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Infrared thermography in bovine mastitis: A preliminary study

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Abstract

The aim of this study was to standardize the use of infrared thermography in bovine mastitis. This study included a total of twenty-nine cross-bred dairy HF cows, comprising of seventeen apparently healthy cows and twelve clinically mastitic cows. A significant ($p<0.05$) increase of 0.91 ° C was observed in clinical quarters when compared to healthy quarters (37.5 ±0.13 vs 36.59 ±0.086). The subclinically mastitic quarters had a USST 0.24 °C higher than the healthy quarters. A highly significant ($p<0.01$) positive correlation was obtained between the USST and CMT ($r=0.477$),EC ($r=0.382$) and a moderate significant($p<0.05$) positive correlation between USST and the Log_{10} SCC ($r=0.245$). Significant positive correlation between USST and milk inflammatory parameters such as CMT and EC ($p<0.01$, $r=0.568$, $r=0.407$) were obtained in clinical quarters. The quarters were divided 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 quarters (37.20 ± 0.10 vs 36.53 ±.070 °C). The apparently healthy quarters (n= 96) were further divided according to their respective somatic cell count into three main groups. 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 study concluded that infrared thermography is a technique that could be successfully correlated to the other diagnostic methods such as CMT, EC and SCC, indicating that IRT has potential to be an excellent non-invasive method to diagnose mastitis and the use of IRT in field conditions must be further evaluated.

Keywords: Infrared thermography, Bovine, Mastitis

Mastitis is a heavy burden for the economy of the dairy sector, not only in India but world-wide. The losses include significant loss of milk production, discarded milk and milk product and their inferior quality, veterinary services and treatment cost, labour cost and culling cost (Bansal and Gupta, 2009). The prevalence of clinical and subclinical mastitis is 15 and 42% worldwide and 18 and 45% in India, respectively (Krishnamoorthy *et al.*, 2017). 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). The most commonly reported pathogen worldwide has been reported to be *Staphylococcus aureus*, which has dreadful effects on the udder due to release of its exotoxins (Hauber *et al.,* 2017). There are various techniques which have been developed over time for the detection of mastitis such as CMT, pH, SCC. Detecting the reduction of milk lactose has been a newer method in detecting subclinical mastitis (Berglund *et al.*, 2007).

However, there is a necessity to develop an easy to perform non-invasive technique to detect subclinical mastitis at the field level. Infrared thermography (IRT) is a revolutionary, sophisticated technique that was originally developed for military and industrial applications (Eugeniusz-Herbut and Mazur, 2006). This technique utilizes the Stefan-law, stating that all objects emit infrared radiation proportional to their temperature in accordance, therefore a thermal camera is utilized which detects the infrared radiation and creates a pictorial representation based on the heat generated without exposing the user to harmful radiation. (Kunc *et al.*, 2007). Thermography in the veterinary field was initially used to detect lesions in the digit for the early detection of hoof lesions such as digital dermatitis. Purohit and McCoy (1980) found that using infrared thermography they could detect an increase in temperature in the hoof indicating early inflammatory signs of the hood two weeks before clear cut clinical signs occured. 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) attempted in using infrared thermography as a method to set as a health

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index for livestock. They found that there were three regions with varying thermal patterns considered as “pain spots” which were the udder, the hooves and in regions around skin injuries. In the case of experimentally induced mastitis in eighty-three lactating dairy cows, Castro-Costa *et al*. (2014) observed that the first change that was visualized was the increase in udder skin surface temperature about four hours post-inoculation. The highest temperature was recorded at 6 hours post-inoculation. Studies done by Polat *et al*. (2010), Samara et al. (2014) and Sathiyabharathi *et al*. (2018) could successfully correlate the USST with milk inflammatory parameters such as CMT, Electrical conductivity and Somatic cell count. Da Silva *et al.* (2019) detected an increase in USST upto 8.5 °C and 2.46 °C in quarters affected with clinical and sub-clinical quarters respectively. Golzarian *et al.* (2016) reported an increase upto 0.45 ˚C in subclinically infected quarters when compared to healthy ones. This indicated that this increase in temperature could be utilized to detect mastitis.

Therefore, this study aimed to standardize the use of infrared thermography to visualize the change in temperature observed in healthy, subclinically and clinically affected animals.

Materials and Methods

This experiment including capturing thermal images and milk sampling were performed as per the guidelines of IAEC of College of Veterinary Science, GADVASU.

Study area and animals

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°C. 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.

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 96 images.

Infrared thermography technique

A FLIR E60 infrared thermographic camera 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.1). 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 analyse the photos, and the temperatures of the animal were compared to the parameters of the quarter foremilk samples.

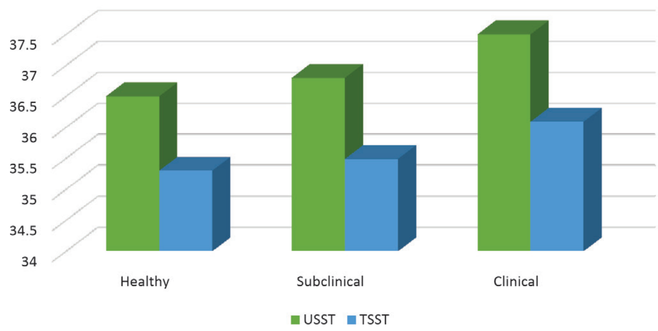

Fig. 1: Udder and teat skin surface temperature with respect to udder health status

Sampling

Quarter samples were collected in quarters to be analysed further. The teats were thoroughly cleaned and dried before collecting the milk samples. 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 the Infrared Thermography findings. The milk samples were subsequently tested in the Department of Veterinary Medicine's mastitis lab.

Infrared thermography images and their readings

The images which were saved to an SD card were 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 analysed. The milk somatic cell count, pH, EC, and bacteriological test were then compared to these temperatures.

Statistical analysis

The SPSS program (IBM) was used to analyse 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.

Results and Discussion

In our study, we examined 29 cows, comprising of 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 quarters were classified into three main groups – Healthy, sub clinical and clinical (Table 1). 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 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 (Fig. 1). Similarly, the temperature of the teat was taken at the

Table 1:Udder and teat skin surface temperature and milk inflammatory parameters w.r.t. udder health status

Type	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	5.188 ±0.126^a	58.01 ±4.80^a
Subclinical	26	36.83 ±0.097^a	35.49 ±0.18^a	1.17 ±0.12^b	6.76 ±0.03	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	6.836 ±0.504^b	-

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

mid-teat level. It was observed that healthy quarters had the lowest TSST of 35.38±0.13 with temperatures ranging from 32.1-36.6 °C. The subclinical quarters were found to have a mean TSST of 35.496±0.19 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 ranging from 33.6- 36.9 °C. The TSST of clinical quarters were significantly higher ($p<0.05$) when compared to healthy and subclinical quarters. Therefore, in our study we found that those that were subclinically mastitic quarters had a USST, 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 in 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.* (2016) who reported that in subclinically 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 difference between these two findings could be due to environmental differences according to geographical regions leading to higher USST in the healthy cattle found in tropical and subtropical regions.

Pearson's correlation test was applied to observe for correlation between USST, TSST and the milk inflammatory parameters, irrespective of the udder health status (Table 2). 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 Log_{10} SCC ($r=0.245$). This indicates that with an increase in the udder skin surface temperature of a quarter, CMT score, E.C. and SCC of that quarter increased simultaneously. Polat *et al.* (2010) determined to find the degree of interrelationship between USST and milk inflammatory parameters such as SCC and CMT score and found a positive correlation ($r = 0.76$; $P < 0.0001$), indicating that quarters afflicted with infections had a higher udder skin surface temperature due to the inflammatory reaction occurring within it. We obtained a strong positive correlation between TSST and the CMT scores of the respective sides ($p<0.01$, $r=0.22$). We recorded a non-significant ($p>0.05$) positive correlation between the TSST and parameters such as EC and SCC.

On perusal of Table 3, we observed a significant positive correlation between USST and milk inflammatory parameters such as CMT and log_{10} SCC in healthy quarters ($p<0.01$, $r=0.369$, $r=0.303$). 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

Table 2: 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)

Table 3: Correlation between the udder and teat skin surface temperature with the milk inflammatory parameters in healthy quarters (n=70)

	USST	TSST	CMT	EC
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

** Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed) parameters such as CMT, log_{10}SCC and EC. In Table 4 when we obtained the correlation between udder skin surface temperature and the milk inflammatory parameters in clinical quarters, we observed a significant positive correlation between USST 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.

A total of 116 quarters belonging to 29 cows under study were divided according to CMT score in Table 5. 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 ones (37.20 ± 0.10 vs 36.53 ±.070), therefore quarters with a CMT positive were found to be 0.67 °C higher than quarters that had a CMT negative. Similarly, the TSST was higher in quarters with a CMT >1, however these changes were not found to be significant ($p>0.05$). Sathiyabharathi *et al*. (2018) reported an increase of upto 0.8 °C and 1 °C in subclinical and clinical quarters. 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. In Table 6, the apparently healthy quarters (n= 96) were further divided according to their respective somatic cell count into three main groups. 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. Samara *et al*. (2014), Bortolami *et al*. (2015) and Sathiyabharathi *et al.* (2018) have all found a fair to strong correlation between USST and SCC. The increase in udder skin surface temperature appears to one of the first detectable change in the udder, Castro-Costa (2014) noted that the first increase in temperature was detected as early as 4 hours post intramammary inoculation of endotoxin into the udder, peaking at 6 hours where the temperature was found to be the highest.

In conclusion, infrared themography is a technique that could be successfully correlated to the other diagnostic methods such as CMT, EC and SCC. There was a significant ($p<0.05$) increase in the clinically affected

Table 4: 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)

Table 5: Udder & teat skin surface temperature according to CMT

CMT	USST (°C)	TSST (°C)
Negative (n=69)	36.53 ± .070[a]	35.37 ± 0.13
Positive (n=47)	37.20 ± 0.10[b]	35.7 ± 0.12

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

Table 6: 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 different superscript differ significantly ($p<0.05$)

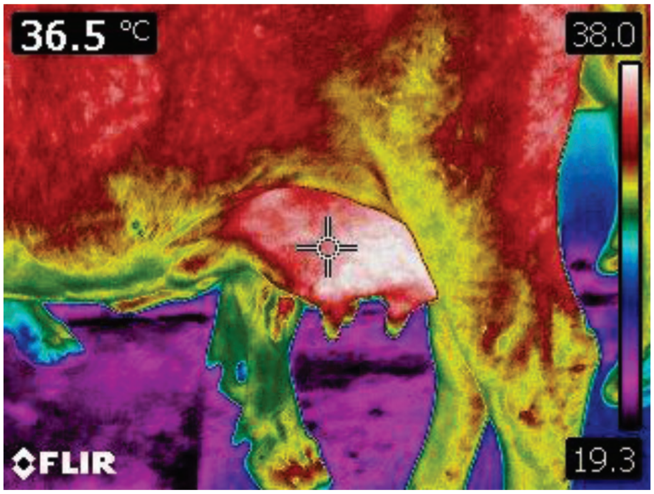

Fig. 2: IRT image of healthy left forequarter

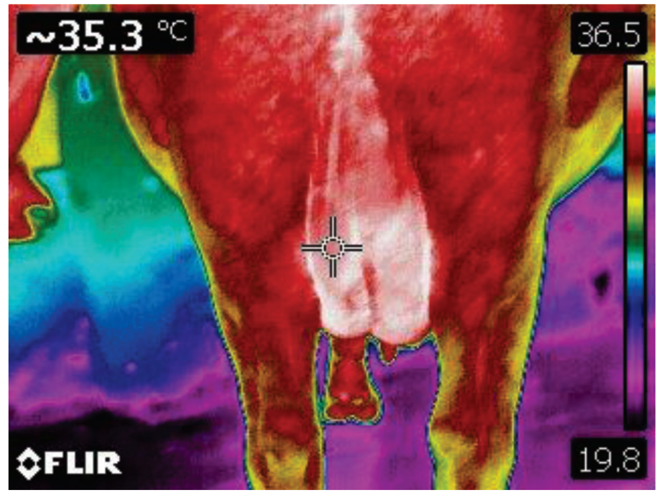

Fig. 3: IRT image of healthy left hind quarter

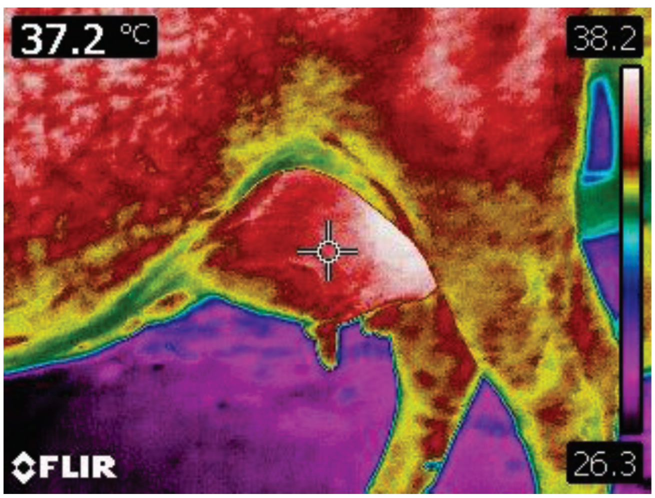

Fig. 4: IRT image of subclinically affected left fore quarter

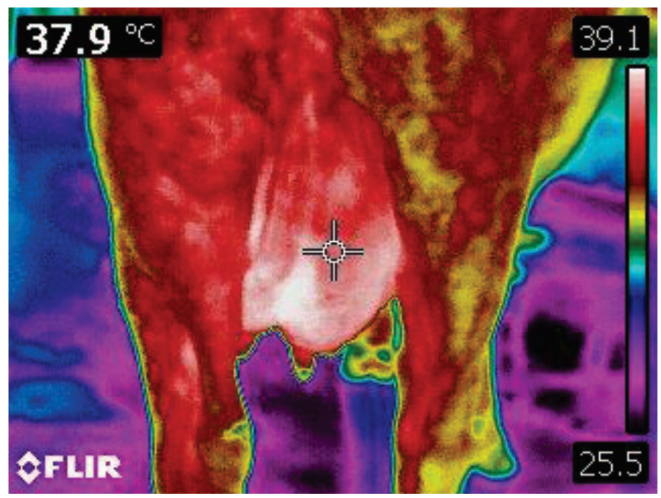

Fig. 5: IRT image of clinically affected right hindquarter

quarters when compared to healthy ones. An increase of 0.91 °C was detected. The increase in temperature was attributed to the status of tissue metabolism and blood circulation which is reflected in skin temperature; aberrant thermal patterns indicated areas of superficial inflammation or circulatory problems. This supports our objective in which we can use infrared thermography as a method for diagnosing subclinical as well as clinical mastitis. However, it must be kept in mind that when employing IRT on a farm, environmental elements like as sunlight, dust, and the distance between the camera and the animal must be taken into account, as these might alter the temperature readings.

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