

Use of thermal imaging in dairy calves: exploring the repeatability and accuracy of measures taken from different anatomical regions¹

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ABSTRACT: Three experiments were undertaken to 1) quantify the repeatability and reproducibility of thermal imaging across day and operator experience and 2) assess the correlation between descriptive infrared (IR) temperature parameters from different anatomical areas and core body temperature in dairy calves under 12 wk of age. In experiment 1, a single operator captured 30 replicate images of both the left and right eyes (defined as the whole eye + 1 cm margin) and the rectal area (defined as the anus +1.5 cm margin) from each of 16 calves. In experiment 2, three operators of varying experience captured images from both the left and right eyes and the rectal area of each of 12 calves. In experiment 3, a single operator captured images of the right eye and rectal area for a period of 5 consecutive days for each of 205 calves. All images were captured between 0900 and 1300 h. Core body temperature, obtained via rectal thermometer, was recorded every day for each of the 205 calves following completion of IR image capture. Ambient temperature and relative humidity were adjusted for each thermal image prior to manual extraction of maximum, minimum, and average temperature parameters. In experiment 1, lowest error variance was found

within the maximum temperature parameter and the right eye was determined as the most repeatable anatomical area, with 80.48% of the total proportion of variance attributed to the calf. Results indicated that capturing at least three replicate images would provide the precision required to identify ill-health in calves. In experiment 2, operator variance was low across anatomical areas, with values of $\leq 0.01^{\circ}\text{C}^2$ for the right and left eyes and $\leq 0.04^{\circ}\text{C}^2$ for the rectal area. In experiment 3, day to day variation of thermal image measurements and core body temperature were minimal across anatomical areas with values of $\leq 0.008^{\circ}\text{C}^2$. Correlations ranging from 0.16 to 0.32, and from 0.31 to 0.47 were found between maximum eye and core body temperature and maximum rectal area and core body temperature, respectively. Results of the present study indicate a low level of variability and high level of repeatability within IR temperature measurements in calves under 12 wk of age, particularly within maximum temperature parameters. Providing operators of varying abilities with a basic standardized protocol is sufficient to limit between-operator variation. Further research is required to investigate whether correlation between IR and core body temperature can be improved.

Key words: accuracy, dairy calves, infrared thermography, repeatability, temperature assessment

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INTRODUCTION

Changes in body temperature have long been used as an indicator of injury, inflammation, or

infection in veterinary medicine (George et al., 2014); however, the use of temperature devices such as rectal thermometers and thermal microchips can be both invasive and time consuming (Johnson et al., 2011). Infrared thermography (IRT) is a remote sensing method which measures alterations in heat production and loss due to changes in blood flow as a result of stress or ill-health (McManus et al., 2016) and has previously been used to explore thermoregulatory processes in human medicine (Jones, 1998) and for the detection of health and welfare issues in farm animals (e.g., Stewart et al. (2007), Polat et al. (2010), and Schaefer et al. (2012)). In particular, infrared (IR) imaging of the eye in cattle has been highlighted as displaying the earliest response to disease challenge and as the body part providing the most consistent IR temperature measurement (Schaefer et al., 2004). Previous research (Knauer et al., 2016; Mahendran et al., 2017) involved the use of a clinical scoring system for diagnosis of ill-health in calves which utilized a predefined rectal temperature threshold ($\geq 39.5^{\circ}\text{C}$) for diagnosis of pyrexia (McGuirk and Peek, 2014). As such, accuracy and sensitivity are key if IRT is to be used as an early indicator of ill-health. In human medicine, application of IRT has shown high levels of repeatability (Petrova et al., 2018) and reproducibility when using predefined regions of interest and a strictly applied protocol for image recording and processing (Ammer, 2008). However, prior to Byrne et al. (2017), little work has assessed the reliability of IRT in farm animals and to the best of the authors' knowledge, no work has assessed the repeatability and reproducibility of IRT in dairy calves under 12 wk of age. The aim of this study, therefore, was to quantify the repeatability and reproducibility of thermal imaging in calves under 12 wk of age when conducted under conventional farm conditions. A further aim was to assess the correlation between descriptive IR temperature parameters of the eye and rectal areas and core body temperature. It was hypothesized that, with the use of a standardized protocol, operators of varying experience could obtain reliable and repeatable IR temperature measurements of the eye and rectal area of dairy calves under 12 wk of age.

MATERIALS AND METHODS

This study was conducted at the Agri-Food and Bioscience Institute (AFBI) research farm in Hillsborough, located in County Down, in Northern Ireland (latitude $52^{\circ}27'$, longitude $6^{\circ}4'$) with all calves sourced from the dairy herd. All

procedures and treatments within this study were conducted under a license from the Department of Health, Social Services, and Public Safety for Northern Ireland in accordance with the Animals (Scientific Procedures) Act 1986. The study was approved by the AFBI Animal Welfare and Ethical Review Body (AWERB).

Three experiments, based on the methodology described by Byrne et al. (2017), were designed to assess the repeatability of eye and rectal temperature as captured using IRT. Experiments 1 and 2 were carried out in October 2017 and experiment 3 was carried out between September 2015 and December 2016.

All images were captured using a calibrated, handheld FLIR E8 thermal camera (FLIR Systems UK, Kent, UK) which had a resolution of 320×240 pixels. The camera had a spectral range of between 7.5 and 13 μm , thermal sensitivity of $<0.06^{\circ}\text{C}$, and an accuracy of $\pm 2\%$ or 2°C . All images were captured when calves were within the rearing accommodation and out of direct sunlight between 0900 and 1300 h. Images were processed using FLIR Tools software (FLIR Systems UK, Kent, UK) with maximum, average, and minimum temperature of images recorded. For each experiment, ambient temperature and relative humidity were recorded on a daily basis using a calibrated EBI 20-TH data logger (ebro Electronic, Ingolstadt, Germany) situated inside the calf rearing accommodation. An average of values obtained during the corresponding time period of image acquisition was entered into the software program during image processing to allow for atmospheric changes during the sampling period.

Experiment 1

This experiment aimed to quantify the repeatability of eye and rectal area temperature as captured by thermal imaging camera and to determine the level of precision that could be achieved by capturing a predetermined number of image replicates. Byrne et al. (2017) defined precision as the 95% confidence interval range within which the average of the measured temperature was expected to lie relative to the average of 30 temperature measurements. As such, the same methodology was used to determine the level of precision that could be achieved in the present experiment for each of the anatomical regions.

Thirty replicate images of both the left and right eyes and the rectal area from each of eight male and eight female calves (17.5 ± 3.1 d of age) were captured by a single operator. Selected calves were

housed in one of two replicate, straw-bedded group pens of 15 calves and were offered milk replacer and solid concentrate feed via automatic feeders. All images of female calves were taken on the same day, approximately 1 h after calves had received their morning milk meal, with the same procedure repeated for the male calves at the same time the following day. Average ambient temperature within the calf house was 8°C ($\pm 0.28^\circ\text{C}$) across both days.

Prior to image capture, and in order to limit the need for operator handling of the calf, a smaller pen (approximately 1.5 m²) was made with hurdles in the corner of the group housing pen. Calves to be imaged were put into the smaller pen in pairs and allowed to acclimatize for 15 min prior to the commencement of imaging. All images were taken at a consistent distance of no less than 0.5 m and just off perpendicular to the anatomical region to be imaged whilst the calf was standing. An image of the calf ear tag was taken to allow identification of each set of individual images; this was followed by 30 consecutive images of the right eye and then 30 consecutive images of the left eye. Once image capture of the eyes was complete, the calf's tail was lifted and held aloft for approximately 1 min to allow dissipation of heat caused by the tail resting on the rectal area. Thirty consecutive images were then taken of the rectal area whilst the tail was raised. Images in which the eye was closed or the calf had moved suddenly causing the image to blur were discarded and a new image taken.

Experiment 2

This experiment aimed to quantify the within- and between-operator variability in thermal images of calves. A total of six female and six male calves (34.3 ± 2.4 d of age) were selected from the calves used in experiment 1. All images in experiment 2 were captured on a single day and image capture occurred within the same location and using the same pen setup as previously described in experiment 1. Images of the right and left eyes and rectal region of each individual calf were taken by three different operators of varying thermal imaging experience: an experienced operator, an operator with limited experience, and an operator with no previous experience of conducting thermal imaging. For the purposes of this study, an operator with limited experience was defined as one which had used the same model of IR camera to capture images of five calves on each of three previous occasions. Each operator was provided with the same standard operating procedure for image capture prior to

starting the experiment which indicated that images should be taken when calves were standing and at a distance of no less than 0.5 m and at an angle just off perpendicular to the anatomical area to be imaged. Operator order was randomly assigned prior to commencement of the experiment and this order was maintained for image capture of each individual calf. Each operator captured images of the required anatomical areas in the same order (i.e., right eye, left eye, and rectal region). Once the first operator had taken the required images, the operator left the pen and the camera passed to the second operator, with the same procedure followed for the third operator, thus ensuring that each operator did not observe any other operator. This procedure was repeated four times for each individual calf resulting in 12 images per calf for each operator.

Experiment 3

This experiment aimed to quantify the effect of day on repeatability of thermal imaging. Data used in this experiment were captured by a single operator between September 2015 and December 2016. Images of the right eye and rectal region were captured over 5 consecutive days for each of 205 individual calves (99 females and 106 males) which ranged from 5 to 77 d of age. Calves in this experiment were either group or individually housed throughout the rearing period. Image capture of group-housed calves occurred using the same method as described in experiments 1 and 2 within the calf-rearing accommodation. For individually housed calves, individual pens (0.9 by 1.8 m) were constructed using hurdles within two concrete-walled housing blocks. A base layer of woodchip was added to the floor of each pen with sawdust bedding material added on top and replenished on a daily basis. Within these pens, calves had visual contact with all other calves within the housing block and nose-to-nose contact with calves from neighboring individual pens. All images were taken whilst the calves were standing and using the same camera distance and angle as previously described in experiments 1 and 2. Core body temperature of each of these calves was taken on a daily basis immediately following image capture using a rectal thermometer (Model FT09, Beurer UK Ltd., Golborne, UK).

Image Analysis

Manual extraction of maximum, minimum, and average temperatures was undertaken using FLIR Tools software (FLIR Systems Inc., UK;

Kent, UK). For each image, emissivity was set to 0.98, and an average of ambient temperature and relative humidity of the calf-rearing accommodation obtained during the time period corresponding to image capture was entered into the software program during image processing to allow for atmospheric changes during the sampling period. Object distance for each anatomical view was set to 0.5 m.

Analysis of eye images focused on the medial, posterior, palpebral border of the lower eyelid, and the lacrimal caruncle as these have been found to be the area of most consistent temperature (Stewart et al., 2008b). The elliptical tool was used to encompass the whole eye plus a 1 cm margin surrounding the eye (Figure 1). The elliptical tool was also used for analysis of rectal area images, with the area for used for temperature extraction including a 1 to 1.5 cm margin surrounding the anus (Figure 2).

Statistical Analysis

All data were analyzed using GenStat (version 18.1, VSN International Ltd) using the methodology as described by Byrne et al. (2017).

Experiment 1. The between-calf and error variances were calculated for the maximum, minimum, and average temperature of the right and left eyes and rectal area using a residual maximum likelihood estimation (REML) mixed model with calf included as a random effect. Proportion of total calf variation (H_{Calf}) was calculated using the following equation:

$$H_{\text{Calf}} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}$$

Within the equation, σ_c^2 represented the between-calf variance and σ_e^2 represented the error variance. The coefficient of variation (CV) was calculated for each anatomical area and descriptive temperature parameter (i.e., maximum, minimum, and average temperature) using the following equation:

$$CV = \frac{\sigma_c}{\mu}$$

where σ_c represents the between-calf standard deviation and μ is the average of the temperature parameters of each of the anatomical regions being examined (i.e., right and left eyes and rectal area). The following equation, wherein n represented the number of image replicates between 1 and 30, was used to calculate the number of images required to achieve a certain precision (P_n) within a 95% confidence interval:

$$P_n = 1.96 \times \sqrt{\frac{\sigma_e^2}{n_{\epsilon(1,30)}}}$$

Temperature measurements for each anatomical area and descriptive parameter were averaged across image replicates for each calf in order to ascertain the stability of temperature measurements over time. Correlation coefficients between temperature measurements from the first image and all subsequent replicate images were then calculated using the Pearson's Product Moment correlation function in GenStat.

Experiment 2

The between-operator, between-calf, and error variances in descriptive temperature parameters

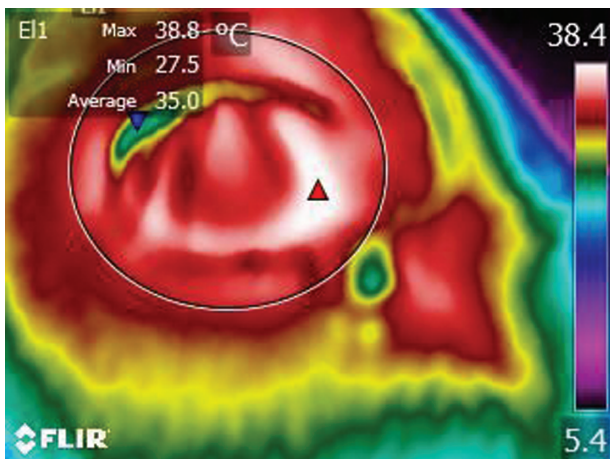


Figure 1. The white ellipse encompasses the area used to determine the maximum (▲), minimum (▲), and average IR temperature measurements of the eye.

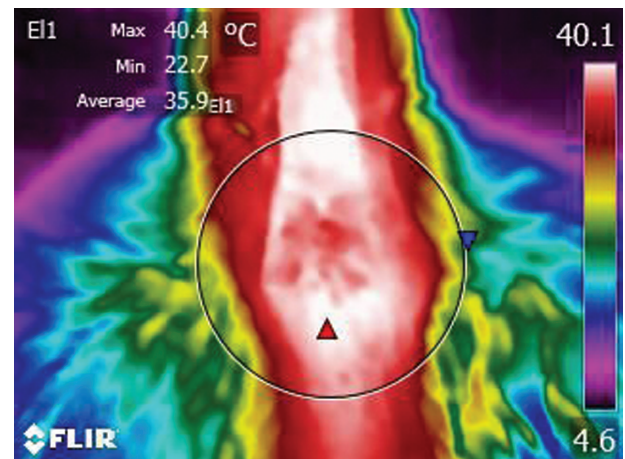


Figure 2. The white ellipse encompasses the area used to determine the maximum (▲), minimum (▲), and average IR temperature measurement of the rectal area.

(i.e., maximum, minimum, and average temperature) for each of the eye and rectal regions were calculated using a REML mixed model with both calf and operator included as random effects. Proportion of total variation as explained by operator ($H_{Operator}$) was calculated using the following equation, wherein σ_o^2 represented the between-operator variance, σ_c^2 the between-calf variance, and σ_e^2 the error variance:

$$H_{Operator} = \frac{\sigma_o^2}{\sigma_c^2 + \sigma_o^2 + \sigma_e^2}$$

The Pearson's Product Moment correlation function in GenStat was used to calculate correlations between each descriptive parameter for each of the anatomical areas.

Experiment 3

The between-day, between-calf, and error variances of maximum and average IR temperature of the right eye and rectal region and of core body temperature were determined using a REML mixed model with calf and day included as random effects. Proportion of total variation as explained by day (H_{Day}) was calculated using the following equation, wherein σ_D^2 represented the between-day variance, σ_c^2 the between-calf variance, and σ_e^2 the error variance,

$$H_{Day} = \frac{\sigma_D^2}{\sigma_c^2 + \sigma_D^2 + \sigma_e^2}$$

Correlation coefficients between descriptive temperature parameters and methods (i.e., IR eye and rectal maximum and average temperature and core body temperature) were determined using the Pearson's Product Moment correlation function in GenStat.

Following analysis of the entire dataset, the data were split into four different age bands to examine whether between-day and error variances, and correlations between temperature methods and descriptive parameters, were affected by calf age. The selected age bands were d5–14, d14–28, d28–56, and d56–77, these representing the first 2 wk of life, the period over which calves commonly received first vaccination, the preweaning period, and the postweaning period, respectively.

RESULTS

Experiment 1

Means (\pm s.d., °C) of the maximum, minimum, and average temperatures were 38.48°C (0.63), 28.45°C (1.89), and 35.11°C (0.80) for the right eye; 38.73°C (0.55), 29.02°C (1.57), and 35.13°C (0.59) for the left eye; and 39.44°C (0.50), 27.40°C (1.59), and 35.44°C (0.91) for the rectal area, respectively. Between-calf and error variance was greatest for the minimum temperature values across all anatomical regions. Greater between-calf variance was observed in the average temperature value of the rectal region in comparison to both the right and left eyes (Table 1). Error variances for maximum temperature were similar across each of the anatomical regions, with values of 0.08°C², 0.10°C², and 0.10°C² for the right and left eyes and rectal region, respectively (Table 1).

As shown in Table 1, a greater proportion of the total variation was ascribed to the calf for maximum temperature of the right eye (80.48%) compared with maximum temperature for both the left eye (67.02%) and the rectal area (62.08%). The lowest proportion of total variation apportioned to the calf for the right eye was observed in the average measurement (44.61%); however, in the left eye and

Table 1. Mean infrared temperature, variances, CV, and proportion of total variation apportioned to calf (H_{Calf}) for the various anatomical and temperature parameters (Temp. Parameter) in Exp. 1

| Anatomical region | Temp. Parameter | Mean, °C | Calf variance, °C ² | s.e. | Error variance, °C ² | s.e. | CV, % | H_{Calf} , % |
|-------------------|-----------------|----------|--------------------------------|-------|---------------------------------|-------|-------|----------------|
| Right eye | Maximum | 38.48 | 0.33 | 0.122 | 0.08 | 0.005 | 0.02 | 80.48 |
| | Minimum | 28.45 | 2.11 | 0.789 | 1.57 | 0.103 | 0.05 | 57.30 |
| | Average | 35.11 | 0.29 | 0.110 | 0.36 | 0.024 | 0.02 | 44.61 |
| Left eye | Maximum | 38.73 | 0.21 | 0.078 | 0.10 | 0.007 | 0.01 | 67.02 |
| | Minimum | 29.02 | 1.12 | 0.425 | 1.42 | 0.093 | 0.04 | 44.07 |
| | Average | 35.12 | 0.22 | 0.081 | 0.14 | 0.009 | 0.01 | 60.32 |
| Rectal area | Maximum | 39.44 | 0.16 | 0.061 | 0.10 | 0.007 | 0.01 | 62.08 |
| | Minimum | 27.40 | 0.74 | 0.292 | 1.83 | 0.120 | 0.03 | 28.73 |
| | Average | 35.44 | 0.56 | 0.208 | 0.30 | 0.020 | 0.02 | 65.24 |

Values derived from the images of the right eye, left eye, and rectal area of each of 16 calves aged 17.5 (\pm 3.1 d) d as captured by a single operator across two consecutive days.

rectal region, the lowest proportion of total variation attributed to calf was found within the minimum temperature measurements, with values of 44.07% and 28.73%, respectively. The CV ranged from 0.010% to 0.051% across the descriptive temperature parameters for each of the anatomical areas, with the greatest CV linked to minimum temperature and lowest to maximum temperature (Table 1). Maximum temperature yielded the most precise results across all anatomical regions with one image, with standard error values of $\pm 0.56^{\circ}\text{C}$, $\pm 0.63^{\circ}\text{C}$, and $\pm 0.62^{\circ}\text{C}$ for the right eye, left eye, and rectal area, respectively (Table 2). Precision of maximum temperature across anatomical areas was improved when five images were captured, with standard error values reduced by more than half to $\pm 0.25^{\circ}\text{C}$, 0.28°C , and 0.28°C for the right eye, left eye, and rectal area, respectively (Table 2). Lowest level of precision across all anatomical regions occurred within the minimum temperature parameter, with standard error values ranging from ± 2.33 to 2.65°C , ± 1.04 to 1.10°C , and ± 0.43 to 0.48°C for one, five, and thirty replicate images, respectively (Table 2).

Figures 3 and 4 display correlations between the first image and all subsequent image replicates for all anatomical areas for maximum and average temperature measurements. Within maximum temperature measurements, correlation between the first image replicate and all other image replicates ranged from 0.71 (replicates 1 and 30) to 0.92 (replicates 1 and 12), 0.37 (replicates 1 and 7) to 0.80 (replicates 1 and 2), and 0.35 (replicates 1 and 17) to 0.78 (replicates 1 and 5) for the right eye, left eye, and rectal area, respectively. Within average temperature measurements, correlation between the first image replicate and all other image replicates ranged from 0.24 (replicates 1 and 18) to 0.91 (replicates 1 and 9), 0.18 (replicates 1 and 20) to 0.82

(replicates 1 and 6), and 0.34 (replicates 1 and 9) to 0.81 (replicates 1 and 2) for the right eye, left eye, and rectal area, respectively.

Experiment 2

The mean (\pm s.d., $^{\circ}\text{C}$) maximum right eye temperature across all calves was 38.04°C (0.70) for the experienced operator, 38.23°C (0.73) for the limited experience operator, and 37.99°C (0.78) for the operator with no prior experience. The mean (\pm s.d., $^{\circ}\text{C}$) maximum left eye temperature across all calves was 37.97°C (0.75) for the experienced operator, 38.10°C (0.74) for the limited experience operator, and 38.07°C (0.63) for the operator with no prior experience. The mean (\pm s.d., $^{\circ}\text{C}$) maximum rectal area temperature across all calves was 39.32°C (0.65) for the experienced operator, 39.37°C (0.72) for the limited experience operator, and 39.38°C (0.65) for the operator with no prior experience.

Operator variance was $\leq 0.01^{\circ}\text{C}^2$ across all temperature parameters for the right and left eyes (Table 3). Operator variance for images of the rectal area was 0°C^2 , 0.44°C^2 , and 0.04°C^2 for the maximum, minimum, and average temperature measurements, respectively. Error variance across anatomical regions was greatest within minimum temperature measurements, this ranging from 1.77% to 2.98% (Table 3). Proportion of total variation explained by operator for the maximum temperature measurements was 1.72% and 0.09% for the right and left eyes, respectively (Table 3). Proportion of total variation explained by operator for the maximum, minimum, and average temperature measurements of the rectal area was 0%, 12.05%, and 6.88%, respectively (Table 3). The CV was 0% across temperature parameters for the right and left eyes and remained $\leq 0.02\%$ for temperature measurements of the rectal area (Table 3). Strong correlations existed between

Table 2. The standard error of one, five, and thirty image replicates for each anatomical area and temperature parameter (Temp. Parameter) in Exp. 1

| Anatomical region | Temp. Parameter | One image, $^{\circ}\text{C}$ | Five image replicates, $^{\circ}\text{C}$ | Thirty image replicates, $^{\circ}\text{C}$ |
|-------------------|-----------------|-------------------------------|---|---|
| Right eye | Maximum | 0.56 | 0.25 | 0.10 |
| | Minimum | 2.46 | 1.10 | 0.45 |
| | Average | 1.18 | 0.53 | 0.22 |
| Left eye | Maximum | 0.63 | 0.28 | 0.12 |
| | Minimum | 2.33 | 1.04 | 0.43 |
| | Average | 0.74 | 0.33 | 0.14 |
| Rectal area | Maximum | 0.62 | 0.28 | 0.11 |
| | Minimum | 2.65 | 1.19 | 0.48 |
| | Average | 1.07 | 0.48 | 0.20 |

Values derived from the images of the right eye, left eye, and rectal area of each of 16 calves aged $17.5 (\pm 3.1 \text{ d})$ d as captured by a single operator across two consecutive days.

the measurements of the right eye and left eye for maximum (0.63) and average (0.71) temperature. Moderate correlations existed between measurements of the right eye and rectal area for maximum (0.31) and average (0.39) temperature, and between measurements of the left eye and rectal area for maximum (0.42) and average (0.48) temperature.

Experiment 3

When examined across all animals and all days, the mean (\pm s.d., °C) maximum and average

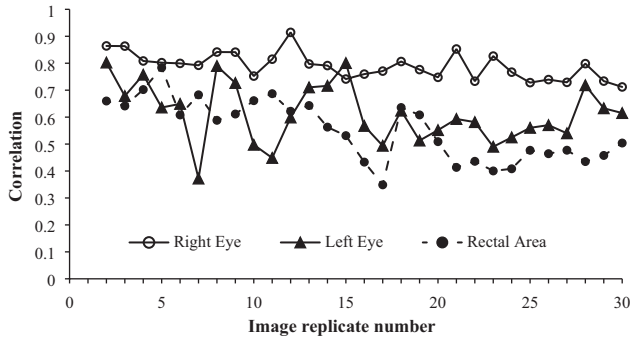


Figure 3. Correlation coefficients of maximum temperature values between the first image and all other replicate images from each of 16 calves as captured by a single operator in Exp. 1.

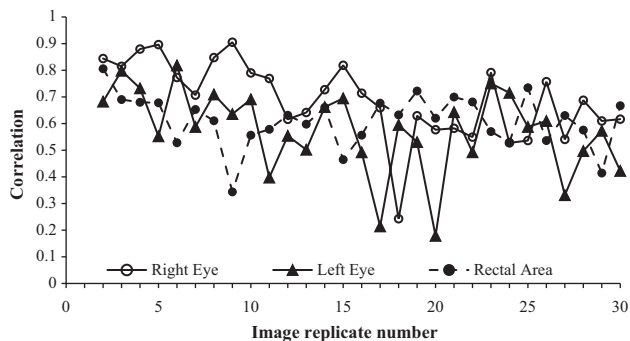


Figure 4. Correlation coefficients of average temperature values between the first image and all other replicate images from each of 16 calves as captured by a single operator in Exp. 1.

temperatures of the right eye were 38.77°C (\pm 0.84) and 35.34°C (1.27), respectively, and the mean (\pm s.d., °C) maximum and average temperatures of the rectal area were 39.79°C (\pm 1.02) and 35.73°C (1.77), respectively. Mean (\pm s.d., °C) core body temperature was 38.94°C (\pm 0.41) across all animals and all days. Correlation between eye and core body was 0.24 for maximum temperature measurements and 0.15 for average temperature measurements (Table 4). Similar results were found between core body and rectal area, with correlation coefficients of 0.38 for maximum and 0.16 for average temperature measurements (Table 4). Moderate correlation existed between the eye and rectal areas for both maximum (0.57) and average (0.51) temperature measurements (Table 4). The CV was \leq 0.004% for the core body, maximum eye, and maximum rectal area temperature measurements (Table 5). Variability in eye and rectal area temperature across days was lower for maximum temperature measurements than average temperature measurements (Table 5). The proportion of total variation as a result of day was less for maximum temperature measurements for both the eye and rectal area compared with average temperature measurements (Table 5).

The mean (\pm s.d., °C) maximum and average eye and rectal area temperatures and core body temperatures for calves from the selected age bands are reported in Table 6. Variability of core body temperature across days was \leq 0.006°C² for all age bands (Table 6). Variability for maximum eye temperature across days was 0.003°C², 0°C², and 0.001°C² between d5–14, d14–28, and d28–56, respectively (Table 6). Increased variability across days was found between d56 and 77 for both maximum average eye and rectal area temperature measurements (Table 6). The CV remained \leq 0.005 for both maximum and average eye and rectal area temperature

Table 3. Operator and error variances, CV, and proportion of total variation apportioned to operator (H_{Operator}) for the various anatomical and temperature parameters (Temp. Parameter) Exp. 2

| Anatomical region | Descriptive parameter | Operator variance, °C ² | s.e. | CV | H_{operator} , % | Error variance, °C ² | s.e. |
|-------------------|-----------------------|------------------------------------|--------|-------|---------------------------|---------------------------------|-------|
| Right eye | Maximum | 0.01 | 0.016 | <0.01 | 1.72 | 0.29 | 0.036 |
| | Minimum | <0.01 | <0.001 | <0.01 | <0.01 | 2.02 | 0.249 |
| | Average | 0.01 | 0.014 | <0.01 | 1.63 | 0.22 | 0.027 |
| Left eye | Maximum | <0.01 | 0.005 | <0.01 | 0.09 | 0.21 | 0.027 |
| | Minimum | <0.01 | <0.001 | <0.01 | <0.01 | 1.77 | 0.218 |
| | Average | <0.01 | <0.001 | <0.01 | <0.01 | 0.22 | 0.027 |
| Rectal area | Maximum | <0.01 | <0.001 | <0.01 | <0.01 | 0.11 | 0.013 |
| | Minimum | 0.44 | 0.505 | 0.02 | 12.05 | 2.98 | 0.370 |
| | Average | 0.04 | 0.050 | 0.01 | 6.88 | 0.25 | 0.031 |

Values derived from the images of the right eye, left eye, and rectal area of each of 12 calves aged 34.3 (\pm 2.4 d) as captured on a single day by an operator of experience, limited experience, and no experience.

Table 4. Pearson correlation coefficients between descriptive temperature parameters and methods (i.e., IR eye and rectal area maximum and average temperature and core body temperature) in Exp. 3

| Temperature parameter | Correlation coefficients | | | | |
|-----------------------|--------------------------|-------------|---------------------|-------------|---------------------|
| | Age range | Maximum eye | Maximum rectal area | Average eye | Average rectal area |
| Core body | d5–14 | 0.16 | 0.31 | 0.06 | 0.17 |
| | d5–77 | 0.24 | 0.38 | 0.15 | 0.16 |
| | d14–28 | 0.21 | 0.32 | 0.17 | 0.23 |
| | d28–56 | 0.31 | 0.47 | 0.23 | 0.23 |
| | d56–77 | 0.32 | 0.38 | 0.21 | 0.16 |
| Maximum eye | d5–14 | | 0.60 | 0.63 | 0.50 |
| | d5–77 | | 0.57 | 0.73 | 0.51 |
| | d14–28 | | 0.59 | 0.71 | 0.52 |
| | d28–56 | | 0.54 | 0.72 | 0.45 |
| | d56–77 | | 0.56 | 0.75 | 0.44 |
| Maximum rectal area | d5–14 | | | 0.39 | 0.71 |
| | d5–77 | | | 0.46 | 0.68 |
| | d14–28 | | | 0.44 | 0.72 |
| | d28–56 | | | 0.43 | 0.66 |
| | d56–77 | | | 0.46 | 0.61 |
| Average eye | d5–14 | | | | 0.47 |
| | d5–77 | | | | 0.54 |
| | d14–28 | | | | 0.44 |
| | d28–56 | | | | 0.44 |
| | d56–77 | | | | 0.56 |

Values derived from the core body temperature (as measured by rectal thermometer) and infrared images of the right eye and rectal area as captured by a single operator over five consecutive days for each of 205 calves aged between 5 and 77 d.

Table 5. Day variances, CV, and proportion of total variation apportioned to day (H_{Day}) for the various anatomical and temperature parameters (Temp. Parameter) in Exp. 3

| Anatomical region | Descriptive parameter | Day variance, °C ² | s.e. | CV | H_{Day} , % |
|-------------------|-----------------------|-------------------------------|-------|-------|---------------|
| Core body | | 0.006 | 0.001 | 0.002 | 3.48 |
| Eye | Maximum | 0.022 | 0.006 | 0.004 | 2.75 |
| | Average | 0.204 | 0.039 | 0.013 | 12.43 |
| Rectal area | Maximum | 0.030 | 0.008 | 0.004 | 2.60 |
| | Average | 0.309 | 0.061 | 0.016 | 9.65 |

Values derived from the core body temperature (as measured by rectal thermometer) and infrared images of the right eye and rectal area as captured by a single operator over five consecutive days for each of 205 calves aged between 5 and 77 d.

measurements between d5–14, d14–28, and d28–56 and ranged from 0.002 to 0.007 during d56–77 (Table 6). The CV for core body temperature was ≤0.002 across all age ranges (Table 6). Proportion of total variation as a result of day ranged from 0% to 3.31% for core body temperature across age ranges (Table 6). An increased proportion of total variation as a result of day for both maximum and average eye and rectal area temperature measurements was found between d56 and 77 compared with all other age ranges (Table 6). Weak-to-moderate correlations, ranging from 0.16 during d5–14 and 0.32 during d56–77, were found between maximum eye temperature and core body temperature (Table 4). Correlation between core body temperature and maximum rectal area temperature

ranged from 0.31 to 0.47, with highest correlation found during d28–56 (Table 4). Moderate correlations were found between maximum eye and rectal area temperatures, with these ranging from 0.54 to 0.60 (Table 4).

DISCUSSION

Changes in core body temperature are often used as an indicator of ill-health, with pyrexia in calves one of the primarily detectable physiological responses to infection and inflammation (Mahendran et al., 2017). Stress, a result of common management practices in early calthood, can also result in increased hypothalamic–pituitary–adrenal (HPA) axis activity, thus causing changes in heat

Table 6. Day variances, CV, and proportion of total variation apportioned to day (H_{Day}) for the various anatomical and temperature parameters (Temp. Parameter) separated into four age ranges in Exp. 3

| Age range | Anatomical region | Descriptive parameter | Mean temperature, °C | s.d. | Day variance, °C ² | s.e. | CV | H_{day} , % |
|-----------|-------------------|-----------------------|----------------------|-------|-------------------------------|-------|-------|----------------------|
| d5–14 | Core body | | 38.92 | 0.39 | 0.005 | 0.004 | 0.002 | 3.31 |
| | Eye | Maximum | 38.57 | 0.86 | 0.003 | 0.006 | 0.001 | 0.34 |
| | | Average | 34.76 | 1.29 | 0.033 | 0.032 | 0.005 | 1.92 |
| | Rectal area | Maximum | 39.28 | 1.15 | 0.021 | 0.018 | 0.004 | 1.41 |
| Average | | 34.75 | 1.99 | 0.000 | 0.000 | 0.000 | 0.00 | |
| d14–28 | Core body | | 38.96 | 0.36 | 0.001 | 0.001 | 0.001 | 0.86 |
| | Eye | Maximum | 38.55 | 0.92 | 0.000 | 0.000 | 0.000 | 0.00 |
| | | Average | 34.86 | 1.26 | 0.017 | 0.014 | 0.004 | 1.03 |
| | Rectal area | Maximum | 39.59 | 1.08 | 0.008 | 0.008 | 0.002 | 0.60 |
| Average | | 35.15 | 1.74 | 0.035 | 0.028 | 0.005 | 1.10 | |
| d28–56 | Core body | | 38.99 | 0.46 | 0.006 | 0.003 | 0.002 | 2.90 |
| | Eye | Maximum | 38.74 | 0.80 | 0.001 | 0.003 | 0.001 | 0.14 |
| | | Average | 35.32 | 1.14 | 0.000 | 0.000 | 0.000 | 0.00 |
| | Rectal area | Maximum | 39.91 | 1.00 | 0.004 | 0.005 | 0.002 | 0.37 |
| Average | | 35.72 | 1.59 | 0.000 | 0.000 | 0.000 | 0.00 | |
| d56–77 | Core body | | 38.85 | 0.35 | 0.000 | 0.000 | 0.000 | 0.00 |
| | Eye | Maximum | 39.05 | 0.75 | 0.032 | 0.014 | 0.005 | 5.47 |
| | | Average | 35.94 | 1.18 | 0.056 | 0.026 | 0.007 | 4.06 |
| | Rectal area | Maximum | 39.99 | 0.86 | 0.008 | 0.007 | 0.002 | 1.02 |
| Average | | 36.57 | 1.60 | 0.066 | 0.033 | 0.007 | 2.58 | |

Values derived from the core body temperature (as measured by rectal thermometer) and infrared images of the right eye and rectal area as captured by a single operator over five consecutive days for each of 205 calves.

production due to blood flow responses (McManus et al., 2016). However, common methods of assessing stress can be invasive and potentially confound results (Stewart et al., 2005). Both stress and disease challenge can reduce immunocompetence and thus have an adverse effect on animal welfare and economic efficiency (Schaefer et al., 2004; Hulbert and Moisa, 2016). This means that the development of methods which can help in the remote and early detection of clinical disease and in the identification of stress as a result of common management practices throughout the early rearing period is of great importance (Theurer et al., 2013).

Within- and Between-Animal Variances

Results of previous studies involving equines (Johnson et al., 2011), cattle (Schaefer et al., 2012), sheep (George et al., 2014), and pigs (Cook et al., 2015) have highlighted IRT as a sensitive, noninvasive method of measuring change in temperature as a result of pathological or physiological factors. Previous work (Johnson et al., 2011; Hoffmann et al., 2013) has indicated that the use of the maximum temperature values improves sensitivity and specificity of results. In the present study, maximum temperature values of the eye and rectal areas were associated with the lowest error variance when compared with minimum and average temperature

measurements, in accordance with Byrne et al. (2017), which indicates a greater level of precision with the use of maximum temperature values.

Okada et al. (2013) reported on the effects of various extraneous environmental and physical factors on IR temperature measurements, including distance from the object to be imaged and ambient temperature. In the present study, a large proportion the total variance was attributed to the calf for maximum temperature values of the eye and rectal area, and this was particularly evident for the right eye. As indicated by Byrne et al. (2017), a large between-animal variance is considered preferable as it means that the temperature of the animal itself has a greater impact on recorded IR temperature than peripheral environmental factors present during image capture. Little work has recorded IR eye temperature values in very young calves, and, to the best of the authors' knowledge, no previously published research has reported IR rectal area temperatures of young calves; however, maximum IR eye temperature values in the present study are within $\leq 1^\circ\text{C}$ of the range reported by Stewart et al. (2008b, 2010), whose research involved 6- to 16-wk old calves. The reason for the 13.46% difference in between-calf variance between the right and left eyes is unknown; however, the operator was right-handed and reported an increased ease of acquisition of images of the right eye, which may have contributed to the difference.

Precision of IR Images

Schaefer et al. (2012) reported a 1.2°C increase in core body temperature and 0.9°C in maximum orbital IR temperature in calves diagnosed as true positive for bovine respiratory disease (BRD) when compared with those diagnosed as BRD negative calves. As difference in maximum orbital IR temperature between disease positive and negative animals can be minimal, if IRT is to be used as health screening tool, it is of high importance that IR measurements are precise. In the present study, maximum temperature measurements of all anatomical areas resulted in the most precise results, with images of the right eye showing particular consistency over the 30 image replicates. As expected, precision improved with increasing repetition, whereby the standard error of a single measurement was reduced by half when the average of five replicates was taken and by half again when the average of 30 measurements was taken. However, if IRT is to be of practical on-farm use, it is important to determine the number of replicates required to obtain sufficient precision for the diagnosis of ill-health. From the results of the present study, we can determine that if only one image was captured across anatomical regions, it is possible for the maximum temperature of two image replicates to differ by over 1°C. As previously discussed, differences in maximum IR orbital temperature between sick and healthy calves have been reported as being below 1°C (Schaefer et al., 2012), meaning that one image does not offer sufficient precision. If using the average value obtained from five replicate images, the potential difference between replicates reduces to below 0.6°C. In agreement with results of Byrne et al. (2017), results of the present study suggest that capturing at least three image replicates would offer the required level of precision. In the present study, images were captured using a handheld camera, meaning that increased replication resulted in increased labor time. As such, if IR were to be used in a practical farm setting and image replication were to be included as part of the protocol, it may be beneficial for the IR camera to be automated and incorporated into a drinking water station such as that described by Schaefer et al. (2012).

Within- and Between-Operator Variances

As previously discussed, IR temperature can be affected by various external factors (Church et al., 2014). On-farm investigations have the potential to involve different housing systems, varying

environmental conditions, and IR camera operators of a wide range in ability, all of which have the potential to reduce the effectiveness of IRT. Implementation of an imaging protocol, similar to that used in IRT investigations in humans (Ammer, 2008), could help standardize the imaging process and minimize variation between images and operators. In the present study, between-operator variance had a limited effect on IR temperatures, and this was particularly evident for images of the right and left eyes. This indicates that providing operators of varying experience with a basic protocol which outlined an approximate distance and angle from the animal to be imaged was adequate to provide image standardization between operators. As highlighted by Byrne et al. (2017), development of specific on-farm protocols for imaging of various anatomical areas could help further reduce the potential for variation and improve the effectiveness of IRT.

Within- and Between-Day Variances

As discussed, measuring rectal temperature is considered a standard method of monitoring animal health status in veterinary medicine (Hoffmann et al., 2013). Previous research by Burfeind et al. (2010) reported a high level of repeatability in rectal temperature measurements of dairy cows. However, manual acquisition of rectal temperature can be laborious for the producer and disruptive for the animal, and the procedure itself, the type of thermometer used, and the depth of thermometer insertion can result in temperature variation of $\pm 0.5^\circ\text{C}$ (Burfeind et al., 2010). Results of the present study were similar, with standard deviation of core body temperature, as measured by rectal thermometer, peaking at $\pm 0.46^\circ\text{C}$ and variability across days remaining low. This indicates a high level of repeatability of core body temperature measurements in calves between 5 and 77 d of age.

Although previously published studies have measured IR temperatures across days (e.g., Schaefer et al. (2012)), they have not included measures of repeatability. In the present study, when all data from calves aged between 5 and 77 d of age were analyzed, maximum temperature measurements yielded the least variation across days, this again highlighting maximum temperature as the most precise measurement. When analyzed over the various age bands, increased variation in IR temperature across days was found in calves aged between 56 and 77 d, which is the period when weaning generally occurred. In the present study, 62.9% of

calves were weaned abruptly at 8 wk of age, with the remaining 37.1% weaned gradually from 56 d of age with complete milk replacer withdrawal occurring at 70 d of age. [Freetly et al. \(2006\)](#) previously indicated that mature cows undergoing periods of feed restriction display a reduction in heat production due to the associated changes in metabolic rate and physiological demands. In addition to this, previous research has indicated that weaning can cause changes in behaviour and levels of activity ([Weary et al., 2008](#)). As environmental and physiological changes can affect body temperature ([Carroll et al., 2012](#)), it is possible that the increased variation in IR temperature across days during this time could be as a result of the combination of physiological, nutritional, and environmental stressors encountered during the weaning period. This highlights the need for further research to examine factors affecting IR temperature in young calves.

Correlation Between Temperature Methods

If IRT is to be considered as a reliable method of measuring body temperature, then it is important to capture images where the surface temperature corresponds to changes in core temperature ([Johnson et al., 2011](#)). Again, no previously published research has addressed the correlation between IR and core body temperature in very young calves; however, correlation between IR eye temperature and core body temperature was similar to that found in Jersey heifers by [Salles et al. \(2016\)](#), but lower than that found in sheep and multiparous cows as reported by [George et al. \(2014\)](#). Reasons for the lower correlation in animals in the present study are unknown; however, as stress can affect IR temperature, the various stressors encountered in early calthood could potentially play a role.

[Nogami et al. \(2013\)](#) and [Hill et al. \(2016\)](#) used wireless data loggers taped to the skin over the tail vein of young calves to examine the relationship between tail skin and rectal temperature, reporting correlations of 0.80 and 0.61, respectively. In the present study, IR rectal area maximum temperature measurements are within the range of tail skin measurements as reported by [Hill et al. \(2016\)](#); however, correlations between maximum IR rectal area temperature and core body temperature peaked at 0.47. As IR rectal temperature is an external measurement, the captured temperature could be affected by activities affecting the proximity of the tail to the rectal area, such as tail-wagging and defecation ([Nogami et al., 2013](#)). Measuring IR rectal area temperature in the present study involved

lifting the calves' tails, whereas research using the tail skin temperature loggers did not require any animal handling during temperature acquisition. Handling methods have previously been shown to affect IR temperature in cattle ([Stewart et al., 2008a](#)); therefore, the process of lifting the tail during imaging could be another reason for the lower correlation between IR rectal area and core body temperature when compared with tail skin and core body temperature. Although a certain level of animal handling is required during IR rectal area temperature acquisition, the procedure itself remains less invasive than insertion of a rectal thermometer and results of the present study suggest that it has the potential to be used as a tool for immediate temperature assessment in young calves.

Results of the study provide evidence of correlation between IR and core body temperature of young calves. However, temperature regulation in young calves is metabolically immature ([NRC, 2001](#)) and can be affected by metabolic changes as a result of physiological and environmental stressors ([Carroll et al., 2012](#)). As such, if it is to be used as a health assessment tool, it may be of more benefit to map IR temperature of individuals on a daily basis to develop a baseline IR temperature from which deviations can be identified.

CONCLUSION

Results of the present study indicate a low level of variability and high level of repeatability within IR temperature values in young calves. As expected, precision of image capture is improved with an increased number of image replicates. In accordance with previous work, results of the present study indicate that capturing at least three image replicates would achieve the precision required to distinguish ill-health in young dairy calves. Providing operators of varying abilities with a basic standardized protocol is sufficient to limit between-operator variation. Further research is required to investigate whether correlation between IR and core body temperature can be improved, and if IR temperature thresholds can be developed to allow identification of pyrexia in young calves.

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