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RESEARCH ARTICLE

Infrared thermography in early detection of subclinical mastitis: regional temperature thresholds and environmental effects

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KEYWORDS

Infrared thermography, subclinical mastitis, temperature-humidity index, threshold temperature, udder skin surface temperature

HIGHLIGHTS

- Early detection of subclinical mastitis (SM) is crucial for minimizing economic losses and improving dairy cow welfare.
- Threshold temperatures for SM detection were identified using thermal imaging across 658 small and medium scale dairy farms in four Sri Lankan regions.
- Significantly higher temperature was observed in mastitis-positive udder quarters than unaffected quarters and a significant relationship was observed between temperature difference and prevalence of SM.
- Threshold temperature differences (ΔT °C) ranged from 1.86 to 2.49 across the four regions and were affected by environmental temperature and temperature-humidity Index.

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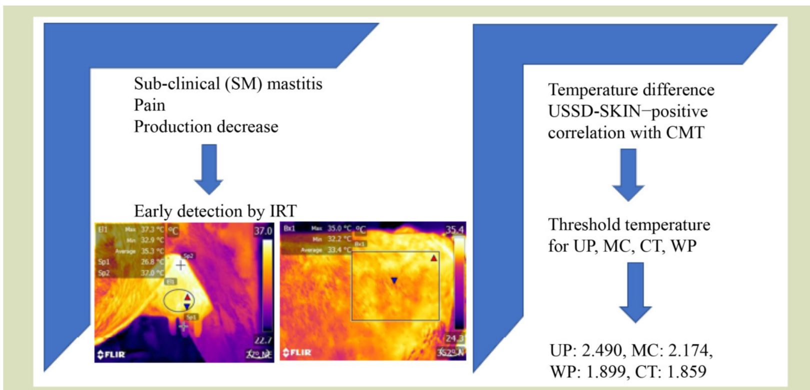
GRAPHICAL ABSTRACT



ABSTRACT

Early detection of subclinical mastitis (SM) in dairy cows is important to minimize economic losses and improve dairy cow comfort. This study focused on the identification of threshold temperature of California mastitis test for being positive for SM and determined if it was affected by temperature-humidity index (THI). Six hundred and fifty-eight small and medium scale dairy farms were selected in four regions of Sri Lanka (UP, Up Country; MC, Mid Country; CT, Coconut Triangle; and WP, Western Province) and 4274 udder quarters were captured using thermal camera. SM positive udder quarters had a higher temperature (as mean ± standard deviation; UP, 36.4 ± 1.79 °C; MC, 36.3 ± 1.79 °C; WP, 37.7 ± 1.84 °C; and CT, 38.3 ± 1.01 °C) compared to SM negative samples. The prevalence of SM was statistically significant for udder skin surface temperature ($P < 0.05$) and the difference between it and flank skin temperature was statistically significant with prevalence of SM ($P < 0.05$). The threshold temperature differences were (in ΔT °C): UP 2.49; MC, 2.17; WP, 1.90; and CT, 1.86, and these were statistically different (B = −0.080, R = 0.957, R^2 = 0.916, $P < 0.05$) from environmental temperature and tended to be significantly related to the THI. Thus, the threshold value of temperature difference can be applied for early detection of SM taking into consideration that threshold temperature varies with the environmental temperature and THI.

1 Introduction

Mastitis is an economically important udder disease[1], and globally estimated economic loss due to mastitis is nearly 35 billion USD, annually[2]. Inflammation of the mammary gland, resulting in mastitis, is predominantly caused by a bacterial infection[3]. Both clinical and subclinical mastitis (SM) caused a decrease in milk production, an increase in treatment cost, increased labor, milk withheld following treatment and premature culling[4]. Also, mastitis reduces well-being of dairy cows due to discomfort and pain[5], and affects the sustainability of farms[6]. Prevalence of mastitis is affected with several factors such as genetic and cow-based factors, udder and teat morphology[7], cow cleanliness[8], improper housing management practices[9], type of bedding[10], stall surface[11] and other inappropriate stock management practices[12]. When clinical mastitis infections result in udder swelling, it is generally visible to farmers. In contrast, SM often has no obvious visible symptoms and can only be identified through specialized diagnostic examinations[13].

Effective treatment of the disease can minimize the effect of SM and therefore, early mastitis diagnosis is important[14]. Numerous biomarkers and tests are used to detect SM[15], and these biomarkers include somatic cell count [16], milk enzymes such as alkaline phosphatase, aspartate aminotransaminase, *N*-acetyl-*β*-d-glucosaminidase, l-lactate dehydrogenase and other milk components. The common tests are the California mastitis test (CMT), electrical conductivity, Wisconsin Mastitis Test and Whiteside Test[15]. According to earlier research, CMT provides a higher overall detection rate[17] than the other tests and a reliable diagnostic method for use as a regular screening test in field conditions even by less trained dairymen[18]. As a most accurate, easy and fast method, many scientists use infrared thermography (IRT) to detect mastitis of dairy cattle[15,19], where thermal camera absorbs infrared radiation and generates an image based on the amount of heat generated[15]. Hence, according to earlier studies, CMT had identified as a reliable, easy diagnostic method for the detection of SMI and IRT applied for the identification of temperature variation. However, no research has been reported for Sri Lanka regarding the threshold value of the CMT being positive for mastitis. Thus, identification of threshold temperature of the CMT using the IRT is the aim of this research and this will fill the research gap regarding CMT and IRT. The threshold value is detected using the temperature difference of udder skin surface temperature (USST) and flank skin temperature (SKIN). Further attention was given to the effect of environmental temperature and temperature-humidity index (THI) on threshold values.

2 Materials and methods

2.1 Selected cows, housing and general farm management

The study was conducted in four regions in Sri Lanka: Up Country (UP), Mid Country (MC), Coconut Triangle (CT) and Western Province (WP). Out of 25 districts in Sri Lanka, UP, includes the districts of Nuwara Eliya, Matale, and Badulla and MC consists with Kandy and Kegalle districts. CT covers Kurunegala, Puttalam, and Anuradhapura districts and WP includes the districts of Colombo, Gampaha, Kalutara, and Ratnapura.

Using an open-ended questionnaire[20], the operators of 658 small dairy farms were interviewed (160 in UP, 184 in MC, 157 in CT and 157 in WP) to gather information on their management practices. According to earlier studies parity, herd size, breed of the animal and lactation stage had affected on the prevalence of mastitis[21]. Thus, 1074 lactating cows (136 in UP, 245 in MC, 306 in CT and 385 in WP) housed in tie-stalls, were selected based on their breed (Friesian-Jersey cross), parity (3.45 ± 0.54, mean ± SD), pregnancy (3.67 ± 1.52 months), lameness score (zero) and body condition score (BCS ⩾ 3.0). During the study period, fresh feed was provided twice per day with ad libitum access to water. Cows were milked two times at around 6–7 a.m. and 3–6 p.m., and sheds were cleaned manually once per day at around 8–10 a.m.

2.2 Infrared thermography, California mastitis test and digital images processing

Thermal images were taken when the cows were in their cattle sheds during the morning. Before capturing infrared images, all animals were encouraged to stand, and attached fecal or bedding residues were removed through light brushing or with minimum warm water. After this cleaning step, the udder was dried with paper towel and given a minimum of 10 min of rest before any imaging commenced[19]. In this way, 4274 (544 in UP, 978 in MC, 1224 in CT and 1528 in WP) images of udder quarters were captured before milking with a thermal camera (IR FlexCam Pro, Infrared solutions, Fluke Company, Everett, WA, USA) held about 50 cm from the udder. The thermograph resolution was calibrated to room temperature for each measurement. The camera used operates at 0–35 °C and can detect temperatures from −20 to 400 °C, in the 8- to 14-μm spectral band. The thermal resolution of the camera was 0.09 °C, and it was calibrated from 0 to 100 °C. The camera used microbolometer detectors and had 60 × 120 focal plane arrays. The camera had an internal recalibration feature, which automatically calibrated the detector to give correct readings for the ambient temperature. The emissivity value was set to 0.98, which was the value used for measuring the temperature of human skin.

In infrared thermal images, the warmest areas appear white or red, whereas the coolest regions appear blue or black[22] as seen in Fig. 1(a). USST was determined using the FLIR tool + program (Teledyne FLIR, Wilsonville, Oregon, USA) by drawing a circle (circle of 40 × 40 pixels) just above the teat (Fig. 1(a)). Using this method, maximum, minimum and average temperatures were measured for the four udder quarters. An earlier study had indicated that infrared thermography (IRT) detected changes in temperature on the right and left flanks, which were found to have strong correlation with heat production[23]. Therefore, for the present study, it was decided to apply that concept by measuring the skin temperature via the right-side flank of the cow and read the values by drawing a square (Fig. 1(b)) using the FLIR tool + program.

After capturing images, all four udder quarters of each cow had scored for the CMT (scores 0–3)[24] and the score udder quarters were regrouped into SM-positive (scores 2 and 3) and SM-negative (scores 0 and 1). Those two groups were compared statistically with the difference between USST and SKIN temperature to detect the prevalence of mastitis

2.3 Environmental temperature, relative humidity and temperature-humidity index

Variation of temperature and relative humidity (RH) inside the cattle shed was recorded throughout the study period using HOBO temperature loggers (HOBO U23 Pro v2 temperature and RH data loggers; Onset Computer Corporation, Bourne, MA, USA) in 5-min intervals of time. Loggers had an effective range of −40 to 70 °C with an accuracy of ± 0.21 °C from 0 to 50 °C of temperature and RH of ± 2.5% from 10% to 90%. The temperature logger was mounted under the roof of the shed about 10–15 cm above the height of the cows[20] and data were downloaded to computer database. Prior to the analysis, air temperature was recorded and the THI was calculated using the following equation[25] where T is the air temperature and relative humidity (RH).

$$THI = (1.8T + 32) - (0.55 - 0.0055 \times RH)(1.8T - 26) \quad (1)$$

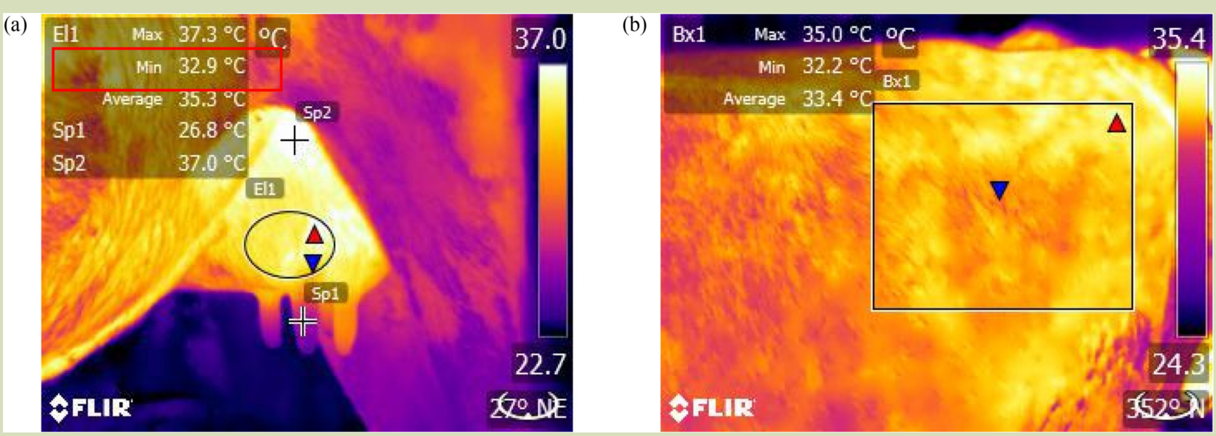


Fig. 1 Calculation of average udder skin surface temperature (a) and flank skin temperature (b) using FLIR Tool(+) software.

3 Statistical analysis

To account for the hierarchical data structure, where udder quarters are nested within individual cows, statistical analyses were revised using mixed-effects models IBM SPSS 23.0 version. A generalized linear mixed model (GLMM) was used to evaluate the relationship between USST and SKIN and their difference were statistically compared with the CMT score using a multinomial logistic regression method to compare the effect of temperature on CMT score. The prevalence of SM (positive or negative) was statistically compared with the difference between USST and SKIN in all four regions via the Mann–Whitney Test. A histogram was plotted for both groups (negative and positive) and an overlapping histogram was obtained for each region. The threshold temperature for being positive for SM is the point that two Gaussian curves were crossed each other.

Using those graphical illustrations, the threshold value could be measured, however, to improve the accuracy of the values and to facilitate advanced data processing, a mathematical routine was run using Python programming language based on the following Gaussian equation for each Gaussian curve to measure the threshold value (γ):

$$\gamma = \frac{1}{\sigma\sqrt{2\pi}}\mathrm{e} - \frac{(\chi-\mu)^2}{2\sigma^2} \tag{2}$$

where, μ is the mean, σ is the standard deviation, π is the constant 3.14159 and e is the constant 2.71828.

Python libraries “Pandas” (for numerical computation based, data grouping and data tabulation)[26] and “SciPy”[27] (optimize segment) (scientific calculations and statistical analysis) were used. For data visualization, the Python library “Matplotlib” was added and the union of “SciPy”, “NumPy” and “Pandas” with “Matplotlib” contributes to a high performance, as mentioned in earlier research[28]. The threshold values obtained were statistically compared with the environmental temperature and THI of the four regions using regression analysis (IBM SPSS 23.0, SPSS Inc., Chicago, IL, USA). Linear regression analysis was also used to examine the relationship between environmental temperature and temperature difference USST minus SKIN. The model reported a significant effect (B = −0.080, R = 0.957, R^2 = 0.916, standard error = 0.104, $P < 0.05$).

$$\gamma = \mathrm{m}\chi + c \tag{3}$$

where, γ is the temperature difference and χ is the THI.

4 Results and discussion

It has been demonstrated that IRT has the potential to assess various physiologic parameters (breathing, stress or estrus)[29], identification of skin/hoof injuries[30], as well as subclinical diseases in calves[31]. Earlier studies have found that the variation of USST is associated with milking, environmental conditions, exercise[19] and also in identifying mastitis, whether subclinical or clinical[15,17]. The temperature of livestock has a certain stability[32]. However, when animals suffer from an inflammatory reaction, the blood flow will change accordingly by indicating higher temperatures on the skin surface of inflamed areas[33]. Through this benchmark, an udder with SM could be successfully detected.

In the present study, SM-positive udder quarters were found to have a higher temperature than non-mastitis quarters and that temperature difference was varied at region level. In UP, USST (mean ± standard deviation, and confidence interval-under 95%) of the SM affected udder quarters of cows was higher (36.4 ± 1.79 °C, 36.1–36.7 °C) than the temperature of non-mastitis udder quarters (34.1 ± 2.02 °C, 33.8–34.4 °C) by 2.27 ΔT °C. In MC, average USST of SM positive udder quarters were (36.3 ± 1.79 °C, 36.1–36.5 °C) slightly higher than the temperature of non-mastitis udder quarters (35.6 ± 1.56 °C, 35.4–35.8 °C) by 0.64 ΔT °C. In CT, 1.09 ΔT °C of temperature difference was observed in between SM-positive (38.3 ± 1.01 °C, 38.2–38.4 °C) and non-mastitis udder quarters (37.2 ± 0.98 °C, 37.1–37.3 °C). In WP also same observation was resulted with slightly higher USST difference (0.80 ΔT °C) in of the SM (37.7 ± 1.84 °C, 37.5–37.9 °C) affected than unaffected udder quarters (36.9 ± 1.14 °C, 36.8–37.0 °C). Thus, in the present study, different USST values were observed in each region.

The environmental conditions and internal factors of animal may be a reason for the variation of USST in each region. However, in all four regions, an increased USST was recorded in cows positive for mastitis than unaffected cows. Thus, according to these results, changes in USST due to SM can be detected using IRT, which is consistent with earlier research[17,19,34]. Also, this study had resulted with higher USST in SM positive cows than the non-mastitis cows. Earlier research has also identified an elevated temperature in mastitis affected compared to unaffected udder quarters[34] and, in some studies, the temperature difference between mastitis-positive and -negative udder quarters was up to 1 °C[35]. Prevalence of SM (positive vs negative) was statistically compared with USST and SKIN and according to the multinomial logistic regression analysis, USST had significant effect on mastitis prevalence (CT: $P < 0.05$, WP: $P < 0.05$, UP: $P < 0.05$ and MC: $P < 0.05$) and the effect of SKIN varied with regions (CT: $P > 0.05$, WP: $P > 0.05$, UP: $P < 0.05$ and MC: $P < 0.05$). Hence, either USST or SKIN may not be able to use as a single measurement to identify the prevalence of SM.

Therefore, attention was focused on applying the difference between those two temperatures (USST and SKIN). A few earlier studies have considered the temperature difference between the body/skin and udder surface for early detection of mastitis[32]. To measure the body/skin temperature, researchers have used different methods such as rectal temperature (RT)[36], virginal temperature[35] and ocular surface temperature (OST)[32]. However, these methods had some difficulties so were not adopted more widely. Errors may occur due to the variety of cow head postures and the detection accuracy of cow head posture on the OST method[32]. The use of digital thermometers to monitor vaginal temperature can reduce the disruption of animal behavior[23] compared to RT. Determination of RT is more laborious, according to some earlier findings. One study indicated that IRT detected changes in temperature on the right and left flanks, and showed a strong correlation with heat production[23]. Therefore, in the current study, it was decided to apply that concept by measuring the skin temperature via the right-side flank of the cow using IRT. According to the Mann–Whitney test, the prevalence of mastitis was statistically significant with the difference between USST and SKIN (right flank) ($P < 0.05$) and SM positive udder quarters had significantly higher temperature compared to SM negatives. This was compatible with earlier research findings[15,34]. It showed that SM-positive cows had significantly higher temperature differences between USST and SKIN, and temperature differences were significant with CMT scores.

Early diagnosis is crucial for the success of mastitis treatments[36] and therefore, establishing a precise temperature difference or threshold is essential for the proper detection of SM. Threshold value of the temperature difference was graphically illustrated using the following Gaussian curves and each threshold value was calculated using Python program.

In Fig. 2, difference threshold values were obtained for each area. In UP where much cooling conditions prevailed, resulted with the threshold of 2.49 ΔT °C and in MC it was observed as 2.17 ΔT °C. In WP the threshold value for being positive for SM was observed as 1.90 ΔT °C and in CT a threshold vale of 1.86 ΔT °C was resulted. Four regions have different threshold values and this may be due to climate conditions, farm management practices or herd composition. When the difference between SKIN and USST was higher than the particular threshold, it was concluded that the observed udder was affected by SM. In an earlier study, mastitis-positive udder quarters were detected when the temperature difference of OST and USST was higher than the threshold value[32] and also SM positive in some earlier studies with a temperature difference of 2.35 ΔT °C. This value is roughly similar to the threshold values from UP and MC whereas WP and CT had smaller values. This might be due to the environmental temperature.

The effect of environmental temperature and THI was compared with the threshold value of each region. The lowest environmental temperature was in UP (22.6 °C) followed by MC (24.3 °C), CT (28.3 °C) and WP (29.7 °C). Throughout the study, the average THI of each region was 71.6 in UP, 74.4 in MC, 80.6 in CT and 82.7 in WP. According to the statistical analysis, environmental temperature had a significant (B = −0.080, $R = 0.957$, $R^2 = 0.916$, standard error = 0.104, $P < 0.05$) relationship with the temperature difference USST minus SKIN. According to R^2, over 90% of the variation in udder quarter temperature was associated with variation in environmental temperature.

The regression equation obtained for the dependent variable of environmental temperature was, $Y = -0.08X + 4.20$, where, Y = temperature difference and X = environmental temperature. Therefore, with the increment of one unit of environmental temperature, the temperature difference will be decreased by 0.08 ΔT °C. This could be due to an increase in both USST and SKIN due to the increment of environmental temperature as demonstrated by earlier research[32]. Environmental conditions can be adverse because the immunological system of dairy cows can be compromised by heat stress[37]. Thus, the immunological capability can decrease and that is likely to affect the biological functions of the body by detrimental to udder health.

The effect of THI on the difference between USST and SKIN was only marginally significant ($P = 0.051$). However, the THI value also had a strong positive correlation with the temperature difference (USST minus SKIN) (B = −0.053, $R = 0.949$, $R^2 = 0.901$, standard error = 0.113). The regression equation obtained for the dependent variable of THI was, $Y = -0.053X + 6.207$ where Y = temperature difference and X = THI. Therefore, with the increment of one unit in the THI, the temperature difference will be decreased by 0.05 ΔT °C. Increased THI was recorded in WP and CT where elevated environmental temperatures are more common. This result was also consistent with the published findings[36] which concluded that the THI can have a significant effect on USST and SKIN. Also, both mammary glands and body use the same

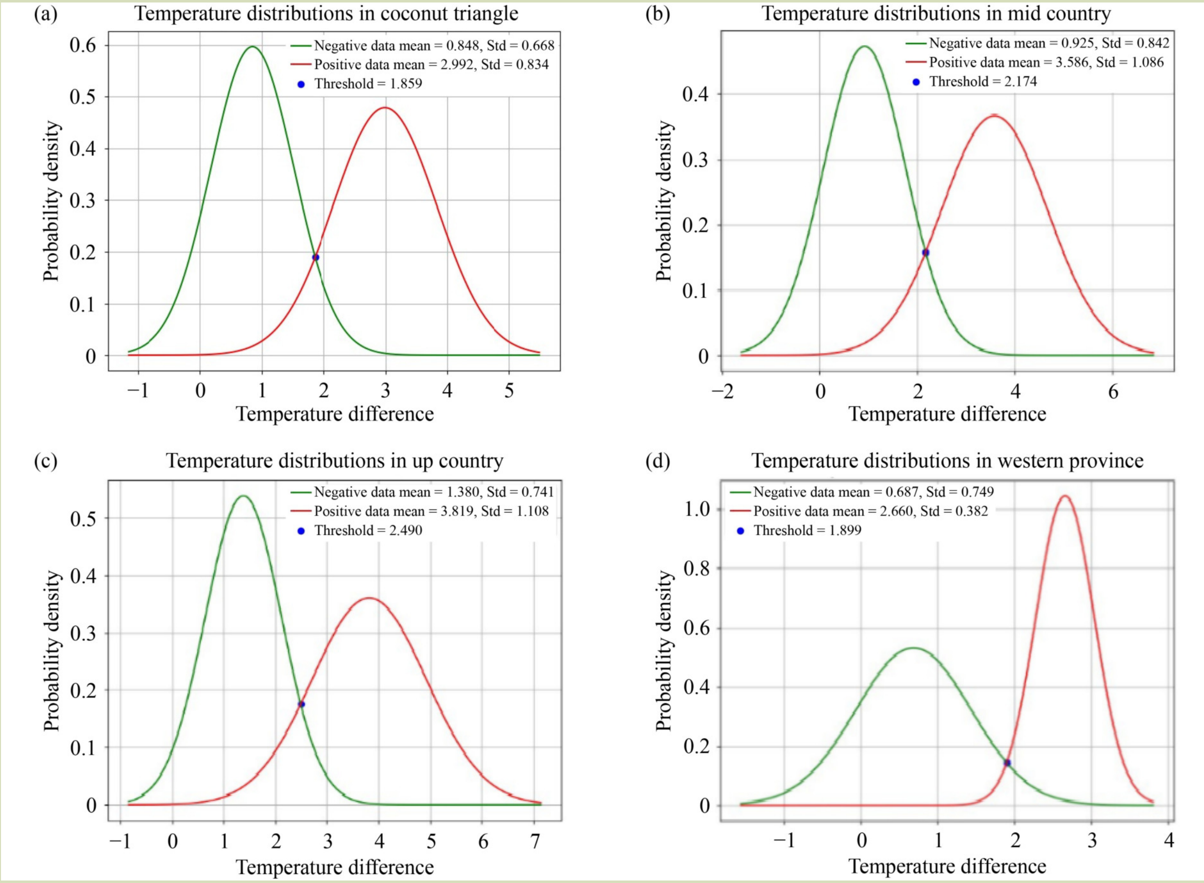


Fig. 2 Gaussian curves and threshold values for udder temperature distributions in four regions of Sri Lanka: mid country (a), coconut triangle (b), up country (c), and western province (d).

mechanisms during high temperature and THI, and therefore the difference between USST and SKIN could be smaller for positive cows. Although, a limited number of reports have been published on the effect of environmental temperature and THI on temperature difference, THI has been identified as a direct factor influencing the USTT and SKIN of cattle.

In addition, USST can be affected by external factors when animals are not kept under controlled environmental conditions[15,19]. Some research findings on early detection of SM found that the application of IRT using several methods was able to detect the threshold temperature for positive SM. However, there can be some potential sources of error related to the use of infrared thermal technology related to distance, angle of measurement, and surface characteristics. Nevertheless, the present study has confirmed that IRT can be used for early detection of mastitis. By calculating the difference between USST and SKIN and comparing it with the particular threshold value, SM can be detected early and efficiently.

SM has a considerable impact on animal welfare, consequently, early detection using IRT can reduce discomfort, inflammation, and the need for antibiotics. Additionally, practical strategies for its implementation could easily be developed, including training programs for farmers, access to user-friendly infrared devices, and collaboration with veterinary professionals. These measures aim to facilitate on-farm adoption and enhance the real-world application of the findings of this study. Therefore, this concept may be a more practicable method for stockowners in future.

5 Conclusions

In addition to diminishing quantity and quality of milk, mastitis also detrimentally influences animal welfare and affects the sustainability of dairy farms. Therefore, early detection of SM is vital. The difference between USST and SKIN can be applied to detect SM and when the value is more than the threshold, it indicates that cows are most likely to be positive for SM. This method (IRT-based detection for SM) could be integrated into herd management software applications in farm and then can facilitate the early detection of threshold temperature. This threshold value is affected by environmental temperature and is often significantly related to the THI. Since the threshold value varies with regional conditions, in the future researchers should focus on building up a model by combining IRT with diagnostic approaches, including somatic cell counts and milk quality metrics, for early detection of SM using neural networks. Also, CMT is an indirect diagnostic tool for SM and lacks sufficient sensitivity for a definitive diagnosis. Therefore, milk sample analysis may be useful for confirmation of early detection of SM. Additionally, future research should focus on longitudinal studies to track temperature variations over time, monitor mastitis progression and refine the threshold values for IRT under contrasting environmental conditions.

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Compliance with ethics guidelines

Weerasinghe Pathirage Chamila Gayani Weerasinghe, Eranda Rajapaksha, and Thusith Semini Samarakone declare that they have no conflict of interest or financial conflicts to disclose. All applicable institutional and national guidelines for the care and use of animals were followed.

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