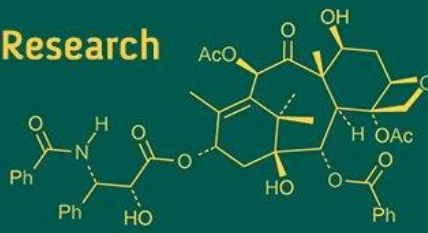


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Quantification of tick infestation in cattle using infrared thermal image processing

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Abstract

In tropical climates, tick infestations are common in bovines and cause significant economic losses worldwide. In this study, ticks were identified using Darvi TI analysis software on a Darvi DTL007 camera, based on the principle that they are cooler than the host animal's body surface temperature. An infrared thermography (IRT) image was auto-analysed for quantification of ticks. The study was conducted to quantify tick infestation using infrared thermography images, coupled with ImageJ-based auto-counting, and to compare the results with visual counting in 112 crossbred cattle on an organised farm. The results showed that the maximum temperature difference ($^{\circ}\text{C}$) was recorded between the tick and skin surface temperatures in the inner thigh region, followed by the perineum, dewlap, and lateral side of the body, where the temperature variation was significant ($p < 0.05$). The IRT image, coupled with auto-analysis in ImageJ, revealed that detection and quantification of ticks were most efficient in the inner thigh region, with no significant ($p < 0.01$) difference in tick count between manual counting and ImageJ analysis. In contrast, in the perineum, dewlap and lateral aspect, the auto-counting was not equally efficient. IRT image capture, coupled with automated analysis in ImageJ, would be an efficient and valuable tool for quantifying tick infestation in dairy animals.

Keywords: ImageJ, infrared thermography, quantification, tick

Introduction

Awareness of the economic losses associated with tick infestation remains limited; however, ticks and tick-borne diseases such as Tick pyemia, Toxicosis, Paralysis, Babesiosis, Theileriosis and Anaplasmosis are globally recognized as significant constraints in livestock production (Ragulraj, S *et al.*, 2023) ^[17]. Economic losses due to ticks primarily arise from reduced productivity, deterioration of hide quality and disease transmission (Gracia, 2003) ^[9]. Each tick causes an average live weight loss of 1.37 ± 0.25 grams in cattle (Jonsson, 2006; Holroyd and Dunster, 1978) ^[12, 10], and infestations of approximately 40 ticks per day can result in annual body weight losses of up to 20 kg per animal (Frisch *et al.*, 2000) ^[8]. The risk for younger animals is higher if their growth gets compromised at an early age due to stress; their growth and production over their lifetime are affected. The collective economic loss from these effects on the global livestock industry is in the billions of dollars (Rodriguez-Vivas *et al.*, 2014) ^[19]. Therefore, it is crucial to quantify the tick infestation level in dairy animals to make effective management decisions.

Ticks are the second most common vectors of infectious diseases worldwide, after mosquitoes, in both livestock and humans. Globally, these ectoparasites affect the livestock industry as approximately one billion bovines are at risk of infestation (Rodriguez *et al.*, 2011) ^[18]. Mortality incidence is less associated with tick infestation, but growth-and production-related losses are much more evident (Marcelino *et al.*, 2012) ^[15]. Generally, tick infestation assessment is carried out manually, which is labor-intensive and time-consuming. With technological advancements, researchers can measure the variation in surface temperature of the tick and the host animal using IRT. For the first time, Cortivo *et al.* (2016) ^[5] reported that the surface temperature of the host animal body is $>34^{\circ}\text{C}$, and the temperature of the tick body is 33°C to 34°C , and the difference between tick and skin surface temperature would be around 1°C . Therefore, there is a need to explore the potential Cortivo *et al.* (2016) ^[5] also compared visual count methods with an image analysis program for automated counting of ectoparasites (ticks and horn flies) captured via infrared thermography.

The visual count and thermography, coupled with an auto-counting program, were highly correlated for horn flies (0.82) and distal-side ticks (0.84), but a correlation of 0.32 was observed for lateral-side ticks. The variation may be due to technical hitches in counting small ticks (diameter < 4 mm), hair length, skin coat colour, and environmental temperature. The surface temperature difference between the tick and the animal's body is used as a principle for detecting ticks. Environmental temperature can also influence tick body temperature. So, it may affect the accuracy of the tick count. Long, excessive hair makes it difficult to distinguish between ticks. A lack of temperature contrast reduces the actual tick count in the animal body (Cortivo *et al.*, 2016) [5]. Sometimes the software may consider the cluster of ticks as a single tick. Capturing the angle of the IRT image can help improve the contrast between the cattle and the tick. Hair length interference can be mitigated by increasing the camera's sensitivity and resolution (Barbedo *et al.*, 2017) [3]. Barbedo *et al.* (2017) [3] also proposed a new algorithm based on colour differences using mathematical morphology operations. They reported that IRT images can provide a rough estimate of tick infestation level, which can be better quantified by manual or proposed algorithms. Still, accurate quantification remains difficult due to various extrinsic and intrinsic factors. It is worth exploring the optimal body location for accurate tick quantification using the IRT image, coupled with automated analysis using a programme under tropical climatic conditions. Further, the concept of detecting and quantifying tick infestation using Infrared Thermography and auto-counting ticks in ImageJ software in crossbred animals under tropical climatic conditions, accounting for differences in climate, was validated.

Materials and Methods

The study was conducted at the Livestock Research Centre (LRC) of the ICAR-National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana, at an elevation (altitude) of 250 meters above mean sea level, latitude 29°43" North and longitude 77°20" East. This is a tropical climate region, and the annual minimum and maximum temperatures range from 4 to 45 °C. During the hot-dry season (April to June), the relative humidity varies from 41 to 85% and the vapour pressure from 7.0 to 25 mmHg. Ethical approval (number 45-IAEC-19-3) was sought from the Institutional Animal Ethics Committee (IAEC) at ICAR-National Dairy Research Institute for the protocols followed and the animals' management in the experiment. A total of 112 crossbred animals aged 12-18 months, infested with ticks, were randomly selected. Then, ticks were counted manually, and values from visual and thermal images, coupled with automated analysis in ImageJ, were evaluated to determine the best region for capturing Infrared thermography images to detect ticks with higher accuracy. Surface temperature data from the animal and the tick were used to determine where the most significant temperature difference occurred between them.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 (IBM Corporation, Armonk, New York, USA). Data on tick and skin surface temperature were analysed by using an independent t-test. Then, the temperature difference between the tick and skin surface was analysed using one-way analysis of variance (ANOVA), followed by the post hoc Tukey test to compare the temperature differences between regions. The data on manual tick counts and ImageJ

analyses of tick counts across various regions were analysed using an independent t-test.

Results and Discussion

Predilection site of ticks in crossbred animals

The maximum population of ticks in crossbred animals was recorded in the lateral side, followed by the fore limb, inner thigh, brisket, perineum and abdomen region (Table 1). The variable results were documented regarding the predilection site of the ticks (Katuri *et al.*, 2013; Eyo *et al.*, 2014; Akthar *et al.*, 2019) [13, 6, 2] as the predilection site of tick infestation and site of tick attachment to the animal body varies with tick species (Abiso *et al.*, 2019; Huruma *et al.*, 2015; Fantahun and Abdul, 2012) [1, 11, 7], crowdedness of the host species, season, time and accessibility of grooming kit (Solomon and Kassa, 1998) [20]. Katuri *et al.* (2013) [13] reported maximum tick infestation under the scrotum, followed by the ano-vulva, perineum, dewlap and brisket region. Eyo *et al.* (2014) [6] reported that the inner thigh was most infested, followed by the armpit, udder, penis, scrotum and vulva in indigenous cattle. Akthar *et al.* (2019) [2] reported the maximum number of tick infestations in the inguinal region (310), followed by abdomen (300), head (200), tail (100) and thorax (40) in cattle. Apart from the lateral side of the body, the forelimb and inner thigh regions have a greater number of ticks because, in general, ticks prefer sites with thin skin, less hair, and a rich blood supply for easy penetration. Ticks prefer moist, warm sites as well as sites protected from environmental temperatures and from birds (Muchenje *et al.*, 2008) [16]. The odour of various predilection sites, especially the armpit and inner thigh, may also be an additional factor for higher tick infestation in the region (Wanzala *et al.*, 2004) [21].

The tick and skin surface temperature (°C) in various regions of the animal body

Based on the findings involving 112 animals, significantly ($p < 0.05$) higher skin surface temperatures (°C) in the dewlap (36.77 ± 0.16 vs. 36.06 ± 0.15), perineum (36.06 ± 0.13 vs. 34.95 ± 0.11), and inner thigh (35.75 ± 0.12 vs. 34.27 ± 0.13) regions were observed compared to the tick surface. In contrast, skin surface temperature was higher on the lateral side (35.93 ± 0.12 vs. 35.62 ± 0.12), but this difference was not significant (Figure 1). Cortivo *et al.* (2016) [5] also reported that tick surface temperature was lower compared to skin surface temperature. The scientific reason behind it has already been discussed in the present paper.

Difference between the tick and skin surface temperature (°C) in various regions of the animal body:

Based on the findings involving 112 animals, the maximum temperature (°C) difference recorded between the tick and skin surface temperature (1.48 ± 0.04) was in the inner thigh region, followed by perineum (1.11 ± 0.06), dewlap (0.71 ± 0.03) and lateral (0.30 ± 0.02) aspect of body ($p < 0.01$) (Figure 2). In a similar line, Cortivo *et al.* (2016) [5] reported that the temperature difference between the tick and the skin surface was approximately 1 °C. The scientific reason behind it has already been discussed in the present paper.

Tick count by Image J and manual of various regions of the animal body

No significant difference was recorded in tick count between Manual count and Image J analysis in the inner

thigh region (42.89 ± 1.59 vs. 43.83 ± 1.61). Still, it was found to be significantly ($p < 0.01$) different in dewlap (21.01 ± 1.59 vs. 15.04 ± 1.25), perineum (19.9 ± 1.32 vs. 15.58 ± 1.34) and lateral side (7.15 ± 0.94 vs. 4.47 ± 0.81) of the body (Figures 3 & 4). The correlation between visual count and IRT image, coupled with ImageJ analysis of the inner thigh region (0.85), dewlap (0.68), perineum (0.75), and lateral side (0.59), was estimated.

In a similar line, Cortivo *et al.* (2016) [5] reported the highest correlation (0.82) between Image J and manual tick count in the distal part of the animal body. This might be due to lower hair density and a greater surface-temperature difference between the tick and the animal's body in the inner thigh region. A significant difference ($p < 0.01$) of Image J count and manual tick count was found in dewlap, perineal region and lateral side of the body, further supported by the findings of Cortivo *et al.* (2016) [5] who reported lowest correlation (0.32) between Image J counting and Manual tick count in the lateral side of the animal body and Barbedo *et al.* (2017) [3] reported poor correlation (0.285) between visual count and developed algorithm in neck region. This might be due to the presence of a lengthy and dense hair coat, as well as direct environmental influences, causing a minor temperature difference between the tick and the skin coat; therefore, the lack of temperature contrast reduced the actual tick count (Cortivo *et al.*, 2016; Barbedo *et al.*, 2017) [5, 3]. The surface temperature difference between the tick and the animal's body was used as a principle for detecting ticks. Tick temperature may be influenced by environmental temperature, potentially affecting the accuracy of the tick count. A wide variation in tick surface temperature was also evident in the present study, but it was always lower than the animal's skin surface temperature. It had also been reported that an error in counting of ticks might be associated with the size of the ticks (less than 4 mm in diameter), skin and hair colour, as well as length of the hair (Cortivo *et al.*, 2016) [5]. Sometimes the software may consider a cluster of ticks as a single tick. Capturing the angle of the IRT image can help improve the contrast between the cattle and the tick. Hair length interference can be mitigated by increasing the camera's sensitivity and resolution (Barbedo *et al.*, 2017) [3]. The quality of the image in capturing the thermal signature of ticks may also influence tick quantification, and image quality can sometimes be compromised by the inevitable

movement of animals even after proper restraint (Barbedo *et al.*, 2017) [3]. Barbedo *et al.* (2017) [3] found a low correlation between visual counting of thermographic images and manual tick counts, corroborating our study. Besides that, no part of the body can be captured by IR images. The present study demonstrated that tick count using IRT image capture, coupled with automated analysis in ImageJ, especially in the inner thigh region, holds greater promise for identifying and quantifying ticks. Another benefit of assessing ticks using an IRT camera is that ticks may fall due to licking or scratching by the animal, as well as at different stages of the tick's life cycle; they may also be assessed, as demonstrated by Cortivo *et al.* (2016) [5].

Despite its shortcomings, IRT can serve as a valuable tool for detecting ticks across various body regions. The non-invasive nature, lower labour intensity, and easier counting are the key points of interest. The visual quantification of ticks has its own limitations; ticks smaller than 4 mm in diameter, skin colour of the animal, length and density of hair, and the skill of the observer may influence the actual tick infestation rate. Similar to our observation, IRT image capture and programme-based auto-analysis quantify tick and horn flies (Cortivo *et al.*, 2016) [5], and the filming method for horn fly quantification has been reported to be superior to visual count (Lima *et al.*, 2002) [14]. Image-based quantification of ticks has several advantages, as described by Cortivo *et al.* (2016) [5]. Besides, ticks can exhibit contrast due to temperature variation. IRT can also capture inflammation caused by the tick bite, as the tick may be detached from the animal's body before image capture due to licking, scratching, the tick's lifecycle stage, or the use of grooming brushes on commercial farms. The study's limitation is that the threshold for quantifying tick infestation in each image is independent, and tick species identification is another challenge.

Table 1: Predilection site of ticks in crossbred animals (n = 112)

Regions	Total Ticks $\pm$ S. E	Percentage (%)
Fore limb	108.41 ± 10.47	23.31
Inner thigh	87.66 ± 6.47	18.84
Brisket & Dewlap	85.16 ± 7.33	18.31
Perineum	27.78 ± 1.84	5.97
Lateral side	128.63 ± 16.25	27.65
Abdomen	27.54 ± 1.85	5.92
Total	465.17 ± 37.08	100

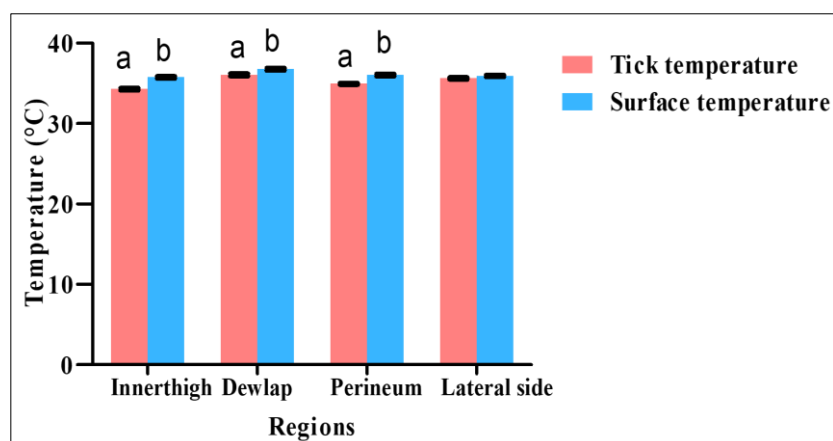


Fig 1: Tick and skin surface temperature (°C) in various regions of animal body and tick species identified; Bars with different superscripts differs significantly ^{ab}($p < 0.05$)

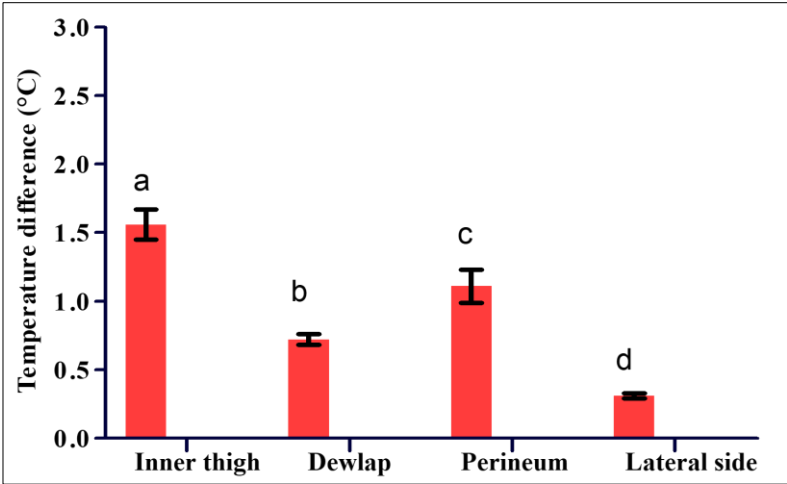


Fig 2: Difference between the tick and skin surface temperature (°C) in various region of animal body; Bars with different superscripts in a row differs significantly ^{ab}($p < 0.05$)

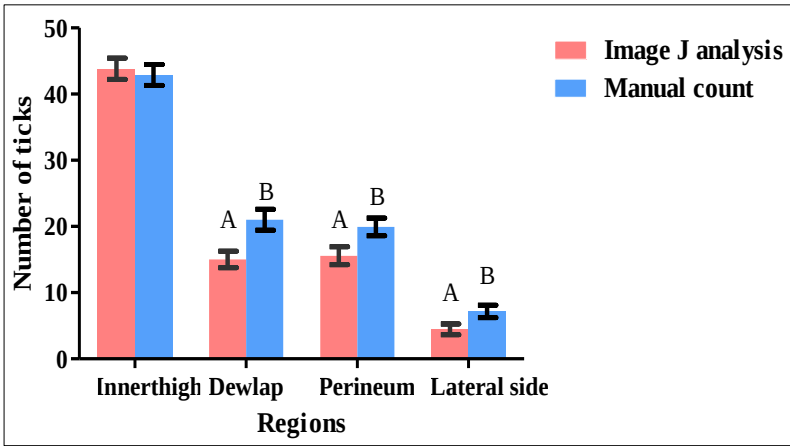


Fig 3: Tick count by Image J and manual of various regions of animal body; bars with different superscripts differ significantly ^{AB}($p < 0.01$)

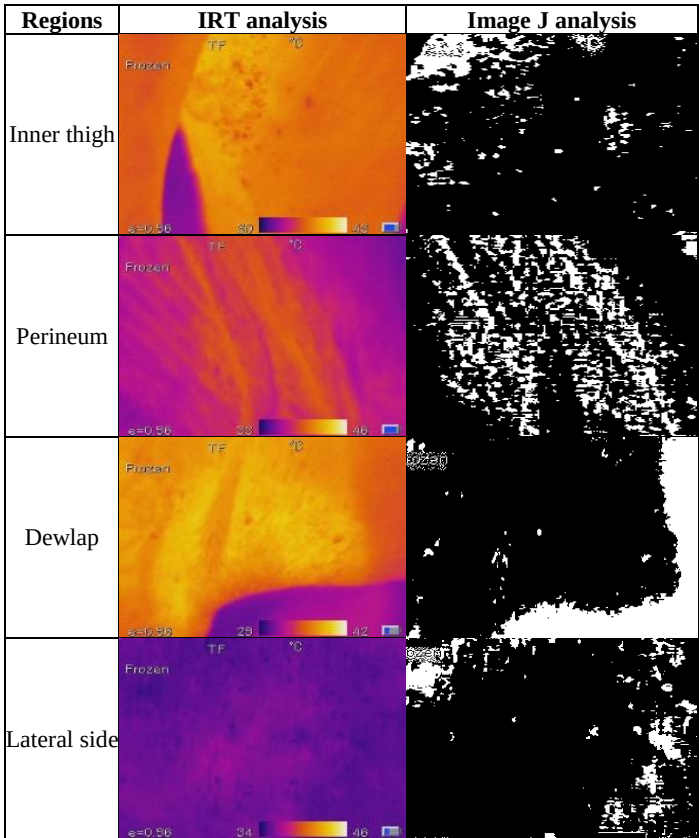


Fig 4: Infrared images and ImageJ analysis images of various regions in crossbred animal
~ 389 ~

Conclusion

The present study has identified that thermal imaging, coupled with automated analysis using ImageJ, is an effective method for accurate quantification of ticks in the inner thigh region of crossbred animals. The potential for accurate quantification in other areas, *e.g.*, the dewlap, perineum, and lateral body regions, is limited. Still, it can provide a rough estimate of the tick infestation rate. Another challenge lies in capturing images of various body parts by IRT, which can be accurately obtained by manual counting of ticks. But, given accuracy, time commitment, speed, workforce involvement, and precision, IRT image capture coupled with automated analysis in ImageJ has greater promise for identifying and accurately quantifying ticks in a specific region of the body. Further, the concept of capturing images across different seasons, various breeds, and all possible body parts to develop a prediction model based on a specific predilection site across a large number of animals is warranted. It can further open new dimensions in the existing automated image-based technologies, such as body condition scoring and lameness.

Conflict of interest

The authors have no conflicts of interest.

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