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Infrared thermal imaging of udder skin surface temperature variations to monitor udder health status in *Bos indicus* (Deoni) cows

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HIGHLIGHTS

- USST variations of *Bos indicus* cows were monitored by using infrared thermography.
- Milk samples were screened for mastitis using SCC, EC and California mastitis test.
- Udder skin surface temperature was positively correlated with somatic cell count and electrical conductivity.
- USST differences as monitored by IRT technique could possibly detect subclinical mastitis-affected quarters of Deoni cows at an early stage.

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ABSTRACT

The study was conducted to evaluate the potential of infrared thermography (IRT) technique for the early detection of subclinical mastitis in indigenous cows. A total of fifty-six quarters of lactating *Bos indicus* (Deoni) cows ($n = 14$) were monitored for ocular surface temperature (OST) and udder skin surface temperature (USST) prior to morning and evening milking using FLIR i5 (forward-looking infrared) camera continuously for 28 days. Milk samples were collected from each quarter and screened for mastitis using Somatic Cell Count (SCC), Electrical Conductivity (EC) and California Mastitis Test (CMT). Thermographic images were analyzed by using FLIR Quick Report 1.2. Data on OST and USST were compiled and analyzed statistically using SPSS 16.0. The mean (±SD) OST (i.e. body temperature) of an individual cow during the study period was 36.10 ± 0.08 °C. The mean (±SD) OST (36.10 ± 0.08 °C) and USST (36.19 ± 0.06 °C) of unaffected cows did not differ significantly, however, the mean (±SD) USST of the subclinical mastitis-affected quarter was 37.61 ± 0.29 °C which was 1.51 °C higher than the body temperature ($P < 0.001$). The increase in USST of subclinical mastitis quarters showed a positive linear relation with the SCC and EC with $R^2 > 0.95$. The maximum udder skin surface temperature of 37.90 was taken and used for the ROC analysis. The ROC curve analysis revealed a higher sensitivity and specificity for difference between OST and USST and their potential for detection of subclinical mastitis with a cut-off value >0.58 °C. Infrared thermal imaging technique could be used as a potential noninvasive, cow-side diagnostic method for screening and detection of subclinical mastitis.

1. Introduction

India ranks first in the world livestock population and milk production. According to 19th Livestock Census [1], total livestock population in India is 512.1 million numbers among this the milch animals comprises of 19.42 million crossbred and 48.12 million indigenous cattle, respectively. Mastitis is a complex multi etiological disease affecting dairy cattle and causes reduced 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 [2]. The incidence of mastitis in Karan Fries, Karan Swiss, Sahiwal, Tharparkar cows was reported to be 36.90, 38.46, 33.98 and 33.44 per cent, respectively. The incidence was highest in fourth parity and above and lowest in first parity [3]. Prevalence of subclinical mastitis among non-descript, Deoni, Jersey and HF cows were 40.8, 36.1, 47.8, and 54.7 per cent, respectively [4].

The global estimated economic loss per year due to mastitis amounts to USD 35 billion and Rs.6000 crore for Indian dairy industry in which subclinical mastitis is responsible for approximately 70–80 per cent (Rs.4365 crore) of economic losses. Delay

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in the detection of subclinical mastitis and lack of appropriate and accurate technique are contributing to the higher incidence of clinical mastitis [5]. Early detection of mastitis is most important to prevent the losses associated with decreased milk production, quality and for quick and effective treatment.

Abnormal thermal patterns in the body surface or skin surface 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 [6–9]. In most of the studies IRT was utilized to detect changes in USST caused by milking, environmental temperature, exercise [10], subclinical and clinical mastitis induced by infusion of *E. coli* lipopolysaccharide [11,7,9]. A perusal of literature revealed that there are scarce reports available to establish whether a natural infection with a common course of events and its associated changes with mastitis are possible for detection by IRT in an organized herd. The present study was undertaken to assess the body and USST differences and its potential for detection of subclinical mastitis.

2. Materials and methods

2.1. Study area and experimental animals

The study was conducted at Livestock Research Centre of the Southern Regional Station of Indian Council of Agricultural Research-National Dairy Research Institute, Bengaluru located at an altitude of 1200 m above sea level on 12°58′N latitude and 77°38′E longitudes. Fourteen lactating multiparous Deoni cows with an average body weight of 451 ± 19 kg and milk yield of 3.2 ± 0.2 kg were taken for the study. Their parity ranged between 1st and 4th and a total of 5, 2, 5, 2 cows were included from I, II, III and IV parities, respectively. The cows were apparently healthy and did not show any clinical condition/symptoms and abnormal behaviour during the period of experiment. Infrared thermal imaging of ocular and udder skin surface temperature (fifty-six udder quarters) of all the fourteen cows were recorded and monitored continuously for 28 days.

The cows were maintained under loose housing system and milked twice daily (morning at 5.00 AM and evening at 5.00 PM) by hand milking. The cows were fed twice a day with sufficient concentrate containing 89 per cent dry matter, 18–19 per cent crude protein (CP), 5.5–6 per cent, crude fibre and 72–75 per cent total digestible nitrogen and roughages (green and dry fodder) one time per day and had free access to water.

2.2. Measurement of temperature and relative humidity (RH)

Temperature and RH were recorded both in the morning and evening during milking time throughout the experimental period using dry and wet bulb thermometer installed at milking shed. The mean temperature and RH during the study period were 21.4 °C and 92.4 per cent in the morning and 26.6 °C and 62.6 per cent in the evening respectively. The ambient temperature and humidity data were fed into the software for analysis while processing and interpreting the thermal image.

2.3. Thermal imaging and analysis

Thermal images of ocular and udder surface were taken using a FLIR i5 camera (FLIR Systems, Inc. 27700 SW Parkway Ave. Wilsonville, OR 97070, USA) to monitor the changes in the USST of the individual udder quarter throughout the experimental period. Prior to capturing the IRT image, the camera was calibrated to ambient temperature, and the temperature measurement was adjusted to degree Celsius and distance to meters. The values of emissivity and reflected apparent temperature were set to 0.98 and 20.0 °C respectively. Before capturing thermal imaging, animal's udder quarters were wiped and given rest period of 15 min to stabilize normal udder surface temperature.

A lateral thermographic image at a distance of 1.0 m from the animal's head around the eye region including the ocular globe, inner canthus, the skin surrounding the ocular cavity and the lacrimal gland was recorded to observe body temperature of the cows. Eye IR-temperature is more consistent than temperature of any other anatomical region to compare the udder skin surface temperature. Thermographic images of udder were taken prior to morning and evening milking at a distance of 1.0 m from the udder. Thermographic images were captured from the lateral side for fore quarters and posterior or lateral side for hind quarters of the udder (Fig. 1). Total of 392 ocular and 1568 udder images in the morning and 392 ocular and 1568 udder images in the evening were taken throughout 28 days of the experimental period. The thermographic images were analyzed by FLIR Quick Report 1.2 software. As observed by the previous workers, due care was taken while recording and processing the IRT image to record mean and ΔT. In the present study multiple points were considered to obtain average temperature and the recordings were done in a shed where influence of external environment/factors was minimal/-controlled. For ocular surface temperature, the temperature of inner canthus of eye and the average temperature (average of three points) of the udder surface in a particular image was recorded and used in the analysis.

2.4. Milk sampling

After capturing thermal images of all the quarters, milk samples from each quarter were collected separately during morning milking in a clean polystyrene tube and subjected immediately to Somatic Cell Count (SCC) (the PortaSCC® somatic cell test, Whittendale Drive, Suite E Moorestown, NJ 08057 USA), Electrical Conductivity (EC) (Draminski Electronic Mastitis Detector, DRAMINSKI Ul. Owocowa 17 10-860 Olsztyn Poland) and California Mastitis Test (CMT) (IMMUCELL CORPORATION, 56 Evergreen Drive, Portland), using standard protocol or as per the manufacturer's guidelines. The milk sample from a quarter of an individual cow which showed SCC of more than 200,000 cells/ml, was considered as subclinical mastitis-affected quarters [12]. Besides, among four quarters examined, the quarters which showed EC value of more than 50 unit difference between quarters and CMT score of trace was considered as subclinical mastitis.

2.5. Statistical analysis

Normality assumption of various parameters studied was checked using normal probability plots and Kalmogrove Simirnov test and Shapiro Wilk test. The OST and USST did not follow normal distribution ($P < 0.05$), might be due to narrow range of variation. On the other hand, EC and SCC followed normal distribution ($P > 0.05$). However, the equivalent non-parametric tests which are free from assumptions (Wilcoxon Signed Rank Test for Paired *t*-test and Mann Whitney *U* Test for independent *t*-test) were used. As both the tests provided similar results and did not affect the conclusions the results of the parametric tests are alone presented.

Since the body and USST of quarters of the same animals are correlated, the Paired *t*-test was employed to test the significance of difference between the mean body and USST of non-mastitis cows. The USST of the quarters was repeatedly measured on different days. Therefore, in order to test the significance of the effect of quarters, days and timing of milking on USST, the repeated measure ANOVA model was employed. Independent *t*-test was used