DOI: 10.1590/1809-6891v24e-76726E

Ciência Animal Brasileira
Brazilian Animal Science

e-ISSN 1809-6891

Section: Veterinary medicine
Research article

Infrared thermography for detection of clinical and subclinical mastitis in dairy cattle: comparison between Girolando and Jersey breeds

Termografia infravermelha na detecção de mastite clínica e subclínica em bovinos de leite: comparação entre as raças Girolando e Jersey

Isabele Pessoa Ribeiro[1], Pablo Henrique Delai Gonçalves[1], Manoela Simionato Rodrigues[1], Guilherme Batista do Nascimento[1], Rafaela Speranza Baptista[1], José Ruben Lacerda Calil Filho[1], Alexandre Wolf[1], Sandra Helena Gabaldi Wolf[1]*

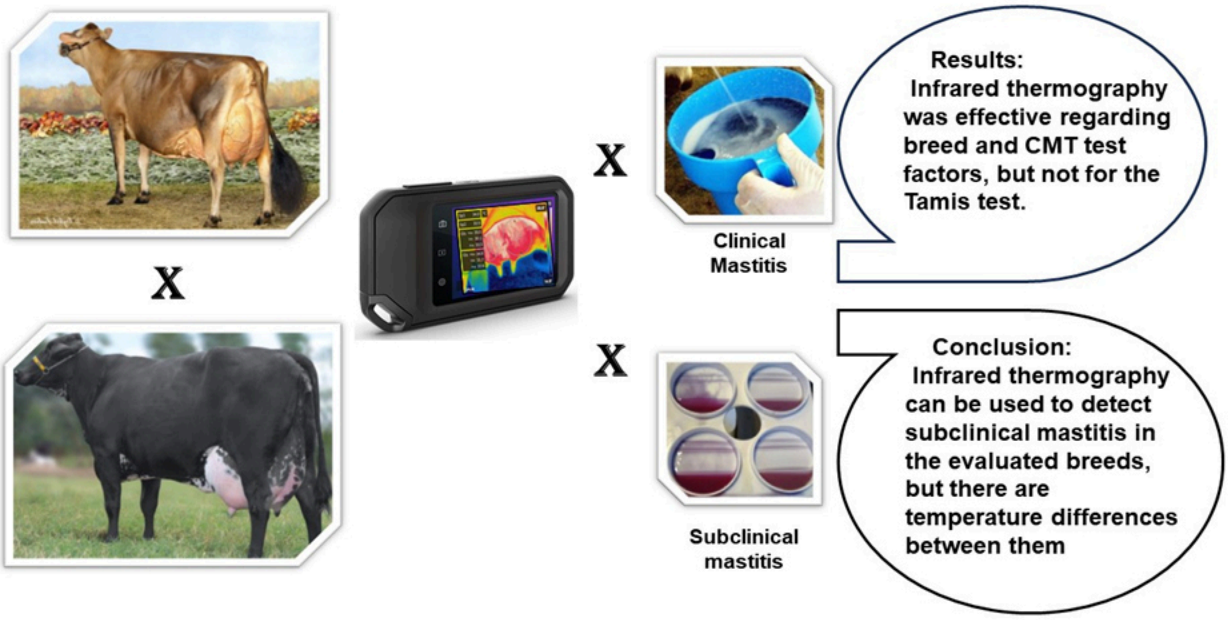
[1]Centro Universitário de Adamantina (UniFAI), Adamantina, São Paulo, Brazil
*Corresponding author: sandra@fai.com.br

Abstract
Mastitis is one of the most prevalent diseases in dairy cattle globally, ranking at the top in terms of prevalence and incidence. It impacts milk production and quality, subsequently decreasing economic returns and farm sustainability. Early diagnosis and treatment of mastitis are crucial to mitigate its detrimental effects on both animals and the dairy industry. Infrared thermography (IRT) in animals serves as a clinically relevant method to detect pathophysiological changes, marked by thermal variations caused by inflammation. This study aimed to evaluate the potential of IRT as a diagnostic tool for clinical and subclinical mastitis in Girolando and Jersey cows. We examined 78 udder quarters from Girolando cows and 104 from Jersey cows, all from farms in the Adamantina region. Differences in IRT image intensities were compared with anterior and posterior udder temperatures at a single central point or area, correlating with results from Tamis and CMT tests. All analyses were conducted in R software, with a significance level set at 5%. When evaluating thermographic images, the effect size was significant for the breed and CMT test, but not for the Tamis test. In conclusion, IRT exhibits potential in screening for subclinical mastitis in the evaluated breeds, demonstrating a predictive diagnostic capability similar to the CMT, albeit with a temperature difference between them. Their measurements, whether at a point or an area of the mammary gland, were found to be equivalent.
Keywords: diagnosis; mammary gland; thermal imaging; inflammation.

Resumo
A mastite é uma das doenças mais comuns do gado leiteiro em todo o mundo, ocupando o primeiro lugar, com alta prevalência e incidência. Afeta a produção e a qualidade do leite, diminuindo o retorno econômico e a sustentabilidade da fazenda. A precocidade do diagnóstico e tratamento da mastite é de extrema importância, visando diminuir os danos, tanto para o animal quanto para o produtor e a indústria. A termografia infravermelha (TI) em animais é um método clinicamente útil para detectar alterações fisiopatológicas, por meio de variações térmicas, causadas pela inflamação. Este trabalho objetivou avaliar o potencial da técnica de TI para o diagnóstico de mastite clínica e subclínica em vacas Girolando e Jersey. Foram avaliados 78 quartos de vacas Girolando e 104 de Jersey pertencentes a propriedades rurais de Adamantina e região. As diferenças das intensidades das imagens por TI foram comparadas com as temperaturas do quarto anterior e posterior, em um único ponto central ou área, em relação aos resultados dos testes de Tamis e CMT. Todas as análises foram realizadas no Software R, sendo adotado um nível de significância igual a 5%. Quando a imagem termográfica foi avaliada, o tamanho do efeito foi significativo para raça e para o teste de CMT, porém não para o teste de Tamis. Em conclusão, a TI tem potencial no rastreamento de mastite subclínica nas raças avaliadas, com capacidade diagnóstica preditiva semelhante ao CMT, mas com diferença de temperatura entre elas, sendo equivalentes suas mensurações em ponto ou área da glândula mamária.
Palavras-chave: diagnóstico; glândula mamária; imagem térmica; inflamação.

Received: July 5, 2023. Accepted: September 4, 2023. Published: October 11, 2023.

https://revistas.ufg.br/vet/index



Graphical abstract - Infrared thermography for detection of clinical and subclinical mastitis in dairy cattle: comparison between Girolando and Jersey breeds

1. Introduction

Mastitis, an inflammation of the mammary gland, leads to temperature rise in the affected area, decreased milk secretion, and altered membrane permeability. It poses a major challenge in dairy farming due to significant economic implications[1-3]. The disease can be categorized as clinical, where observable changes occur in the mammary parenchyma and milk secretion, or subclinical, where the only indication is an elevated somatic cell count[2, 3].

Clinical and subclinical mastitis in dairy cattle results in significant economic losses for producers. To monitor mastitis, the Tamis test (popularly known as *caneca preta*) is used for clinical cases while the California Mastitis Test (CMT) is used for subclinical ones, both performed in the milking parlor. Subclinical mastitis can also be identified by somatic cell counting (SCC) in the laboratory. However, more efficient diagnostic tools are needed for timely detection, especially outside milking parlors, in heifers, and during dry periods, especially in the subclinical form[4].

Infrared thermography (IRT) is gaining traction for its potential in diagnosing inflammation, both in human and veterinary medicine. Since all objects emit infrared radiation proportional to their temperature, it can be captured in a thermogram and expressed as a thermal gradient in a color pattern[5, 6]. This non-invasive method measures skin-emitted heat, revealing underlying tissue metabolism and blood flow. When there is pain (hypersensitivity), swelling, and hyperthermia in the initial phase of inflammation and infection, the skin's surface temperature can reflect the underlying tissue metabolism and blood flow[5].

This new tool has proven to be interesting in veterinary research, as it is extremely sensitive, simple, and effective in detecting skin surface temperature changes [7] through images. These are used to diagnose inflammation of mammary glands with non-invasive portable equipment[8]. IRT is practical, precise, fast, and has no need for restraint, and it can be used outside milking parlors[9]. Hovinen et al.[10] showed IRT's ability to identify temperature increases (> 1 °C) in the udder of cows with clinical mastitis, experimentally induced by *E. coli* strains; therefore, the health status of mammary glands can be assessed by the skin's surface temperature.

In a study conducted on Holstein and Brown Swiss cows in a temperate climate, Colak et al.[6] used IRT to measure skin surface temperature changes. They found that these changes correlated with the severity of mammary gland infections, as indicated by the scores for subclinical mastitis obtained through the CMT test. Specifically, as the CMT scores rose, there was a corresponding linear increase in the skin surface temperature of the cow's quarters. This suggests that thermography, being a non-invasive method, might be effective for mastitis screening in dairy cows.

Polat et al.[7] demonstrated in Brown Swiss cows that mammary gland surface temperatures measured by IRT were correlated with somatic cell counts (SCC) associated with subclinical mastitis. Quarters with subclinical mastitis showed a skin surface temperature 2.35 °C higher than healthy quarters, proving IRT is sensitive to detect thermal changes in the udder skin caused by subclinical mastitis; Zaninelli et al.[11] corroborated with similar results in Holstein cows. Moreover, Chakraborty et al.[12] observed that the diagnostic capacity of IRT was similar to the CMT test and could distinguish clinical mastitis from subclinical cases, being considered portable diagnostic equipment.

In a semi-arid environment, Silva et al.[13] studied the surface temperature variations of the mammary glands in Girolando cows, both with and without mastitis. Imaging took place between 05h00 and 07h00, where three shots were taken per cow, capturing the right anterolateral, left anterolateral, posterior, and inferior views. Mammary quarters with subclinical mastitis displayed a higher average surface temperature (33.2 ± 0.67 ºC and 34.64± 1.07 ºC) compared to those that tested negative (29.3 ± 1.78 ºC and 32.24 ± 0.62 ºC) and those that were healthy (29.3 ± 1.78 ºC and 31.58 ± 0.62 ºC). Cows with clinical mastitis had even higher udder surface temperatures, ranging between 34.0 and 37.5 °C. The authors concluded that the mammary gland's temperature rises with the severity of inflammation.

Porcionato et al.[14] employed IRT to identify subclinical mastitis in Gir cows (*Bos indicus*) during their second and third lactations. They measured the mammary gland's surface temperature at three levels: upper, middle, and lower. These measurements were then compared with microbiological tests and milk somatic cell counts. The upper region showed higher temperatures than the other regions. However, no significant correlation was found between udders with subclinical mastitis or positive microbiology results. The study concluded that IRT could detect temperature variations at different mammary gland heights in Gir cows, but it was not effective in diagnosing subclinical mastitis.

Bortolami et al.[4] identified a significant correlation ($p<0.05$) between an elevated gland temperature, as shown by IRT images, and the milk's somatic cell count. This suggests IRT is a valuable tool for detecting clinical mastitis. However, its effectiveness in diagnosing subclinical mastitis remains unconfirmed, indicating the need for further research in this area.

In a study by Velasco-Bolaños et al.[15], IRT was used to assess the udder surface temperature of Holstein cows in high-altitude tropical regions (Caldas/Colombia, 2,100 m above sea level), to diagnose both clinical and subclinical mastitis. Despite considering environmental factors like wind speed, atmospheric temperature, relative humidity, and the temperature-humidity index, no correlation with mammary gland surface temperature was found. The study concluded that while IRT is effective in detecting clinical mastitis, it falls short in diagnosing subclinical mastitis.

Poikalainen et al.[16] and Yang et al.[17] investigated udder temperatures pre- and post-milking. They found no significant temperature difference between the left and right udder quarters. They concluded that udder surface temperature is not influenced by the milking process or by specific mammary quarters. However, Yang et al.[17] noted that post-milking IRT might be affected by the amount of milk produced.

Although there is no standardized method for capturing IRT images yet, review articles highlight its potential for diagnosing mastitis and other conditions [18-20]. In Brazil, where breeds are more climate-adapted and experience less thermal stress, further research on mammary gland IRT is required to determine its effectiveness in diagnosing both clinical and subclinical mastitis in a tropical climate.

This study emphasizes the value of IRT as a quick, cost-effective method to detect clinical and subclinical mastitis in the field, thereby reducing milk production losses. Notably, there is a research gap in comparing breed differences. Hence, our objective was to assess the efficacy of IRT in detecting mastitis by correlating it with the Tamis and CMT tests and to explore potential diagnostic differences between Jersey (*Bos taurus*) and Girolando (Holstein-Gir) breeds.

2. Material and methods

Our research, approved by the Ethics Committee on the Use of Experimental Animals (CEUA/FAI - approval no. 22005), examined 102 mammary quarters from Jersey cows and 78 from Girolando cows. These cows were reared semi-intensively with mechanical milking in the Adamantina region, São Paulo, Brazil. The study was conducted during November and December 2022 in a high-altitude tropical climate, with temperatures between 15°C and 32°C, characterized by a wet summer and dry winter. The lactation stage, calving numbers, and milk production were not factors in our evaluation.

We subjected the quarters to the Tamis Test for clinical mastitis detection[2] and the California Mastitis Test (CMT) for subclinical mastitis, following the classification by Schalm and Noorlander[21]. Given that mammary quarters operate independently regarding blood circulation and inflammation, our experimental unit was the mammary quarter itself. We measured the skin temperature in two quarters (anterior and posterior) on one side of the udder, either right or left, based on the cow's milking parlor position. Images were captured during morning milking (05h00-08h00) before conducting pre-dipping and the Tamis and CMT tests.

We utilized the FLIR C5sc thermal imaging camera, featuring a 160 x 120 thermal capture (19,200 pixels), MSX™ (Multi-Spectral Dynamic Imaging) technology, and a 5-megapixel camera. Images were analyzed with FLIR RESEARCH IR software. For each mammary quarter, three thermal images were taken, positioning the camera 50-100 cm away at a 180º angle. During editing, temperature was gauged in two ways: across the mammary quarter's area and at a central point within it. Anterior and posterior mammary quarter images were edited independently due to their distinct nature.

We tracked temperature and relative humidity during data collection days, ensuring they remained within the thermal comfort range for dairy cows (*Bos taurus*: 0-16 ºC; Crossbreeds: 5-31 ºC)[22] using the Thermotool™ app. Temperature variables in the study underwent descriptive analysis, focusing on mean and standard deviation values. The breed effect, along with each Tamis and CMT test, was determined using a two-way ANOVA, with in-factor contrasts derived from the Bonferroni multiple comparisons test[23]. This approach was guided by a specific variance analysis equation:

$$y_{ij} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ij}$$

where: yij = the value of the dependent variable for the observation at the intersection of the i-th level of the first factor and the j-th level of the second factor, μ = overall mean, ai = fixed effect of the i-th level of the first factor, βj = fixed effect of the j-th level of the second factor, $\alpha\beta ij$ = fixed effect of the interaction between the i-th level of the first factor and the j-th level of the second factor, and εij = random residual.

We checked for multicollinearity using the variance inflation factor (VIF). The model's standardized residuals passed normality (*Shapiro-Wilk* test) and homoscedasticity criteria (Levene's test). Any data with residuals exceeding three times the interquartile range were seen as outliers and excluded. To assess the treatment's impact on response variability, we used Eta squared (η^2), which gauges the treatment variance against the total variance. Effect sizes of 0.10-0.30 were seen as low, 0.31-0.50 as moderate, and over 0.51 as high[24, 25]. The correlation between area and central point temperatures was assessed with *Pearson* correlation, given the data's normality confirmed by the *Shapiro-Wilk* test. All analyses were done in R software[26], with a 5% significance level.

3. Results

On the day we took thermographic images with the infrared camera in the Adamantina region, we measured the ambient temperature and air humidity with the Thermotool™ app (Table 1).

Table 1. Average (± SD) ambient temperature and relative humidity during image capture for each breed

Variable	Breed	n	Average ± SD	Minimum	Maximum
Temperature (ºC)	Girolando	39	14.50 ± 1.97	13	17
	Jersey	51	17.00 ± 0.00	17	17
Humidity (%)	Girolando	39	71.80 ± 5.42	65	76
	Jersey	51	62.30 ± 0.95	62	65

SD: standard deviation; **n**: number of observations.

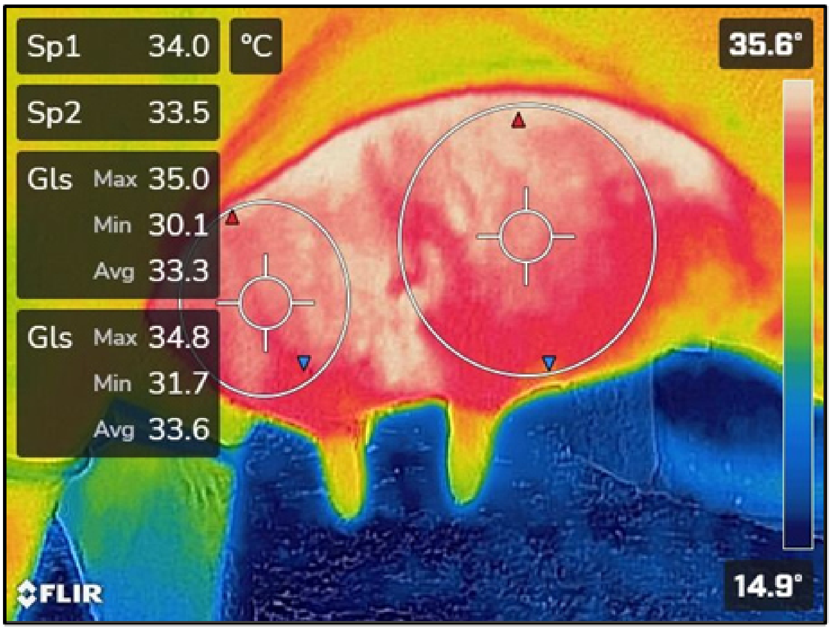


Figure 1. Infrared thermography camera image of a Girolando cow. Blue = lower temperatures; yellow and green = intermediate temperatures; red = higher temperatures. The temperature scale is displayed on the right side.

In the evaluated dairy farms, 6 out of 78 (7.7%) udder quarters showed clinical mastitis in Girolando cows, and 3 out of 102 (2.9%) in Jersey cows, based on the +, ++, and +++ results in the Tamis test. Meanwhile, in the CMT test using the same scale, 36 out of 78

(46.1%) udder quarters in Girolando cows and 15 out of 102 (14.7%) in Jersey cows tested positive (subclinical mastitis). Individual results can be found in Tables 2 and 3 (Tamis Test) and Tables 4 and 5 (CMT Test).

Images of the front and back udder quarters were edited separately since they are independent of each other (Figure 1). For some Jersey cows, due to the morphology, the back mammary gland could not be captured from the side. Thus, images were taken from two different angles: the front quarter from the side and the back quarter from behind (Figure 2), according to Colak et al.[6].

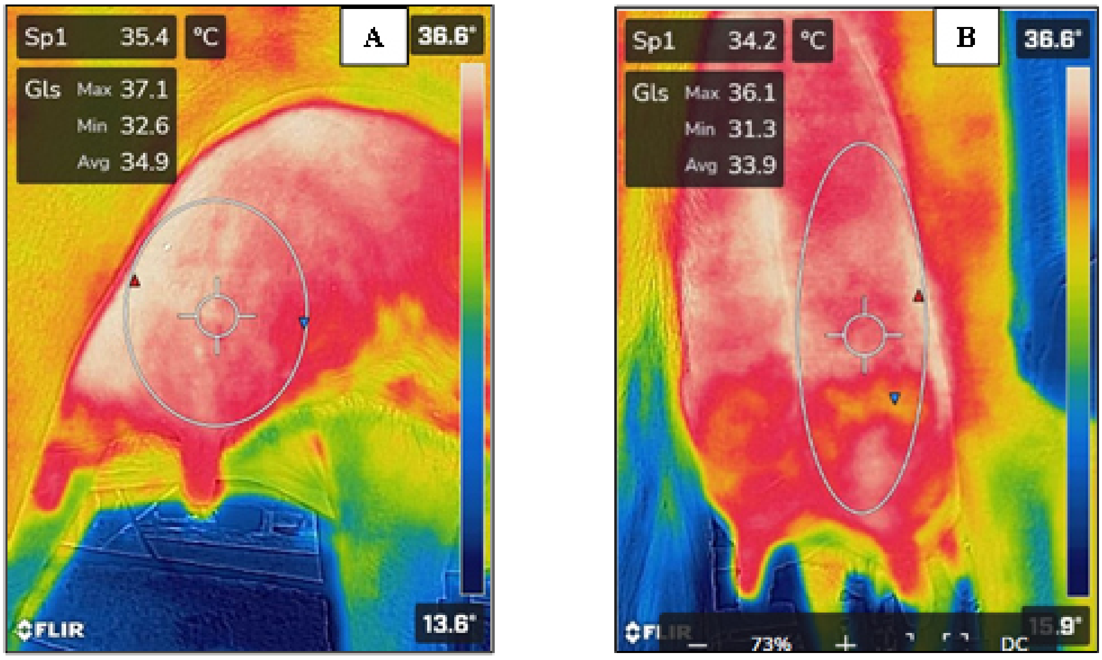


Figure 2. Infrared thermography camera images of Jersey cows. A: side view, B: rear view. Blue = lower temperatures; yellow and green = intermediate temperatures; red = higher temperatures. The temperature scale is displayed on the right side.

When evaluating the thermographic image at the central point (Table 2), no significant difference (P<0.05) was found between the Tamis averages within the front and rear points. However, within each point, the breed factor showed significant differences (P<0.05). The Eta squared (η^2) value was used to measure the effect size of this difference. Based on this, we can state that the breed accounted for 31.8% of temperature variation at the front point and 25.9% at the rear point.

Table 2. Descriptive analysis and effect of breed and Tamis test on the central point temperature variable (Mean ± SD)

Breed	Point	Tamis	n	Average ± SD	Minimum	Maximum
Girolando	Front	0	33	$31.03 \pm 2.01^{A,a}$	26.8	34
		+/-	3	$32.87 \pm 0.64^{A,a}$	32.5	33.6
		+++	3	$33.33 \pm 0.90^{A,a}$	32.4	34.2
	Rear	0	36	$30.91 \pm 1.74^{A,a}$	27.7	34.5
		+/-	-	-	-	-
		+	-	-	-	-
		++	3	$31.20 \pm 0.20^{A,a}$	31.0	31.4
Jersey	Front	0	39	$34.17 \pm 1.61^{B,a}$	29.8	36.1
		+/-	12	$34.05 \pm 3.58^{B,a}$	28.6	37.8
		+++	-	-	-	-
	Rear	0	36	$34.17 \pm 1.55^{B,a}$	31.5	36.7
		+/-	12	$33.85 \pm 2.12^{B,a}$	31.1	36.5
		+	3	$31.37 \pm 0.12^{B,a}$	31.3	31.5
		++	-	-	-	-

SD: standard deviation; **n**: number of observations. Uppercase letters indicate a significant difference ($p<0.05$) between the breed averages within each point. Lowercase letters indicate a significant difference ($p<0.05$) between the Tamis test averages within each point. The residual of the regression model was considered normal according to the *Shapiro-Wilk*.

Besides measuring the central point of thermographic images, we also checked the average temperature of the entire photographed area (Table 3). In these scenarios, no significant differences (P<0.05) were observed between the Tamis averages as well. Moreover, just as in the point-by-point analysis, there were differences between breeds within each area. In this area-based assessment, breed accounted for 40.0% of temperature variation at the front point and 31.02% at the rear point.

Table 3. Descriptive analysis and effect of breed and Tamis test on the temperature of the evaluated area (Mean ± SD)

Breed	Area	Tamis	n	Average ± SD	Minimum	Maximum
Girolando	**Front**	0	33	30.35 ± 2.32$^{A.a}$	24.4	33.3
		+/-	3	32.80 ± 0.20$^{A.a}$	32.6	33
		+++	3	33.30 ± 0.44$^{A.a}$	32.8	33.6
	Rear	0	36	30.85 ± 1.73$^{A.a}$	27.3	34.3
		+/-	-	-	-	-
		+	-	-	-	-
		++	3	31.57 ± 0.29$^{A.a}$	31.4	31.9
Jersey	**Front**	0	39	34.24 ± 1.42$^{B.a}$	30.7	35.9
		+/-	12	33.64 ± 3.22$^{B.a}$	28.7	36.8
		+++	-	-	-	-
	Rear	0	36	34.16 ± 1.21$^{B.a}$	32.1	36.7
		+/-	12	33.84 ± 1.88$^{B.a}$	32.0	36.2
		+	3	31.97 ± 0.06$^{B.a}$	31.9	32.0
		++	-	-	-	-

SD: standard deviation; **n**: number of observations. Uppercase letters indicate a significant difference (p<0.05) between the breed averages within each area. Lowercase letters indicate a significant difference (p<0.05) between the Tamis test averages within each evaluated area. The residual of the regression model was considered normal according to the *Shapiro-Wilk*.

In Table 4, when analyzing the central point in the thermographic image and the CMT, we observed that the effect size (η^2) of the breed was 41.84% in the assessment of the front quarter point. This means the temperature variation was due to different breeds. However, when comparing the Tamis averages within the front point, there was a significant difference (P<0.05) between Jersey animals with CMT +++ and the other animal groups. Using the SD measure of effect size (η^2), we noted a low effect (0.0104), meaning only 1.04% of the temperature variation was due to the CMT categories within the front point.

Table 4. Descriptive analysis and effect of breed and CMT test on the central point temperature (Mean ± SD)

Breed	Point	CMT	n	Average ± SD	Minimum	Maximum
Girolando	**Front**	-	18	29.45 ± 2.67$^{A.a}$	24.1	33.3
		+/-	6	32.00 ± 1.85$^{A.a}$	29.8	34.0
		+	3	32.87 ± 0.64$^{A.a}$	32.5	33.6
		+++	12	32.24 ± 1.52$^{A.a}$	29.6	34.2
	Rear	0	9	31.72 ± 1.70$^{A.ab}$	29.5	33.7
		+/-	9	30.43 ± 1.95$^{A.a}$	27.7	33.1
		+	6	32.77 ± 0.38$^{A.b}$	32.3	33.3
		++	3	31.73 ± 0.21$^{A.ab}$	31.5	31.9
		+++	12	32.58 ± 1.44$^{A.b}$	31.0	34.5
Jersey	**Front**	-	36	34.40 ± 1.44$^{B.a}$	29.8	36.1
		+/-	12	32.53 ± 3.00$^{B.a}$	28.6	36.5
		+	-	-	-	-
		+++	3	37.47 ± 0.49$^{B.b}$	36.9	37.8
	Rear	0	24	33.79 ± 1.68$^{B.ab}$	31.1	36.2
		+/-	15	33.17 ± 1.46$^{B.a}$	31.3	35.2
		+	9	34.80 ± 1.85$^{B.b}$	32.2	36.7
		++	3	36.30 ± 0.20$^{B.b}$	36.1	36.5
		+++	-	-	-	-

SD: standard deviation; **n**: number of observations. Uppercase letters indicate a significant difference (p<0.05) between the breed averages within each point. Lowercase letters indicate a significant difference (p<0.05) between the CMT test averages within each point. The residual of the regression model was considered normal according to the *Shapiro-Wilk*.

In the assessment of the rear point, thermography proved to be more efficient, as there was a significant difference (P<0.05) between the CMT averages with an effect of 11.72%. Notably, there was a difference of 5.87 between the rear point of Girolando animals (+/-) and Jersey (++).

When analyzing the temperature area and CMT (Table 5), the effect size (η^2) of the breed was 47.07% in the front quarter, i.e., this temperature variation was due to the breed factor, and 0.71% of temperature variation was due to the CMT categories. Meanwhile, in the rear area, the effect size (η^2) of the breed was 34.63%. Thus, this temperature variation was due to different breeds, and 11.06% of the temperature variation was derived from the CMT categories.

Table 5. Descriptive analysis and effect of breed and CMT test on the temperature variable of the evaluated area (Mean ± SD)

Breed	Area	CMT	n	Average ± SD	Minimum	Maximum
Girolando	Front	-	18	$29.45 \pm 2.67^{A.a}$	24.1	33.3
		+/-	6	$32.00 \pm 1.85^{A.a}$	29.8	34.0
		+	3	$32.87 \pm 0.64^{A.ab}$	32.5	33.6
		+++	12	$32.24 \pm 1.52^{A.b}$	29.6	34.2
	Rear	0	9	$31.78 \pm 1.71^{A.ab}$	29.4	33.7
		+/-	9	$30.46 \pm 2.21^{A.a}$	27.3	33.2
		+	6	$32.67 \pm 0.41^{A.b}$	32.2	33.1
		++	3	$31.90 \pm 0.1^{A.b}$	31.8	32.0
		+++	12	$32.47 \pm 1.2^{A.b}$	30.9	34.3
Jersey	Front	-	36	$34.40 \pm 1.44^{B.a}$	29.8	36.1
		+/-	12	$32.53 \pm 3.00^{B.a}$	28.6	36.5
		+	-	-	-	-
		+++	3	$37.47 \pm 0.49^{B.b}$	36.9	37.8
	Rear	0	24	$33.82 \pm 1.18^{B.a}$	32.0	35.5
		+/-	15	$33.37 \pm 1.14^{B.a}$	31.9	34.9
		+	9	$34.57 \pm 1.94^{B.ab}$	32.0	36.7
		++	3	$36.17 \pm 0.06^{B.b}$	36.1	36.2
		+++	-	-	-	-

SD: standard deviation; **n**: number of observations. Uppercase letters indicate a significant difference (p<0.05) between the breed averages within each area. Lowercase letters indicate a significant difference (p<0.05) between the CMT test averages within each evaluated area. The residual of the regression model was considered normal according to the *Shapiro-Wilk*.

For both measurements — front point vs. rear point (correlation 0.80; P<0.001) and front area vs. rear area (correlation 0.90; P<0.001) —, the correlations were high, positive, and significant (P<0.05). This indicates that the temperature of the front quarter was close to the results of the rear quarter.

4. Discussion

In this study, the thermal imaging of the udder was obtained between 05h00 and 08h00, when the ambient temperature and relative humidity (Table 1) were within the thermal comfort zone for animals. In this sense, Velasco-Bolaños et al.[15] found no influence from temperature in high-altitude tropical regions and Berry et al.[5] observed that the udder temperature remained within the detectable range for inflammation despite ambient temperature variations throughout the day. Despite that, both studies emphasized that the same might not be true for animals under extreme seasonal temperatures, suggesting the need for further research. Moreover, most studies referenced in the literature[5-7, 10, 11] were conducted in temperate climates. Still, our findings indicate the possibility of application in tropical climates as well.

The temperature could not be measured on the side of the rear udder quarters of some Jersey cows due to their position. As a result, the images were taken from the back, as done for Holstein cows by Colak et al.[6]. In the Gir breed, Porcionato et al.[14] noted that thermal imaging showed temperature differences on the udder skin. Furthermore, Poikalainen et al.[16] and Yang et al.[17] noted that the udder surface temperature is not dependent on the milking time but rather on the measurement time (pre- and post-milking) and is not related to udder quarters since they are independent.

In our study, within each breed, the temperatures detected by thermal imaging, whether from the front or rear quarter and assessed at the central point or over the area, were highly correlated (correlation 0.96; P<0.001). This finding means that temperature readings were consistent whether taken from a point or the entire area. Therefore, if there is any dirt on the udder in the field, a cleaner spot can be chosen for reading, a potential interference mentioned in the literature[4, 8, 11, 15]. While the temperatures between the front and rear quarters were similar within each breed, aligning with findings from Colak et al.[6], Poikalainen et al.[16], and Yang et al.[17], differences emerged between the breeds for both point and area assessments.

When considering only the breed effect within the rear point, the effect size was 30.04%. This outcome suggests that breed played a dominant role in the temperature average differences across all scenarios examined. Therefore, when using thermal cameras to identify cases of subclinical mastitis, the animal breed must be considered. Conversely, no studies in the literature have compared different breeds within a single research.

The Girolando breed, adapted to tropical climates, showed a lower mammary gland temperature than the Jersey breed (*Bos taurus*). This difference is attributed to Girolando's better heat dissipation, with more sweat glands and a lower metabolic heat rate[22]. Still, diagnosing subclinical mastitis was feasible in both breeds. Therefore, when interpreting thermal imaging results for mastitis, the breed's origin, whether *Bos taurus*, *Bos indicus*, or crossbred, should be considered.

Infrared thermography could not distinguish quarters with clinical mastitis based on Tamis test gradings, differing from findings by Hovinen et al.[10], Silva et al.[13], and Velasco-Bolaños et al.[15]. This inconsistency might have been caused by the small number of quarters with clinical mastitis. On the other hand, temperature differences between healthy quarters and those with clinical mastitis were not conclusive in both breeds studied, likely due to the low count of quarters with clinical mastitis. In contrast, some studies in the literature[4, 10] induced clinical mastitis using *E. coli* strains for detection. This low prevalence was because the herds in this study had good udder health, so the lack of clinical forms reduced the method's diagnostic potential.

For detecting subclinical mastitis as measured by the CMT, the technique proved efficient. There was a positive correlation between the CMT test grading and increased surface skin temperature in quarters with subclinical mastitis. This aligns with findings from Colak et al.[6], Polat et al.[7], Hovinen et al.[10], Zaninelli et al.[11], Chakraborty et al.[12], and Silva et al.[13]. However, it contrasts with the results described by Bortolami et al.[4], Porcionato et al.[14], and Velasco-Bolaños et al.[15], who found no temperature differences for subclinical mastitis.

Our study revealed that IRT detected subclinical mastitis in both front and rear quarters (correlation 0.80 at one point and 0.90 across the entire area; $P<0.001$). Silva et al.[13] also confirmed such an outcome. However, Wang et al.[20] pointed out a limitation: if a cow has mastitis in more than one udder quarter, the temperature difference between those sides might be reduced, leading to potential diagnostic errors for mastitis.

When considering the temperature difference between quarters with subclinical mastitis, on the front and rear sides, there were increases of 2.79 °C and 0.86 °C, respectively, compared to healthy quarters in Girolando cows, and 3.07 °C and 2.51 °C in Jersey cows. Other researchers reported skin temperature differences of 2.35 °C[7] or only 0.72 °C[8]. On the other hand, thermal imaging produced remarkably similar temperatures in point or whole area measurements, both in the front and rear quarters, when determining subclinical mastitis (CMT Test) in Girolando and Jersey breeds.

In the literature reviewed, each study used a different model of infrared thermography equipment and image capture method. However, the temperatures recorded in this study, regardless of the position, were similar to those in published works. Velasco-Bolaños et al.[15] mentioned a lack of standardization in thermal imaging, concerning capture distance and angle, anatomical location, and whether the view was front, rear, or side for both the front and rear quarters.

In this study, we found that the infrared thermography camera was easy to use, practical, precise, and quick for data collection, without needing to restrain the animals. This aligns with findings from Berry et al.[5], Colak et al.[6], Zheng et al.[9], Hovinen et al.[10], Chakraborty et al.[12], McManus et al.[18], and Neculai-Valeanu e Ariton[19], thus supporting the viability of using this new technology.

5. Conclusion

Infrared thermography shows promise for detecting subclinical mastitis in both Girolando and Jersey breeds. It offers a non-invasive, portable, and fast diagnostic tool in the field, with predictive diagnostic capabilities similar to those of the CMT. However, one must account for the distinct udder skin temperature differences between breeds. Breed origin, whether Jersey or Girolando, should be considered for a more accurate mastitis diagnosis. Whether measuring at a central point or across the entire mammary gland area, the method is effective for detecting subclinical mastitis in dairy breeds.

Conflict of interests

The authors declared no conflict of interest.

Authors contributions

Conceptualization: S. H. G. Wolf. *Data curation*: G. B. do Nascimento. *Formal analysis*: I. P. Ribeiro, P. H. D. Gonçalves, and G. B. do Nascimento. *Investigation*: I. P. Ribeiro, P. H. D. Gonçalves, M. S. Rodrigues, and S. H. G. Wolf. *Methodology*: G. B. do Nascimento and S. H. G. Wolf. *Project management*: I. P. Ribeiro and S. H. G. Wolf. *Visualization:* I. P. Ribeiro, R. S. Baptista, and J. R. L. Calil Filho. *Resources*: R. S. Baptista, J. R. L. Calil Filho, and S. H. G. Wolf. *Supervision*: A. Wolf and S. H. G. Wolf. *Writing (original draft)*: G. B. do Nascimento, A. Wolf, and S. H. G. Wolf. *Writing (proofreading and editing)*: A Wolf and S. H. G. Wolf

Acknowledgments

The authors would like to thank the Office of Research and Graduate Studies of the Adamantina University Center (FAI) for the Undergraduate Research Fellowship Program (PROBIC) and the Voluntary Undergraduate Research Program (PIVIC).

References

1. Santos RDL, Alessi AC. Patologia veterinária, 3ª ed. Grupo GEN; 2023. ISBN: 09788527738972.

2. Constable PD. Clínica veterinária - Um tratado de doenças dos bovinos, ovinos, suínos e caprinos. 11ª ed. Grupo GEN; 2020. ISBN-10: 8527736926.

3. Santos MV, Fonseca FL. Controle da mastite e qualidade do leite - desafios e soluções. São Paulo: Edição dos Autores; 2019. ISBN: 9788591591312.

4. Bortolami A, Fiore E, Gianesella M, Corro M, Catania S, Morgante M. Evaluation of the udder health status in subclinical mastitis affected dairy cows through bacteriological culture, somatic cell count and thermographic imaging. Pol J Vet Sci 2015; 18(4):799–805. https://doi.org/10.1515/pjvs-2015-0104

5. Berry RJ, Kennedy AD, Scott SL, Kyle BL, Schaefer AL. Daily variation in the udder surface temperature of dairy cows measured by infrared thermography: Potential for mastitis de-

tection. Can J Anim Sci 2003; 83(4):687–93. https://doi.org/10.4141/A03-012

6. Colak A, Polat B, Okumus Z, Kaya M, Yanmaz LE, Hayirli A. Short Communication: Early detection of mastitis using infrared thermography in dairy cows. J Dairy Sci 2008; 91(11):4244–8. https://doi.org/10.3168/jds.2008-1258

7. Polat B, Colak A, Cengiz M, Yanmaz LE, Oral H, Bastan A, Kaya S, et al. Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. J Dairy Sci 2010; 93(8):3525–32. https://doi.org/10.3168/jds.2009-2807

8. Sathiyabarathi M, Jeyakumar S, Manimaran A, Jayaprakash G, Pushpadass HA, Sivaram M, et al. Infrared thermography: A potential noninvasive tool to monitor udder health status in dairy cows. Vet World 2016; 9(10):1075–81. https://doi.org/10.14202/vetworld.2016.1075-1081

9. Zheng S, Zhou C, Jiang X, Huang J, Xu D. Progress on Infrared Imaging Technology in Animal Production: A Review. Sensors 2022; 18; 22(3):705. https://doi.org/10.3390/s22030705

10. Hovinen M, Siivonen J, Taponen S, Hänninen L, Pastell M, Aisla AM, et al. Detection of clinical mastitis with the help of a thermal camera. J Dairy Sci 2008; 91(12):4592–8. https://doi.org/10.3168/jds.2008-1218

11. Zaninelli M, Redaelli V, Luzi F, Bronzo V, Mitchell M, Dell'Orto V, et al. First evaluation of infrared thermography as a tool for the monitoring of udder health status in farms of dairy cows. Sensors 2018; 18(3):862. https://doi.org/10.3390/s18030862

12. Chakraborty S, Dhama K, Tiwari R, Yatoo MI, Khurana SK, Khandia R, et al. Technological interventions and advances in the diagnosis of intramammary infections in animals with emphasis on bovine population—a review. Vet Q 2019; 39(1):76–94. https://doi.org/10.1080/01652176.2019.1642546

13. Silva RAB, Pandorfi H, Luiz Pontes de Almeida G, Antônio de Assunção Montenegro A, da Silva MV. Spatial dependence of udder surface temperature variation in dairy cows with healthy status and mastitis. Rev Bras Saúde Prod Anim 2019; 20(1): 1-15. http://dx.doi.org/10.1590/s1519-99402001102019

14. Porcionato AM, Canata TF, Oliveira CEL, Santos, MV. Udder thermography of Gyr cows for subclinical mastitis detection. Rev Bras Eng Biossistemas 2009; 3(3), 251–257. https://doi.org/10.18011/bioeng2009v3n3p251-257

15. Velasco-Bolaños J, Ceballes-Serrano CC, Velásquez-Mejía D, Riaño-Rojas JC, Giraldo CE, Carmona JU, et al. Application of udder surface temperature by infrared thermography for diagnosis of subclinical mastitis in Holstein cows located in tropical highlands. J Dairy Sci 2021; 104(9):10310–23. https://doi.org/10.3168/jds.2020-19894

16. Poikalainen V, Praks J, Veermae I, Kokin E. Infrared temperature patterns of cow's body as an indicator for health control at precision cattle farming. Agro Res Biosystem Eng 2012, 1:187-194. https://agronomy.emu.ee/vol10Spec1/p10s121.pdf

17. Yang C, Li G, Zhang X, Gu X. Udder skin surface temperature variation pre- and post- milking in dairy cows as determined by infrared thermography. J Dairy Res 2018; 85(2):201–3. https://doi.org/10.1017/S0022029918000213

18. McManus R, Boden LA, Weir W, Viora L, Barker R, Kim Y, et al. Thermography for disease detection in livestock: A scoping review. Front Vet Sci 2022. https://doi.org/10.3389/fvets.2022.965622

19. Neculai-Valeanu AS, Ariton AM. Udder health monitoring for prevention of bovine mastitis and improvement of milk quality. Bioeng 2022; 9(11):608. https://doi.org/10.3390/bioengineering9110608

20. Wang Y, Li Q, Chu M, Kang X, Liu G. Application of infrared thermography and machine learning techniques in cattle health assessments: A review. Biosyst Eng 2023; 230:361–87. https://doi.org/10.1016/j.biosystemseng.2023.05.002

21. Schalm OW, Noorlander DO. Experiments and observations leading to development of California Mastitis Test. J Am Vet 1957; 130(5):199-207.

22. Pereira C.C.J. Fundamentos de bioclimatologia aplicados à produção animal. Belo Horizonte: FEPMVZ; 2005.

23. Haynes W. Bonferroni Correction. In: Dubitzky W, Wolkenhauer O, Cho KH, Yokota, H (eds) Encyclopedia of systems biology. New York:Springer; 2013. https://doi.org/10.1007/978-1-4419-9863-7_1213

24. Box GEP; Cox DR. An analysis of transformations. J Royal Soc 1964; 26(2):211-252.

25. Tomczak M, Tomczak E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. Trends Sport Sci 2014; 1(21):19-25.

26. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/