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DIGITAL INFRARED THERMAL IMAGING OF BODY AND HOOF SKIN SURFACE TEMPERATURE PROFILE IN MURRAH BUFFALOES (*Bubalus bubalis*): A PRELIMINARY REPORT

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ABSTRACT

The aim of the present study was to generate thermographic profile of foot region *viz*. body temperature (BTºC), coronary band temperature (CBTºC), hoof skin surface temperature (HSSTºC) and mean temperature difference (ΔTºC) in Murrah buffaloes (*Bubalus bubalis*) and its relation with ambient temperature. A total of 60 hooves of lactating Murrah buffaloes (n = 15) maintained in a farm, were monitored once a day continuously for 15 days before evening milking using forward-looking infrared (FLIR) i5 camera. Total of 1125 thermograms were analyzed using FLIR Quick Report 1.2 image analysis software. The mean ± SD (°C) of BT, CBT, HSST and ΔT of all animals throughout the experimental period were 37.39±0.05, 34.93±0.13, 34.16±0.13 and 0.76±0.26 respectively. The CBT was around 0.5 to 1°C higher than HSST. Statistical analysis revealed that ambient temperature is highly and positively correlated with CBT (ºC) (r = 0.95) and HSST (ºC) (r = 0.99), indicating greater influence of ambient temperature on surface temperature of foot region. Therefore, CBT (ºC) and HSST (ºC) have a tendency to follow variations in ambient temperature (R^2 = 0.91 and 0.98 respectively). However, significant difference was observed between BT vs. CBT, BT vs. HSST and CBT vs. HSST (ºC). The mean ± SD (°C) of ΔT of all animals, throughout the observation period was 0.96±0.41. A significant difference was observed in ΔT between animals (P<0.001). However, significant difference was not observed in ΔT between

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days and among four hooves within animal. It is concluded that, to the best of our knowledge this is the first report on baseline thermographic information on BT, CBT, HSST and ΔT differentials for buffalo and its clinical significance in monitoring hoof health and management.

Keywords: *Bubalus bubalis*, buffaloes, Infrared thermography, Murrah buffaloes, ambient temperature, hoof, temperature, hoof health

INTRODUCTION

India is the largest producer and consumer of milk in the world and also holds the world's highest bovine population. Water buffalo accounts for around 53 % of the total output of milk and annually the production is growing around 4.4% annually (Landes *et al*., 2017). India is very famous for its riverine buffaloes and Murrah breed comes under one of the subgroup Murrah under riverine buffaloes.

Hoof health status is important in animal welfare and economic point of view (Green *et al*., 2002; Whay and Main, 2003). As the pain and severity of hoof lesions were increased, milk production was decreased. After appearance of hoof disorders the average loss in milk was 3.8 liters/day/animal and after treatment and hoof trimming, improvement of average milk production was 0.6 liters/day/animal. In the whole lactation loss of milk up to 31.66% was seen due to disorders of hoof (Bagate *et al*., 2012). Bovine hoof health is given less importance under field condition by the farmers due to difficulties associated with early diagnosis, treatment, examination of lame animals and ignorance (Hess, 1904). The major cause of lameness is due to hoof problems like white line abscess, sole ulcer and sole hemorrhage (Smilie *et al*., 1996). Cattle and buffalo are the two different species having differences in their morphology and anatomy, they perform distinctly dissimilar behaviors in the bodily function of heat release (Koga *et al*., 2004). Buffaloes are well known for their hardiness, and they cope up well in hostile situations (Damasceno *et al*., 2010). Mohsina *et al*., (2014) studied incidence of lameness in different species and found 5.47% in buffaloes compared to 7% in cattle. Bagate *et al*. (2012) surveyed the incidence of hoof disorders in dairy animals at Gujarat, India and found that, among total hoof cases found 31.79% were buffaloes and 68.21% were cattle. About 14.02% of overall average incidence of hoof diseases was seen, among which 14.88% were cattle and 12.16% were buffaloes. Under different management systems it was reported that, the incidences of hoof disorders were variable in overall population (4.65 to 16.59%) and amongst buffaloes (2.75 to 12.88%) and cattle (8.57 to 21.94%) (Joshi, 2006). Highest breed incidence among buffaloes was noticed in Mehsana (22.26%), followed by Surti (18.75%), Murrah (15.04%), non-descript (15.21%) and Jafrabadi (10.36%).

Infrared thermography (IRT) is used in veterinary medicine as a non-invasive tool in the early diagnosis of diseases and studying thermal physiology. IRT is generally employed in mass screening of animals for early detection of febrile diseases (Lutz *et al*., 2011), assessing stress levels (Stewart *et al*. 2005), diagnosing bovine viral diarrhoea in calves (Schaefer *et al*., 2004), early detection of mastitis in cows (Sathiyabarathi *et al*. 2016a; Sathiyabarathi *et al*. 2016b; Sathiyabarathi *et al*., 2016c; Sathiyabarathi *et al*., 2018; Polat *et al*., 2010; Poikalainen *et al*., 2012), diagnosing bovine respiratory disease in beef cattle (Schaefer *et al*., 2007). In addition, IRT is also employed in measuring methane production from ruminants (Montanholi *et al*., 2008) and for monitoring oestrus in buffaloes (de Ruediger *et al*., 2018), oestrous climax determination (Hellebrand *et al*., 2003), assessing fertility index in bulls by measuring scrotal surface temperature and profile (Lunstra and Coulter, 1997; Ahirwar *et al*., 2017; Ahirwar *et al*., 2018), to study the influence of machine milking on teat and udder surface temperature in cows (Sathiyabarathi *et al*., 2017), finding autonomic reactions to painful procedures performed on cattle (Stewart *et al*., 2010), and for assessing the transport stress in cattle (Schaefer *et al*., 1998), etc.

To the best of our knowledge, no studies have been published till date on the use of IRT to generate thermographic profile for assessing hoof health status in buffaloes. The present study reports body temperature and foot surface temperature and their association with ambient temperature. Outcome of this study provides baseline thermographic profile on body temperature and foot surface temperature. In future studies on pathological buffalo hooves, this database could be helpful in early detection and comparison of diseased hoof with healthy hooves. The aim of the study was to generate temperature pattern (thermographic profile) of foot region and its relation with ambient temperature for experimental and clinical interpretation in Murrah buffaloes (*Bubalus bubalis*).

MATERIALS AND METHODS

Ethical approval

The experiment was carried out as per the guidelines of Institutional Animal Ethics Committee (IAEC) and approved by IAEC (CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2017/No. 17).

Study area

This study was conducted on a buffalo farm located in India (16.35ºN and 75.28ºE; 549 m above mean sea level). Mudhol is located at and classified under Northern Dry Agro-climatic zone of Karnataka. The climate is warm and dry throughout the year and rainfall is also scarce. Annually, the average rainfall in this region is around 318 mm. September and December months account for about 52% of the total annual rainfall. The mean temperature ranges from 32 to 36ºC and the highest temperature is observed during April to June (Figure 11). The ambient temperature and relative humidity (Figure 12) were recorded prior to capturing thermal images.

Experimental animals and their management

Fifteen apparently healthy multiparous lactating Murrah buffaloes (*Bubalis bubalis*), with an average body weight ranging from 450 to 500 kg were selected for the studies. The wet average (total milk yield/total number of lactating buffaloes) of the farm was 8.5 kg per day. The overall health status was confirmed by observing general behavior and measuring the rectal temperature of buffaloes using digital rectal thermometer throughout the study. Buffaloes were milked in morning at 6 am and evening at 5 pm by hand milking. The buffaloes were fed twice a day with sufficient concentrate feed and roughages as per the requirement, with ad libitum access to drinking water. Animals were reared in intensive system and cleaning of concrete floor along with manual manure disposal was done twice per day (8 am and 4:30 pm). The roof of the shed was 5 m high, with 1.22 m high sidewalls along the longitudinal axis. All the animals were maintained under intensive system of management.

Thermal imaging and analysis of thermograms

The methodology of infrared thermal imaging was established by keeping in mind, the routine practices of management carried out in the farm, with the aim of causing minimal stress for animals, which may increase their body temperature. All infrared images were captured inside the shed. In total, 225 eyes and 900 hoof thermograms were taken, before the evening milking using forward-looking infrared (FLIR) i5 camera (FLIR Systems, Inc. 27700 SW Parkway Ave. Wilsonville, OR 97070, USA) for fifteen consecutive days. The infrared camera with accuracy = ±2^{o}C (or) 2%; sensitivity = 0.1^{o}C; temperature range = -20 to 250^{o}C; center spot; thermal image quality: 100x100 pixels; field of view: 21^{o} (horizontal) x 21^{o} (vertical) was used. Before capturing the image, the camera was calibrated to ambient temperature, and the temperature measurement was adjusted to degree celsius and distance to meters. The value of emissivity and reflected apparent temperature were kept constants for all the thermograms as 0.98 and 20°C, respectively. Thermograms of eye were captured at a distance of 1.0 to 1.5 m from the lateral side of animal's head as a measure of core body temperature (BT). Thermographic images of hooves were taken from lateral side before milking at a distance of 1.0 m from the hoof. The thermographic images were analyzed by FLIR Quick Report 1.2 software. The temperature of the inner canthus of eye, coronary band temperature (CBT) and hoof skin surface temperature (HSST) in a particular image was recorded and used in the analysis (Figure 7 and 8). In each image, average temperature of three points above coronary band region was taken as HSST (Figure 6). Ambient temperature and humidity at the time of thermal imaging were recorded daily throughout the experimental period, which were entered for processing of every image. The thermographic images of animals with dirty and wet feet were not captured, which might alter the temperature readings.

Statistical analysis

A one-way analysis of variance was carried out to compare the variation of mean temperature difference (ΔT) (i.e. CBT-HSST) between animals and between days. Repeated measures analysis of variance was carried out to assess the variation of CBT, HSST and ΔT among all four healthy hooves, throughout the experimental period. For comparing BT, CBT and HSST, paired t-test was performed. Boxplots were created to compare the distribution pattern of CBT and HSST among four healthy hooves. Temperature data from hoof sites were compared with ambient temperature and CBT was compared with HSST using Pearson's correlation and linear regression. For assessing the influence of ambient temperature on CBT and HSST linear regression was performed. Data on BT, CBT, HSST and ΔT were compiled and analyzed statistically using SPSS 16.0 (IBM Corporation, Armonk, New York, USA), Rstudio (Version 1.1.453 – 2009 to 2018 Rstudio, inc.) and Microsoft Excel 2013.

RESULTS AND DISCUSSIONS

During the study period, the ambient temperature and relative humidity were ranging from 21 to 31°C and 25 to 30% respectively. The mean ± SD (°C) of BT, CBT, HSST and ΔT of all animals throughout the study period were 37.39±0.05, 34.93±0.13, 34.16±0.13 and 0.76±0.26

respectively (Table 2).

Influence of ambient temperature on coronary band and hoof skin surface temperature

Environmental factors like ambient temperature have its impact on the temperature readings of thermograms. As the ambient temperature increased, the claw temperature was increased from 12 to 20°C (Turner, 2001). Similarly, in a study by Alsaaod *et al*. (2014) found that, overall means (± SEM) of HSST and CBT in healthy claws were 27.3±2.9°C and 30.3±3.2°C; 28.6±2.1°C and 32.1±1.7°C; and 29.9±1.8°C and 33.8±1.3°C, at ambient temperature of 12.2, 15.7 and 20.3°C, respectively. In distal limbs of equines, temperature changed proportionally with ambient temperature, along with substantial variation between horses (Palmer, 1983). Skin temperature of the distal limb was significantly not influenced by weather, humidity, or atmospheric pressure (Kameya and Yamaoka, 1968). However, in the present study, the overall means of CBT (°C) in healthy hooves were 34.6, 34.88, 35.49 and 35.53 at ambient temperatures (°C) of 21, 25, 28 and 31°C respectively. Similarly, the overall means of HSST (°C) in healthy hooves were 33.86, 34.08 and 34.54 for ambient temperatures 21, 25, 28 and 31°C, respectively (Figure 3).

In the present study, linear regression and Pearson's correlation analysis were employed to assess the relationship between ambient temperature with CBT and HSST. The linear correlation co-efficient for CBT and HSST were 0.95 and 0.99 respectively, which indicate both CBT and HSST were highly and positively correlated with ambient temperature. The co-efficient of determination for CBT and HSST were 0.91 and 0.98 respectively, which indicates data is very closely related to regression line and majority of total variation in CBT and HSST is explained by ambient temperature (Figure 3). Therefore, CBT and HSST have a tendency to follow variations in ambient temperature. The increase in hoof surface temperature could be accredited to vasodilatation, where there is flow of warm blood from arteries to skin and vice versa. Mogg and Pollitt (1992) observed that, changes in surface temperature over a short period may also be due to pyrexia, but those variations are likely to be symmetrical. The highest temperature changes were seen in hoof region, where density of arteriovenous anastomoses is greater, signifying that arteriovenous anastomoses could influence the surface temperature of hoof significantly.

Variation of coronary band and hoof skin surface temperature between hooves

In the present study, analysis was carried out to find out difference of CBT and HSST between four healthy hooves within animal. Both CBT and HSST did not show significant difference between four hooves (Figure 4 and 5). Therefore, no within animal variation was seen between the four healthy hooves ($P>0.05$). The mean ± SE (°C) of ΔT of left-fore, left-hind, right-hind and right-fore hooves, throughout the observation period were 0.89±0.16, 1.05±0.29, 0.99±0.16 and 0.87±0.22 respectively. No significant difference was observed in ΔT among four hooves ($P>0.05$) throughout the experimental period. While recording the HSST, one must consider region of alopecia on lateral aspect of foot due to constant and regular pressure in that region. However, significant difference was observed between body parts *viz*. BT vs. CBT, BT vs. HSST and CBT vs. HSST (Figure 1 and Table 1).

Variation of ΔT (CBT-HSST) between animals and days

The mean ± SD (°C) of ΔT of all animals, throughout the observation period was 0.96±0.41. A significant difference was observed in ΔT between animals (P<0.001) (Figure 9). Alsaaod and Buscher (2012) carried out an experiment on Holstein cows and studied the effect of stage of lactation for all healthy hooves in cows ≤200 days in milk than in cows >200 days in milk and found that the CBT and HSST were significantly higher in cows ≤200 days in milk (early/mid lactation) when compared to cows >200 days in milk (late lactation). They found higher sensitivity to heat stress in lactating animals compared to non-lactating cows (Purwanto *et al*., 1990). Further, as there is positive relationship between heat production and milk yield, cows yielding more milk are more susceptible to heat stress than low yielders (Spiers *et al*., 2004).

In our study, the thermograms showed comparatively higher temperature in the region of alopecia (common in old, aged animals and lateral aspect of feet), than in the region with hairs. This could be the reason for getting a significant difference of ΔT between animals. Therefore, it is better to measure the HSST other than lateral aspect of foot in future studies for assessing hoof health in buffaloes. However, ΔT did not show significant difference between days (P>0.05) (Figure 10).

Relation between coronary band and hoof skin surface temperature

The CBT was 0.5 to 1°C higher than HSST. The lesser temperature difference between coronary band and skin above hoof in buffaloes could be due to the reason that, the coronary band area is partially covered by long hairs. Our results are similar to the report of Turner (2001); in horses the warmest area above the hoof was seen in coronary band region and the skin temperature above hoof was 1 to 2°C lesser than CBT. Similarly in a study by Alsaaod *et al*. (2014) reported that the HSST was 2 to 3°C lesser than that of CBT. Pearson’s correlation and linear regression between CBT and HSST in healthy hooves revealed that, HSST was highly and positively correlated with CBT (r = 0.94), regardless of ambient temperature. It indicates that, variation (positive or negative) between CBT and HSST within a foot occur simultaneously (Figure 2).

CONCLUSION

Our study for the first time provides information related to establishment of baseline thermographic information on BT, CBT, HSST and ΔT differentials for buffaloes. It is conclude that, CBT and HSST were influenced by ambient temperature. There was no within animal variation of CBT and HSST among four hooves. However, significant difference was observed between different body parts of buffalo *viz*. BT, CBT and HSST. Similarly, between buffaloes significant difference in ΔT was seen. However, no significant difference in ΔT was observed between days. Regardless of the ambient temperature, CBT is strongly and positively correlated with HSST in healthy buffalo hoof. Further, thermographic profile would be useful in developing thermographic signature for individual animal and developing algorithm based predictive model for early detection of affected hooves in precision dairying for automatic monitoring of hoof health in large herds. Further research is needed to study the influence of breeds, age, milk yield, days-in-milk, management condition and various pathological

Table 1 Comparison of body, coronary band and hoof skin surface temperature of healthy hooves in Murrah buffaloes using paired t-test (n = 15 buffaloes; 900 hooves).

Temperature (°C)	**Mean±SD (°C)**	**ΔT±SD (°C)**		
		BT vs. CBT	**BT vs. HSST**	**CBT vs. HSST**
BT	37.40±0.21	2.47±0.62	3.24±0.62	0.76±0.26
CBT	34.93±0.51			
HSST	34.15±0.52			
t-value		15.47	20.34	11.29
Level of significance		P<0.0001	P<0.0001	P<0.0001

BT: Body temperature (eye temperature); CBT: Coronary Band Temperature;
HSST: Hoof Skin surface Temperature; vs.: Versus; ΔT: Mean temperature difference.

Table 2. Descriptive statistics of body, coronary band and hoof skin surface temperature (°C) of hooves in Murrah buffaloes.

Descriptive measures	**Murrah buffaloes**		
	BT	**CBT**	**HSST**
Mean	37.40	34.93	34.16
Median	37.32	34.88	34.05
SD	0.21	0.42	0.30
Range	0.69	1.15	0.91
Minimum	37.16	34.36	33.69
Maximum	37.85	35.51	34.60

BT: Body temperature (eye temperature); CBT: Coronary Band Temperature;
HSST: Hoof Skin surface Temperature.

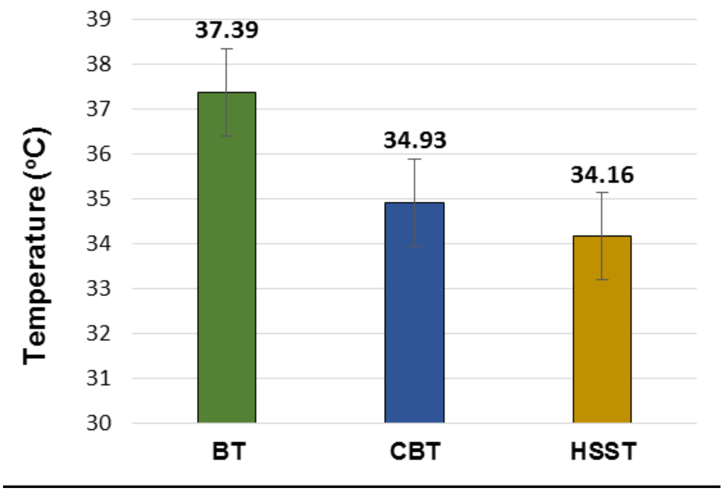

Figure 1. Comparison of body temperature (BT), coronary band temperature (CBT) and hoof skin surface temperature (HSST). Significant difference was observed between BT vs. CBT, BT vs. HSST and CBT vs. HSST (P<0.0001).

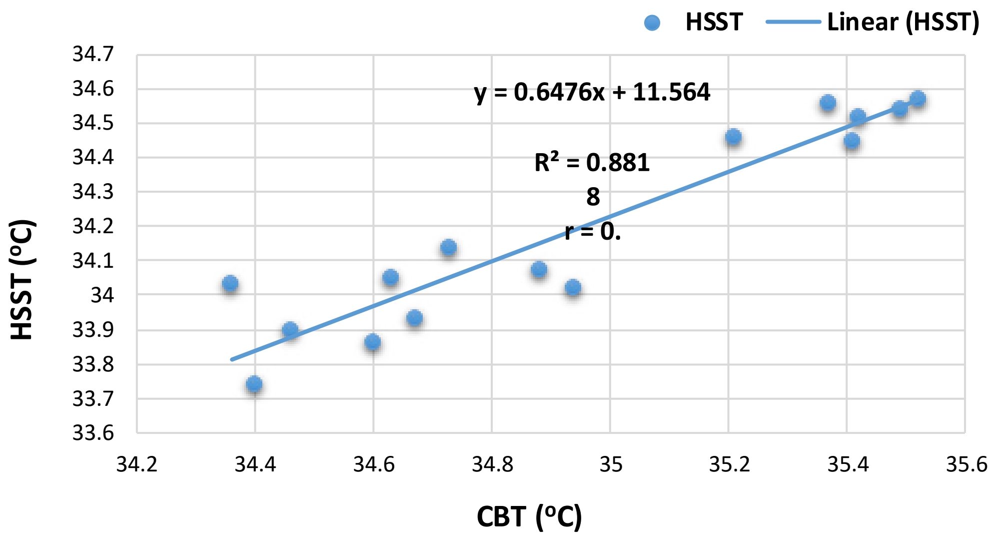

Figure 2. Pearson's correlation and linear regression between coronary band temperature (CBT) and hoof skin surface temperature (HSST) in healthy hooves. Hoof skin surface temperature was positively correlated with coronary band temperature, regardless of ambient temperature.

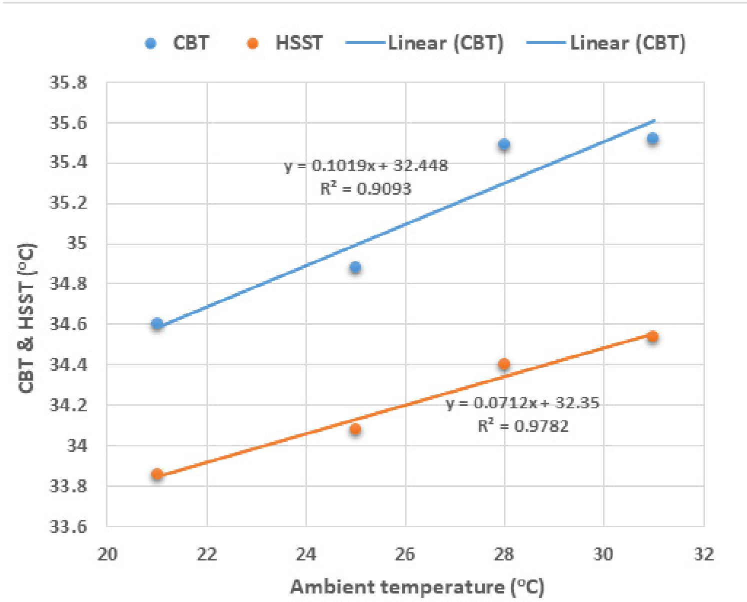

Figure 3. Pearson's correlation and linear regression relating ambient temperature with CBT and HSST in healthy hooves of Murrah buffaloes. CBT: Coronary band temperature; HSST: Hoof skin surface temperature.

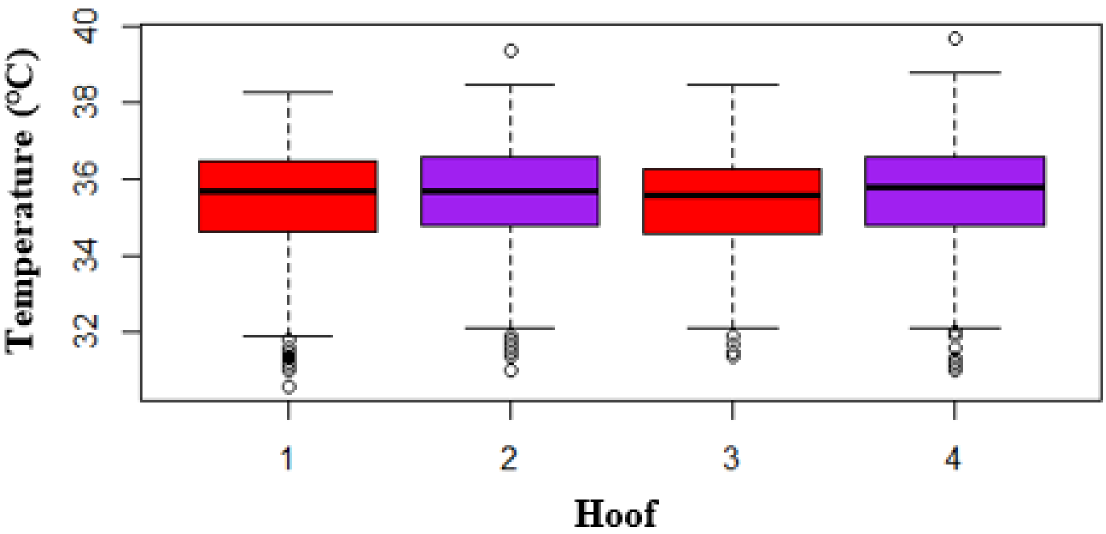

Figure 4. Box plot for comparing the distribution pattern of coronary band temperature (CBT) between four healthy hooves.

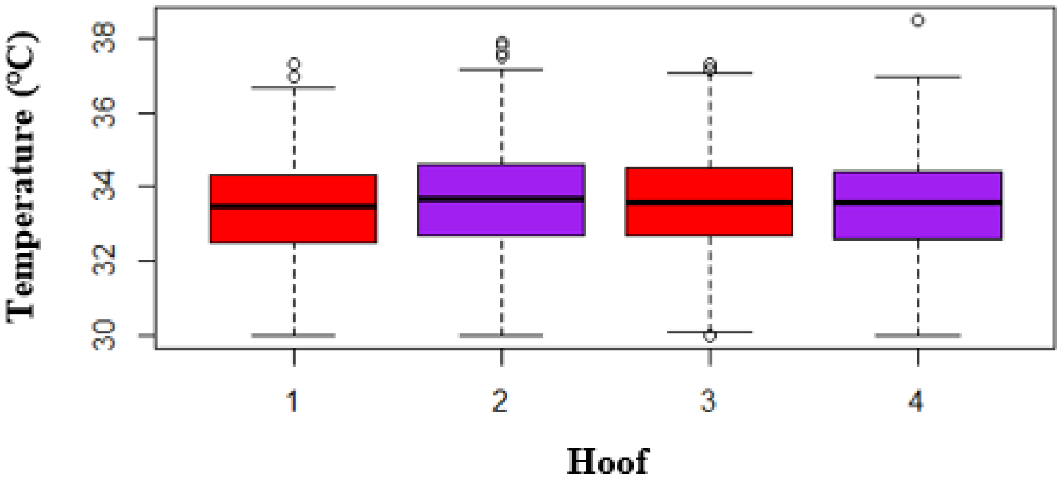

Figure 5. Box plot for comparing the distribution pattern of hoof skin surface temperature (HSST) between four healthy hooves.

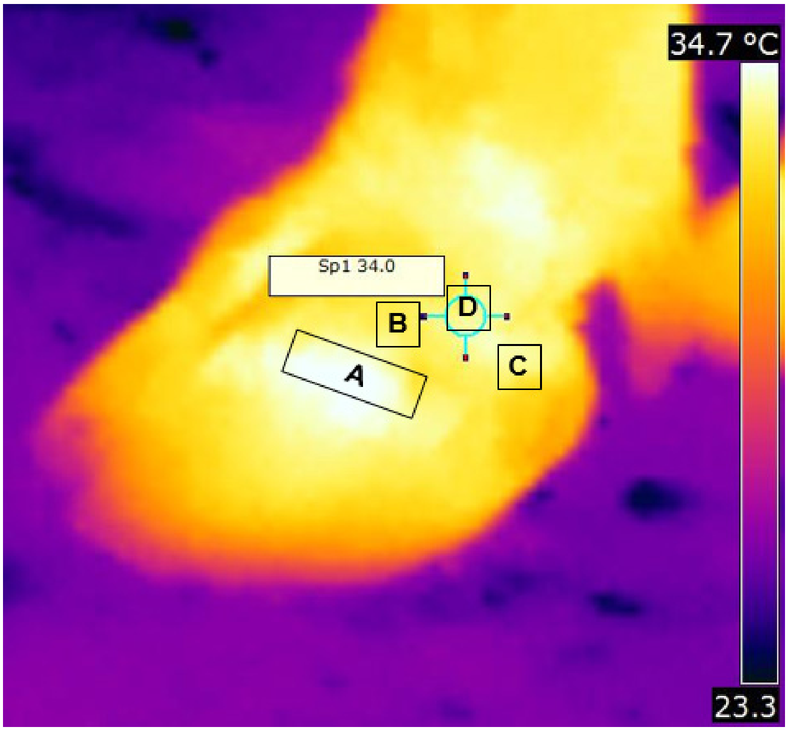

Figure 6. Point of interest for measuring coronary band temperature (A) and hoof skin surface temperature (mean of B, C and D).

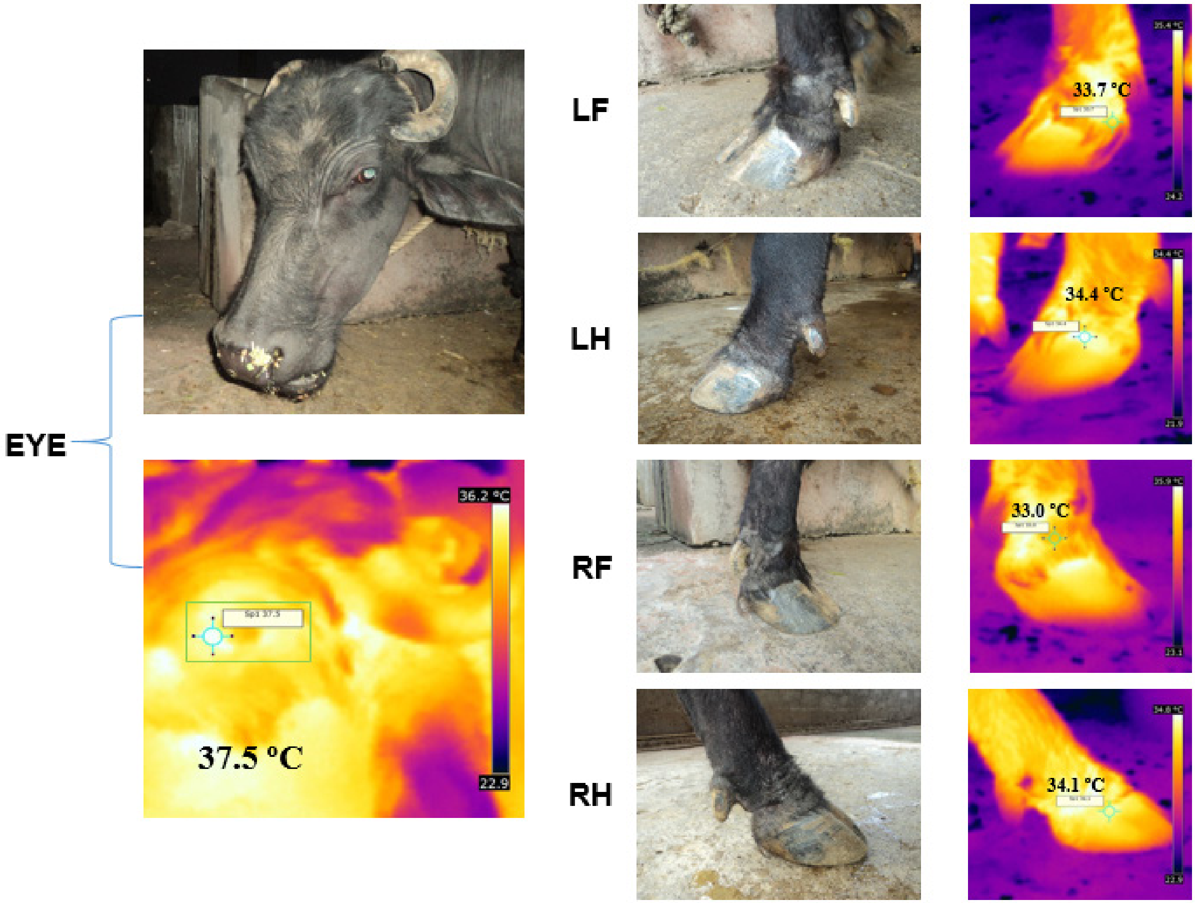

Figure 7. Visual and infrared images of eye and four healthy hooves of a Murrah buffalo. LF: Left fore; LH: Left hind; RF: Right fore and RH: Right hind foot.

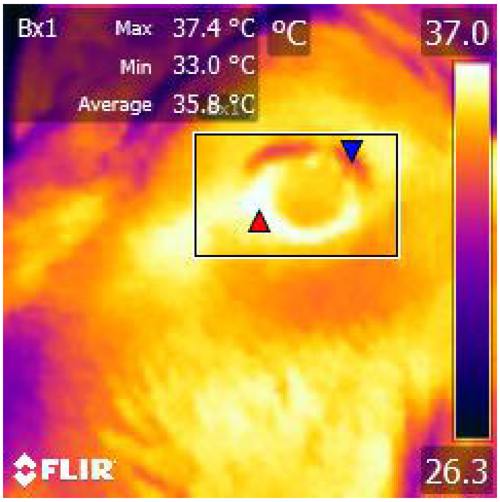

Figure 8. Point of interest for measuring eye temperature (inner canthus).

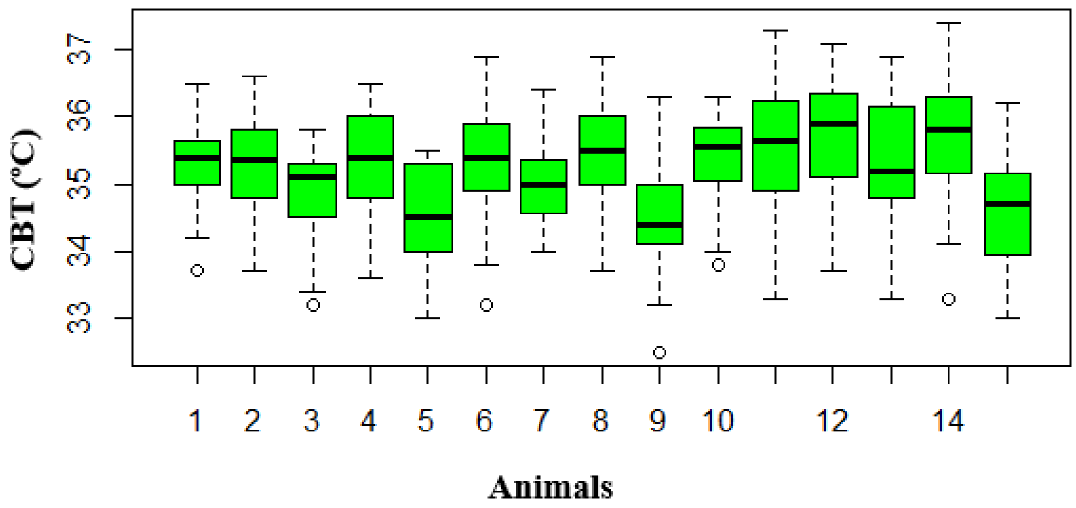

Figure 9. Box plot indicating variation of coronary band temperature (CBT) between different animals during the experimental period.

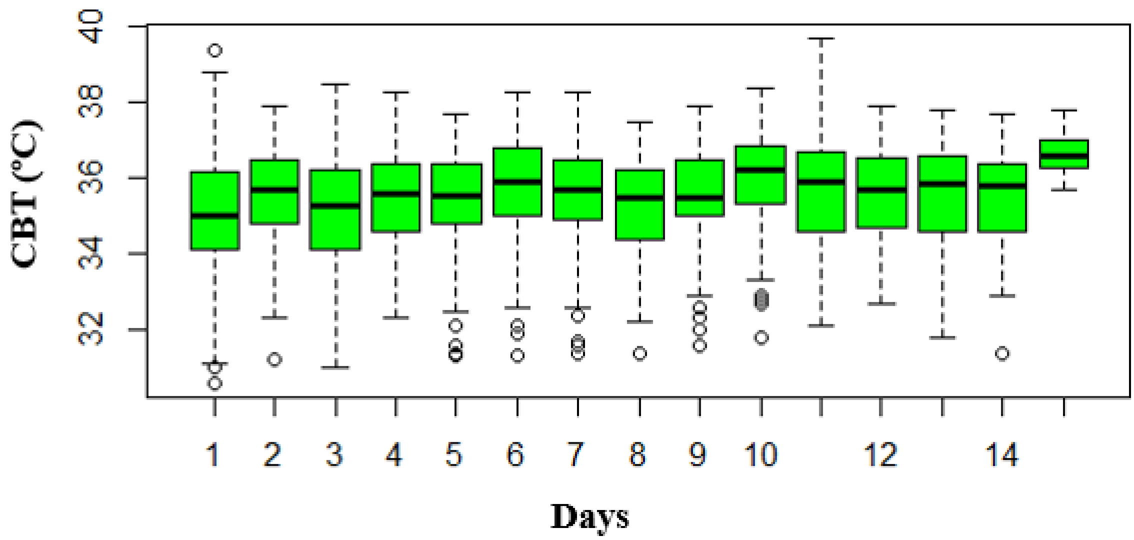

Figure 10. Box plot indicating variation of coronary band temperature (CBT) between different days during the experimental period.

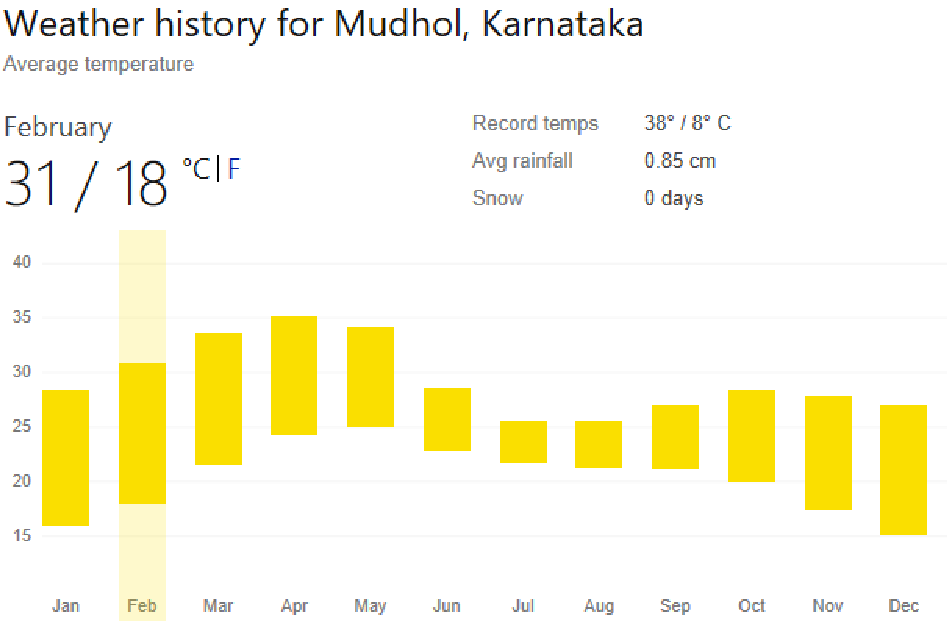

Figure 11. Weather history of present study area throughout the year (https://www.bing.com/Accessed online on 29th, June, 2018).

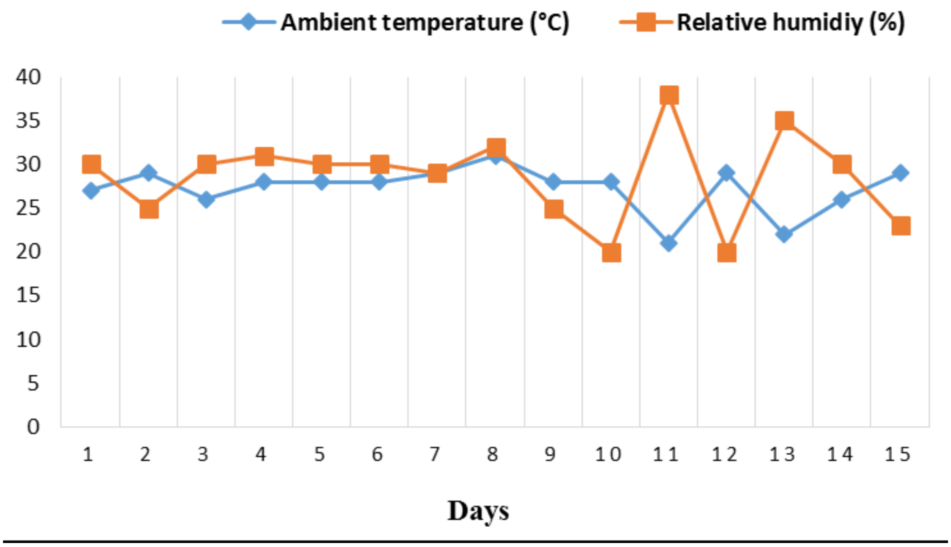

Figure 12. Ambient temperature (°C) and relative humidity (%) during the study period.

conditions of hooves using IRT in buffaloes.

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