation could be found with an increase in the udder skin temperature and an increase in somatic cell count of the respective quarter.

Hence, they concluded that infrared thermography is an effective technique in the detection of subclinical infections.

CHAPTER-III

MATERIALS AND METHODS

This study involved the evaluation of the use of two diagnostic techniques for the early and non-invasive detection of mastitis and to observe the changes produced in the udder due to clinical as well as subclinical mastitis. The study involved two parts- the first being the ultrasonography of the udder, teat and supramammary lymph nodes of apparently healthy animals. Fifty-two lactating animals in the Livestock Dairy farm, GADVASU, Ludhiana were examined ultrasonographically to detect the changes in the teat, udder and supramammary lymph node in subclinically affected animals. The same was done in thirty cattle with clinical mastitis to observe the changes in the udder and teat tissue as well as the supramammary lymph nodes.

The second objective was to standardize the use of infrared thermography in the case of mastitis in which they were divided into two groups – healthy and clinical. The temperature variation between healthy, subclinical and clinical quarters were observed and statistically analyzed to observe if there was a significant difference between the three groups and to evaluate if there was a correlation present between the temperatures of the respective quarters and the milk inflammatory parameters of the ipsilateral quarters.

3.1 Ultrasonography (USG) of udder, teat and the supramammary lymph node

3.1.1 USG of udder, teats and supramammary lymph node of apparently healthy cows

The study was conducted from the period of December 2020 to April 2021 at DLF (University Dairy Farms)

3.1.1.1. Animals

The study included 52 cows of the dairy university farm. Ultrasonography was used to scan all four teats, the udder parenchyma, and the supramammary lymph node. The experiment was carried out to examine the ultrasonographic alterations in subclinically afflicted udders, teats, and supramammary lymph nodes in dairy cows as a result of intramammary infection.

The health and infection status of the cows were determined through the analysis of milk samples using tests such as the California mastitis test, somatic cell count, electrical conductivity, and bacteriological findings, which were then correlated with ultrasonographic changes of the udder, teat, and supramammary lymph node.

3.1.1.2. Ultrasonography technique

The ultrasound (US) was performed with a 10-5 Mhz linear transducer (L38, Sonosite, serial number: 03RQ5k) on a portable ultrasound scanning equipment (Sonosite M-Turbo, Fig:1). The mammary gland parenchyma was ultrasonographically examined with a 10-5 MHz linear probe utilizing the direct contact technique (transcutaneous echography) (Cartee et al., 1986). The water-bath approach (Franz et al., 2009) or the cup method, in which the teat is completely immersed in a clear plastic container filled with warm water (30–35°C) and the transducer is positioned in contact with the container, is the principal technique utilized for echography of the teat structures (Fig.7). To improve the quality of the image obtained, a large amount of gel is applied to the transducer. A substantial quantity of gel is placed on the transducer to improve the quality of the image obtained and increase the contact with the plastic glass. The probe was moved vertically until a clear image of the teat emerged on the USG console. A vertical cross-section image of the teat was captured and then stored in the system (Singh, 2012). All four quarters were scanned and evaluated for ultrasonographic alterations using a similar approach. Teat dimensions such as the overall teat diameter (OTD), teat canal length (TCL), cistern diameter of the teat (CD), Teat wall thickness (TWT), the teat diameter at the level of the Furstenberg rosette (FTD), as well as other abnormalities and changes in the udder and teats, were noted. When taking measurements of the dimensions, certain standards were followed, such as measuring the teat diameter at the level of the Furstenberg rosette (FTD) at a distance of 1 cm from the measurement taken for the Overall Teat Diameter (OTD). The direct contact gel technique has been used to conduct ultrasonography of the udder. The gland cistern was scanned after gel was placed over the base of the teat. When the image was taken, it was saved on to the console, so that the different parameters could be measured later using Image J software.

Fig. 1: USG equipment used for scanning udder and teat

The supramammary lymph nodes were then examined. The lymph node has a highly hyperechoic capsule due to the presence of dense connective tissue, a hypoechoic cortex, and a highly echogenic inner medulla, the supramammary lymph nodes that are placed superficially are stated to be rather clearly delineated ultrasonographically from the surrounding fat and udder tissue (Bradley et al., 2001). The size and structure of the lymph node in dairy cattle vary depending on the animal's health and the size of the udder, which increases in size in proportion to the level of infection present. The lymph nodes are first identified between the udder and the thigh, on the dorsal and caudal aspect. After applying isopropyl alcohol to the area, the gel is applied on to the probe and then scanned (Fig.9). The length and depth of the lymph node is measured using the Image J software and then further correlated with the ultrasonographic findings of the teats and udder and milk parameters.

3.1.1.3. Sampling

Quarter foremilk samples were collected for further analysis. The teats were thoroughly wiped and dried before collecting the milk samples. The teat orifice was cleaned in a centrifugal direction with 70% alcohol (spirit). The first few streaks of milk sample were discarded, and separate quarter samples (about 10 ml) were collected in sterile marked test tubes, stored in an ice box, and immediately transferred to the laboratory. The milk samples were subsequently tested in the Department of Veterinary Medicine's mastitis lab.

3.1.1.4 Parameters studied

3.1.1.4.1 Ultrasonographic readings

Other abnormalities and changes in the udder and teats were detected using ultrasonography, including the overall teat diameter (OTD), teat canal length (TCL), cistern diameter (CD), Teat wall thickness (TWT), the teat diameter at the level of the Furstenberg rosette (FTD), and other abnormalities and changes in the udder and teats. The size of the supramammary lymph node was also measured and noted, with the length and depth noted

3.1.1.4.2. Isolation and identification of pathogenic bacteria

Microbial organisms were isolated and identified from milk samples according to standard procedures of National Mastitis Council (Brown et al., 1969).

Fig. 2: Inoculation of milk samples

The milk samples were well mixed before being streaked on 5 percent defibrinated sheep blood agar plates at the mastitis laboratory. Each plate was divided into eight equal sections, each quarter was marked, and 0.05 ml of quarter milk sample was streaked with a platinum loop (Fig 2). The plates were then incubated aerobically at 37°C for 24 hours before being re-evaluated for the presence of any bacterial growth after 48 hours. If there were more than five colonies isolated, a quarter was declared infected. If three or more bacterial species were isolated from each sample and no significant udder pathogens were found, the samples were considered contaminated. According to Hargital et al. (1992) the suspect colonies were recognized morphologically, microscopically, and biochemically.

3.1.1.4.2.1 Gram staining

Using the conventional Gram's staining procedure (Cruickshank et al.,1982), organisms were classified as Gram-positive or Gram-negative.

3.1.1.4.4.2.1.1 Identification of Gram-positive (coagulase positive bacteria and its differentiation from coagulase negative bacteria)

The aseptically collected milk samples were properly shaken before being streaked onto the 5 percent defibrinated sheep blood agar (BA) with the use of a 4 mm diameter platinum loop. After that, each plate was separated into four equal sections for the inoculation of samples from each cow's separate quarters. They were then incubated at 37°C for 24 to 48 hours, but not longer than that to avoid contamination.

The organisms were identified by the grams stain that the organism took up, as well as shape and colony characteristics after inoculation. To identify the organism, additional tests such as catalase and oxidase tests were done. The catalase test distinguishes between Gram positive cocci *Staphylococcus* spp. and *Streptococcus* spp. *Staphylococcus* spp. is catalase positive as it has the ability to catalyze hydrogen peroxide to form oxygen and water, which can be seen as frothing. Staphylococcus spp. is oxidase negative due to the absence of the cytochrome oxidase enzyme. Both enzymes are absent in Streptococcus, making it catalase and oxidase negative.

When grown on blood agar, *Staphylococcus* can be identified by their characteristic round smooth, golden-colored colonies that are mostly surrounded by an area of haemolysis known as beta haemolysis, although there may also be no haemolysis around the colonies or gamma haemolysis. Inoculation of *Staphylococcus epidermis* on blood agar results in smaller, white cohesive colonies that are non-hemolytic. Gram positive cocci are detected in large clusters when these colonies are stained with Gram’s stain. By inoculating the colonies grown on Mannitol Salt Agar, a selective and differential media, *Staphylococcus aureus* can be distinguished from other staphylococcal species such as *Staphylococcus epidermis* and *Staphylococcus saprophyticus*. Due to mannitol fermentation, *Staphylococcus aureus* turns the media yellow and forms yellow colonies, whereas the other Staphylococcus spp do not have the ability to ferment mannitol and produce pink or red colonies.

3.1.4.2.1.1.1 Coagulase Test

Staphylococci coagulase activity was examined and seen in rabbit plasma. (Mumbai, India: HiMedia Laboratories Pvt. Ltd.)

Slide Coagulase Test (Clumping factor)

The experiment began with two drops of saline placed in two distinct positions on a slide to symbolize test and control. After that, an individual colony was added to each of these sites using an inoculating loop. A drop of rabbit plasma was added to the test and stirred carefully and gently for 10 to 15 seconds. The clumping was seen within 10 seconds in the test and was considered positive, but there was no clumping in the control.

Tube Coagulase Test

For the tube coagulase test, 500 µL of rehydrated rabbit plasma was pipetted into a serial test tube, followed by 50 µL of broth culture of the test organism and incubated at 37 °C. The tubes were checked at regular intervals and were first viewed at 4 hours, then again at 24 hours for formation of clumps or clots. Those found to have clumping were considered as coagulase positive.

3.1.1.2.1.1.2 Catalase test

The slide catalase test was used to distinguish *Staphylococcu*s spp. from *Streptococci* spp. (Fig. 3) It works on the principle that *Staphylococcus* spp., an aerobic organism, can use hydrogen peroxide and break it down into oxygen and water due to the presence of the catalase enzyme. This process is depicted in the image below by the formation of froth or bubbles. A small bacterial colony was transferred from the plate to a slide using a glass rod for this test. Following that, one to two drops of 3 percent hydrogen peroxide were applied to the colony. The presence of *Staphylococcus* was indicated by the formation of bubbles or froth because of the rapid breakdown. The presence of *Staphylococcus* was visualized with the formation of bubbles or froth due to the rapid break down of hydrogen peroxide into oxygen. *Streptococci* showed no bubbling as they do not possess this enzyme.

Fig. 3: Catalase test

3.1.4.2.1.1.3 Identification of *Streptococcus* spp. organism

Streptococcus organisms were first detected using Gram's stain, where they were identified based on colony characteristics and morphology. They form small translucent colonies on blood agar, which may be surrounded by a zone of β-haemolysis. These colonies were then tested for catalase and oxidase activity, and they were discovered to be catalase and oxidase negative.

3.1.4.2.1.2 Identification of Gram-negative bacteria

When milk samples are inoculated on blood agar, the colony characteristics of *E. coli* are large, circular, moist, and grey in colour. They have a β-hemolytic nature to them. They appeared as gram-negative rod-shaped bacterium on Gram staining. The colonies were subsequently inoculated on EMB agar to confirm the presence of *E. coli*. Due to the metachromatic qualities of the dye, this agar is a selective agar for Gram negative bacteria, and *E. coli* produces a distinctive metallic green sheen. Catalase is present in *E. col*i, although it lacks oxidase.

3.1.4.3 California Mastitis Test/ Sodium Lauryl Sulphate Test

The CMT kit, which was developed by the university, is used for this test. Sodium lauryl sulphate (3%) and bromocresol purple make up the reagent (0.5 %). The reagent is used to identify an increase in the number of somatic cells present in the milk in this test. It breaks down the membranes of the cells present, allowing the reagent to react with it and causing the gel to develop. This reaction was analysed using Pandit and Mehta's conventional technique (1969). The results were categorized based on the gel formation and scored as Negative (0), Trace (0.5), 1, 2 or 3 according to the degree of gel formation which could provide an overview about the severity of the condition.

3.1.4.4 Electrical conductivity

The electrical conductivity was measured using a Mettler-Toledo AG Electrical Conductivity Meter (Fig: 4) The data was given in mS/cm (milli Siemens per cm). sample.

3.1.4.5 pH

A digital pH meter, Mettler-Toledo AG FEP20, serial number B511709055, was used to record the pH of milk.

3.1.4.6. Somatic Cell Count

A somatic cell counter by DELTA Instrument, BV Kelvinlaan 3, 9207 JB Drachten, The Netherland, was used to analyze milk samples for SCC (Fig.5). Each measurement takes around 30 seconds and the machine takes approximately 1 ml of the sample. The SomaScope Smart cell counter counts somatic cells from a laminar flow of cells through the flow cell using a dual detection system that utilizes a light emitting diode (LED) as the light source. Results were expressed in x10^3 cells/ml.

Fig. 4: Measurement of EC and pH

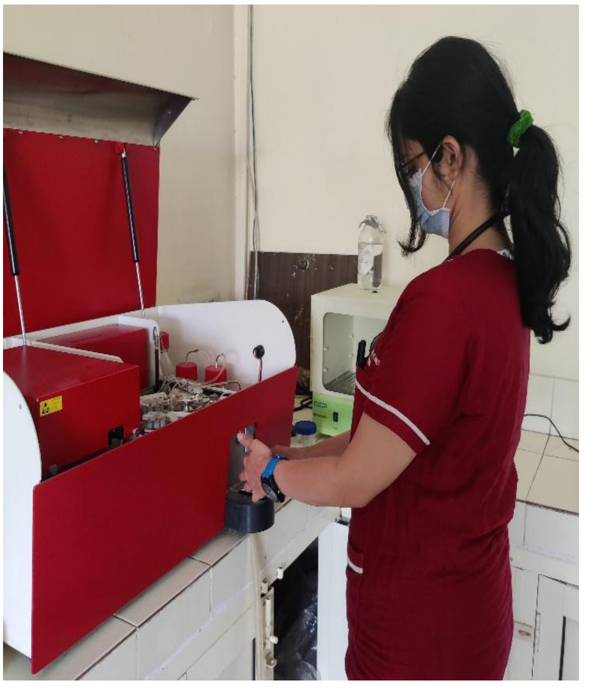

Fig. 5: Measurement of SCC

3.1.1.5 Definition of quarter health status

The health status of individual quarters was assessed and defined based on bacteriology and somatic cell count of quarter foremilk samples, based on International Dairy Federation (IDF) criteria.

Milk SCC (Cells/ml)	Microbial Pathogen	Health Status
≤ 200,000	Not detected	Healthy
≤ 200,000	Detected	Latent Infection
> 200,000	Not detected	Non-Specific mastitis
> 200,000	Detected	Specific Mastitis

3.1.2 Ultrasonography of udder, teats and supramammary lymph nodes of clinical cows affected with clinical mastitis

The study was conducted from September 2020 to May 2021 at the Large Animal Clinic.

3.2.1 Animals

A total of 30 lactating cows were scanned (120 quarters clinically affected). This experiment was done to observe the ultrasonographic changes in the udder, teats and supramammary lymph nodes in cows affected with clinical mastitis. This was done in animals brought to the Large Animal Clinic, GADVASU, Ludhiana.

3.2.2 USG technique and changes

The udder, teat, and suprammamary lymph node were inspected as mentioned earlier. Other abnormalities and alterations in the udder and teats were recorded, including overall teat diameter (OTD), teat canal length (TCL), cistern diameter of the teat (CD), Teat wall thickness (TWT), and teat diameter at the level of the Furstenberg rosette (FTD). In addition, the length and depth of the supramammary lymph node were measured. These were also compared to healthy animals, and the results were correlated to the tests performed on the milk samples collected.

Fig. 6: Linear probe

Fig. 7: USG of teat: water-bath technique

Fig. 8: Convex probe

Fig. 9: USG of supramammary lymph node: direct contact method

3.2.3 Sampling

Quarter foremilk samples were aseptically collected as defined earlier.

3.2.4 Parameters to be studied.

Same parameters were studied as described earlier

3.2.5 Use of software for evaluation of USG images

The teat scans were then saved in JPEG format on the embedded flash drive and then transferred to a computer via USB for further image processing. Using an open-source software application, images were analyzed and parameters were measured (ImageJ, National Institutes of Health, Bethesda, MD). Wie land et al. (2018) measured teat dimensions using the same software and found no significant differences between the two techniques. We used this software to measure the teat and its dimensions, as well as an ultrasound machine, and discovered no significant differences between the two methods. The teat dimensions measured were – Overall teat diameter (OTD), teat wall thickness (TWT), Teat diameter at level of rosette of Furstenberg (FTD), cistern diameter (CD) and teat canal length (TCL) was measured. The measurements taken for the two supramammary lymph node were length and depth (LxD)

3.2.6 Statistical analysis

The SPSS package (IBM) was used to analyze the data. To characterize numeric data, descriptive statistics have been used, including frequency, mean, and standard error (SE). To compare the mean of normally distributed numeric data, the independent sample student’s T test was used. Pearson's correlation coefficient for numeric variables was used to examine the relationship between milk inflammatory parameters and ultrasonographic changes in the udder and teat was done. The link between supramammary lymph node dimensions and milk inflammatory parameters, as well as teat tissue measurements, was investigated using Spearman's rank correlation. To establish normalcy, the SCC values were transformed to Log_{10}. P values ≤0.05 were regarded as statistically significant.

3.2 To standardize the use of infrared thermography in healthy and mastitis cows

3.2.1 Infrared thermography (IRT) of udder

The research took place at Livestock Dairy Farm, from March to April 2021. This research was also carried out on clinical cases at Large Animal Clinic, GADVASU, where the climate ranges from warm to temperate during these months. The farm is located at 30.8919° N, 75.8003° E, at a height of 253 m. above sea level. The annual average temperature is 23.5 ℃. June is the warmest month of the year, with an average temperature of 32.4°C, while January is the coldest, with an average temperature of 8°C. The average annual rainfall is approximately 860 mm, with July receiving the most rain and November being the driest month. During the study period in March, the average temperature was 20.5 °C, with a mean relative humidity of 56 %. The temperature measurement was corrected to degrees Celsius and the distance to meters after the camera was calibrated to ambient temperature. For all the images, the emissivity and reflected apparent temperature were 0.95 and 20.0°C, respectively. The image was captured at a distance of 1 m from the animal, and the experiment was carried out within a period of one week, in a covered shaded area to avoid direct sunlight which might affect the equipment as well as the udder skin surface temperature.

3.2.2 Animals

During the study period, a total of 29 animals were examined, which comprised of 17 apparently healthy and 12 clinically mastitic cows. The animals having a rectal temperature within the normal range were regarded to be apparently healthy. The purpose of the study was to measure temperature differences between affected and unaffected quarters, to distinguish healthy from mastitic animals based on these differences, and to standardize the use of infrared thermography as a rapid and non-invasive diagnostic approach for mastitis diagnosis.

Infrared thermography was conducted on the following quarters – left fore (LF), right fore (RF), left hind (LH) and right hind (RH), with four images per animal amounting to a total of 116 images.

Fig. 10: Infrared thermal camera; FLIR E60

3.2.3 Infrared thermography technique

A FLIR E60 infrared thermographic camera (Fig. 10) was utilized to obtain the infrared images (FLIR Systems Inc, 27700 SW Parkway Ave. Wilsonville, OR 97070, USA). The FLIR E60 features a 20 x 240 60Hz infrared detector with a thermal sensitivity of 0.05°C and a temperature range of -20 to 650°C. The healthy animals were selected from the GADVASU dairy farm, which yielded 812.5 L. of milk, with a wet average of 15.9 L. and a peak yield of roughly 32.4 L.

Udder skin temperature was taken before milking in the shed avoiding direct sunlight at an approximate temperature of 25°C (Fig.11). The quarters were divided into left fore (LF), right fore (RF), left hind (LH) and right hind (RH). The animal was restrained to avoid temperature variations due to activity and then the thermal camera was pointed to the udder beginning with the left forequarter, then the left hind, followed by the right hindquarter and finally the right forequarter. This was repeated for all the other animals as well. To ensure accuracy, the image was captured from a distance of around 1 metre. The images from the camera were saved onto the SD card which was then transferred to the computer. The FLIR Report Studio tool was used to transfer and analyze the photos, and the temperatures of the animal were compared to the parameters of the quarter foremilk samples.

Fig. 11: Capturing thermal image using infrared thermal camera

3.2.4 Sampling

Quarter samples were collected in quarters to be analyzed further. The teats were thoroughly cleaned and dried before collecting the milk samples. The teat orifice was cleaned in a centrifugal direction with 70% alcohol (Spirit). The first few streaks of milk sample was discarded, and separate quarter samples (about 10 ml) was collected in sterile marked test tubes, stored in an ice box, and immediately transferred to the laboratory. The milk samples were subsequently tested in the Department of Veterinary Medicine's mastitis lab.

3.2.5 Parameters studied

3.2.5.1 Infrared thermography images and their readings

For each animal, four images were taken, representing the four quarters of the animal. These images were saved to an SD card and then transferred to a computer. After viewing the photos, the temperatures of the animals' various udder quarters were recorded in degrees Celsius. These images were then transferred to the FLIR Report Studio application, where they were processed and analyzed. The milk somatic cell count, pH, EC, and bacteriological test were then compared to these temperatures.

3.2.5.2 Isolation of pathogenic bacteria

The isolation of pathogenic bacteria was done as in a similar fashion to experiment number one.

3.2.6 Statistical analysis

The SPSS program (IBM) was used to analyze the data. To characterize numeric data, descriptive statistics were used, including frequency, mean, and standard error (SE). The mean of normally distributed numeric variables was compared using the independent sample student’s T test. Pearson's correlation coefficient for numeric variables was used to examine the relationship between milk inflammatory characteristics and temperature changes seen in infrared thermography. To establish normalcy, the SCC values were transformed to Log_{10}. P values ≤ 0.05 were regarded as statistically significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Ultrasonography of udder and teats

4.1.1. Ultrasonography of udder and teats of apparently healthy cows

In our study, a total of 52 apparently healthy cows comprising of 208 teats and quarters were examined ultrasonographically. The quarter health status was determined according to International Dairy Federation (IDF) criteria. Of fifty-two animals, 30 cows (57.69%) were completely healthy with unaffected quarters whereas subclinical infection in at least one quarter was found in 22 (42.3%) of the cows. Out of 208 quarters, 123 (59.14%) quarters were healthy, 41 (19.71%) with latent infection, 7 (3.37%) of the quarters had non-specific infection and 37 (17.79%) with specific subclinical infection (Table 1). These findings were similar to Mir et al. (2014) who observed that the prevalence of SCM, 30.73% and 57.8 % on quarter and animal basis, respectively in the districts of Punjab. Krishnamoorthy et al. (2017) also found a pooled prevalence of subclinical mastitis of 41% on animal basis. Another metanalysis by Bangar et al. (2015) reported quarter and animal-wise prevalence of mastitis as 23.25% and 46.35% respectively.

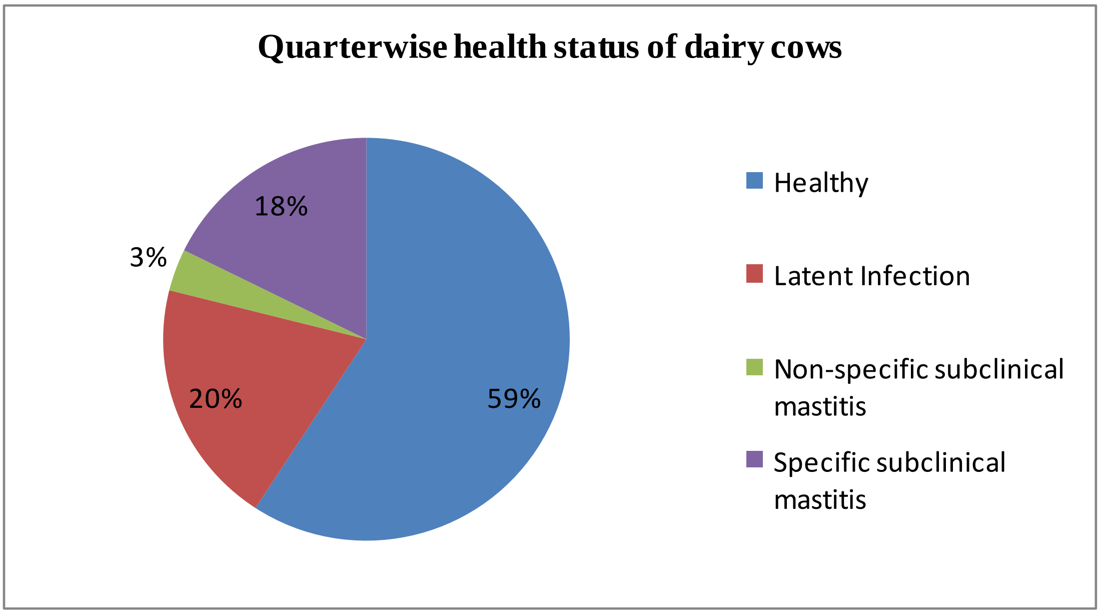

Fig. 12: Quarter wise health status of apparently healthy cows

Table 1: Quarter wise health status of dairy cows

Type	Number	Percentage
Healthy	123	59.14
Latent Infection	41	19.71
Non-specific subclinical mastitis	7	3.37
Specific subclinical mastitis	37	17.7
Total	208	100

Table 2: Distribution of quarters w.r.t quarter health status

Teat	Healthy	Latent Infection	Non-specific subclinical mastitis	Specific Subclinical mastitis
LF	31	9	2	10
LH	32	8	1	11
RF	33	11	3	6
RH	27	13	1	10
Total	**123**	**41**	**7**	**37**

Out of 208 quarters examined, 85 quarters had atleast one type of intramammary infection – latent, specific, or non-specific. Perusal of table 2 revealed that the prevalence of the infection in LF, LH, RF and RH subclinical quarters was observed to be 24.7, 23.53, 23.53 and 28.23 percent (%), respectively. The proportion of specific subclinical infection in the forequarters was 16/41 (39.02%) and the hindquarters had a proportion of 21/44 (47.73%) (Table 2). This observation is like the findings reported by Swami et al. (2017) where the quarter wise prevalence of subclinical mastitis quarter wise in left fore, left hind, right fore and right hind were 20.45, 27.27, 24.44 and 29.54 %, respectively with right hind quarter having the highest prevalence of subclinical mastitis. The proportion of specific subclinical mastitis in the present study was, in forequarter 56.77% (21/37) and in 43.24 % (16/37) in hindquarters Our findings however, differed from Singh (2019) who reported the proportion of forequarters in specific mastitis as 23/44 (52.27%) and the proportion of hind quarters in specific mastitis was 21/44 (47.72%). But he also observed high proportion of specific mastitis in forequarters so did we.

Table 3: Overall distribution of organisms isolated from quarters of apparently healthy cows – quarter- wise distribution

Organism	Frequency	LF	LH	RF	RH
No growth	130	33	33	36	28
Coagulase positive staphylococcus (CS)	45 (57.69%)	13	8	10	14
Coagulase Negative staphylococcus (CNS)	30 (38.46%)	4	10	7	9
E. coli	2 (2.56%)	1	1	-	-
***Streptococcus* sp.**	1 (1.28%)	1	-	-	-

Out of the 208 quarters, a total of 78 quarters, were found culturally positive on bacteriological examination. Of the total quarters, 7 quarters with SCC (>2 lakh cells/ml but culturally negative and were categorized as non-specific subclinical mastitis. 130 quarters had no growth, out of 78 culturally positive quarters, 45 (57.69%) of the positive quarters had coagulase positive staphylococci (CS), 30 (38.46%) coagulase negative staphylococci (CNS), 2 (2.56%) *E. coli* and only 1(1.28%) *Streptococcus* sp. This is similar to the findings reported by Sukur & Esendal (2020) who reported that 87 out of 235 samples were bacteriologically positive for staphylococci. Out of these 87 samples, the prevalence of coagulase positive staphylococci was 60.90% (53/87) and of coagulase negative staphylococci was 39.10% (34/87).

4.1.1.1. Ultrasonographic changes in the udder and teats w.r.t. quarter health status

The teat tissue measurements were categorized according to the udder health status into healthy and subclinically infected. The healthy quarters included those that had latent infection along with the completely healthy quarters, whereas the subclinical quarters included specific and non-specifically affected quarters. In the present study, we observed a significant ($p<0.05$) increase in the OTD of subclinical quarters when compared to the healthy ones. This was in corroboration with the studies by Franz et al. (2009) who noted that quarters affected with mastitis, had a larger diameter and larger TWT due to the proliferation of tissues and mucosal lesions. They also noted that in some cases stenosis of the teat cistern was also present. Singh et al. (2019) observed the changes produced in the teat tissue structures brought about by machine milking such as an increase in the overall teat diameter and an increase in the teat wall thickness and narrowing of the cistern diameter. They also reported that these teat tissue parameters would require a minimum of 4 hours to recover back to their physiological state after machine milking.

There was also about 7.5 and 3.6% increase in the TWT and TCL of subclinical quarters, respectively. No other teat tissue measurements changed significantly. Studies done by Kotb et al. (2014), on ultrasonographic examination of quarters with mastitis, revealed a thickened teat wall with a complete loss of the three-layered appearance which are consistent with our findings. Ismail & Nazive (2016) reported that a strong linear relationship between somatic cell count (SCC) and teat wall thickness in clinical quarters. Our findings about TCL coincide with findings by Gulyas and Ivansics (2001) who noted a positive correlation between TCL and the SCC of the ipsilateral quarter, indicating that quarters with intramammary infection had a longer TCL. This however contradicts with the findings by Grindal et al. (1991) who reported that quarters with subclinical and clinical mastitis had a shorter teat cistern length as compared to healthy uninfected animals.

Table 4: Teat tissue measurements (Mean±S.E.) in relation to quarter health status w.r.t apparently healthy cows

Quarter	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
Healthy (n=164)	2.60 ± 0.02[a]	0.94 ± 0.01[a]	2.33 ± 0.02[a]	0.84 ± 0.03[a]	1.39 ± 0.02[a]
Subclinical (n=44)	2.80 ± 0.07[b]	1.01 ± 0.04[a]	2.25 ± 0.08[a]	0.80 ± 0.05[a]	1.44 ± 0.06[a]

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

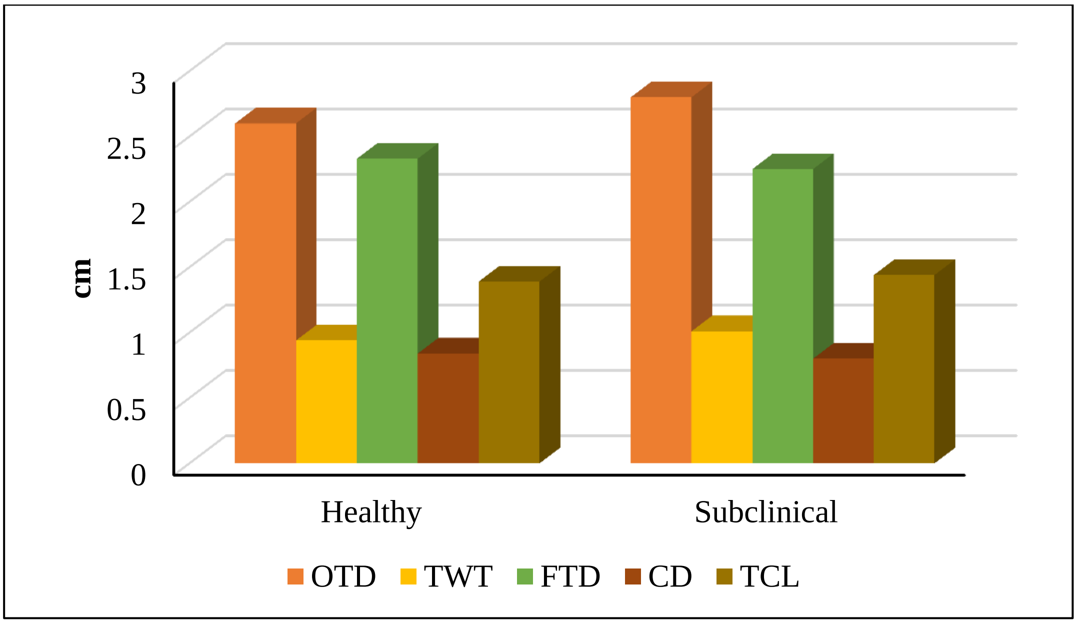

Fig. 13: Teat tissue measurements in relation to quarter health status

Table 5: Changes in milk inflammatory parameters (Mean±S.E.) in relation to quarter health status

Quarter	CMT	pH	EC (mS/cm)	SCC ($\times 10^3$ cells/ml)	Log_{10} SCC
Healthy (n=164)	0.13 ± 0.02^a	6.72 ± 0.01^a	5.23 ± 0.08^a	43.74 ± 3.61^a	1.31 ± 0.04^a
Subclinical (n=44)	1.43 ± 0.10^b	6.87 ± 0.01^b	6.40 ± 0.27^b	448.09 ± 27.99^b	2.61 ± 0.02^b

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

On perusal of Table 5, it revealed that in subclinically affected quarters, all the milk inflammatory parameters were significantly elevated when compared to the healthy quarters. As discussed by Dasohari et al. (2018) it was noted that the accuracy of CMT in detecting mastitis was found to be 76.52%, making it one of the most reliable field side tests. The elevation of the milk inflammatory parameters such as pH and EC was in concordance with the studies done by Kitchen (1980) who noted an elevation in the pH and EC due to a significant increase in the blood supply to the udder following intramammary infection, which was proceeded by an increased permeability in the blood vessels leading to an increased influx of sodium and chloride ions into the milk. A depletion of lactose in the milk has also been attributed to the increase in mastitic milk. Shahid et al. (2011) who also observed milk pH of higher than 6.8 in quarters found positive for subclinical mastitis. Sathiyabharathi et al. (2018) also reported that EC is an effective diagnostic tool, as it can detect the intramammary infection at an early stage.

A similar trend was observed with SCC was also as found in EC, when SCC was transformed to Log_{10} SCC. Petzner et al. (2017) reported that SCC below 200,000 cells/ml of milk is widely used as the threshold level to differentiate healthy udders from sub clinically affected quarters, and these were consistent with our classification and findings as well. The cause of a higher SCC has been known to be due to inflammation that further leads to widened endothelial and epithelial intercellular junctions which allow the migration of inflammatory cells from the blood stream into the extracellular space and into the milk. Godkin & Leslie (1993) explained how a positive correlation could be obtained between a higher SCC and subclinical mastitis and how it could be a useful technique in screening herds for major pathogens

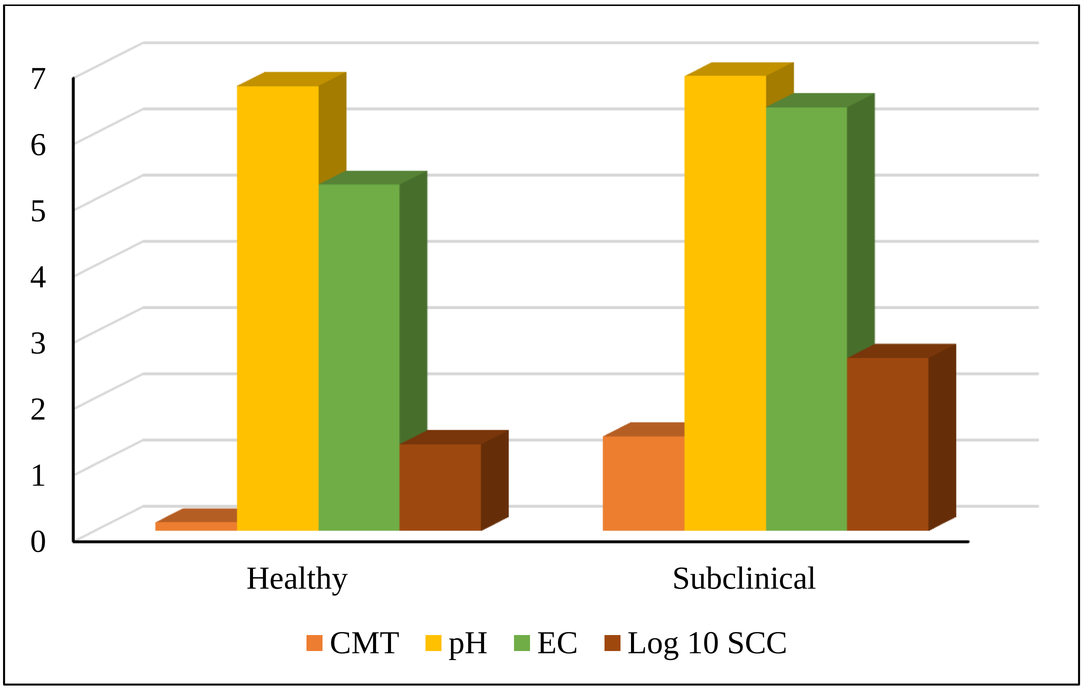

Fig. 14: Changes in milk inflammatory parameters in relation to quarter health status

Table 6: Teat tissue measurements (Mean± SE) with respect to infection status of apparently healthy cows

Health status	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
Uninfected (n=130)	2.61 ±0.02[a]	0.95 ±0.02[a]	2.34 ±0.03[a]	0.87 ± 0.036[a]	1.41 ± 0.02[a]
Infected (n=78)	2.69 ± 0.05[a]	0.97 ± 0.03[a]	2.26 ± 0.05[a]	0.76 ± 0.03[a]	1.38 ± 0.04[a]

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

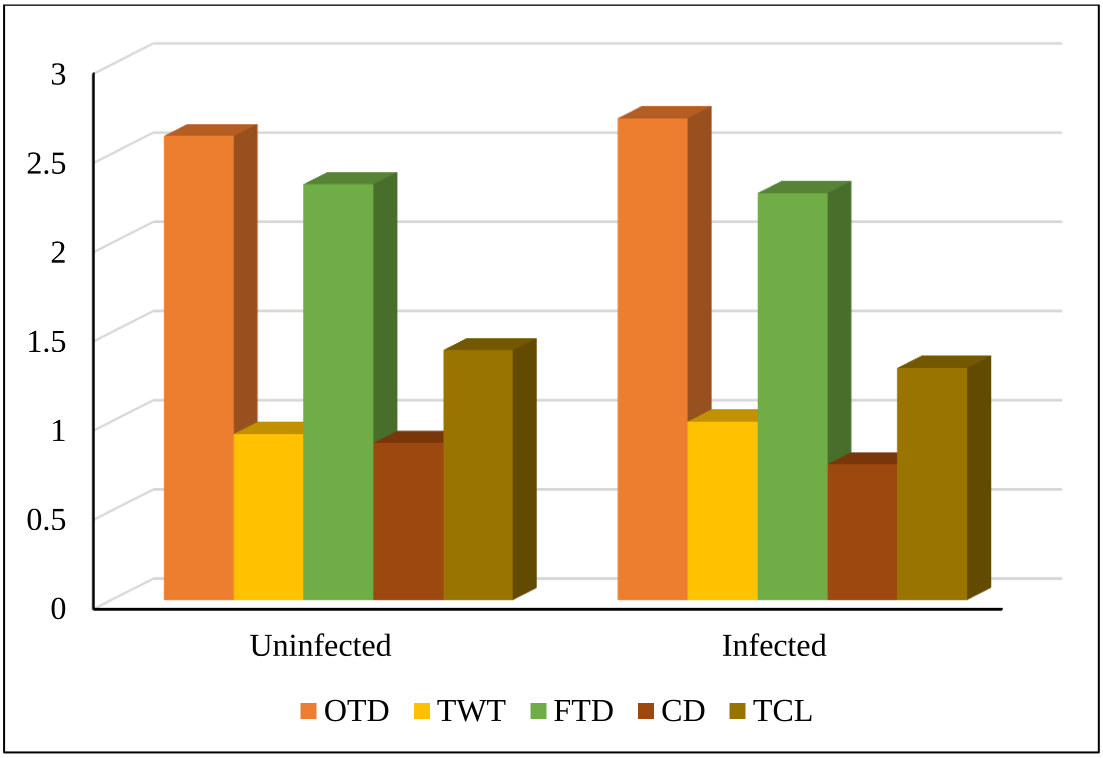

Fig. 15: Teat tissue measurements with respect to infection status of apparently healthy cow

On comparing, the teat tissue measurements based on intramammary infection, no significant variation in any of the parameters was recorded. The TWT in infected quarters swelled 4.3% more than those of uninfected ones. This is in concordance with the findings reported by Singh (2019) who reported a significant ($p<0.05$) increase in the TWT in infected quarters when compared to uninfected quarters.

Table 7: Teat tissue measurements in relation to infection with major pathogens

Organism	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
No growth (n= 130)	2.61 ±0.02[a]	0.95 ±0.02[a]	2.34 ±0.03[a]	0.87 ± 0.036[a]	1.41 ± 0.02[a]
Coagulase positive staphylococci (CS) (n= 45)	2.64 ±0.065[ab]	0.96 ±0.04[a]	2.23 ±0.07[a]	0.73 ± 0.037[b]	1.35 ±0.07[a]
Coagulase negative staphylococci (CNS) (n=30)	2.75 ± 0.09[b]	0.99 ±0.46[a]	2.37 ±0.07[a]	0.82 ±0.08[a]	1.40 ± 0.07[a]

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

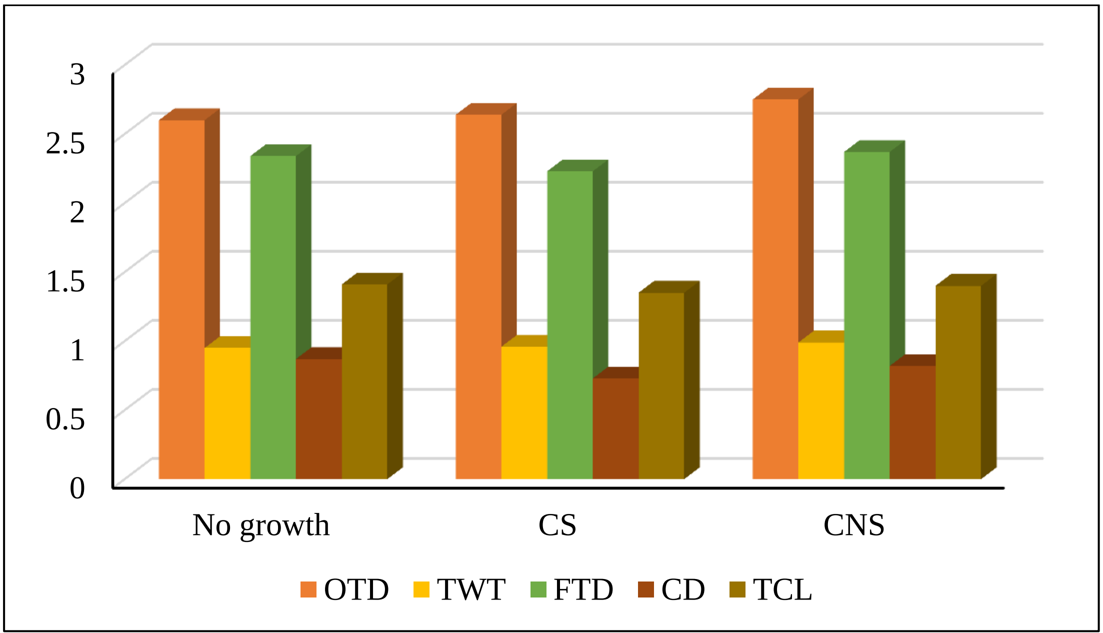

Fig. 16: Teat tissue measurements in relation to infection with major pathogens

The teat tissue measurements were further classified according to the organism that was isolated in the respective quarter (Table 7). Quarters affected with CNS means had significantly higher ($p<0.05$) OTD as compared to quarters with no growth. The CD in quarters afflicted with CS were significantly ($p<0.01$) smaller as compared to quarters with no growth. TWT was thicker in quarters where CNS and CS were isolated, however these findings were not significant ($p>0.05$). Studies done by Seker et al. (2009) reported that FTD was influenced by the age of the animal and based on the studies they conducted they found that those animals found to be CMT positive had a slightly smaller FTD when compared to those with CMT negative quarters which could probably be due to the disappearance of the typical appearance of the rosette of Furstenberg in the case of clinical mastitis. This could explain the reason why the quarters in our study affected with CNS and CS had a shorter FTD.

Table 8: Teat tissue measurements w.r.t. SCC of apparently healthy cows

Group	SCC (cells/ml)	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
1	**< 2 lakh (n=164)**	2.60 ± 0.03[a]	0.94 ± 0.01[a]	2.33 ±0.03[a]	0.84 ±0.03[a]	1.39 ±0.02[a]
2	**2-4 lakh (n=23)**	2.58 ± 0.08[a]	0.98 ±0.04[a]	2.21 ±0.13[a]	0.80 ±0.09[a]	1.60 ±0.07[b]
3	**>4 lakh (n=21)**	3.05 ±0.11[b]	1.05 ± 0.018[a]	2.30 ±0.09[a]	0.79 ±0.06[a]	1.27 ± 0.02[a]

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

Teat tissue measurements of apparently healthy cows were compared according to milk SCC. Three groups of SCC such as <2, 2-4 and >4 lakh cells/ml were formed. The OTD between group 1 and 3 differed significantly ($p<0.05$) (2.60 ±0.03 vs 3.05 ±0.11 cm). The OTD of the second group also differed significantly ($p<0.05$) from the third group, however the OTD did not vary significantly between group 1 and 2. The TWT was found to be the highest in the group 3, which could be attributed to the inflammatory changes due to mastitis, however no significant ($p>0.05$) differences were found between the three groups. FTD and CD did not differ significantly between various groups. TCL was observed to be significantly longer in group 2 as compared to group 1 and group 3, whereas no significant difference in TCL was found between group 1 and group 3. This corroborates with findings reported by Singh (2019) who reported that the TWT was found to be significantly more in quarters with SCC 5-10 lac cells/ml of milk (0.882±0.039 cm). Our findings are in accordance with previous studies where healthier animals had longer TCL and animals with subclinical or clinical infections were found to have shorter TCL. In this experiment the other teat tissue measurements like TWT, FTD and CD had statistically non-significant difference in case of all the remaining groups.

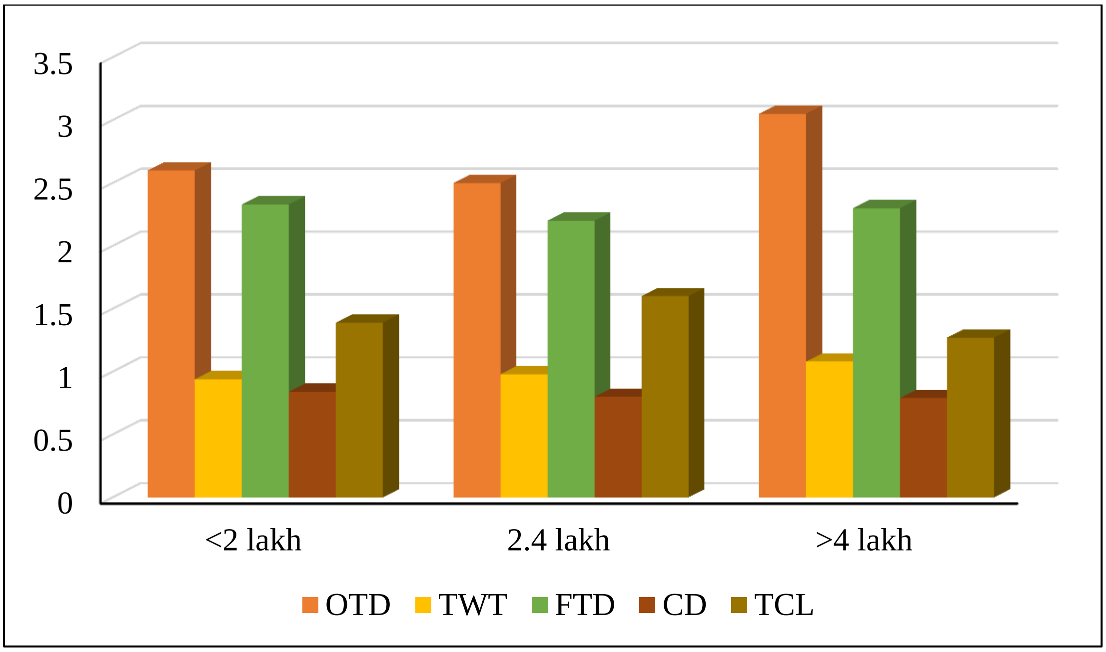

Fig. 17: Teat tissue measurements w.r.t SCC

On comparing teat tissue measurements with CMT, it was observed that CMT positive quarters had a significant increase ($p<0.05$) in OTD and TWT when compared to those which were CMT negative. The FTD and CD did not vary significantly between CMT positive and negative groups. Although TCL did not vary significantly ($p>0.05$) between CMT negative and positive groups it was found that TCL was shorter in the case of the CMT positive quarters. These findings are in concordance with studies done by Kotb et al. (2014) who reported that the most significant ultrasonographic findings on examination of those quarters affected with subclinical and clinical mastitis was the disappearance of the three-layered appearance of the teat wall, an irregular appearance of the mucosal layer of the teat and a significant thickening of the teat wall.

Table 9: Teat tissue measurements w.r.t. CMT

CMT	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
Negative (n=131)	2.59 ± 0.02[a]	0 .92 ± 0.01[a]	2.33 ± 0.03[a]	0.83 ± 0.03[s]	1.42 ± 0.02[a]
Positive (n=77)	2.73 ± 0.05[b]	1.01 ± 0.03[b]	2.29 ± 0.05[a]	0.83 ± 0.04[a]	1.36 ± 0.05[a]

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

Further, CMT score was expanded, and a similar analysis was made to compare the CMT score of the quarter milk samples and the corresponding teat tissue measurements. We found that group 2 and group 4 varied significantly ($p<0.05$) from group 1 which were the healthy quarters. TWT, FTD and CD varied non-significantly ($p>0.01$) between the four groups. TCL in group 2 was shorter and varied significantly ($p<0.1$) from group 1, there was a significant difference between group 2 and 3 in TCL, as well. This could be in accordance with the studies done by Kotb et al. (2014) in which they reported that in the case of subclinical mastitis in buffaloes a significant feature happened to be an overlapped appearance of the papillary duct and the Furstenberg rosette.

Table 10: Teat tissue measurements w.r.t. CMT score of apparently healthy cows

Group	CMT	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
1	**0 (n= 130)**	2.59 $\pm$ 0.02^{a}	0.92 $\pm$ 0.01^{a}	2.33 $\pm$0.03^{a}	0.83 $\pm$ 0.03^{a}	1.42 $\pm$ 0.02^{a}
2	**0.5 (n=30)**	2.58 $\pm$0.06^{a}	0.99 $\pm$ 0.05^{a}	2.34 $\pm$ 0.07^{a}	0.86 $\pm$0.07^{a}	1.27 $\pm$0.06^{b}
3	**1 (n=26)**	2.69 $\pm$ 0.08^{abd}	1.04 $\pm$ 0.05^{a}	2.28 $\pm$ 0.10^{a}	0.86 $\pm$0.08^{a}	1.53 $\pm$ 0.06^{a}
4	**2-3 (n=21)**	3.00 $\pm$0.12^{c}	0.99 $\pm$ 0.06^{a}	2.23 $\pm$ 0.10^{a}	0.76 $\pm$ 0.06^{a}	1.30 $\pm$ 0.08^{a}

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

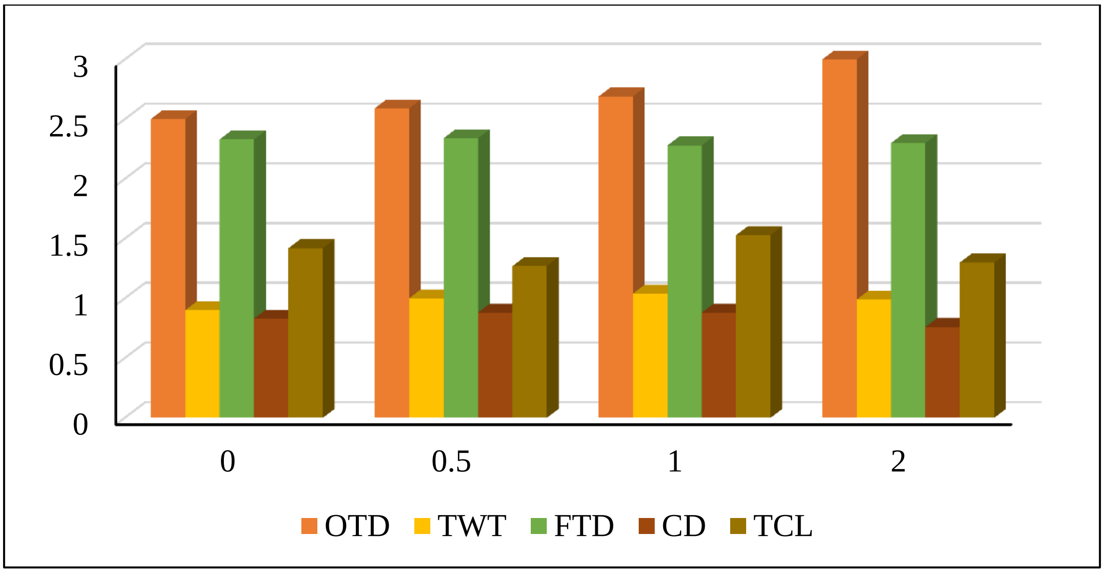

Fig. 18: Teat tissue measurements w.r.t. CMT score of apparently healthy cows

The teat tissue measurements and the milk inflammatory parameters were correlated applying Pearson's correlation. Teat tissue measurements were also correlated to each other. According to our studies, we found that OTD could be positively correlated ($p<0.01$) with parameters such as TWT, FTD and CD. TWT was significantly ($p<0.01$) correlated to OTD, FTD and TCL. CD was negatively correlated with parameters such as TWT, FTD, CMT and SCC, however these findings were not significant.

CMT had a strong significant correlation ($p<0.01$) with OTD and ($p<0.05$) TWT. pH was significantly ($p<0.01$) correlated with OTD, as well. EC and SCC also had a significant ($p<0.01$) positive correlation with OTD. This indicated that an increase in the milk inflammatory parameters such as CMT, pH, EC and SCC could lead to a simultaneous increase in OTD. Singh (2019) reported the CMT score was significantly correlated with TWT ($p<0.01$), which is similar to our findings. There was linear correlation between these diagnostic methods, but they were not fully correlated.

All milk inflammatory parameters showed positive significant correlation with each other. This is in accordance with studies done by Norberg et al. (2004) who reported 80.6% of clinical and 45.0% of subclinical cases which were screened could be classified correctly based on electrical conductivity, and of the cows classified as healthy, 74.8% were classified correctly. They concluded by noting the electrical conductivity, is a good diagnostic technique to detect subclinical mastitis and its efficiency can be increased using other additional diagnostic techniques. SCC which has been used as one of the most important diagnostic technique. According to our findings we found that SCC was positively correlated ($p<0.01$) with OTD, CMT, pH and EC further revealing to us that SCC was an important technique which could be used to detect subclinical mastitis. This is in concordance with the studies done by Langer et al. (2014). They employed bacteriological examination, CMT, pH, EC, and SCC as diagnostic procedures to evaluate and study the compatibility between each of these diagnostic tests in milk samples from 266 quarters of 69 dairy cow. They discovered that the findings of the SCC, impregnated pH paper strip, CMT, EC-meter, pH-meter, Hand-held mastitis detector, and bacteriological culture examination (reference test) were 64.4, 63.5, 59, 59, and 53, respectively.

Table 11: Pearson correlations among various milk inflammatory parameters and ultrasonographic measurements of apparently healthy cows (n=52)

	OTD	TWT	FTD	CD	TCL	CMT	pH	EC	SCC	Log_{10} SCC
OTD	1									
TWT	0.241**	1								
FTD	0.528**	0.253**	1							
CD	0.170*	-0.130	-0.022	1						
TCL	0.110	0.203**	0.301**	-0.071	1					
CMT	0.301**	0.146*	-0.089	-0.034	-0.060	1				
pH	0.186**	0.124	0.029	0.022	0.084	0.387**	1			
EC	0.221**	-0.010	0.123	0.131	-0.041	0.328**	0.092	1		
SCC	0.247**	0.113	-0.043	-0.027	-0.061	0.881**	0.335**	0.421**	1	
Log_{10} SCC	0.175*	0.061	-0.026	0.094	-0.059	0.703**	0.384**	0.370**	0.780**	1

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

Our studies which revealed a strong correlation between SCC, CMT, pH and EC ($p<0.01$) were in concordance with studies done by Sahu et al. (2018) in which they evaluated the use of SCC, pH and EC as diagnostic markers for the detection of subclinical mastitis and found that quarters with subclinical and clinical mastitis appeared to have a higher EC and SCC when compared to healthy animals and found a positive correlation ($p<0.05$) between SCC, EC and pH of milk.

4.1.2 Ultrasonographic changes in the supramammary lymph node

The supramammary lymph nodes were identified and located based on the studies done by Bradley et al. (2001). The lymph nodes were located in the caudo-dorsal aspect of the udder. They were not palpable or visible by the naked eye except for certain clinical cases. A total of 104 lymph nodes of 52 apparently healthy cows were scanned with a 5 Mhz convex transducer which provided us a more accurate depiction and a wider field as compared to the 7.5 MHz linear transducer with a depth of field 8 cm that was recommended by Bradley et al. (2001). There were two superficial supramammary lymph nodes present, one that was present laterally and one present deeper and medially. The lymph node was well demarcated from the tissue around it, as described by Kofler et al. (1998) in which they noted that the superficial supramammary lymph node was very similar to the other lymph nodes found in cattle. These lymph nodes appeared to have a well demarcated hyperechoic capsule, a hypoechogenic cortex and a hyperechogenic central line that indicated the medulla as seen in Fig 26. The medulla appears to be hyperechogenic either due to the incoming drainage of the lymphatic vessels or due to the presence of fat within. It has been noted that both of these reasons could also contribute to the hyperechogenicity of the medulla. The shape of the lymph node was however variable, ranging from ovoid, to triangular to kidney shaped.

The herd in which the lymph nodes were scanned had a mean CMT score of 0. 40 ± 0.046 (0-3) and mean somatic cell count of 129.27 ± 13.17 cells/ml (1-956 lakh cells/ml). The mean pH and EC of the herd was found to be 6.75 ± 0.01(5.94- 7.52) and 5.55 ± 0.09 mS/cm (2.3-10.71 mS/cm) respectively. The mean left length of the lymph node was found to be 7.22 ± 0.29 cm (3.49 – 12.6 cm) and the mean left depth was found to be 5.11 ± 0.22 cm (2.69 – 8.71 cm), whereas the mean right length and depth of the lymph node was found to be 7.20 ± 0.24 cm (4.7 – 13.4 cm) and 5.04 ±

0.21 cm (2.11 – 8.73 cm), respectively. Our findings corroborated with the findings by Bradley et al. (2001) the mean length of lymph nodes of 54 cows to be 7.4 cm and depth 2.5 cm. It is possible that our findings for the width of the lymph node was found to be slightly increased as compared to the above findings because of the use of a convex transducer instead of a linear one.

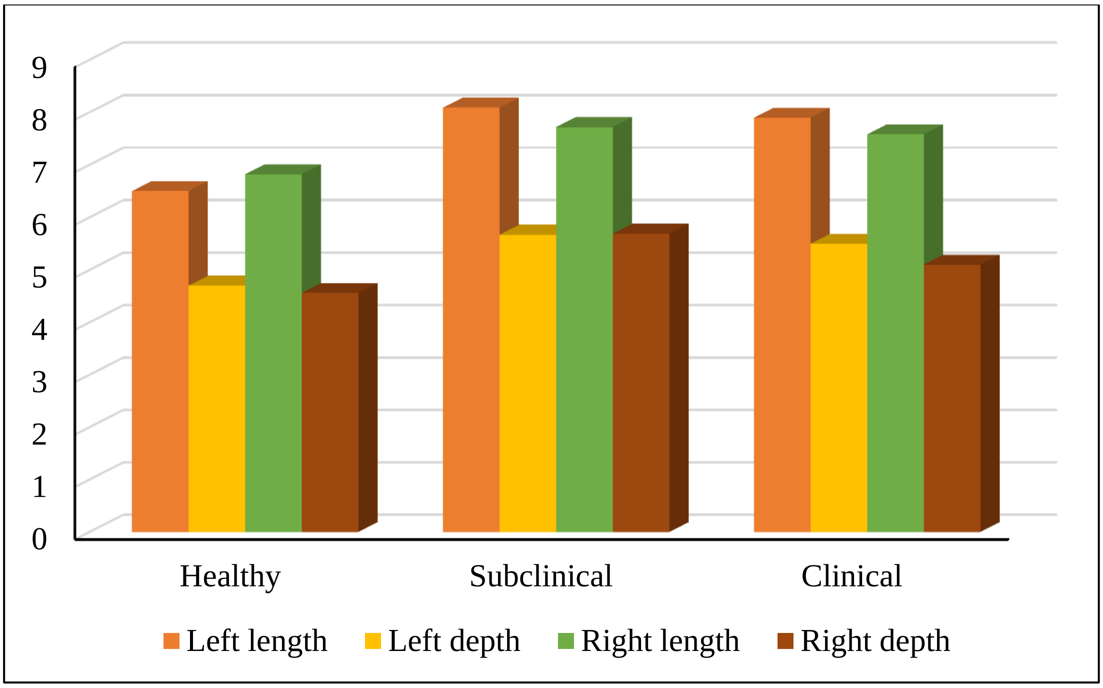

Fig. 24: Supramammary lymph nodes dimensions w.r.t udder health status

The cows were then classified as healthy and subclinical based on the milk somatic cell count and culture. Independent sample student's t-tests were carried out to observe the significant differences between these two groups. Those that had a somatic cell count more than 2 lakh cells/ml even in one quarter were categorized into the subclinical category and those that had a lower cell count were categorized into the healthy group. There were found to be 30 (57.69%) healthy cattle and 22 (42.30%) subclinically affected animals. It was observed that in case of healthy animals, the length of the left of the lymph node was significantly ($p<0.05$) smaller when compared to those that were sub clinically affected (6.59 ± 0.29 vs 8.09± 0.52), the depth was also found to be significantly ($p<0.05$) smaller when compared to those that were sub clinically affected (4.71± 0.23 vs 5.67± 0.39). The right lymph node length was noted to be larger as compared to the healthy animals as well (7.72± 0.42 vs 6.82± 0.22) however, these changes were not significant ($p>0.05$). The right lymph node depth was observed to be significantly ($p<0.05$) larger in subclinically affected animals as compared to healthy ones (4.56 ± 0.26 vs 5.69± 0.22). These findings are in concordance with Khoramian et al. (2015) who reported the mean length of the lymph node 9.2 (±0.27) cm (range 5.77-12.90) and their mean depth was 4.03 (±0.23) cm (range 2.07-7.41) in a herd with a history of chronic *Staphylococcus aureus.* The increase in the size of the lymph node could be due to an infection in any of the respective quarters. As each quarter is a separate entity but both the lymph nodes of one side i.e., the fore and hindquarter of each side drain into the respective lymph node on that side. This indicates that an intramammary infection in even one quarter could lead to a significant increase in the size of the lymph node which could be used as a diagnostic aid.

Table 12: Supramammary lymph nodes dimensions w.r.t udder health status animal wise

Udder health	Left length (cm)	Left depth (cm)	Right length (cm)	Right depth (cm)
Healthy (n= 30)	6.59 ± 0.29^{a}	4.71 ± 0.23^{a}	6.82 ± 0.22^{a}	4.56 ± 0.26^{a}
Subclinical (n= 22)	8.09 ± 0.52^{b}	5.67 ± 0.39^{b}	7.72 ± 0.42^{a}	5.69 ± 0.22^{b}

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

Table 13: Correlation of supramammary lymph node dimensions with milk inflammatory parameters in apparently healthy cows

	Length	Depth	CMT	pH	EC	Log_{10} SCC
Length	1					
Depth	0.702**	1				
CMT	0.353**	0.331**	1			
pH	0.104	0.184	0.376**	1		
EC	0.031	0.065	0.233	0.024	1	
Log_{10} SCC	0.280**	0.158	0.80**	0.388**	0.377**	1

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

Having applied Spearman's rank correlation coefficient (rs) on combined values of both the right and left lymph done dimensions and comparing them to the milk inflammatory parameters, we observed a significant (p<0.01) correlation between the length and the CMT (r=0.353) as well as the somatic cell count (r=0.280). We also obtained a non-significant positive correlation between the lymph node length and EC (0.031) and pH (r=0.104). This coincides with the studies done by Gürbulak et al. (2009) and Risvanli et al. (2019) who observed that the supramammary lymph node increased in size with an increase in the milk inflammatory parameters such as CMT, EC, pH and SCC due to the inflammatory processes that occurred in the glandular parenchyma. This indicated that lymph nodes with higher milk inflammatory readings had the tendency to have larger lymph nodes.

Our studies are also in accordance with studies done by Khoramian et al. (2015) where on observing correlation, they obtained a positive significant (p=0.008) correlation between CMT scores and the ipsilateral supramammary lymph node dimensions. They also obtained a positive correlation between the lymph node length and depth and the mean log_{10} SCC. They reported a linear positive relationship between Log_{10} SCC and the depth of the lymph nodes on both sides.

Table 14: Correlation of supramammary lymph node dimensions with teat tissue measurements in apparently healthy cows

	Length	**Depth**	**OTD**	**TWT**	**FTD**	**CD**	**TCL**
Length	1						
Depth	0.702**	1.000					
OTD	0.250*	0.154	1				
TWT	0.213*	0.283**	0.222*	1			
FTD	0.141	0.121	0.759**	0.024	1		
CD	0.172	0.015	0.274**	-0.330**	0.377**	1	
TCL	0.046	0.178	0.226*	0.433**	0.374**	-0.161	1

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

On obtaining the correlation between the lymph node dimensions (left and right) and the teat tissue measurements we obtained a significant ($p<0.05$) correlation with OTD (r=0.250) and TWT (r=0.213). This indicates that any affliction to the mammary parenchyma is reflected not only in the milk inflammatory parameters, but also leads to a simultaneous increase in the lymph node dimensions as well as the teat tissue measurements. This can be explained by studies done by Soltys and Quinn (1999) who explained that mastitic cows had larger lymph nodes which could be attributed due to the activation of the lymphocytes followed by proliferation and migration into the supramammary lymph node to fight the intramammary infection in the glandular parenchyma. Studies done by Yang et al. (2019) who observed that after 48 hours of experimentally inducing *S.aureus* endotoxin, intramammary, there were clear and distinct histological changes in the glandular parenchyma.

This is in corroboration with the findings reported by Al-Galil & Ahmed (2016) who reported a significant increase in the lymph node dimensions such as the length and depth. They also noted a significant ($p<0.05$) increase in the volume and elapse area of the superficial supramammary lymph node and a significant ($p<0.05$) increase in teat wall thickness in a herd of buffaloes affected with subclinical mastitis. They also noted that on ultrasonography, the sensitivity of lymph node length, depth and elapse area to sub-clinical mastitis was 96, 92 and 94% respectively, in contrast the sensitivity of SCC was 94% and CMT was 68.8%, which concluded that ultrasonography of the supramammary lymph node could be a more sensitive and easy method for the diagnosis of subclinical mastitis.

Table 15: Supramammary lymph node size categorized according to the age in healthy cow (n=30)

Age (years)	Length (cm)	Depth (cm)
Upto 3 (n= 17)	6.35 ± 0.15^{a}	4.75 ± 0.17^{a}
4-5 (n=8)	6.55 ± 0.27^{a}	4.53 ± 0.25^{a}
>5 (n=5)	7.95 ± 0.27^{b}	5.50 ± 0.25^{b}

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

Out of the 52 animals that were screened ultrasonographically, the cows were first separated into two groups – healthy (n=30) and those with subclinical infection (n=22). Within these groups, the animals were categorized according to their age, parity and stage of lactation to visualize changes in the supramammary lymph node according to these parameters and to observe for any changes due to these parameters. On perusal of Table 15 we observed that animals that were upto three years had smaller lymph node dimensions when compared those cows that were more than five years old. The length and the depth increased significantly ($p<0.05$) according to the age of the cow.

Table 16: Supramammary lymph node dimensions categorized according to parity in healthy cow (n=30)

Parity	Length (cm)	Depth (cm)
1 (n=16)	6.38 ± 0.14^{a}	4.46 ± 0.20^{a}
>1 (n=14)	7.00 ± 0.20^{b}	5.22 ± 0.20^{b}

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

Similarly, when the healthy cows were categorized according to the parity of the cow into two groups (Table 16). A significant increase ($p<0.05$) in the length of the supramammary lymph node was observed in those cows that had calved more than once when compared to those that had calved only once (7.00 ±0.20 vs 6.38 ± 0.14). A significant increase ($p<0.05$) in the depth of the lymph node was also observed. Therefore, there did appear to be an increase in the supramammary lymph node dimensions according to the parity of the animal.

Table 17: Supramammary lymph node dimensions categorized according to stage of lactation in healthy cow (n=30)

Stage of lactation	Length (cm)	Width (cm)
Early (n=12)	7.11 ± 0.17^{a}	5.17 ± 0.18^{a}
Mid (n=9)	6.50 ± 0.30^{a}	4.14 ± 0.21^{b}
Late (n=9)	6.21 ± 0.16^{b}	4.75 ± 0.12^{a}

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

Table 18: Supramammary lymph node size categorized according to the age in cows with subclinical mastitis (n=22)

Age (years)	Length (cm)	Depth (cm)
2-3 (n= 11)	7.82 ± 0.39^{a}	5.18 ± 0.19^{a}
4-5 (n=5)	8.41 ± 0.40^{a}	5.27 ± 0.19^{a}
>5 (n=5)	7.96 ± 0.52^{a}	5.65 ± 0.25^{a}

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

The healthy animals were lastly, categorized according to the stage of lactation into three groups – early, mid and late lactation. This was done to observe any changes that maybe caused by stage of lactation on the supramammary lymph node. It was observed that the lymph node significantly (p<0.05) decreased in length according to the stage of lactation. During early lactation, the supramammary lymph node appeared to have the largest length and depth (7.11 ±0.17 cm; 5.17 ± 0.18 cm) when compared to late lactation, in which the lymph node had a significantly (p<0.05) smaller length when compared to early lactation (6.21 ± 0.16 vs 7.11 ± 0.17 cm). The depth also decreased in size with the stage of lactation however, this was not found to be significantly reduced. This is in corroboration with the findings reported by Bradley et al. (2001) who noted that but there was a positive correlation between the lactation number and their size and that the supramammary lymph nodes tended to be larger in older cows. There was, however no literature available on the size of the lymph node and the stage of lactation.

Similarly, the 22 cows that had subclinical infection were classified according to the age, parity, and stage of lactation to observe for the effect of these parameters on the lymph node. There was an increase in size (length and depth) of the supramammary lymph node, however these changes were not found to be significant (p<0.05)

Table 19: Supramammary lymph node dimensions categorized according to parity in cows with subclinical mastitis (n=22)

Parity	Length (cm)	Depth (cm)
1 (n=9)	7.80 ± 0.14[a]	5.14 ± 0.29[a]
>1 (n=13)	8.21 ±0.29[a]	5.46 ±0.21[a]

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

Table 20: Supramammary lymph node dimensions categorized according to stage of lactation in cows with subclinical mastitis (n=22)

Stage of lactation	Length (cm)	Depth (cm)
Early (n=6)	8.10 ± 0.27[a]	5.60 ± 0.22[a]
Mid (n=9)	7.87 ± 0.43[a]	5.30 ± 0.28[a]
Late (n=7)	8.04 ± 0.45[a]	5.13 ± 0.22[a]

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

In table 19 and table 20, when the subclinically infected cows were classified according to the parity and stage of lactation. There was a non-significant increase in the length and depth of the lymph node with the parity of the animal. On perusal of table 20, no particular changes of the lymph node were observed as in the case of healthy cows, this could be due to the udder health status of each individual cow or the severity of infection in each individual cow.

Table 21: Correlation of supramammary lymph node dimensions with age, parity and stage of lactation of healthy cows (n=30)

	Length	Depth	Age	Parity	Stage of lactation
Length	1				
Depth	0.641**	1			
Age	0.262**	0.020	1		
Parity	0.283**	0.210*	0.885**	1	
Stage of lactation	-0.297**	-0.188*	0.005	-0.089	1

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

On examination of Table 21, Spearman's correlation was applied to enumerate the correlation between the supramammary lymph node dimensions and the age, parity as well as stage of lactation of the animal. A significant ($p<0.01$) positive correlation was obtained between the lymph node dimensions (length and depth) and the age (r= 0.262) and parity (r= 0.283) of the animals, indicating that older cows had larger supramammary lymph nodes. This is in corroboration with findings reported by Bradley et al. (2001) who found a significant ($p<0.05$) positive correlation between the lymph node dimensions and the age as well as lactations of the animal. We also obtained a significant ($p<0.01$) negative correlation between the lymph node dimensions and the stage of lactation (r=-0.297) indicating that the size of the lymph node decreases towards the end of lactation.

4.1.3. Ultrasonographic changes in udder and teats of clinical mastitic dairy cows

Ultrasonographic evaluation was done of 30 clinical mastitic cows, that were brought to the large animal clinic, GADAVSU. These 30 animals included 120 functional quarters out of which 40 (33.3%) where found to be healthy quarters, 18

(15%) were found to be nonclinical quarters (no visible signs but affected with specific subclinical mastitis) and a total of 62 (51.67%) clinical quarters. The occurrence of clinical mastitis however was found to be 54.84% in the hindquarters, with the left and right hind having an equal number of clinical quarters (17) and 45.16% in the forequarters indicating that there is a possibility that hindquarters are more prone to clinical mastitis when compared to forequarters. This is in concordance with studies done by Thakur et al. (2021) who noted that hindquarters (32.31%) were the most prone to clinical mastitis when compared to forequarters (15.46%). This has been attributed to the fact that since the hindquarters have a larger mass, they are more prone to direct trauma, closer to the floor when compared to the forequarters and a higher chance of contamination with urine, faeces and environmental contamination (Hase et al., 2013).

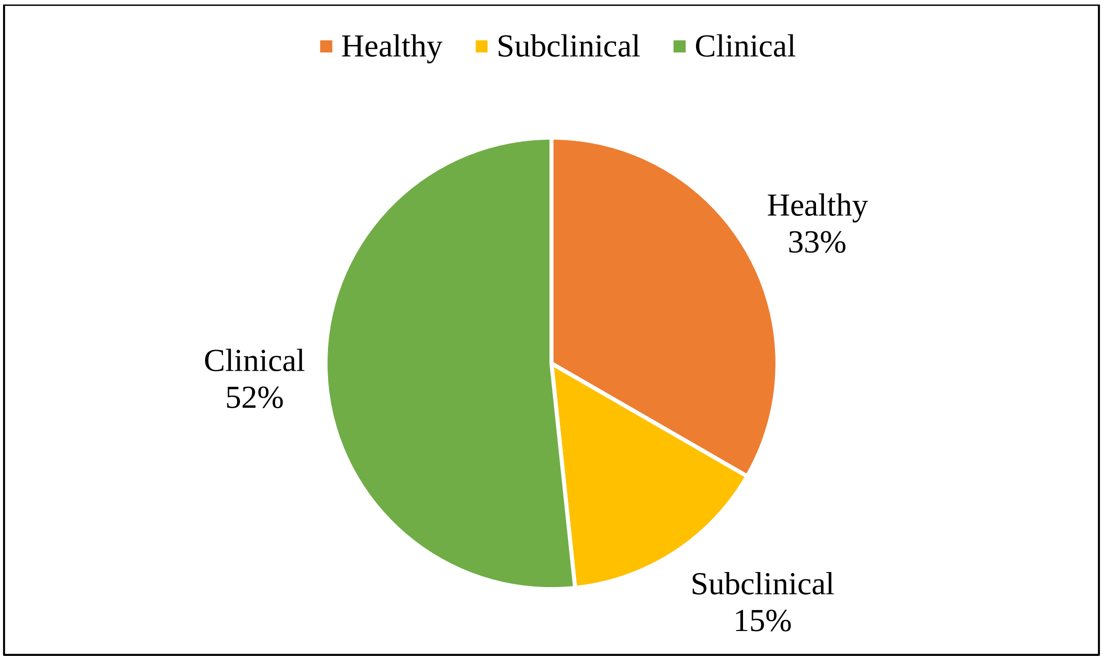

Fig. 33: Quarter wise health status of dairy cows

Table 22: Quarter wise health status of clinically mastitic dairy cows

Type	Number	Percentage
Healthy	40	33.33
Nonclinical	18	15
Clinical	62	51.67
Total	**120**	**100**

Table 23: Distribution of quarters in clinically mastitic animals

Teat	Healthy	Nonclinical	Clinical
LF	9	6	16
LH	9	4	17
RF	11	6	12
RH	11	2	17
Total	**40**	**18**	**62**

Table 24: Teat tissue measurements (Mean± SE) in relation to quarter health status of clinically mastitic cows

Type	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
Healthy (n=40)	2.71 $\pm$ 0.08^{a}	0.86 $\pm$0.09^{a}	2.30 $\pm$0.07^{a}	1 $\pm$0.08^{a}	1.28 $\pm$ 0.06^{a}
Nonclinical (n=18)	2.84 $\pm$0.09^{a}	0.94 $\pm$0.07^{a}	2.31 $\pm$0.14^{a}	0.93 $\pm$0.1^{a}	1.29 $\pm$ 0.13^{a}
Clinical (n=62)	2.98 $\pm$ 0.07^{b}	0.92 $\pm$0.03^{a}	2.48 $\pm$0.05^{a}	1.1 $\pm$0.08^{a}	2.4 $\pm$ 1.10^{a}

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

All the quarters were divided into three groups, healthy, non-clinical and clinical and independent samples t-test was done to observe variations between the groups. OTD in clinical quarters was found to be significantly higher ($p<0.05$) when compared to healthy quarters, however non-clinical quarters did not differ significantly ($p>0.05$) from the healthy and clinical quarters. A non-significant ($p>0.05$) increase in the TWT was observed in non-clinical and clinical quarters when compared to healthy quarters. FTD, CD and TCL showed no significant variations between the three groups. Our findings are in corroboration with Flock & Winter (2006) in which they reported an increase in the TWT and the changes in the teats of the mammary gland with intramammary infection which are due to mild inflammation, proliferation of tissues and mucosal lesions. They reported that on ultrasonography the teat wall thickness increases and stenosis of the channel or teat cistern in mastitic quarters is present.

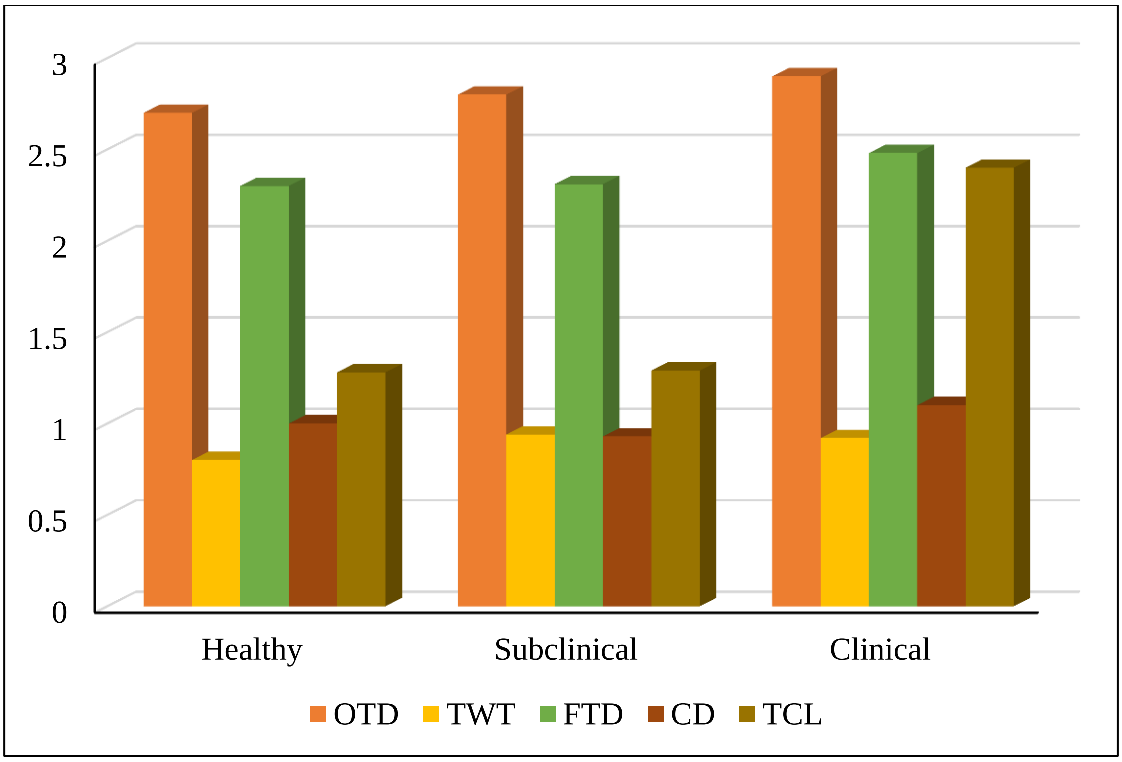

Fig 34: Teat tissue measurements w.r.t. quarter health status of clinical mastitis cases

Table 25: Alterations in milk parameters (Mean± SE) w.r.t. udder health status of clinically mastitic cows

Udder Health Status	CMT	pH	EC (mS/cm)	SCC (× 10^3 cells/ml)	Log_{10} SCC
Healthy (n=40)	0.42 ± 0.13^a	6.83 ± 0.05^a	5.71 ± 0.18^a	40.20 ± 6.3^a	1.57 ± 0.06^a
Nonclinical (n=18)	1.33 ± 0.15^b	6.95 ± 0.12^a	6.92 ± 0.55^b	163.4 ± 39.48^b	2.51 ± 0.04^b
Clinical (n=62)	2.36 ±0.39^b	6.88 ±0.03^a	7.55 ±0.22^b	-	-

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

Independent sample t-test was done to compare and observe the changes observed in the milk inflammatory parameters. A significant (p<0.05) increase in the CMT score was observed in the clinical (2.36 ±0.39) and non-clinical quarters (1.33 ± 0.15) when compared to the healthy quarters (0.42 ± 0.13). There was a significant elevation in the EC and SCC of the non-clinical and clinical quarters when compared to healthy quarters, however no significant difference was detected between clinical and non-clinical quarters. Sood et al. (2008) reported that the increase in EC in the case of quarters with mastitis is due to several factors such as an increase in the blood supply to the udder, an increased permeability of the blood vessels leading to an increase influx of Na and Cl into the milk henceforth increasing the electrical conductivity of the milk.

There was no significant variation in pH between the three groups (p>0.05). This could be explained by findings by previous studies, which reported that pH may be an efficient technique to diagnose subclinical or clinical mastitis only as an adjunct and must be used while comparing it with other diagnostic markers. The fact that the inflammatory reaction and leukocyte population in SCM are insufficient to increase the alkalinity beyond the buffering capacity of milk buffers is responsible for the negligible increase in pH (Schalm et al., 1971). These changes could also be due to the supposed increase in the citrate and bicarbonate level of milk during subclinical mastitis (Ogola et al., 2007).

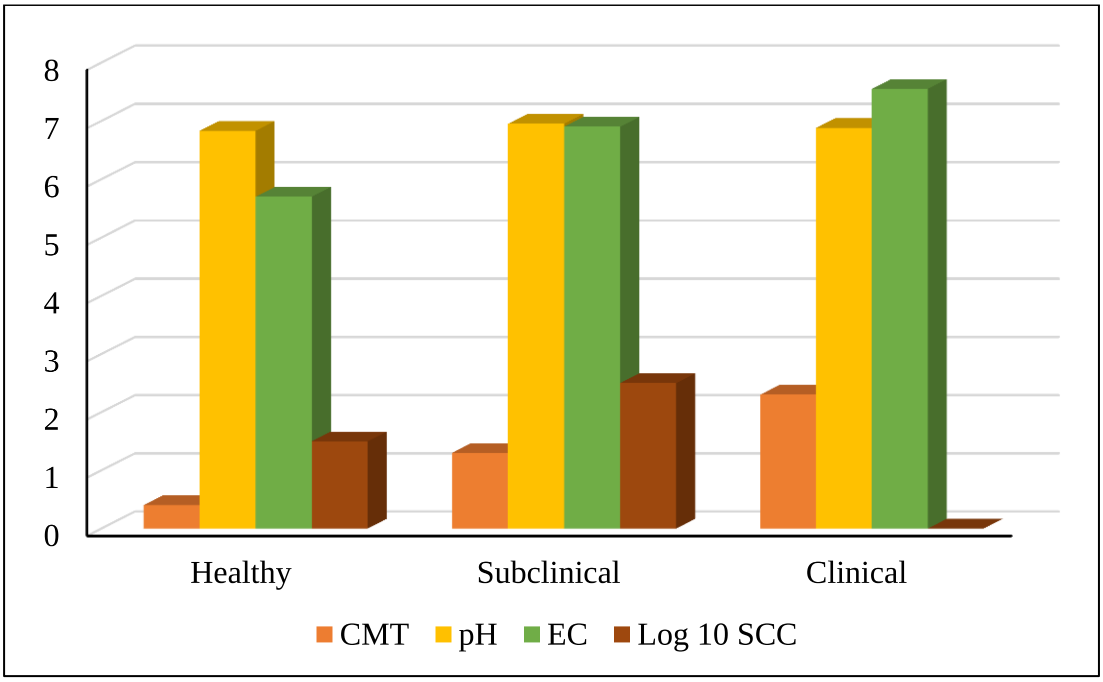

Fig 35: Alterations in milk parameters in relation to udder health status of clinically mastitic cows

Table 26: Teat tissue measurements in relation to infection with major pathogens isolated from clinically mastitic animals (n=30)

Organism	OTD (cm)	TWT (cm)	FTD (cm)	CD (cm)	TCL (cm)
No growth (n= 45)	2.57 ± 0.05[a]	0.85 ± 0.03[a]	2.22 ± 0.06[a]	0.88 ±0.06[a]	1.21 ± 0.07[a]
Coagulase positive staphylococci (n= 36)	2.92 ± 0.08[b]	0.95 ±0.04[a]	2.39 ± 0.08[b]	1.04 ± 0.09[b]	1.37 ± 0.07[a]
Coagulase negative staphylococci (n=30)	3.19 ± 0.1[b]	0.91 ± 0.05[a]	2.60 ± 0.07[b]	1.41 ± 0.12[c]	1.18 ± 0.08[a]

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

Independent sample t-test were done to observe if there were any significant teat tissue changes observed caused by a particular organism. 45 of the quarters had no growth including thirteen quarters with non-specific infection. A total of 74 quarters were found to be culturally positive, In the 74 quarters, 36 (43.9%) were coagulase positive staphylococci (CS) and 30 quarters were affected with coagulase negative staphylococci (CNS) (36.6%). 4 (4.9%) of the quarters were affected with *E. coli* and 4 (4.9%) of the quarters were affected with *Streptococcus* spp. These findings were similar to Sharma et al. (2012) in which they found a prevalence of 38.6% in the case of coagulase positive staphylococci and 29.33% coagulase negative staphylococci. Our findings were also in corroboration to the findings reported by Waage et al. (1999) as well, in which they reported the prevalence of *Staphylococcus aureu*s (44.3%), coagulase-negative staphylococci (12.8%), *Escherichia coli* (6.4%). They also reported some minor pathogens such as *Streptococcus* spp. and *Arcanobacteriacae* spp.

OTD was significantly higher in quarters affected with CS and CNS when compared to healthy quarters. TWT was non-significantly increased in quarters affected with CS and CNS. Quarters affected with CS and CNS had a higher FTD when compared to quarters which had no growth, however no variation was observed between quarters affected with CS and CNS. CD in the affected quarters were significantly ($p<0.05$) larger compared to those quarters with no growth. There was significant variation between the quarters affected with CS and CNS as well. No significant variations were obtained between the quarters in TCL.

Our findings are similar to that of Nak et al. (2005) who reported that in the case of thelitis the three-layered appearance of the teat wall was completely replaced, and all layers of the teat wall appeared as hyperechoic. The increase in the echogenicity of the teat wall coincided with the severity of the condition. Studies done by Kotb et al. (2014) reported that in the quarters affected with *Staphylococcus aureus* there appeared to be an obstruction in the teat canal with a destruction of the rosette of Furstenberg leading to a larger FTD, which are like some of the findings that we have observed in some of the quarters that were affected with CS.

Pearson's correlation was done to correlate the teat tissue measurements with milk inflammatory parameters. The teat tissue parameters were correlated with each other as well. OTD was significantly ($p<0.01$) correlated with TWT, FTD and CD. TWT had a significant ($p<0.01$) negative correlation with CD. TCL had non-significant ($p>0.05$) negative correlation with all teat tissue parameters such as OTD, TWT, FTD and CD.

Table 27: Pearson correlations among various milk inflammatory parameters and ultrasonographic measurements of clinically mastitic cows (n=30)

	OTD	**TWT**	**FTD**	**CD**	**TCL**	**CMT**	**pH**	**EC**	**SCC**	**Log_{10} SCC**
OTD	1									
TWT	0.224*	1								
FTD	0.711**	0.262**	1							
CD	0.571**	-.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	-.203*	-0.012	-0.152	-0.039	1			
EC	0.054	-0.019	-0.028	0.058	-0.050	.401**	0.160	1		
SCC	0.004	-0.037	-0.067	-0.048	-0.049	.494**	.369**	.316*	1	
Log_{10} SCC	0.215	0.018	0.094	0.040	0.007	.361**	.376**	.395**	.865**	1

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

4.1.4. Ultrasonography of supramammary lymph node in clinically mastitic cows

A similar examination in 30 clinically mastitic cows was also carried out to observe the changes in the supramammary lymph nodes using ultrasonography. A total of 60 clinically affected supramammary lymph nodes were scanned. In this, at least one of the lymph nodes was affected however, there were cases in which group, all four quarters were affected as well. In some of the cases where the intramammary infection was severe, it was observed that the lymph nodes could be visualized and palpated externally. This was not possible in all the cases. In these cases, most of the cows had at least one clinical quarter with variable number of subclinical and healthy quarters depending on the severity of the infection. The mean CMT score of this group was 1.769 ± 0.15, the mean pH was 7.09 ± 0.09, an EC of 6.68 ± 0.17 mS/cm and the SCC of 138.99 ± 20.02 lakh cells/ml (only of healthy and subclinical quarters). SCC of clinically affected quarters was not done. The mean length of the left lymph node was observed to be 7.97±0.42 (3.49 – 12.7 cm) which was significantly increased in size when compared to those of the apparently healthy cows in the previous study. The mean depth of the left lymph node was 5.5± 0.42 (2.14 – 13.4 cm). The mean length of the right lymph node was observed to be 7.58±0.38 (3.63- 12.3 cm) and the mean depth of the right lymph node was 5.1±0.38. (1.79 – 10.3 cm). The left lymph node depth, right lymph node length and depth were increased in size, when compared to the apparently healthy animals screened before, but the difference was not significant ($p>0.05$). Our results are in concordance with the studies by Al-Galil & Ahmed (2016) who reported that in the case of subclinical mastitis, the lymph node had a significant increase in length, depth, elapse area, elapse volume and teat wall thickness and a significant ($P\leq0.05$) decrease in gray scale analysis of lymph node. Hussein et al. (2015) also measured the ultrasonographic length, depth and area of the superficial inguinal lymph nodes and observed them to be significantly increased, in the infected groups ($P<0.05$) and positive correlation was found between the SCC values and the ultrasonographic length ($r = 0.59$, $P<0.01$) and depth ($r = 0.64$, $P<0.01$).

Table 28: Comparison of supramammary lymph nodes dimensions (Mean± SE) in healthy and mastitic cows

Type	Left Length (cm)	Left depth (cm)	Right length (cm)	Right depth (cm)
Healthy (n= 30)	6.59 ± 0.29[a]	4.71 ± 0.23[a]	6.82 ± 0.22[a]	4.56 ± 0.26[a]
Clinical (n=30)	7.97 ±0.42[b]	5.5 ± 0.42[a]	7.58 ±0.38[a]	5.1 ±0.38[a]

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

Table 29: Correlation of supramammary lymph node dimensions with milk inflammatory parameters in clinically mastitic cows

	Length	Depth	CMT	pH	EC
Length	1				
Depth	0.743**	1.000			
CMT	0.265*	0.217	1		
pH	-0.138	-0.136	0.376**	1	
EC	-0.022	-0.029	0.233	0.024	1

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

Similarly, Spearman's correlation was assessed to observe the correlation between the supramammary lymph node dimensions and the milk inflammatory parameters in clinically mastitic animals. We obtained consistent findings in which we obtained a significant ($p<0.05$) correlation between the length and the CMT ($r=0.265$). This is consistent with our previous findings as well in which we observed that those quarters which had a higher CMT score had larger lymph node dimensions. This is also in corroboration with studies done by Hussein et al. (2015) who found significant alterations in the dimensions, structure and physiological ultrasonographic pattern of supramammary lymph node of cattle with subclinical mastitis.

This is similar to the findings reported by Risvanli et al. (2019) who reported that on the doppler ultrasonographic examination of the supramammary lymph node, those quarters that were CMT negative were smaller and showed an avascular type of image whereas quarters which were CMT positive and clinically mastitic appeared larger and had distortion-type vascular morphology. They also found a significant ($p<0.05$) correlation between the lymph node dimensions and CMT score.

Table 30: Correlation of supramammary lymph node dimensions with teat tissue measurements in clinically mastitic cows

	Length	Depth	OTD	TWT	FTD	CD	TCL
Length	1						
Depth	0.743**	1					
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)

In a similar way, we obtained the Spearman's correlation between the lymph node dimensions and the teat inflammatory measurements to establish their relationship. A consistent finding, on perusal of all the tables above was the notable significant ($p<0.01$) relationship of the length with OTD ($r=0.360$). We also obtained a significant ($p<0.05$) correlation between length and the FTD ($r=0.277$) and CD ($r=0.291$). Szencziova and Strapak (2012) explained that the clear ultrasonographic changes observed in the glandular parenchyma were directly attributed to the effects of bacterial, mycotic and viral agents. Ultrasonography of the supramammary lymph node could be an excellent technique to overcome the limitations that came with conventional techniques such as CMT and SCC. (Hussein et al. 2015)

Our studies have revealed a positive correlation between the dimensions of the supramammary lymph node and the teat tissue measurements such as OTD, TWT and FTD, indicating an increase in the lymph node dimensions would invariably lead to an increase in the size of the OTD and the TWT as well as the FTD. This indicates that these parameters could act as inflammatory markers and would help in the detection of subclinical mastitis. Our studies are in accordance with the studies done by Gürbulak et al. (2009) who reported that an increase in size of the supramammary lymph node could be correlated with an increase in the somatic cell count, electrical conductivity, and bacteriological examination of the milk. It was also determined that *Staphylococcus aureus* was the most common etiological agent for mastitis. In our study as well CS was one of the most common bacteriological organism present followed by CNS and of minor importance were *E. coli* and *Streptococcus* spp. There are other factors also that must be considered, as the lymph node size tends to increase with the age and number of lactations. Bradley et al. (2001) also reported that ultrasonographically the nodes were slightly shorter and deeper than the direct measurements. These discrepancies may have been due to the node having being scanned obliquely, and small inaccuracies in measuring the length may also have resulted from the measurements having been made over more than one field of view. This indicates that the actual measurements of the lymph node maybe slightly more when measured in real when compared to ultrasonographic measurements.

4.3. Infrared thermography of apparently healthy and clinically mastitic animals

There have been several methods and diagnostic markers that have developed over the years for the purpose of diagnosis of mastitis. California mastitis test, somatic cell count, detection of pH and EC, molecular techniques such as PCR, serological tests such as ELISA, detection of certain enzymes such as N-Acetyl glucosaminidase and lactate dehydrogenase are used for the early diagnosis of mastitis, the gold standard is still the bacteriological culture obtained from the milk samples. Infrared thermography is the method that could ideally be used to detect mastitis at field level, as it is non-invasive, convenient method that may help us diagnose and detect early cases of mastitis. In the present study, an attempt has been made to standardize infrared thermography to detect changes in the udder skin surface temperature in different forms of mastitis.

Table 31: Udder and teat skin surface temperature and milk inflammatory parameters w.r.t. udder health status

Udder health status	Number	USST (°C)	TSST (°C)	CMT	pH	EC (mS/cm)	SCC ($\times 10^3$ cells/ml)
Healthy	70	36.59 ±0.086[a]	35.38 ±0.1325[a]	0.20 ±0.05[a]	6.73 ±0.01[a]	5.188 ±0.126[a]	58.01 ±4.80[a]
Sub-clinical	26	36.83 ±0.097[a]	35.49 ±0.18[a]	1.17 ±0.12[b]	6.76 ±0.03[a]	5.525 ±0.256[a]	348.91 ±21.37[b]
Clinical	20	37.5 ±0.139[b]	36.09 ±0.14[b]	1.82 ±0.19[c]	6.73 ±0.05[a]	6.836 ±0.504[b]	-

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

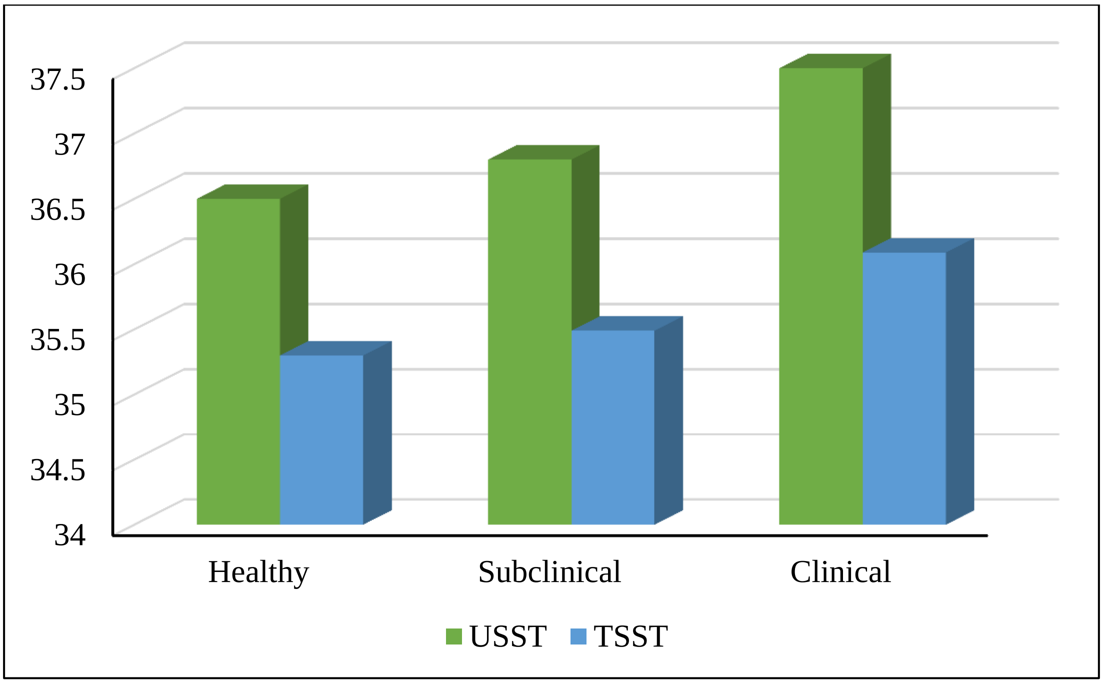

Fig. 50: Udder and teat skin surface temperature with respect to udder health status

In our study, we examined a total of 29 cows (Table 31), comprising 116 quarters. Out of these twenty-nine cows, 17 cows were apparently healthy with no visible changes in the milk or quarter and 12 cows were clinically affected with either one or more clinical quarters. The mean USST and TSST of these 29 cows were determined and found to be 36.80± 0.06°C and 35.5 ± 0.09°C, respectively. The quarters were classified into three main groups – Healthy, sub-clinical and clinical. The healthy included all those that had a somatic cell count less than 2 lakh cells/ml, those quarters that had a somatic cell count more than 2 lakh with or without bacteriological growth were classified into the sub-clinical group and the clinical group consisted of only the quarters with visible changes. The present study revealed that healthy quarters had the lowest udder temperatures with a mean USST of 36.59 ±0.087°C with temperatures ranging from 34.9-38 °C, the sub-clinical quarters had a visibly increased temperature of 36.83±0.09 °C with temperatures ranging from 35.1-38.1 °C, however this did not significantly ($p>0.05$) vary from that of the healthy quarters. The clinically affected quarters had a mean temperature of 37.5±0.14 °C with temperatures varying from 36.6 – 38.9 °C. The USST of the clinical quarters varied significantly ($p<0.05$) when compared to healthy and sub-clinical quarters. Similarly, the temperature of the teat was taken at the mid-teat level. It was observed that healthy quarters had the lowest TSST of 35.38±0.13 °C with temperatures ranging from 32.1-36.6 °C. The sub-clinical quarters were found to have a mean TSST of 35.496±0.19 °C with temperatures ranging from 33.2-36.2 °C and the clinical quarters were found to have a mean TSST of 36.09±0.14 °C ranging from 33.6- 36.9 °C. The TSST of clinical quarters were significantly higher ($p<0.05$) when compared to healthy and sub-clinical quarters. Therefore, in our study we found that those that were sub-clinically mastitic quarters had an udder skin surface temperature 0.24 °C higher than the healthy quarters. The USST in clinical quarters in our study was found to vary from the healthy quarters by approximately 0.91°C. The TSST the subclinical and clinically mastitic cows were 0.12 °C higher when compared to healthy quarters. Our findings are in corroboration with the observations by Golzarian et al. (2017) who reported that in sub-clinically infected quarters there was an elevation of USST upto 0.45 °C when compared to healthy ones. However, Samara et al. (2014) reported that subclinical mastitic udders had an average udder skin temperature of 1·42 °C greater than healthy udders. The reason could be due to environmental differences according to geographical regions leading to higher USST in the healthy cattle found in tropical and subtropical regions.

Table 32: Correlation between the udder and teat skin surface temperature with the milk inflammatory parameters in all quarters (n= 116)

	USST	**TSST**	**CMT**	**EC**	**Log_{10} SCC**
USST	1				
TSST	0.387^{**}	1			
CMT	0.477^{**}	0.227^{*}	1		
EC	0.382^{**}	0.136	0.531^{**}	1	
Log_{10} SCC	0.245^{*}	0.076	0.735^{**}	0.245^{*}	1

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

Pearson's correlation test was applied to observe any correlation between USST, TSST and the milk inflammatory parameters, irrespective of the udder health status. A highly significant ($p<0.01$) positive correlation was obtained between the USST and CMT ($r=0.477$) as well as EC ($r=0.382$). There was also a statistically significant ($p<0.05$) positive correlation between USST and the mean log_{10} SCC (r=0.245). This indicates that with an increase in the udder skin surface temperature of a quarter, CMT score electrical conductivity and SCC of that quarter increased simultaneously. This is in concordance with studies done by Polat et al. (2010) who determined to find the degree of interrelationship between USST and milk inflammatory parameters such as SCC and CMT score and found a positive correlation (r = 0.76; p<0.0001). We obtained a strong positive correlation between TSST and the CMT scores of the respective sides (p<0.01, r=0.227). We also recorded a positive correlation between the TSST and parameters such as EC and SCC, however these were not found to be significant. This indicates that with an increase in the USST, there will be a simultaneous increase in the milk inflammatory parameters such as CMT, EC and SCC. This supports our objective in which we can use infrared thermography as a method for diagnosing subclinical as well as clinical mastitis.

Table 33: Correlation between the udder and teat skin surface temperature with the milk inflammatory parameters of healthy quarters (n=70)

	USST	**TSST**	**CMT**	**EC**	**Log_{10} SCC**
USST	1				
TSST	0.348**	1			
CMT	0.369**	0.088	1		
EC	0.211	-0.021	0.121	1	
Log_{10} SCC	0.330**	0.088	0.720**	0.147	1

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

The quarters were further divided into the same category and then Pearson's correlation was applied to find a relation between USST, TSST and the milk inflammatory parameters to observe the strength of the relation in the different groups. In case of healthy quarters, we found a significant positive relation between USST and the TSST (p<0.01, r=0.348). A strong positive correlation was also found between USST and the milk inflammatory parameters such as CMT score and mean log_{10} SCC (p<0.01, r=0.369, r=0.330). A positive non-significant (p>0.05) correlation was also observed between USST and EC. This is similar to the findings reported by

Castro-Costo et al. (2014) who noted an increase in USST and SCC, 6 hours post intramammary infusion of *E. coli* endotoxin.

Table 34: Correlation between the udder and teat skin surface temperature and the milk inflammatory parameters of subclinical quarters (n=26)

	USST	**TSST**	**CMT**	**EC**	**Log_{10} SCC**
USST	1				
TSST	0.369	1			
CMT	0.057	0.001	1		
EC	0.369	0.124	0.062	1	
Log_{10} SCC	0.079	-0.185	0.529**	0.119	1

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

Table 35: Correlation between the udder and teat skin surface temperature with the milk inflammatory parameters of clinical quarters (n=20).

	USST	**TSST**	**CMT**	**EC**
USST	1			
TSST	0.386**	1		
CMT	0.568**	0.360**	1	
EC	0.407**	0.165	0.670**	1

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

In subclinical quarters, we found a positive correlation between the USST and milk inflammatory parameters such as CMT, EC and log_{10}SCC, however it was found to be non-significant ($p>0.05$) as seen in Table 34. On perusal of table 35, we observed a significant positive correlation between USST and milk inflammatory parameters such as CMT and EC. ($p<0.01$, r=0.568, r=0.407). This is in par with the findings reported by Sathiyabharathi et al. (2018b) and Sarubhi et al. (2020) who have reported a positive correlation between the USST and milk inflammatory parameters such as CMT, log_{10}SCC and EC.

4.3.1. Infrared thermography in apparently healthy animals

To observe individual changes in apparently healthy animals, we categorized the 17 apparently healthy animals that we had screened to observe the temperature and milk inflammatory changes in them. We found that out of 17 apparently healthy cows that, 54 quarters were healthy and 14 sub-clinically affected. This classification was done based on the somatic cell count and CMT scores. In the healthy quarters, the mean pH, EC and SCC was found to be 6.73±0.01, 4.96±0.32 mS/cm and 45.6±4.23 x10^3 cells/ml respectively. The USST and TSST of these quarters was found to be 36.39± 0.07 °C and 35.23±0.16 °C respectively. In the subclinical quarters the mean pH, EC and SCC was found to be 6.82±0.03, 5.01±0.14 mS/cm and 347.55±31.8 x 10^3 cells/ml, respectively. The USST and TSST was found to be 36.62± 0.02 °C and 35.20±0.25 °C, respectively. The SCC count of the subclinical quarters differed significantly ($p<0.05$) from the healthy quarters. The USST and TSST however did not differ significantly ($p>0.05$). However, in case of the subclinical quarters we observed that the USST was slightly higher comparatively by 0.23 °C, this is very similar to our previous findings where the difference between healthy and unhealthy quarters was found to be 0.24 °C.

Table 36: Correlation between udder and teat skin surface temperature and milk inflammatory parameters of healthy quarters (Healthy+Latent quarters; n=54)

Healthy quarters	USST	TSST	CMT	pH	EC	Log_{10} SCC
USST	1					
TSST	0.282*	1				
CMT	-0.052	-0.053	1			
pH	-0.078	0.167	0.211	1		
EC	-0.017	-0.128	-0.167	-0.226	1	
Log_{10} SCC	-0.306*	-0.065	0.162	-0.247	-0.074	1

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

Pearson’s correlation was done to observe the relation between the USST, TSST and the milk inflammatory parameters of the subclinical quarters. In these findings, we found that there was a positive correlation between USST and TSST, CMT, EC and mean log SCC. However, these were not found to be significant. ($p>0.05$). The relation between the USST and EC and mean log SCC was found to be particularly stronger compared to that of CMT (r=0.264, r=0.292). This was in concordance with the studies done by Pampariene et al. (2016) who reported that when infrared thermographic images were taken of the teats at the level of the area of Furstenberg rosette and the level of the udder, it was then analysed and correlated with the findings found in CMT and SCC and it was found that there was a correlation to that of subclinical mastitis. Infrared thermography is an excellent tool to identify and isolate a quarter or animal which may have subclinical mastitis; therefore it is a good tool for early detection of subclinical mastitis, however it must be supplemented with other milk inflammatory parameters such as CMT, EC, SCC and bacteriological examination to confirm the presence of subclinical mastitis.

Table 37: Correlation between udder and teat skin surface temperature and milk inflammatory parameters of subclinically affected cows (Specific + Non-specific; n=14)

Subclinical	USST	TSST	CMT	pH	EC	Log_{10} SCC
USST	1					
TSST	0.290	1				
CMT	0.076	0.287	1			
pH	0.035	0.240	0.48	1		
EC	0.264	-0.095	-0.438	-0.025	1	
Log_{10} SCC	0.292	-0.164	0.602*	0.123	-0.202	1

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

4.3.2. Infrared thermography in clinical mastitic cows

A total of 12, clinically mastitic cows that were brought to the large animal clinic were also analysed (Table 38) and the udder and teat skin surface temperature was taken accordingly. They were classified into healthy (16), sub clinical (12) and clinical quarters (20) to observe the changes within the clinical cases. It was observed that the healthy quarters in the clinical cases appeared to have a higher USST than the healthy quarters of the completely healthy animals (37.28± 0.20). The clinical quarters had a higher temperature compared to healthy quarters however there was no significant difference ($p>0.05$) between these groups. The difference between the healthy and clinically affected quarters was found to be 0.22 °C. This is similar to the findings reported by Scott et al. (2000) who detected an increase upto 2.3 °C in quarters, six hours after they were infused with endotoxin. They also noted an increase in temperature in the contralateral quarter, which would explain the increase in temperature in healthy quarters.

It was observed, that the subclinical (1.25±0.16) as well as the clinical quarters (2.12± 0.14) appeared to have a significantly different ($p<0.05$) CMT score when compared to healthy animals. The EC of the clinical quarters appeared to be significantly ($p<0.05$) higher when compared to the healthy quarters (7.44±0.49 vs 5.77±0.14). The SCC of subclinical quarters was also found to be significantly higher ($p<0.05$) when compared to healthy quarters (350.2±29.33 vs 99.8750±21.2). This is in concordance with the studies done by Sathiyabharathi et al. (2018a) who reported the mean (±SD) USST of the subclinical quarters was reported be 37.9 °C and those quarters which were clinically affected were 38.2 °C. They noted a 1.1 °C difference between non-mastitic and mastitic cows. The findings observed by Da Silva et al. (2016) was that there could be upto an increase of 8.55 and 2.46 °C in clinical and subclinical cases. This differed significantly from our findings. This difference could be due to the use of different thermal cameras, the sensitivity of the thermal camera, geographical location, and the influence of ambient temperature on the infrared thermal gun.

Table 38: Udder and teat skin surface temperature w.r.t. quarter health status in clinically mastitic cows

Quarters	USST (°C)	TSST (°C)	CMT	pH	EC (mS/cm)	SCC ($\times 10^3$ cells/ml)
Healthy (n= 16)	37.28 ± 0.20 ^{a}	35.8 ±0.11 ^{a}	0.43 ±0.19^{a}	6.74 ±0.02 ^{a}	5.77 ±0.14^{a}	99.8750 ±21.2^{a}
Subclinical (n= 12)	37.08 ±0.14 ^{a}	35.83 ±0.22 ^{a}	1.25 ±0.16^{b}	6.69 ±0.04 ^{a}	6.17 ±0.322 ^{a}	350.2 ±29.33^{b}
Clinical (n= 20)	37.51 ± 0.13 ^{a}	36.09 ±0.14 ^{a}	2.12 ± 0.14^{b}	6.733 ±0.05 ^{a}	7.44 ±0.49^{b}	

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

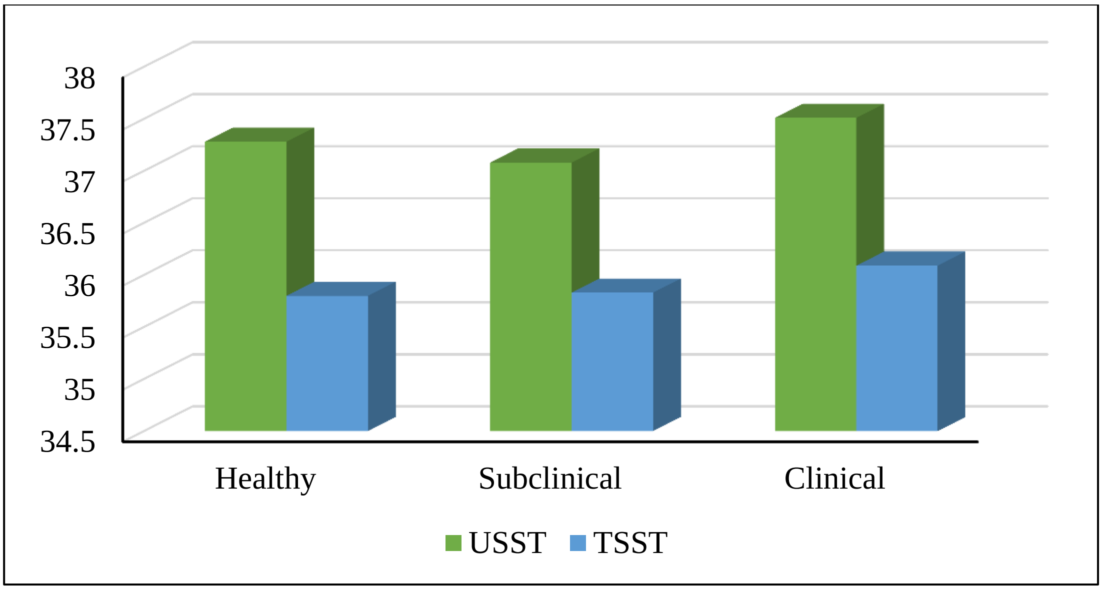

Fig. 51: Udder and teat skin surface temperature w.r.t. quarter health status in clinically mastitic cows

Table 39: Correlation between udder and teat skin surface temperature and milk inflammatory parameters in clinical mastitic cows (n=16)

Healthy quarters	USST	TSST	CMT	pH	EC	Log_{10} SCC
USST	1					
TSST	0.433	1				
CMT	0.568*	0.343	1			
pH	0.106	0.221	0.646**	1		
EC	0.485	0.428	0.458	0.094	1	
Log_{10} SCC	0.558*	0.227	0.825**	0.423	0.383	1

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

Table 40: Correlation between udder and teat skin surface temperature and milk inflammatory parameters in clinical mastitic cows (n=20)

Clinical	USST	TSST	CMT	EC
USST	1			
TSST	0.055	1		
CMT	0.073	0.121	1	

EC	0.093	-0.057	0.799**	1

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

Pearson's correlation was done to observe the degree of correlation between the healthy, clinical quarter and the milk inflammatory parameters. In the healthy quarters, a positive significant correlation was obtained between USST and CMT ($p<0.01$, r= 0.568) as well as the mean $\log_{10}$ SCC ($p<0.01$, r=0.558). A positive correlation was also observed between USST and EC, however this was not found to be significant ($p>0.05$). There was a positive correlation between the TSST and the milk inflammatory parameters as well, however these were non-significant ($p>0.05$). In the clinical quarters as well, we noted a positive but non-significant correlation ($p>0.05$) between the TSST and the milk inflammatory parameters such as pH, EC and log mean SCC.

Our findings are in corroboration with the findings of (Barth et al., 2000, Polat et al., 2010 and Sarubhi et al., 2020). They reported a positive correlation between the USST and the milk inflammatory parameters such as EC, CMT and SCC. This indicates that infrared thermography which is an extremely sensitive equipment that detects the slightest increase in temperature, could be used to detect and diagnose cases of early subclinical mastitis. However, as the equipment is variable according to the external ambient temperatures, variations will occur geographically disabling us from creating regular thermal patterns of the udder. However, infrared thermography appears to be good method for the early, non-invasive detection of subclinical mastitis that can serve as a supplement to the other methods of diagnosis

Table 41: Correlation between udder and teat surface temperatures and milk inflammatory parameters in apparently healthy quarters (n=96)

	USST	TSST	CMT	EC	$\mathbf{Log_{10}}$ SCC
USST	1				
TSST	0.353**	1			
CMT	0.307**	0.077	1		
EC	0.256*	0.026	0.095	1	
$\mathbf{Log_{10}}$ SCC	0.245*	0.076	0.735**	0.177	1

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

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

Pearson's correlation was done to observe the correlation between the USST, TSST and milk inflammatory parameters in apparently healthy quarters. We obtained a significant positive correlation between the USST and the TSST ($p<0.01$, $r=0.353$). A highly significant ($p<0.01$) correlation was found between the USST and the CMT scores as well ($r=0.307$). Similarly, statistically significant ($p<0.05$) correlation was obtained between USST and the milk inflammatory parameters such as EC ($r=0.256$) and $\log_{10}$SCC (r=0. 245). This indicated that an increase in USST leads to a simultaneous increase in the milk inflammatory parameters such as CMT, EC and SCC which is near to the findings reported by Barth (2000) and Polat et al. (2010) who found a significant correlation between USST and SCC. We obtained a positive correlation between TSST and milk inflammatory parameters such as CMT, pH, EC and SCC, however these were not found to be significant ($p>0.05$) which is in concordance with the findings reported by Sathiyabharathi et al. (2018b) who noted a positive correlation between USST and SCC (r= 0.93) as well as EC (0.76) They also noted that the increase in USST was linear with increase in SCC (R2 = 0.9583) and EC (R2 = 0.9748).

Table 42: Udder and teat surface skin temperature according to milk somatic cell count (n=96)

Group	SCC (cells/ml)	USST (°C)	TSST (°C)
1	**<2 lakh (n=68)**	36.58 ± 0.08^{a}	35.37 ± 0.13^{a}
2	**2-4 (n=22)**	36.85 ± 0.13^{a}	35.50 ± 0.19^{a}
3	**>4 (n=6)**	36.86 ± 0.14^{a}	35.46 ± 0.48^{a}

Values in columns with same superscript do not differ significantly ($p < 0.05$)

The apparently healthy quarters (n= 96) were further divided according to their respective somatic cell count into three main groups. Number of quarters with, <2, 2-4 and >4 lakh cells/ml involved 68, 22 and 6 quarters, respectively. The group with the highest SCC (>4 lakh cells/ml) had the highest USST (36.86 ± 0.14 °C), among the three groups followed by the group containing SCC of 2-4 lakh cells/ml (36.85 ± 0.13 °C). The first group with SCC <2 lakh cells/ml had the lowest USST among the three groups. However, there was no significant difference ($p>0.05$) among these three groups. This is in accordance with the studies by Sathiyabharathi et al. (2018) who reported that the healthy udder tissue had an approximate temperature of about 36.10 ± 0.06 and noted a linear relationship between an increase in USST and SCC.

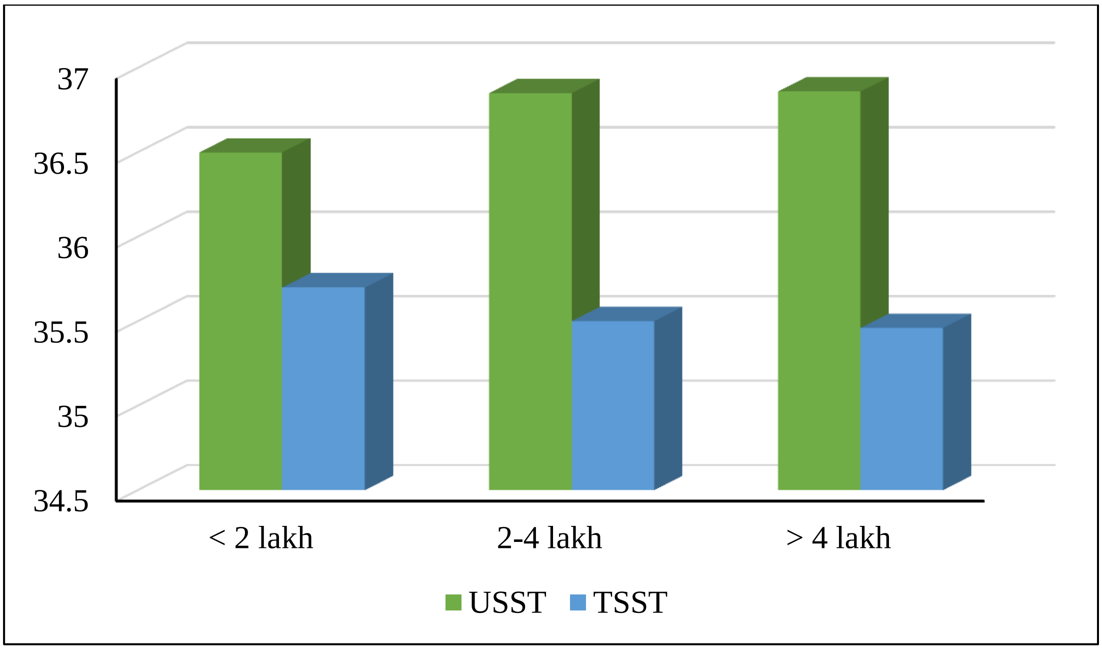

Fig. 52: Udder and teat surface skin temperature according to milk somatic cell count (n=96)

Table 43: Udder and teat skin surface temperature according to CMT

CMT	USST (°C)	TSST (°C)
Negative (n=69)	36.53 ± 0.070^{a}	35.37 ± 0.13^{a}
Positive (n=47)	37.20 ± 0.10^{b}	35.7 ± 0.12^{a}

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

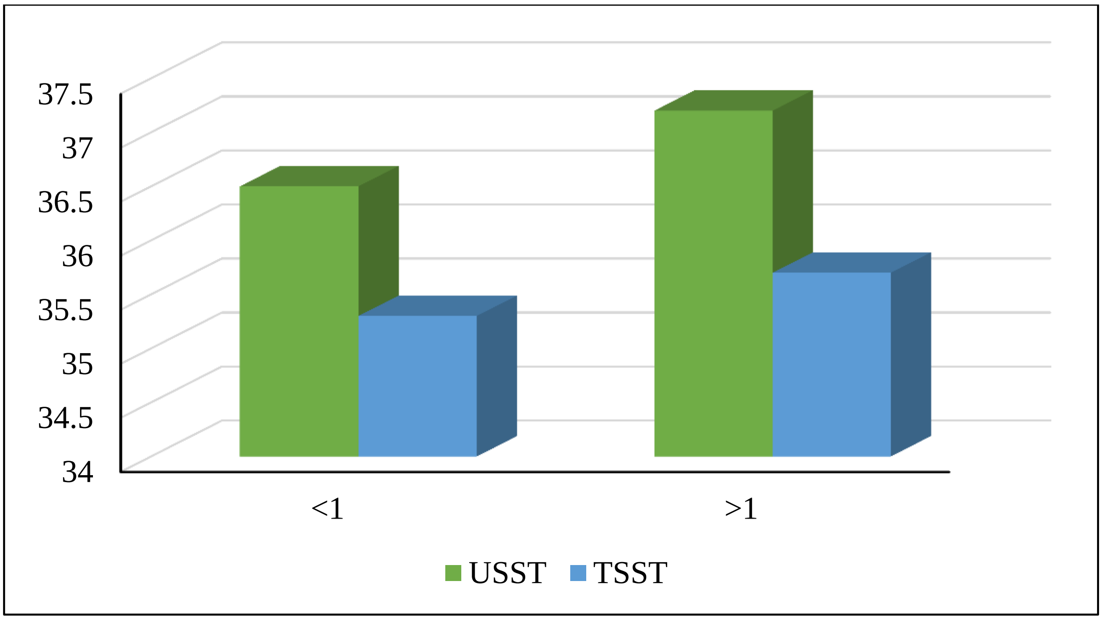

Fig. 53: Udder & teat skin surface temperature according to CMT

A total of 116 quarters belonging to 29 cows under study were divided according to CMT score. The USST in the group with CMT positive quarters had a significantly ($p<0.05$) higher USST as compared to the group with CMT negative quarters (37.20 ± 0.10 vs 36.53 ±.070). Similarly, the TSST was higher in quarters with a CMT positive, however these changes were not found to be significant ($p>0.05$). This is in corroboration with the findings of Kennedy (2004) who found that intramammary infections would cause an increase in udder skin surface temperature far before manifestation of other clinical signs. She noted an increase of 2.3°C in the udder of experimentally induced mastitis.

CHAPTER-V

SUMMARY AND CONCLUSIONS

Our study consisted of two main parts, the first part was to evaluate the ultrasonographic changes due to intra-mammary infection in udder, teats and supramammary lymph nodes of cross bred dairy cows and the second to standardize the use of infrared thermography (IRT) in mastitis cases. For our first objective, a total of 52 apparently healthy cows were ultrasonographically scanned, in which there were a total of 208 functional teats. Along with this, 104 supramammary lymph nodes of these 52 apparently healthy cows were also scanned. This part of the objective was done in the Livestock Dairy Farm, GADVASU. Besides this, 30 clinical cases of mastitis were also taken up in the Large Animal Clinic, GADAVSU, in which 120 functional teats and 60 lymph nodes were scanned ultrasonographically.

The second part was to standardize the use of infrared thermography for the detection of mastitis. A total of 29 cows were present in this study, out of which 17 apparently healthy cows were screened for their udder skin surface temperature and teat skin surface temperature in the university dairy farm, GADAVSU and 12 cows with clinical mastitis which were brought to the Large Animal Clinic, GADVASU were involved. Among these 29 cows, a total of 116 quarters were examined for temperature changes and were further divided into three main groups based on the somatic cell count and bacteriological organism isolated– Healthy, sub-clinical and clinical.

Out of 208 quarters, 123 (59.14%) quarters were healthy, 41 (19.71%) with latent infection, 7 (3.37%) quarters had non-specific infection and 37 (17.79%) with specific subclinical infection. The prevalence of the infection in LF, LH, RF and RH subclinical quarters was observed to be 24.7, 23.53, 23.53 and 28.23 percent, respectively. The proportion of specific subclinical infection in the forequarters was 16/41 (39.02%) and the hindquarters had a proportion of 21/44 (47.73%).

Out of the total 208 quarters, a total of 130 quarters had no growth. The 78 culturally positive quarters comprised of 45 (57.69%) coagulase positive *staphylococci* (CS), 30 (38.46%) coagulase negative *staphylococci* (CNS), 2 (2.56%) *E. coli* and only 1(1.28%) *Streptococcus* sp.

Upon comparison of teat tissue measurements in relation to the udder quarter health status, we observed a significant ($p<0.05$) increase in the OTD of subclinical quarters when compared to the healthy ones. There was also about 7.5 and 3.6% increase in the TWT and TCL of subclinical quarters, respectively. This was, however, non-significant. No other teat tissue measurements changed significantly.

All milk inflammatory parameters in subclinically affected quarters were significantly ($p<0.05$) elevated when compared to the healthy quarters. Further, teat tissue measurements with respect to infection with major pathogen was carried out. Quarters affected with CNS had significantly higher ($p<0.05$) OTD as compared to quarters with no growth. The CD in quarters afflicted with CS were significantly ($p<0.05$) smaller compared to quarters with no growth. TWT was thicker in quarters where CNS and CS were isolated, however these findings were not significant ($p>0.05$).

Teat tissue measurements were compared based on SCC (<2 lakh, 2-4 lakh and >4 lakh cells/ml) to observe the effect of SCC on tissue changes. The OTD of the second group (SCC 2-4 lakh cells/ml) also differed significantly ($p<0.05$) from the third group (SCC > 4 lakh cells/ml). The TWT was found to be the highest in the group containing SCC of >4 lakh cells/ml, however no significant ($p>0.05$) differences were found between the three groups. FTD and CD did not differ significantly between various groups. TCL was observed to be significantly longer in the second group (2-4 lakh cells/ml) as compared to group 1 (SCC <2 lakh cells/ml) and group 3 (> 4 lakh cells/ml).

Teat tissue measurements with respect to CMT score were compared and it was observed that CMT positive quarters had a significant increase ($p<0.05$) in OTD and TWT when compared to those which were CMT negative. The FTD and CD did not vary significantly between CMT positive and negative groups. Further, quarters were grouped according to the CMT score into four groups- 0, 0.5, 1 and 2-3 and results were compared. It was observed that the group with the CMT score of 0.5 had significantly ($p<0.05$) increased OTD when compared to healthy quarters. The OTD of the fourth group was significantly ($p<0.05$) increased and varied from all other groups. TCL of the second group (CMT score of 0.5) was significantly reduced when compared to healthy quarters (1.27 ± 0.06 vs 1.42 ± 0.02).

Among teat tissue measurements, OTD was found to be significantly ($p<0.01$) positively correlated with other parameters such as TWT, FTD and CD. TWT was significantly ($p<0.01$) correlated to OTD, FTD and TCL. CD correlated negatively with parameters such as TWT and FTD.

When teat tissue measurements were correlated with milk inflammatory parameters it revealed that, CMT had a strong significant correlation with OTD ($p<0.01$) and TWT ($p<0.05$) .pH, EC and SCC also had a significant ($p<0.01$) positive correlation with OTD. This indicated that an increase in the milk inflammatory parameters such as CMT, pH, EC and SCC could lead to a simultaneous increase in OTD.

The herd in which the supramammary lymph nodes were scanned had a mean CMT score of 0. 40 ± 0.046 (0-3) and mean somatic cell count of 129.27 ± 13.17 cells/ml (1-956 x10^3 cells/ml). The mean pH and EC of the herd was found to be 6.75 ± 0.01(5.94- 7.52) and 5.55 ± 0.09 mS/cm (2.3-10.71) respectively. The mean left length of the lymph node was found to be 7.22 ± 0.29 cm (3.49 – 12.6 cm) and the mean left depth was found to be 5.11 ± 0.22 cm (2.69 – 8.71 cm), whereas the mean right length and depth of the lymph node was found to be 7.20 ± 0.24 cm (4.7 – 13.4 cm) and 5.04 ± 0.21 cm (2.11 – 8.73) cm, respectively.

There were found to be 30 (57.69%) healthy cattle and 22 (42.3%) subclinically affected ones. This was done based on the SCC count and presence of bacteriological organism. It was observed that in case of healthy animals, the length of the left of the lymph node was significantly ($p<0.05$) smaller when compared to those that were sub clinically affected (6.59 ± 0.29 vs 8.09± 0.52), the depth was also found to be significantly ($p<0.05$) smaller when compared to those that were sub clinically affected (4.71± 0.23 vs 5.67± 0.39). The right lymph node length was noted to be larger as compared to the healthy animals as well (7.72± 0.42 vs 6.82± 0.22) however, these changes were not significant ($p>0.05$). The right lymph node depth was observed to be significantly ($p<0.05$) larger in subclinically affected animals as compared to healthy ones (4.56 ± 0.26 vs 5.69± 0.22).

On finding the correlation of the lymph node dimensions with the milk inflammatory parameters. On performing a combined Spearman’s correlation test including both the right and left lymph node dimensions and comparing them to the milk inflammatory parameters, we observed a significant ($p<0.01$) correlation between the length and the CMT (r=0.353) as well as the somatic cell count (r=0.280). We also obtained a non-significant correlation between the lymph node length and EC (0.031) and pH (r=0.104).

On obtaining the correlation between the lymph node dimensions (left and right) and the teat tissue measurements we obtained, a significant ($p<0.05$) correlation with OTD (r=0.250) and TWT (r=0.213) indicating an increase in the size of the lymph node usually led to a significant ($p<0.01$) increase in the OTD and TWT as well.

Spearman’s correlation was applied to enumerate the correlation between the supramammary lymph node dimensions and the age, parity as well as stage of lactation of the animal. A significant ($p<0.01$) positive correlation was obtained between the lymph node dimensions (length and depth) and the age (r= 0.262) and parity (r= 0.283) of the animals, indicating that older cows had larger supramammary lymph nodes. We also obtained a significant ($p<0.01$) negative correlation between the lymph node dimensions and the stage of lactation (r=-0.297) indicating that the size of the lymph node decreases towards the end of lactation.

A total of 30 animals having clinical mastitis with 120 functional teat and udder tissue presented to Large Animal Clinical complex were ultrasonographically scanned, out of which 40 (33.3%) were healthy quarters, 18 (15%) nonclinical quarters (no visible signs but affected with specific subclinical mastitis) and a total of 62 (51.67%) clinical quarters.

A significant ($p<0.05$) increase in the CMT score, EC and SCC was observed in the clinical (2.36 ±0.39) and non-clinical quarters (1.33 ± 0.15) when compared to the healthy quarters (0.42 ± 0.13), however, no significant difference was detected between clinical and non-clinical quarters. There was no significant variation in pH among the three groups ($p>0.05$).

On comparing means of teat tissue measurements of clinical mastitis quarters, it was noted that the OTD in clinical quarters was found to be significantly higher ($p<0.05$) when compared to healthy quarters, however non-clinical quarters did not differ significantly ($p>0.05$) from the healthy and clinical quarters. A non-significant ($p>0.05$) increase in the TWT was observed in non-clinical and clinical quarters when compared to healthy quarters. FTD, CD and TCL showed no significant variations among the three groups.

Besides, ultrasonography of supramammary lymph nodes in 30 clinically mastitic cows was also carried to observe the changes owing to infection. A total of 60 clinically affected supramammary lymph nodes were scanned. The mean CMT score of this group was 1.769 ± 0.15, the mean pH was 7.09 ± 0.09, an EC of 6.68 ± 0.17 mS/cm and the SCC of 138.99 ± 20.02 lakh cells/ml (only of healthy and subclinical quarters). SCC of clinically affected quarters was not done.

The mean length of the left lymph node was observed to be 7.97±0.42 (3.49 – 12.7cm) which was significantly increased in size when compared to those of the apparently healthy cows in the previous study. The mean depth of the left lymph node was 5.5± 0.42 (2.14 – 13.4 cm). The mean length of the right lymph node was observed to be 7.58±0.38 (3.63- 12.3 cm) and the mean depth of the right lymph node was 5.1±0.38 (1.79 – 10.3 cm).

Similarly, Spearman's correlation was assessed to observe the correlation between the supramammary lymph node dimensions with the milk inflammatory parameters and teat tissue measurements in clinically mastitic animals. We obtained a significant positive correlation between the length and the CMT scores of the respective sides ($p<0.01$, $r=0.265$). The width was observed to have a positive correlation with the CMT scores of the ipsilateral sides ($p<0.05$, $r=0.217$), however this was non-significant.

We obtained a notable significant ($p<0.01$) relationship of the length with OTD ($r=0.360$). We also obtained a significant ($p<0.05$) correlation between length and the FTD ($r=0.277$) and CD ($r=0.291$). This is in concordance with our previous studies that in cases of clinical and subclinical mastitis, there is a significant increase in the OTD as well as the FTD.

For infrared thermography we examined a total of 29 cows, comprising 116 quarters. Out of these twenty-nine cows, 17 cows were apparently healthy with no visible changes in the milk or quarter and 12 of the cows were clinically affected with either one or more clinical quarters. The mean USST and TSST of these 29 cows were determined and found to be 36.80± 0.06 °C and 35.5 ± 0.09 °C.

The present study revealed that healthy quarters had the lowest udder temperatures with a mean USST of 36.59 ±0.087°C with temperatures ranging from 34.9-38 °C, the nonclinical quarters had a visibly increased temperature of 36.834±0.09 °C with temperatures ranging from 35.1-38.1 °C, however this did not significantly vary from that of the healthy quarters ($p>0.05$). The clinically affected quarters had a mean temperature of 37.5±0.14 °C with temperatures varying from 36.6 – 38.9 °C. The USST of the clinical quarters varied significantly ($p<0.05$) when compared to healthy and subclinical quarters. Therefore, in our study we found that those that were subclinically mastitic quarters had udder skin surface temperature 0.24 °C higher than the healthy quarters. The USST of clinical quarters in our study was found to vary from the healthy quarters by approximately 0.91°C.

Pearson's correlation test was applied to observe any correlation between udder and teat skin surface temperature and the milk inflammatory parameters, irrespective of the udder health status. A highly significant ($p<0.01$) positive correlation was obtained between the USST and CMT ($r=0.477$) as well as EC ($r=.382$). There was also a statistically significant ($p<0.05$) positive correlation between USST and the mean $\log_{10}$ SCC ($r=0.245$). We obtained a strong positive correlation between TSST and the CMT scores of the respective sides ($p=0.01$, $r=0.22$).

The apparently healthy quarters (n= 96) were further divided according to their respective somatic cell count into three main groups. Number of quarters with, 2, 2-4 and >4 lakh cells/ml involved 68, 22 and 6 quarters, respectively. The group with the highest SCC (>4 lakh cells/ml) had the highest USST (36.86 ± 0.14 °C), among the three groups followed by the group having SCC of 2-4 lakh cells/ml (36.85 ± 0.13). Further, on comparing USST according to CMT the USST in the group with CMT positive quarters had a significantly ($p<0.05$) higher USST as compared to the group with CMT negative (37.20 ± 0.10 vs 36.53 ±.070 °C).

Conclusions:

- Ultrasonographic teat tissue measurements (OTD and TWT) were well correlated with milk inflammatory parameters so they can be taken into consideration while assessing for infection.

- Supramammary lymph node size increases remarkably in both clinical and subclinical mastitis so it can be taken as an early indicator for diagnosis of subclinical mastitis cases.
- Infrared thermography could detect changes of USST in affected quarters and correlated fairly with SCC, however future studies with large number of subjects need to be carried out to establish strong conclusion.

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