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Evaluation of Usefulness of Infrared Thermography for the Detection of Mastitis Based on Teat Skin Surface Temperatures in Dairy Cows

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ABSTRACT

Given the severe economic losses due to mastitis, the identification of a diagnostic tool that can rapidly screen cows suspected of mastitis immediately before milking would be especially advantageous for farmers owning a large number of dairy cows. It is important to detect mastitis at an early stage to ensure effective and successful prevention and management of intramammary infections. Therefore, this study evaluated utility of Infrared Thermography (IRT) as a diagnostic tool for mastitis based on the heat patterns of thermal images. We found that 49 (1–4 teats in 16 cows) of the 252 teats were suspected of mastitis and 203 teats appeared normal. Our results showed that cows suspected of mastitis presented teat skin surface temperature (TSST) values that were 5.05°C higher, on average, then those of healthy cows. Moreover, TSST values were well correlated with both high somatic cell count values (r=0.99; P<0.001) and California mastitis test scores (r=0.59; P<0.001), indicating that TSST temperature monitoring using IRT could represent an easy and reliable method for screening for mastitis in dairy cows breeding farms.

Key words: Dairy cow, Infrared thermography, Mastitis, Milk quality, Teat skin surface temperature.

INTRODUCTION

Mastitis is one of the most common disorders that affects the dairy cow's industry, causing economic losses and significant effects on milk quality (Bar et al. 2008; Hiitiö et al. 2017; Aghamohammadi et al. 2018; Azooz et al. 2020; Chen et al. 2021; Hu et al. 2021; Velasco-Bolaños et al. 2021). Economic losses are shown in various ways, such as a decrease in milk production and milk quality, a loss of milk, a shorter in lactation life, and culling of the affected cow (Sathiyabarathi et al. 2016; Aghamohammadi et al. 2018; Azooz et al. 2020).

According to a survey by Erskine et al. (2003), the total annual cost of mastitis-associated in the USA was $126 for cow and $45 for lactation. Similar indicators can be seen in other countries, so effective mastitis control programs, including early diagnosis of mastitis, are more important than treatment (Halasa et al. 2007; Polat et al. 2010). Puerto et al. (2021) reported that in the case of mastitic cows, the drops in production was significant due to a significant decrease in cumulative milk value (-287 to 591 Can$, -228 to -470 US$), margin over feed cost (-243 to -540 Can$, -193 to -429 US$) and gross profit (-649 to -908 Can$, -516 to -722 US$) for mastitic cows at all stages.

Mastitis has caused severe economic losses among dairy farmers in Korea for decades. As of December 2018, a total of 410,000 dairy cows were reported in Korea, with each farm had managing approximately 62 heads of livestock, which produced 25,987 L of milk annually (Korean Statistical Information Service, KOSIS). The incidence of clinical mastitis in Korea was 6.4 - 8.6% in the 1980s (Seok et al. 1981; Jang and Kim 1984) and 7.8% in the 2000s (Nam 2010). The National Mastitis Control Program has been conducted in Korea since the early 2000s to control mastitis in dairy cattle (Nam 2010). Nam (2010) reported a study conducted from 2004 to 2010, based on the California mastitis test (CMT), where 13.8% and 7.8% of dairy cows were infected with subclinical mastitis (SCM) and clinical mastitis (CM), respectively.

The ability to quickly screen cows suspected of suffering from mastitis immediately before milking would be especially advantageous for farmers with larger dairy cows. The early diagnosis of mastitis is very important for successful therapy of intra-breast infection. Therefore, the development of potentially applicable efficient and rapid

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diagnostic tools in the field is essential to monitor the occurrence of mastitis (Clark and Cena 1977; Purohit et al. 1980; Turner et al. 1986). Several skills are useful for the early detection of SCM-associated changes in milk including CMT. However, these assessments can be subjective and have a wide variety of predictive abilities. In addition, these examinations are not routinely applied to the monitoring of animal health on-farm, due to the associated costs and time requirements (Hovinen et al. 2008; Pyörälä and Taponen 2009; Polat et al. 2010; Matzer et al. 2014). Additionally, these tests represent indirect measurements of infection, and the abilities and sensitivities of these detection technologies should be considered (Hovinen et al. 2008). Therefore, in this study, both teat skin surface temperature (TSST) and CMT scores were hypothesized to increase in cows suspected of mastitis.

Thermal imaging cameras absorb infrared rays emitted from an object and generate images according to the amount of heat generated without being reflected (Eddy et al. 2001; Mazur and Eugeniusz-Herbut 2006). In general, in thermal images, the warmest spot looks white or red, while the coldest part appears blue or black (Eddy et al. 2001; Colak et al. 2008). Skin surface temperature (ST) represents the status of metabolism and blood circulation of tissues, and abnormal ST patterns can indicate areas of surface inflammation or circulatory disorders (Sathiyabarathi et al. 2016). Infrared thermography (IRT) was found to be sufficiently susceptible to detect shifts in udder skin surface temperature (USST) caused by milking, environmental temperatures, and physical movement (Berry et al. 2003). This study aimed to evaluate whether IRT could be useful as a diagnostic tool to identify mastitis, based on TSST measurements.

MATERIALS AND METHODS

Ethical Approval

The protocol and conduct of this study were approved by the Kyungpook National University of Animal Ethics Committee, Republic of Korea (KNU2019-0091).

Animals

This study was conducted on 252 teats of 63 Holstein-Fresian (HF) cows (Bos taurus) [mean±SD, days in milk=115±42; milk yield=14.8±4.9kg, parity=2.3±1.9], on a private farm. To avoid uncertain TSST data, cows with disorders other than SCM were excluded from this study. These animals were maintained in a typical housing system and milked twice a day (06:30 and 16:30). The cows were provided with the recommended amount of concentrated feeds (corn, etc) and roughages (straw, etc), TMR feed, and water was freely used. In this study, no cows experimentally induced mastitis, and IRT images were obtained from all participating cows to detect mastitis naturally caused by infection and environmental factors (unclearness and no regular disinfection of the barn, insufficient straw replacement, temperature, humidity, and stress due to insufficient milking management, etc).

IRT

A total of 252 TSST thermal images (from 63 cows) were obtained before milking, using an infrared radiation camera (T420, FLIR Systems, Inc., Wilsonville, Oregon, USA). Before milking, teats soiled with manure are washed hygienically, with clean water, and dried with a dry towel. After calibrating the camera according to the atmosphere temperature, the temperature measurement and distance were adjusted to Celsius and meters, respectively. At this time, the emissivity was 0.97, a distance of 0.5m from the cow was maintained, and all four teats were photographed from the ventral side, on a single screen, to facilitate comparisons between the temperatures of the teats. The thermal images were analyzed using specialized software (FLIR Tools Professional, Teledyne FLIR Systems Inc., Oregon, USA), and the highest temperature was recorded for each teat for analysis.

Milk Sampling, Somatic Cell Count and California Mastitis Test

Teats that appeared red or white in the thermal image were classified as suspected of mastitis. Milk was collected from all 252 teats including suspected mastitis-infected teats for somatic cell count (SCC) (Porta SCC® Somatic Cell Test, Suite E Moorestown, NJ, USA) and CMT (ImmuCell Corporation, 56 Evergreen Drive, Portland) analyses.

Various definitions for identifying SCM based on the SCC cut-off value have been known. Sears and McCarthy (2003) defined healthy and SCM as healthy when SCC ≤400,000 cells/mL, and SCM when SCC >400,000 cells/mL. Because there are differences in defining SCM based on SCC cut-off, De Vliegher et al (2001) and Chagunda et al. (2006) evaluated healthy and SCM based on 200,000 cells/mL as cut-off. However, according to the Korean Animal and Plant Quarantine Agency's guidelines, the evaluations (healthy vs. SCM) in this study were based on the following cut-off values: samples with SCC values of ≤250,000 cells/mL were considered healthy; 250,000-350,000cells/mL without clinical mastitis symptoms (edema, pain, and redness) were considered SCM; and ≥350,000 cells/mL were regarded clinical mastitis (if the CMT score was false positive culture and the SCC values were ≥350,000 cells/mL, it was considered clinical mastitis). CMT was conducted immediately after taking the image with a thermal camera, and the results were recorded. CMT results were scored as 0 (negative), ± (false positive), +1 (+), +2 (++) and +3 (+++) (White et al. 2005).

Statistical Analysis

All statistical analysis were conducted using SPSS software (version 25, IBM SPSS Statistics, USA), with the significance level established as P<0.05. The main trends for all 252 teats were estimated using unpaired t-test with Welch's correction or Mann–Whitney U test. To determine the suitability of the sample size, power analysis (version 3.1.9.7, G*Power, Germany) (Faul et al. 2007; Faul et al. 2009) was conducted with α (significance level) set as 0.05 and power as 0.8. Results were calculated to obtain descriptive statistics from SCC values. The correlation between TSST and mastitis indicators of non-mastitis (healthy) and mastitis affected (SCM or CM) cows was analyzed using Spearman test. Correlation of <0.3 was considered weak, 0.3 ~ 0.7 was considered moderate, and of ≥0.7 was considered strong.

RESULTS

Infrared Thermography

Based on the heat patterns of thermal images, 49 (1–4 teats in 16 cows) of 252 teats were suspected of mastitis, and 203 teats appeared normal. Based on TSST measurements, teats suspected of mastitis (34.62±3.59°C) were 5.05°C hotter than normal teats (29.57±1.84°C) (Fig. 1). Even after washing, the thermal images (A) of teats of non-mastitis cows that were washed just before milking appear blue or black. However, the thermal image (B) showed red and yellow color distribution patterns in teats, indicating potential infection (Fig. 2).

Statistical Interpretation

Based on the research results of Polat et al. (2010), the positions of the teats were not considered in this study because temperatures did not differ according to the teat position. The mean SCC values (□103 cells/ml) were 52, 145, 263 and 2,144 for normal, false positive culture, SCM, and clinical mastitis samples, respectively. The mean TSST values (°C) of 29.57, 31.24, 32.46 and 35.48, corresponded with CMT score of negative (n=203), false positive culture (n=13), +1 (n=25), and +2 (n=11), respectively. No CMT scores of +3 were recorded. The descriptive statistics for mastitis indicators are showed in Table 1. All mastitis indicators were interrelated. TSST values were positively correlated with SCC values (r =0.99; P<0.001) and CMT scores (r =0.59; P<0.001) (Fig. 3 and 4).

DISCUSSION

Infrared thermography (IRT) can detect infrared radiation emitted from an object, which is a measure of its surface temperature (ST) and can present it as a heat map (Travain et al. 2015; Radigonda et al. 2017; Harrap et al. 2018; Menegassi et al. 2018; Qu et al. 2020; Isola et al. 2020; Schmitt and O′Driscoll 2021; Zheng et al. 2022). However, an individual's average body temperature may vary slightly when IRT is used to measure temperature. When diagnosing mastitis using IRT, the temperature measurement and the thermographic image of the emitted infrared ray must be confirmed, as shown in Fig. 2. This is the simplest method that can be visually confirmed immediately through an image and easily monitored periodically. When the distribution of color in the IRT image of the teats appears red or white, which differs from the distribution of color observed for healthy cow teats, the occurrence of the disease can be suspected. Therefore, our study results suggested that non-invasive monitoring in the field may be possible using IRT as several authors have reported (Zaninelli et al. 2018; Byrne et al. 2018).

Sathiyabarathi et al. (2016) demonstrated that the USST values measured in SCM and CM-affected HF crossbred cows were 0.86–1.02°C higher than those of healthy cows. In this study, however, the average TSST value of mastitis-affected cows was 5.05°C higher than that of healthy cows. The observed differences in temperatures between USST and TSST may be because the udder temperature can be affected by hair and blood vessels during IRT.

Most previous studies (Mazur and Eugeniusz-Herbut 2006; Colak et al. 2008; Sathiyabarathi et al. 2016; Velasco-Bolaños et al. 2021) have used IRT to diagnose mastitis in dairy cows based on USST. However, Berry et al. (2003) suggested that IRT was limited and only available for back-quarter monitoring. In particular, since the surface temperature may be affected by heat radiation emitted from the inside of the adjacent leg, the pattern of the surface temperature may be different in the front forequarters. Additionally, the rear quarters are more exposed to environmental temperatures than the

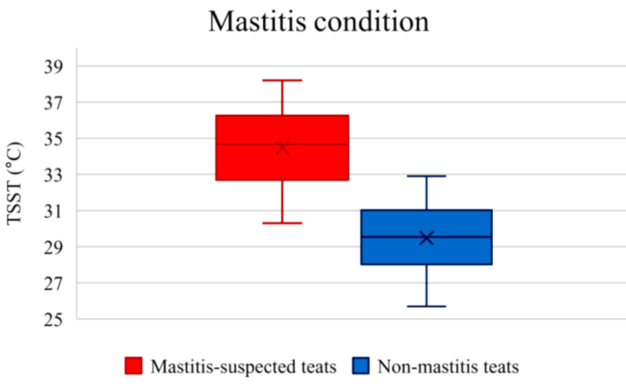

Fig. 1: Box plot visually showing the temperature of dairy cows' teats. The left box plot shows the temperatures of animals suspected of mastitis, whereas the right box plot shows the temperature of animals without mastitis. The average difference was 5.05°C, with cows affected by mastitis displaying a higher teat temperature.

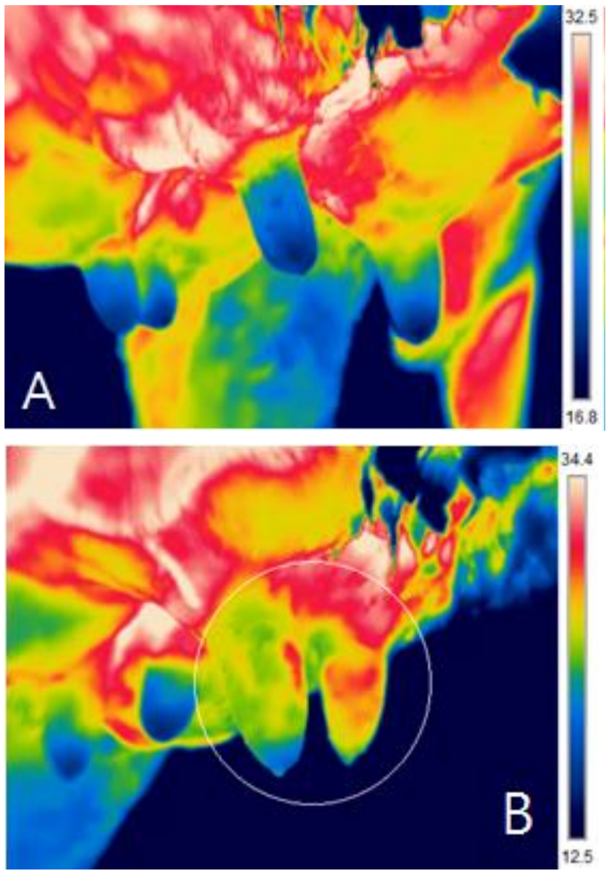

Fig. 2: Examples of infrared thermographic images for the examined regions. All shots were taken under the same conditions such as angle, distance, washing condition, and ambient temperature. A: Teat skin surface temperature of a non-mastitis cow. B: Teat skin surface temperature of a mastitis-affected cow.

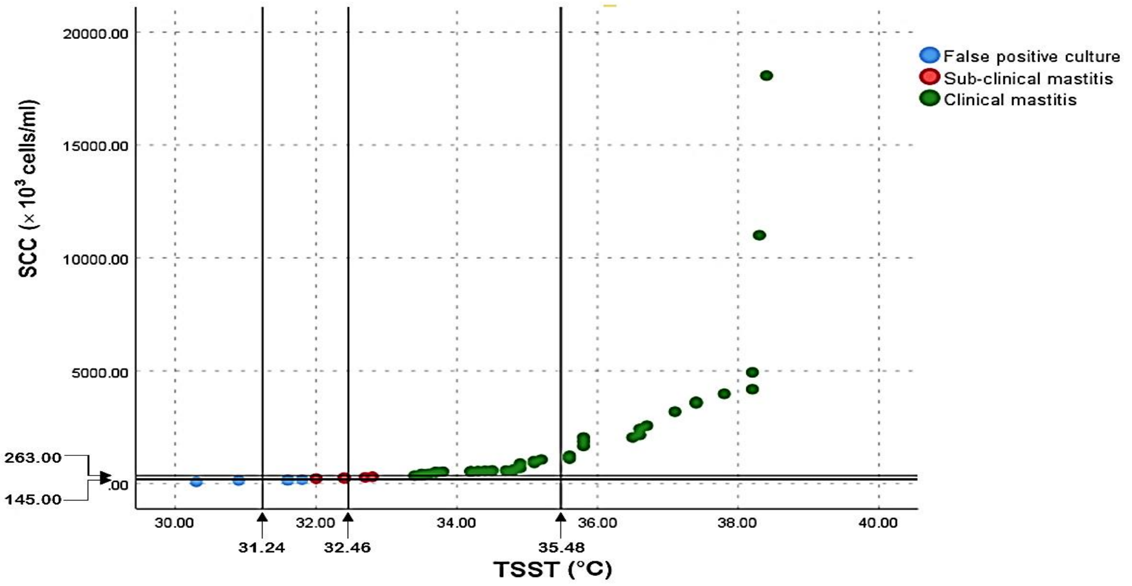

Fig. 3: A scatter plot showing the relationship between somatic cell count (SCC) and the teat skin surface temperature (TSST), as determined by infrared thermography [TSST, °C].

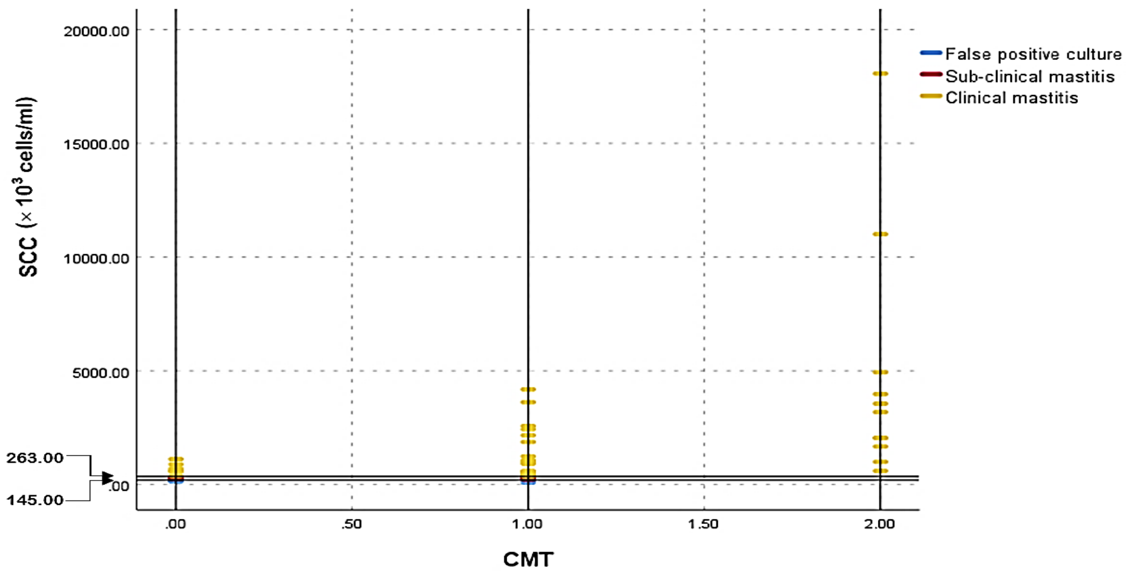

Fig. 4: A scatter plot showing the relationship between somatic cell count (SCC) and California mastitis test (CMT). 0: CMT score = ±, 1: CMT score = +1, 2: CMT score = +2.

Table 1: Descriptive statistics regarding somatic cell counts (SCC, $\times 10^3$ cells/mL) and teat skin surface temperature (TSST, °C), according to the California Mastitis Test (CMT) scores

	Negative (n=203)			False positive culture (n=5)			Subclinical mastitis (n=5)			Clinical mastitis (n=39)		
Descriptive measure	CMT	SCC	TSST	CMT	SCC	TSST	CMT	SCC	TSST	CMT	SCC	TSST
Mean	0	52	29.57	0.2	144.8	31.24	0.6	263	32.46	1.10	2143.92	35.48
Median	0	24	29.4	0	145	31.6	1	265	32.4	1	993	35.1
SD	0	9.50	1.32	0.4	33.45	0.56	0.49	28.45	0.28	0.67	3228.16	1.50
Var	0	63.61	1.74	0.16	1118.96	0.31	0.24	809.2	0.08	0.45	10420996	2.24
Maximum	0	159	30.9	1	178	31.8	1	305	32.8	2	18070	38.4
Minimum	0	1	25.7	0	84	30.3	0	228	32	0	357	33.4

Linearity test, P<0.05. SD=standard deviation; Var=variance; n=the number of tests.

forequarters. Furthermore, larger, denser udders can limit thermography (Gautherie 1983). Also, Hovinen et al. (2008) could not detect local inflammatory changes of the udder, which appear earlier than the rectal temperature increase using IRT.

Machado et al. (2021) reported that IRT is a practical technology that can detect cases of mastitis in dairy cows with high-precision using thermal images from the anatomical site in front quarters of the udder. Other options, including TSST, should be explored because USST can be obtained potentially unreliable data when measuring USST using IRT.

Many biomarkers can be used to detect SCM, although none of these methods is perfect or fully accurate. SCM is clearly treated as normal milk because SCC increases up to 400,000 cells/mL due to the influx of white blood cells (Sears and McCarthy 2003). SCC is considered the best indicator of inflammatory response because it is associated with reduced milk production capacity and changes in milk quality and is detectable starting at 50,000 cells/mL (Hamann et al. 2005). Intra-breast infections are associated with increased SCC and CMT scores (McDermott et al. 1982; Sargeant et al. 2001). In our study, increases in CMT scores and SCC values were positively correlated with increased TSST values. However, even though the SCC values increased (SCC ≥200,000 cells/mL), and a significant color distribution (red or white) demonstrated high TSST value, some CMT results were classified as false positives, which results in the rejection of our null hypothesis, although these cases could potentially represent SCM. In addition, the CMT results may vary with sampling time, and because of the high probability of false positives, more testing is necessary.

Conclusion

This study was conducted using non-invasive IRT to diagnose and monitor mastitis in HF cows. IRT is considered a simpler and more sensitive test tool than conventional SCC and CMT tests, and may be very useful for monitoring and diagnosing mastitis in HF cows in the future and is expected to help promote welfare in Korea.

Author Contributions

All research protocols and animal experiments in this study designed, conducted experiments by SM Kim, KY Eo, TM Park and contributed to data acquisition. GJ Cho contributed to the interpretation of the experimental results and the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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