A comprehensive evaluation of microchips to measure temperature in dairy calves

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ABSTRACT

Elevated temperature is often an indicator of an immune response and used in the diagnosis of illness in dairy calves; however, measuring rectal temperature is labor intensive and often not measured daily on the farm. The objective of this study was to measure body temperature using a microchip and determine an appropriate implant site that would passively read body temperature in dairy calves. First, the precision of the temperature microchips and the rectal thermometer were tested *ex vivo*. Then, Holstein bull calves ($n = 12$) at 14 ± 12 d (mean $\pm$ SD) of age were implanted with microchips subcutaneously by the scutulum of the ear, subcutaneously in the upper scapula (SCAP), and intramuscularly in the trapezius muscle of the neck. One week after implantation, a temperature reading was taken for every microchip implant site using a radiofrequency ID (RFID) reader, as well as rectally and in the tympanic membrane using a digital thermometer every 60 min for 24 h in each calf (hereafter, the hourly study). Additionally, microchip readings and rectal temperatures were taken daily at 0800 h from 8 wk of age ($n = 9$; 57 ± 12 d of age) until 2 wk postweaning for a subset of the bull calves used in the hourly study (hereafter, the daily study). In the *ex vivo* trial, the microchip readings were very highly correlated with the rectal thermometer ($r = 0.96$), and the average coefficient of variation between microchip readings was very low ($0.12 \pm 0.03\%$). The relationships between the microchip readings within ear, SCAP, and neck and rectal and tympanic temperatures were analyzed using Pearson correlations and Bland-Altman plots. The ear and neck readings were strongly correlated for the hourly study [individual animal correlation; median (Q1, Q3), $r = 0.78$ (0.73, 0.84)] and for the daily study [$r = 0.79$ (0.73, 0.89)] across calves. However, rectal temperature was not significantly correlated with ear, SCAP, neck, or tympanic temperature for the daily and

hourly studies. Results suggest that temperature microchips measure temperature appropriately, but temperature is dependent upon the implant site in calves, and temperature measured at ear, SCAP, and neck implant sites cannot be used to estimate rectal temperature. Future research should determine thresholds for fever that are specific to implant sites in calves.

Key words: fever, precision dairy technology, thermometer

INTRODUCTION

Measuring temperature is often used to assess the health status of cattle. When an immune response occurs, body temperature often increases to combat infection (see review by Hart, 1988). Indeed, an increase in temperature that deviates by approximately 1.5°C from the normal baseline is an indicator of an immune response and a possible disease bout in calves (McGuirk and Peek, 2014). Automation of data collection that can be used to detect deviations during the early stages of a disease bout are fundamental to the development of functional precision technology tools. It is necessary to assess temperature technologies that are highly repeatable and accurate for measuring temperature in calves. One repeatable method of measuring temperature in calves is using a rectal thermometer, which is currently considered the gold standard but has only been validated for repeatability in adult cattle (Burfeind et al., 2010). Additionally, taking a rectal temperature requires manual restraint of the animal, and rectal temperatures can vary with degree of nervousness exhibited by the calf (Fisher et al., 2014) and genetic variation in the population (Dikmen et al., 2012). Therefore, alternate methods for measuring temperature in calves should be investigated.

One alternative technology that can measure temperature is radiofrequency identification (RFID) microchips, which can be injected into an animal either subcutaneously or intramuscularly to provide an identification number and a temperature. Research has shown that a microchip implanted behind the ear had lower standard deviations in temperature measurement

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compared with that implanted halfway between the neck and withers in 12 calves (Ude et al., 2010). However, a limitation to Ude et al. (2010) was that correlations and biases were not calculated. To appropriately assess the accuracy of this microchip technology, correlations and biases need to be assessed. Previous work has shown that under immune challenged conditions (i.e., LPS challenge), microchips implanted behind the ear had a moderate correlation with rectal temperature but not with microchips implanted behind the poll and within the umbilical fold in 4 weaned steers (Reid et al., 2012). Similar findings in adult cattle showed a moderate correlation between rectal temperature and microchips implanted behind the ear, but not with those implanted in the upper neck, the udder, or the shank in 5 lactating cows (Huťu et al., 2009); however, repeatability and correlations between implant sites were not calculated. Furthermore, temperature assessment via microchip implantation in the ear was accurate, precise, and repeatable in pigs (Lohse et al., 2010). Together, these studies suggest that the ear may be a reliable microchip implantation site for estimating temperature in dairy calves.

Promising relationships for temperature microchips in relation to rectal temperature have also been associated with implants in the upper scapula in cattle. For example, Lee et al. (2016) observed that the upper scapula, compared with lower scapula or neck implants, had the smallest mean difference in temperature from rectal temperature across the circadian temperature rhythm of 7 Holstein steers. Moreover, in horses, microchip implant sites in the neck were observed to have minimal deviations in temperature between individual animals, although correlations between neck and rectal temperature were negligible to low (Holje, 2012). Thus, in addition to the ear, the upper scapula and neck are implantation sites worth investigating in dairy calves, as is the influence of the individual on temperature estimation at each of these sites.

Although rectal temperature is considered the gold standard for measuring temperature in cattle (Burfeind, et al., 2010), tympanic membrane temperature is the only validated measure of core body temperature in calves (Wiersma and Stott, 1983). However, tympanic temperature has primarily been used as an indicator of stress response by measuring temperature response to environmental stressors (i.e., heat stress) in cattle (Guidry and McDowell, 1966; Hahn et al., 1990, 1992; Macauley et al., 1995). To our knowledge, limited research is available on the relationship between tympanic temperature and temperature reported by implanted microchips, and updated research is needed on the relationship between tympanic temperature and rectal temperature in calves.

Our study objective was to evaluate the use of microchips to estimate the body temperature of dairy calves. We determined the accuracy and repeatability of temperature microchips between calf and implant site and across time for microchips implanted subcutaneously and intramuscularly in dairy calves.

MATERIALS AND METHODS

Bull calves ($n = 12$; 14 ± 12 d of age) were sourced from one commercial dairy herd and raised at the University of Kentucky's Coldstream Research Dairy facilities (Lexington) under the University of Kentucky's Institutional Animal Care and Use Committee IACUC (2018–2971). All calves were assessed for passive transfer after arrival to the research facility. Passive transfer was defined as greater than 8.4% Brix (Bielmann et al., 2010).

Farm Management Procedures

Calves were placed in pens containing an automated milk feeder (DeLaval, Combi, Tumba, Sweden), a calf starter feeder (DeLaval) with calf starter (Baghdad Feeds, Shelby, KY), and a water trough ($1.83 \text{ m} \times 0.33 \text{ m} \times 0.16 \text{ m}$) containing chopped hay (sampled each week and averaged; % DM basis 90.5% DM, 13.4% protein, 51.1% ADF, 61.5% NDF, 12.9% ash, 0.7% crude fat). Calves were fed the first meal with human assistance and offered assistance every 12 h thereafter until independent milk consumption was observed. Calves were housed in group pens ($4.57 \text{ m} \times 10.67 \text{ m}$) bedded with sawdust shavings for the duration of the study until 2 wk post-weaning (84 feeder days). Calves were allotted up to 10 L/d of milk replacer (Cow's Match; 27% CP, 20% fat; Land O'Lakes, Arden Hills, MN) for 49 feeder days, reduced to 50% allotment across 14 feeder days, and then reduced to 20% allotment for an additional 7 feeder days until weaning at 70 feeder days.

Validation of Microchips and Rectal Thermometer in a Water Bath

Before implantation, the RFID temperature-reporting microchips were validated in an ex vivo trial using a water bath for accuracy, precision, and repeatability between readings; quadrants of the water bath were assigned using a Latin square for 8 h. Microchips ($n = 8$) and temperature loggers ($n = 8$; iButtons, DS1921H, Maxim Integrated, San Jose, CA) were assigned in pairs to random starting locations in the water bath. Microchip readings were taken using an RFID scanner (GPR+, Allflex USA Inc., Irving, TX) at 0:00

and 0:30 of each hour, and microchip and temperature logger pairs were moved each hour for spatial difference in the temperature of the water bath. To verify the rectal thermometer used in the calf observational study, digital rectal thermometer readings (GLA M700 Thermometer, GLA Agricultural Electronics., San Luis Obispo, CA) were also taken at each hour in each location to record water bath temperature. Randomly generated temperature settings (between 35 and 43°C) of the water bath were used to verify consistent temperature readings of the microchips. The temperature was kept in the range of expected body temperatures for dairy calves. The temperatures read for each hour were as follows: 1 h (42.5°C), 2 h (41.5°C), 3 h (39°C), 4 h (41°C), 5 h (42°C), 6 h (38.5°C), 7 h (36.5°C), and 8 h (35.5°C).

Repeatability of Microchips

Bull calves ($n = 12$; 14 ± 12 d old) were implanted with microchips (Bio-Thermo, Allflex USA Inc.) after arrival using a sterile 14-gauge syringe after cleaning the site with a surgical iodine solution (7%). The microchips were implanted on the right side of the calf in the following implantation sites: subcutaneously by the scutulum of the ear, subcutaneously in the upper scapula (SCAP), and intramuscularly in the trapezius muscle of the neck. To assess repeatability of microchip readings at each implantation site, calves were placed in a chute and provided a 10-min acclimation period; then, microchip readings were taken consecutively (mean $\pm$ SD: 33.6 ± 9.6 times each for each implantation site) for 2 min while rectal temperature was simultaneously measured.

Hourly Temperature Readings

To identify circadian temperature rhythm and to verify microchip readings between each implantation site, rectum, and tympanic membrane, temperatures were taken for every calf every 60 min for 24 h on d 10 after implantation (hereafter, the hourly study; $n = 12$; 22 ± 12 d old). Once per hour, rectal temperature using a digital thermometer with an angular probe (GLA M700 Thermometer, GLA Agricultural Electronics), tympanic temperature (Vet-Temp Instant Ear Thermometer, Advanced Monitors Corp., San Diego, CA), and microchip temperature readings of the ear, SCAP, and neck using the RFID scanner were taken for each bull calf. Moreover, we investigated the effect of ambient environmental on microchip location sites by collecting temperature and humidity (Hobo, Onset Computer Corp., Bourne, MA) in the pen hourly for

comparison with location sites by calf across the 24-h period.

Daily Temperature Readings

On d 42 after implantation of the microchips, a subpopulation of the bull calves from the previous study (total $n = 9$; 57 ± 12 d old) had rectal temperature and neck, ear, and SCAP temperatures recorded by scanner once daily at 0800 h (hereafter, the daily study). Three bull calves from the hourly study were not used in the daily study due to respiratory disease. Tympanic temperature was not measured because of the difficulty of positioning the tympanic thermometer to obtain an accurate reading during the hourly study. Calves were enrolled in the daily study until 84 feeder days, or 2 wk postweaning.

Descriptive Population Statistics

Bull calves ($n = 12$) weighed (mean $\pm$ SD) 44.1 ± 5.4 kg at arrival, 54.1 ± 10.8 kg at the time of the hourly study ($n = 12$), and 80.3 ± 13.3 kg at the start of the daily study ($n = 9$). At the conclusion of the daily study, calves weighed 118.7 ± 19.1 kg and were 90 ± 6.0 d of age.

Microchip Retrieval

At the conclusion of our research, all calves ($n = 12$) were transported for euthanasia by retractable bullet at the University of Kentucky Meat Laboratory. Calves were concurrently enrolled on another study at the time of euthanasia. All microchips were retrieved and no inflammation, microchip migration, or abscesses were observed at any of the implantation sites.

Statistical Analysis

Descriptive analyses were performed and data verified for normality using PROC UNIVARIATE in SAS (version 9.3; SAS Institute Inc., Cary, NC) and with probability distribution plots. No outliers were detected (data points beyond 3 SD from the mean) and no transformations were deemed necessary. First, for the ex vivo study, following Grinter et al. (2019), precision of the microchip in the laboratory setting was analyzed utilizing Pearson correlation coefficients (r) and linear regression coefficients of determination (R^2). These tests were performed to compare microchip temperature readings with those of the temperature loggers and the handheld rectal thermometer from the water bath. Regressions for statistical analyses were performed in

SAS and Pearson correlations in R (version 3.5.2; <https://www.R-project.org/>). Correlation coefficients and a linear regression coefficient of determination were categorized following Hinkle et al. (1988) (0.00 to 0.30 = negligible; 0.30 to 0.50 = low; 0.50 to 0.70 = moderate; 0.70 to 0.90 = high; and 0.90 to 1.00 = very high). Measures of temperature were considered precise if the r and R^2 were high (>0.70). Calf was the experimental unit. Next, to test reading consistency throughout time, the coefficient of variation (CV) was calculated for each implantation site by calf and averaged for the experimental population. Correlations and Bland-Altman plots were performed to compare readings from each implantation site of the microchips (ear, neck, SCAP) with the rectal and tympanic thermometer readings during the hourly and daily studies. Finally, to ensure no environmental influence of temperature on microchip readings during the hourly study, barn ambient temperature and temperature-humidity index were compared with ear, SCAP, and neck, as well as with temperature differences from rectal temperature to ear, SCAP and neck using regressions.

RESULTS

Validation of Microchips and Rectal Thermometer in a Water Bath

The coefficients of determination for the microchips against the temperature logger ($R^2 = 0.92$; $P < 0.001$) and the microchips against the rectal thermometer ($R^2 = 0.99$; $P < 0.001$) were very high (Figure 1A, C). The coefficient of determination for the temperature logger against the rectal thermometer was high ($R^2 = 0.88$; $P < 0.001$; Figure 1B). Pearson correlations between paired microchips and temperature loggers ($r = 1.00$; $P < 0.001$), microchips and rectal thermometer ($r = 0.96$; $P < 0.001$), and temperature loggers and rectal thermometer ($r = 0.95$; $P < 0.001$) were also very high in the ex vivo (water bath) study.

The Bland-Altman plots of agreement showed most points bound within the 95% CI of agreement for the temperature loggers, rectal thermometer, and microchips in the water bath study, and differences between measurements were close to zero (Figure 2ABC). Specifically, the Bland-Altman plot for the microchip and temperature logger had a mean difference of -0.45 ($\pm 0.20^\circ\text{C}$), with a negative bias, whereas zero was not included in the limits of agreement (Figure 2A). The Bland-Altman plot for the microchip and rectal thermometer had a mean difference of 0.16 ($\pm 0.37^\circ\text{C}$; Figure 2B) and that for the temperature logger and rectal thermometer had a mean difference of -0.29 ($\pm 0.44^\circ\text{C}$; Figure 2C).

Repeatability of Microchips

Microchips were read, on average (mean $\pm$ SD), 33.6 ± 9.6 times each for each implantation site in a 2-min period for each calf, and the average difference between reading locations for calves was small (mean difference $\pm$ SD: $0.1 \pm 0.04^\circ\text{C}$, range 0 to 0.4°C). The CV between readings for all microchips was very low ($0.12 \pm 0.03\%$ CV). Across calves, ear ($0.13 \pm 0.07\%$ CV), SCAP ($0.15 \pm 0.16\%$ CV), neck ($0.07 \pm 0.07\%$ CV),

Figure 1. Regression of temperature readings ($^\circ\text{C}$) over 8 h of (A) temperature logger against microchips, (B) rectal thermometer against microchips, and (C) temperature logger against rectal thermometer, when thermometers were assigned locations in a water bath using a Latin square design.

Figure 2. Bland-Altman plots of agreement comparing the differences in mean temperature readings (°C) over 8 h of (A) temperature logger against microchips, (B) microchips against rectal thermometer, and (C) temperature logger against rectal thermometer, when thermometers were assigned locations in a water bath using a Latin square design. The dotted lines represent the upper and lower limits of agreement (2 SD from the mean difference).

and rectal ($0.15 \pm 0.14\%$ CV) temperatures had small CV values (Supplemental Table S1; <https://doi.org/10.3168/jds.2019-17999>).

Hourly Temperature Readings

Mean, standard deviation, and minimum and maximum temperatures (°C) of the microchip, tympanic, and rectal thermometer readings by calf for the hourly study are reported in Supplemental Table S2 (<https://doi.org/10.3168/jds.2019-17999>). Briefly, we observed that hourly microchip temperatures varied across calves and depended on location.

For the regressions of environmental influence of ambient temperature, no association of ambient temperature was observed (rectal, $P = 0.17$), relationships tended to be negligible (SCAP, $R^2 = 0.15$, $P = 0.05$; neck, $R^2 = 0.23$, $P = 0.05$), or the relationship was negligible (ear, $R^2 = 0.16$, $P = 0.03$). Similarly, the temperature difference between rectal temperature and SCAP, ear, and neck was not associated with temperature-humidity index ($P > 0.10$).

Pearson correlations between neck, ear, SCAP, rectal, and tympanic temperatures for the hourly study are shown in Table 1, and the strength of the relationship was shown to vary by calf (Figure 3). Rectal temperature had negligible correlations with neck, SCAP, and ear for the hourly study, and only ear compared with neck was highly and significantly correlated across all calves [mean (quartile Q1, Q3)] [$r = 0.78$ (0.73, 0.84); $P \leq 0.003$; Table 1].

The Bland-Altman plots of agreement between rectal and tympanic temperature in the hourly study suggested that tympanic temperature was biased and underestimated rectal temperature, with a mean difference of $1.49 \pm 1.98^\circ\text{C}$ (mean difference $\pm$ SD) for the hourly study (Figure 4A). Bland-Altman plots between rectal and ear temperature had mean differences of $0.91 \pm 1.33^\circ\text{C}$ (Figure 4B), demonstrating a slight bias for ear to underestimate rectal temperature in the hourly study. The rectal to neck mean temperature difference was $0.73 \pm 1.53^\circ\text{C}$ (Figure 4C), and rectal to SCAP mean temperature difference was $0.83 \pm 1.44^\circ\text{C}$ (Figure 4D), suggesting that both neck and SCAP had low bias, with strong, narrow limits of agreement between the 95% upper and lower limits of agreement for the hourly study.

Daily Temperature Readings

Mean, standard deviation, and minimum and maximum temperatures (°C) for ear, neck, and SCAP and rectal temperature for the daily study by calf are reported in Supplemental Table S3 (<https://doi.org/10.3168/jds.2019-17999>). We observed again that temperatures varied across calves and depended on location.

Pearson correlation relationships between daily neck, ear, SCAP, and rectal temperatures varied by calf (Fig-

Table 1. The median and interquartile range (Q1, Q3) of Pearson correlations for microchip temperature (°C) read once on the hour for 24 h in 12 dairy bull calves¹

Item	Tympanic–rectal	Ear–rectal	SCAP–rectal	Neck–rectal	Ear–tympanic	SCAP–tympanic	Neck–tympanic	Neck–ear	SCAP–ear	SCAP–neck
Median	0.19	0.10	0.12	0.12	0.4	0.19	0.34	0.78	0.58	0.75
Q1	0.07	0.07	0.10	0.07	0.35	0.1	0.32	0.73	0.45	0.6
Q3	0.22	0.19	0.18	0.19	0.45	0.41	0.44	0.84	0.75	0.84
<i>P</i> -value	≤0.81	≤0.85	≤0.93	≤0.97	≤0.59	≤0.75	≤0.71	≤0.003	≤0.44	≤0.17

¹Microchips were implanted subcutaneously behind the ear and upper scapula (SCAP) and intramuscularly in the neck. Tympanic temperature and rectal temperatures were also recorded.

ure 5). Similar to the hourly study, the daily study detected no significant Pearson correlations of rectal temperature [median (Q1, Q3)] for SCAP [$r = 0.24$ (0.09, 0.34), $P \leq 0.97$], neck [$r = 0.17$ (0.6, 0.28), $P \leq 0.98$], or ear [$r = 0.06$ (0.03, 0.14); $P \leq 0.99$]. The only significant Pearson correlation found across all calves was between ear and neck and it was high [$r = 0.79$ (0.73, 0.89); $P \leq 0.02$].

As in the hourly study, the Bland-Altman plots of agreement between daily rectal and ear temperatures were strongly biased; ear readings underestimated rectal temperature, with a mean difference of $1.32 \pm 2.5^\circ\text{C}$ (mean difference $\pm$ SD; Figure 6A). For rectal compared with neck and SCAP temperatures, the plots included zero and the majority of the points were within the 95% CI upper and lower limits of agreement, suggesting no bias, with a mean difference for neck of $0.61 \pm 2.1^\circ\text{C}$ (Figure 6B) and for SCAP of $0.53 \pm 1.66^\circ\text{C}$ (Figure 6C).

DISCUSSION

The objective of our research was to validate a passive temperature-reporting microchip to determine an implant site for estimating temperature in calves either subcutaneously behind the ear or upper scapula or intramuscularly within the middle neck. We found that the implantable microchips were accurate, precise, and repeatable ex vivo, and repeatable within all implant sites in our calves. However, our implant sites were not correlated with rectal temperature. A strong relationship was observed between readings at the neck and ear implantation sites across calves, suggesting that temperature readings were related in proximal locations.

We first aimed to determine whether the microchips were accurate, precise, and repeatable ex vivo, before implanting in the calves. Temperature-measuring technologies must have high accuracy to true value, repeatability, high precision with low standard error, and low bias; this is especially important for identifying illness in calves, such as scoring for bovine respiratory disease, which relies upon a single temperature reading to contribute to a weighted scale for diagnosing a calf

as sick (typically a temperature threshold of 39.4°C ; McGuirk, 2008; McGuirk and Peek, 2014). Indeed, we found that the microchips and the rectal thermometer were accurate, precise, and repeatable in a controlled setting during the ex vivo trial, which agreed with similar studies assessing microchip accuracy and precision using water baths (Huțu et al., 2009; Ude et al., 2010). We also found low bias for the microchips against the rectal thermometer in the ex vivo trial, which adds to the existing literature describing the validity of this temperature technology in ex vivo settings.

After validating the microchips in ex vivo settings, we aimed to validate the microchip temperature readings at various implantation sites against rectal temperature of calves. We determined that the microchips had high repeatability within each implant site for all 12 calves. Ude et al. (2010) also found low temperature variation and high repeatability of microchip readings implanted at the base of the ear, under the scutulum of the ear, and in the neck. However, Ude et al. (2010) did not report correlations with rectal temperature. Additionally, microchip temperature readings at the implant sites in our study did not correlate with rectal temperature. Other work in adult dairy cows found that temperature measured at the ear implantation site was moderately correlated with rectal temperature and was a better option than measurements at the neck, shank, or within the udder (Huțu et al., 2009). Our results may have differed because we used growing calves and monitored temperature readings hourly (reflecting circadian rhythm) and daily across several weeks, whereas the Huțu et al. (2009) study had a much shorter monitoring duration (5 d) in adult cattle. Another study in immune-challenged calves found a moderate correlation between temperature measured by microchips implanted behind the ear and rectal temperature compared with temperature measured by implants behind the poll and within the umbilical fold in 4 weaned steers (Reid et al., 2012). These findings suggest that ear temperature readings using microchips may not be a proxy for estimating rectal temperature in healthy calves, but it is possible that peripheral temperature readings may be correlated with rectal temperature during illness (or

at least during an induced immune response as in Reid et al., 2012), which deserves future research.

An alternative measure of body temperature in calves is tympanic temperature (Wiersma and Stott, 1983); thus, we also aimed to validate the microchip implantation site temperature with tympanic temperature. However, we found that tympanic temperature was not correlated with microchip temperature in the ear, neck, or scapula, and tympanic temperature was not correlated with rectal temperature. To our knowledge, the relationship of rectal temperature with core temperature has not been assessed in dairy calves, although it has been validated in goats (Torrao et al., 2011). The discrepancy in tympanic versus rectal and microchip temperatures in our study may be explained

by a study that observed tympanic temperature to be sensitive to rapid changes in body temperature because of its proximity to the hypothalamus (Guidry and McDowell, 1966). These findings suggest that tympanic temperature may not be an appropriate comparison for ear, neck, or scapula microchip sites, or for rectal temperature as the gold standard for core body temperature. Future research should determine whether rectal temperatures are similar to core temperatures in dairy calves.

Implantable microchips may serve as an alternative for reporting temperature in calves due to their high repeatability in our studies, but correlations between temperatures at the implantation sites in vivo were highly variable among individuals. Other studies on

Figure 3. Boxplots showing median (line) and interquartile range (box) of Pearson correlations for microchip temperature (°C) read hourly on the hour for 24 h in 12 dairy bull calves. Each colored dot represents an individual calf. Microchips were implanted subcutaneously behind the ear and upper scapula (SCAP) and intramuscularly in the neck. Tympanic temperature (TYM) and rectal temperatures (REC) were also recorded.

temperature microchip technology in cattle have only reported group averages (Hu $\mathring{t}$ u et al., 2009; Ude et al., 2010; Reid et al., 2012; Lee et al., 2016), but individual temperature variation has affected correlations between microchip implant sites and rectal temperature in other species such as goats (where individuals did not show consistent upper scapula implant site and rectal temperature correlations; Torrao et al., 2011), in horses (where the intramuscular neck implant site had individual variability; Holje, 2012), and in pigs (where individuals showed consistent correlations between ear and neck implant sites; Lohse et al., 2010). Our study is similar to the latter, where we found that ear and neck implant sites were well correlated in calves with

low individual variability. However, we caution against comparing our findings in calves to those in other species. For example, Goodwin (1998) found that the strength of the relationship between rectal temperature and that from a microchip implanted in the elbow was greatly influenced by species (negligible in horses, moderate in sheep, and strong in goats). This work suggests that individual variation in temperatures recorded at implantation sites likely contributes to the variability observed in correlations between microchip implant site and rectal temperature. Therefore, the application of this microchip technology must consider individual variation when considering relationships within and between temperature sites.

Figure 4. Bland-Altman plots of agreement comparing the differences in mean temperature readings (°C) of thermometers read hourly on the hour for 24 h in 12 dairy bull calves for (A) tympanic against rectal temperature, (B) microchip implanted behind the ear against rectal temperature, (C) microchip placed intramuscularly in the neck against rectal temperature, and (D) microchip placed subcutaneously in the scapula (SCAP) against rectal temperature. The dotted lines represent the upper and lower limits of agreement (2 SD from the mean difference). The y-axis represents the difference between the 2 thermometer readings, and the x-axis represents the mean between 2 readings at a point in time.

Figure 5. Boxplots showing median (line) and interquartile range (box) of Pearson correlations for microchip temperature ($^{\circ}\text{C}$) read once in the early morning daily for 30 d until 2 wk post-weaning (84 d) for 9 dairy bull calves. Each colored dot represents an individual calf. Microchips were implanted subcutaneously behind the ear and upper scapula (SCAP) and intramuscularly in the neck. Rectal temperatures (REC) were also recorded.

The ability to capture individual variability in temperature readings carries large potential for temperature technologies, especially to detect illness using individual baseline temperatures within implant site. Temperatures between ear and neck implantation sites were strongly correlated across calves and over time, so these locations would be a useful starting point for investigating how microchips detect illness at the individual animal level. Future research should determine whether microchip technologies in different implant sites, such as ear, scapula, and neck, can detect changes from baseline temperature in a similar manner to changes from baseline rectal temperature. Future research should determine whether monitoring deviations in temperature behind the ear and middle neck from

basal temperatures can detect sickness in dairy calves, because these microchips were accurate and precise in the ex vivo trial. However, future work should also consider that the established thresholds for fever using rectal temperature in calves (McGuirk and Peek, 2014) may not be an appropriate threshold for fever at other implant sites.

CONCLUSIONS

Our results indicate that temperature microchips were accurate and precise in an ex vivo trial and were repeatable within calves. Although the neck and ear implant temperature readings were strongly correlated, none of the readings at implant sites were correlated

Figure 6. Bland-Altman plots of agreement comparing the differences in mean temperature readings ($^{\circ}\text{C}$) of thermometers read once in the early morning daily for 30 d until 2 wk post-weaning (84 d) for 9 dairy bull calves. Thermometers were microchips implanted (A) subcutaneously behind the ear, (B) intramuscularly in the neck, and (C) subcutaneously in the upper scapula (SCAP). Tympanic temperature and rectal temperatures were also recorded. The dotted lines represent the upper and lower limits of agreement (2 SD from the mean difference). The y-axis represents the difference between the 2 thermometer readings, and the x-axis represents the mean between 2 readings at a point in time.

with rectal temperature. Moreover, a strong correlation between ear and neck implant sites suggests that implant site variation was not a result of ambient temperature influence, but simply reflected poor estimations of rectal temperature. Strong correlations between the neck and ear temperature microchips across all calves and several days in our study may suggest that temperature microchips in calves have potential to determine baseline temperatures and deviations associated with illness in calves (e.g., fever). However, a power analysis that considers individual animal variation in temperature for these location sites should follow a cohort of animals to ensure that population variation is captured because temperature baselines in the neck and ear are unknown. We suggest that, although microchip temperature technology does not serve as a proxy for rectal temperature, there is potential to passively monitor temperature, and future research is needed to determine what thresholds of change are required to detect fever in the intramuscular region of the neck and subcutaneous implant behind the ear of dairy calves.

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