Mastitis is the most common infectious disease in dairy cattle which causes low milk yield and poor quality of milk. Mastitis ranks first among the diseases of dairy cows with high prevalence and incidence rate, which causes severe economic losses to the dairy farmers. In India, two decades back, the incidence of clinical mastitis and subclinical mastitis were ranging from 1 to 10% and 10 to 50%, respectively in cows. Recent studies showed the higher incidence of subclinical mastitis ranging from 20 to 83% in cows. The incidence of subclinical mastitis amongst 123 crossbred cattle was 17.33% (Saini et al. 1994) while in Karan Fries and Karan Swiss was 36.9 and 38.46%, respectively (Jingar et al. 2014). The economic losses due to mastitis in India have increased about 115 folds (6,000 crore) in last five decades. Delay in the detection of subclinical mastitis and lack of appropriate and accurate technique are contributing to the higher incidence of clinical mastitis. The losses are either due to loss of milk production (temporary or permanent), poor milk quality, discarding of milk from affected animals and reduced productive life or pre-mature culling of the cow. The loss due to subclinical mastitis is higher than the clinical mastitis and milk yield loss due to mastitis ranged from 100 to 500 kg/cow/lactation (Srivastava 2015).

Early detection of mastitis is most important to prevent the losses associated with decreased milk production, quality and make decisions for quick and effective treatment. Skin temperature reflects the state of tissue metabolism and blood circulation; abnormal thermal patterns can signify areas of superficial inflammation or circulatory impairments. IRT is employed as a diagnostic tool and shown to be sensitive enough to detect changes in USST of healthy and mastitis affected quarters (Sathiyabarathi et al. 2016, Polat et al. 2010, Hovinen et al. 2008, Colak et al. 2008, Metzner et al. 2014). In most of the studies, IRT was utilized to detect changes in USST caused by milking, environmental temperature, exercise (Berry et al. 2003, Kennedy et al. 2003), subclinical and clinical mastitis induced by infusion of E. coli lipopolysaccharide (Willard et al. 2007, Polat et al. 2010,
Hovinen et al. 2008, Colak et al. 2008, Metzner et al. 2014). Sathiyabarathi et al. (2016) and (2018) used IRT for early detection of natural infection with a common course of events and its associated changes with mastitis in an organized herd of HF crossbred and Deoni cows. The present study was undertaken to assess the body and USST differences in naturally occurring subclinical and clinical mastitis in KF crossbred cows reared under subtropical conditions.

MATERIALS AND METHODS

Thermal imaging and milk sampling were performed as per the guidelines of the National Dairy Research Institute Animal Ethical Committee for care and use of experimental animals.

Study area and experimental cows: The study was conducted at Livestock Research Centre of ICAR-NDRI, Karnal located at an altitude of 250 m above sea level on 28°16′N latitude and 77° 05′E longitudes of the Trans-Gangetic plain region of India. The climatic condition of the farm is of subtropical where temperature raises up to 42°C in summer and comes closer to 7°C during the winter season. Average rainfall ranges from 760–960 mm and the maximum is received during July to August. The mean temperature and relative humidity (RH) during the study period in the month of February was 21°C and 60%, respectively.

In the present study, a total of 200 quarters of lactating Karan Fries (Holstein Friesian × Tharparkar) cows (50) from first to fifth parity with an average body weight of 350 to 450 kg, 142±14 days in milk and milk yield of 15.6±0.76 kg per cow were used. The animals were maintained under loose housing system and milked thrice daily with herringbone machine milking system. The cows were provided with sufficient concentrate feed by automatic feeding system and animals had free access to water.

Thermal imaging and milk sampling: Total of 50 eyes and 200 individual udder quarter surface thermal images were taken using an FLIR i5 infrared camera (FLIR Systems Inc., 27700 SW Parkway Ave. Wilsonville, OR 97070, USA) to monitor the changes in the USST of the individual udder quarter.

Thermographic images were captured from the lateral side for fore quarters and posterior or lateral side for hind quarters of the udder (Fig. 1). After capturing, the images were transferred to computer and analyzed by using FLIR Quick Report 1.2 image analysis software. The temperature of the inner canthus of the eye and the average temperature of the udder skin surface in a particular image was recorded and used in the analysis.

Milk samples from each quarter were collected to screen for subclinical mastitis using CMT (ImmuCell Corporation, California mastitis test (CMT) score for milk samples (Mohanty et al. 2015)

<table>
<thead>
<tr>
<th>CMT score</th>
<th>Visible reaction</th>
<th>Total cell count</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (Negative)</td>
<td>Milk fluid and normal, no thickening</td>
<td>0–200,000</td>
<td>Healthy quarter</td>
</tr>
<tr>
<td>T (Trace)</td>
<td>Slight thickening, reaction disappears in 10 seconds</td>
<td>200,000–400,000</td>
<td>Early subclinical mastitis</td>
</tr>
<tr>
<td>1 (Weak +ve)</td>
<td>Distinct thickening, no gel formation</td>
<td>400,000–1,200,000</td>
<td>Subclinical mastitis</td>
</tr>
<tr>
<td>2 (Distinct +ve)</td>
<td>Thickens immediately, begins to gel, levels in the bottom of cup</td>
<td>1,200,000–5,000,000</td>
<td>Clinical mastitis</td>
</tr>
<tr>
<td>3 (Strong +ve)</td>
<td>Gel is formed, surface elevates, with a central peak above the mass</td>
<td>Over 5,000,000</td>
<td>Severe clinical mastitis</td>
</tr>
</tbody>
</table>

Fig. 1. Infrared thermographic image of udder quarters of non-mastitis KF crossbred cow. LFQ, left fore quarter; RFQ, right fore quarter; LHQ, left hind quarter; RHQ, right hind quarter.
Portland), EC (Draminski Electronic Mastitis Detector, Draminski U1. Owocowa Olszyn Poland) and SCC (PortaSCC® somatic cell test, Whittendale Drive, Suite E Moorestown, NJ, USA) using standard protocol. The quarter which showed SCC of more than 400,000 cells per ml of milk in a crossbred cow was considered as mastitis affected animals (Saravanan et al. 2015). Besides, the animals with CMT score of trace and more than one (Table 1) and cows with more than 50 units difference of EC values in between four quarters were also considered as mastitis-affected animals.

Statistical analysis: Paired t-test was employed to test the significance of the differences between the mean body and USST of the non-mastitis cows (180 quarters) and subclinical and clinical mastitis affected cows. An analysis of variance (ANOVA) was employed to compare the non-mastitis, subclinical and clinical mastitis quarters with respect to body temperature, USST, and EC. The correlation between the USST and SCC of subclinical mastitis affected cows were performed using Pearson’s test. Correlation less than 0.3 was considered weak, between 0.3 and 0.7 moderate and above 0.7 as strong. The regression model was employed relating USST with EC difference for non-mastitis, subclinical and clinical mastitis affected cows. All the above statistical analysis was made using SPSS 16.0 (IBM Corporation, Armonk, New York, US).

RESULTS AND DISCUSSION

Mastitis is the most common multi-etiological disease and has a substantial effect on quality and quantity of milk and udder health of dairy cattle. For early detection of mastitis, there are many methods viz. color, pH, SCC, CMT, EC, detection of enzymes (N-Acetyl glucosaminidase and lactate dehydrogenase), bacterial culture and advanced techniques like PCR, ELISA, proteomics and mass spectrometry for biomarker detection, biosensor system and chip based diagnostic techniques that are in use under field condition and in precision dairying (Mohanty et al. 2015). However, these diagnostic methods are laboratory oriented, lack full accuracy and needs a considerable time of farm staff or milker (Polat et al. 2010, Hovinen et al. 2008 and Metzner et al. 2014). Therefore, a cost effective, rapid, non-invasive cow side diagnostic technique with a potential application in field is essential for monitoring udder health.

IRT is a non-invasive diagnostic tool for assessing changes in skin surface temperature which are influenced by internal conditions of tissues and organs accompanied by fluctuations in the amount and rate of capillary blood flow (Purohit and McCoy 1980, Hurnik et al. 1984, Metzner et al. 2014). IRT has recently been studied by various authors for early detection of subclinical mastitis and E. coli lipopolysaccharide-induced mastitis in various dairy breeds, viz. Brown Swiss, Ayrshire, and Holstein-Friesian under temperate condition (Willard et al. 2007, Polat et al. 2010, Hovinen et al. 2008, Colak et al. 2008, Metzner et al. 2014).

In the present study, we have demonstrated that body and USST differences monitored by IRT technique could possibly detect subclinical and clinical mastitis-affected udder quarter of a Karan Fries crossbred cow showing the temperature difference of 0.7°C.

Skin surface temperature reflects the underlying tissues physiological status (Berry et al. 2003) and changes in udder circulation are brought about by sympathetic and noradrenergic sympathetic neurons in the mammary gland (Paulrud et al. 2005). IRT could detect minute changes in udder skin surface temperature in relation to health status which is accompanied by alterations in vascularization. A thermal camera absorbs infrared radiation and generates pictorial images based on the amount of heat generated, without causing radiation exposure (Kunc et al. 2007). In cattle, body temperature is relatively constant and USST is positively correlated with body temperature (Bitman et al. 1984) and infrared thermogram of an eye was more consistent than any other anatomical region for comparison of body temperature with USST (Poiklainen et al. 2012,
Schaefer et al. 2003). In this study, it was found that the mean (±SD) body (37.1±0.07°C) and USST (37.1±0.06°C) of non-mastitis cows did not differ significantly.

In this study, out of 200 udder quarters, eleven quarters were diagnosed as subclinical mastitis and nine as clinical mastitis. The mean USST of subclinical (37.9±0.09°C) and clinical mastitis affected quarters (38.2±0.10°C) were significantly higher (P<0.001) than the body temperature (37.1±0.08°C) of the cows by 0.8 and 1.1°C, respectively (Figs 2, 3). This was similar to the observation of Polat et al. (2010) who found that subclinical mastitis quarters had 2.35°C greater USST than healthy quarters and Hovinen et al. (2008) observed an increase of 1 to 1.5°C in USST associated with lipopolysaccharide (LPS) induced clinical mastitis by using infrared thermography. In HF crossbred cows, subclinical and clinical mastitis affected udder quarters showed 0.72 and 1.05°C higher USST than body and non-mastitis quarters (Sathiyabarathi et al. 2016). Similarly, indigenous Deoni cows with subclinical mastitis showed 1.51°C higher USST than body and non-mastitis quarters (Sathiyabarathi et al. 2018). A temperature increase of +2.3°C of USST was detectable through IRT in experimentally induced mastitis by infusion of bacterial endotoxin (Scott et al. 2000). The thermal camera detects increased USST both in experimentally induced (Hovinen et al. 2008, Scott et al. 2000) and in natural course of infection (Sathiyabarathi et al. 2016 and 2018). Udder with subclinical or clinical mastitis may show increased USST due to the result of hyperemia at the infection (Jones and Plassmann 2002).

In the present study, affected quarters showed a higher temperature than the non-mastitis quarters and the temperature difference was 1.1°C. Willits (2005) and Kennedy (2004) observed that mastitis causes USST to rise often prior to other clinical signs are visible. Scott et al. (2000) detected a clear rise in temperature of the

Table 2. Descriptive statistics of the udder skin surface temperature (USST, °C), electrical conductivity (EC, unit), electrical conductivity difference value (unit) and somatic cell count (SCC, lakhs) by California mastitis test (CMT) score

<table>
<thead>
<tr>
<th>CMT score</th>
<th>USST (°C)</th>
<th>EC (unit)</th>
<th>EC difference (unit)</th>
<th>SCC (×10³/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Mean 37.1±0.08</td>
<td>395.02±60</td>
<td>20±10</td>
<td>180±30</td>
</tr>
<tr>
<td>Trace (1)</td>
<td>Median 37.2</td>
<td>57.20</td>
<td>10</td>
<td>168±30</td>
</tr>
<tr>
<td>+1 (9)</td>
<td>Median 37.7</td>
<td>14.63</td>
<td>10</td>
<td>168±30</td>
</tr>
</tbody>
</table>

Table 3. Udder skin surface temperature (USST), electrical conductivity (EC), and electrical conductivity difference value between non-mastitis, subclinical and clinical mastitis affected udder quarters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean (±SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>USST (°C)</td>
<td>Non-mastitis</td>
<td>37.1±0.08a</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Subclinical</td>
<td>37.9±0.09b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>38.2±0.10c</td>
<td></td>
</tr>
<tr>
<td>USST Δ (°C)</td>
<td>Non-Mastitis and SCM</td>
<td>0.80±0.33</td>
<td>0.00</td>
</tr>
<tr>
<td>USST Δ (°C)</td>
<td>Non-Mastitis and CM</td>
<td>1.1±0.36</td>
<td>0.00</td>
</tr>
<tr>
<td>USST Δ (°C)</td>
<td>SCM and CM</td>
<td>0.30±0.48</td>
<td>0.01</td>
</tr>
<tr>
<td>EC (unit)</td>
<td>Non-mastitis</td>
<td>390±60</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Subclinical</td>
<td>370±80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>320±30</td>
<td></td>
</tr>
<tr>
<td>EC difference (unit)</td>
<td>Non-mastitis</td>
<td>20±10</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Subclinical</td>
<td>70±20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clinical</td>
<td>180±30</td>
<td></td>
</tr>
</tbody>
</table>

Non-mastitis (180 quarters), subclinical mastitis (11 quarters) and clinical mastitis (9 quarters). Lowercase letters in the same column indicate a significant difference (P<0.001). Δ = difference.
In mastitis, there is a substantial increase in leukocytes which may indicate the severity of infection and causative organism (Viguier et al. 2009). Subclinical mastitis is characterized by apparently normal milk but with an increase in SCC of up to 400,000 cells per ml (Sears and McCarthy 2003) which further reflects the severity of the infection. Diagnosis of subclinical mastitis can be made through many methods including direct measurement of somatic cell count or CMT as an indirect method at cow side level which may reflect the severity of udder infection (accessed online https://ahdc.vet.cornell.edu/programs/NYSCHAP).

Detection of clinical mastitis is mainly based on abnormal milk with SCC of >500,000 per ml and inflammatory changes, viz. swelling, pain and consistency, which also depends on the severity of infection and causative organism (Mc Dougall 1998). Besides, increased SCC and CMT, the score was associated with isolation of causative organism upon the microbiological culture of milk sample (Mc Dermott et al. 1982, Sargeant et al. 2001). Alterations in EC are of critical importance to assess the quality of milk. The EC of normal milk from healthy quarters varies between 4 to 5.5 mS/cm at 25°C (Hillerton and Wallton 1991) and differential values of EC between four quarters are compared to find out abnormal values related to mastitis affected quarters. In udder infection, milk of affected quarter shows elevated electrical conductivity due to increase in sodium (Na+) and chloride (Cl-) ions in milk (Sathiyabarathi et al. 2016, 2018). Commercially available handheld portable EC measuring system (Druminski mastitis detector, Digital mastitis detector, Mas-D-Tech) are widely employed for initial screening of freshly collected milk sample (Mc Dermott et al. 2001). The Pearson correlation coefficient between SCC and EC, and EC difference value between normal, subclinical and clinical mastitis affected quarters had average SCC of 8.7 lakhs and above 30 lakhs cells per ml of milk, respectively. The regression analysis revealed that an increase in 1°C of USST indirectly reflects an increase in EC difference value by 92.07 units. Other mastitis indicators, viz. CMT score (Table 2) and the EC value, EC difference value obtained between normal and affected quarters, and SCC was interrelated with USST.

The results of ANOVA showed a significant difference in USST and EC, and EC difference value between normal, subclinical and clinical mastitis affected quarters (Table 3). The Pearson correlation coefficient between USST and SCC was 0.574 (P<0.025), indicating a moderate correlation between the USST and SCC. For 1°C increase in USST indirectly reflects an increase in the EC difference by 92.07 units (P<0.001).

Infrared thermal imaging technique could detect the transient increase in udder skin surface temperature of quarters affected with naturally occurring subclinical and clinical mastitis conditions. IRT hand held camera is a portable, non-invasive device which can potentially be employed as a quick cow-side diagnostic technique for early detection of mastitis and consequently for effective treatment in field conditions by veterinarians or herdsmen. Future studies should include large no. of cows with different characteristics such as severity of mastitis, parity, stage of lactation and seasonal influence if any to determine precisely the sensitivity and specificity of IRT to detect mastitis in dairy cows maintained in varied agroecosystem.

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The authors thank the Head, Southern Regional Station and Director, Indian Council of Agriculture Research–National Dairy Research Institute for providing needful facilities and post-graduate research fellowship support from National Dairy Research Institute. The authors are grateful to the staff of milking parlour and Incharge, Livestock Research Centre of ICAR-NDRI, Karnal for their permission and assistance to carry out the study.

REFERENCES


Colak A, Polat B, Okumus Z, Kaya M, Yanmaz L E and Hayirli experimentially induced quarters than the control quarters. In cows with experimentally induced clinical mastitis condition resulted in transient rise in body and USST temperature (Hovinen et al. 2008). However, using IRT, they could not detect local inflammatory changes of the udder, appearing earlier than the rectal temperature increase. Metzner et al. (2014) demonstrated a good correlation between USST and rectal temperature in relation to mastitis-affected hind quarters.

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Polat et al. (2010) and Sathiyabarathi et al. (2018) studied the interrelationships between USST and other mastitis indicators, viz. SCC, EC and CMT score. The USST were positively correlated with SCC and the CMT score (r = 0.86; P<0.0001) (Polat et al. 2010). The USST was positively correlated with SCC and EC in HF crossbred (0.93 and 0.95) and Deoni (0.76 and 0.93) cows. As the EC and SCC values increased, USST linearly increased to 0.79 and 0.76 and 0.95 and 0.97 in HF crossbred and Deoni cows, respectively (Sathiyabarathi et al. 2016, 2018). This is similar to the present results of our study where a moderate correlation of USST with SCC was observed. In this study, the subclinical and clinical mastitis affected quarters had average SCC of 8.7 lakhs and above 30 lakhs cells per ml of milk, respectively. The regression analysis revealed that an increase in 1°C of USST indirectly reflects an increase in EC difference value by 92.07 units. Other mastitis indicators, viz. CMT score (Table 2) and the EC value, EC difference value obtained between normal and affected quarters, and SCC was interrelated with USST.

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