Correlation of rectal temperature and peripheral temperature from implantable radio-frequency microchips in Holstein steers challenged with lipopolysaccharide under thermoneutral and high ambient temperatures

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ABSTRACT: Early detection of disease can speed treatment, slow spread of disease in a herd, and improve health status of animals. Immune stimulation increases rectal temperature (RT). Injectable radio-frequency implants (RFI) can provide temperature at the site of implantation. The fidelity of peripheral site temperature, determined by RFI, relative to RT is unknown in cattle. We hypothesized that during lipopolysaccharide (LPS) challenge, temperature at 3 peripheral sites would be similar to RT in steers (n = 4; BW 77 ± 2.1 kg). The 3 sites were 1) subcutaneous (SC) at the base of the ear (ET); 2) SC posterior to the poll (PT); and 3) SC beneath the umbilical fold (UT). Steers were housed in controlled temperature (CT) rooms (between 18 and 21°C; n = 2/room). Rectal temperature, ET, PT, and UT were recorded every 8 h daily. On d 7, 21, 22, 36, and 37, RT and RFI were taken every 5 min for 6 h, every 15 min for 3 h, and every 30 min for 15 h. To test RFI during a simulated immune challenge, LPS (E. coli 055:B5) was injected intravenously (IV) at 1300 h on d 22 and 37. Basal temperatures (°C) were RT (38.7 ± 0.20), ET (37.1 ± 0.86), PT (36.7 ± 0.57), and UT (36.3 ± 0.97). Rectal temperature increased to 39.9 ± 0.30°C after LPS, but ET, PT, and UT decreased. Heat stress also increases RT, which makes it difficult to identify sick animals using RT. The second hypothesis tested was that ET positively correlates to RT and negatively correlates to RT during LPS under heat stress. Four steers (127 ± 7.3 kg) were housed in CT chambers (n = 2/chamber), implanted with a RFI, and allowed 2 wk to acclimate. One chamber remained at 20°C, the other was increased to 34°C starting at 0800 h for a period of 48 h. The LPS was administered IV to all steers at 1000 h on d 2. After a 2-wk recovery at 20°C, the temperature was increased in the other chamber, resulting in a crossover design with each steer serving as its own control. Pearson’s correlation coefficients for ET and RT were 0.30 (P < 0.01) during heat stress, 0.20 (P > 0.05) during heat stress with LPS challenge, 0.34 (P < 0.01) during thermoneutrality, and −0.42 (P < 0.01) during thermoneutrality with LPS. These data refute the hypothesis that RT and peripheral temperature move in synchrony after LPS challenge. These data suggest that individual response be considered when identifying models for use of ET, but these RFI have potential for use in the early detection of diseases that alter basal temperature.

Keywords: fever, heat stress, lipopolysaccharide, radio-frequency implant

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INTRODUCTION

Production losses due to a variety of illnesses can affect the profitability of production units. For example, financial losses due to illness in dairy cattle averaged $172/cow annually (Miller and Dorn, 1990). Currently, methods of identifying sick animals rely on visual and physical assessments and use of body temperature (BT) to identify animals outside of steady state (i.e., a fever). The ability to use BT as a diagnostic tool has lead to the commercial development of radio-frequency identification devices that also provide real-time...
temperature monitoring. One system is the injectable Biothermo microchip (Digital Angel Corp., St. Paul, MN). The objective of the first experiment was to determine whether the microchips, located at 3 sites, yield data that correlate with rectal temperature (RT) during thermoneutral conditions and an induced fever event of lipopolysaccharide (LPS) challenge. We hypothesized that the microchips would yield similar patterns relative to those obtained by conventional rectal thermometer.

Heat stress costs the U.S. livestock industry $2.5 billion per year due to decreased performance, increased mortality, and decreased reproduction (St-Pierre et al., 2003). Temperature-humidity index (THI; Mayer et al., 1999) has been used to identify periods of heat stress. At high THI (93 ± 3.1), an increase in RT of 0.47°C has been reported (Srikandakumar and Johnson, 2004). Increases in RT due to heat stress can interfere with disease monitoring programs that use RT to identify animals experiencing a health event. A system that measures BT with high frequency may be more accurate in distinguishing increased RT due to environmental heat stress from an immune challenge because more data points would better account for variation. A second objective was to determine whether temperatures from microchip implants are correlated to RT during high ambient temperature and during a LPS challenge during high ambient temperature.

MATERIALS AND METHODS

The use of animals in these experiments was approved by the University of Illinois Institutional Animal Care and Use Committee.

Experiment 1

Animal Description and Sampling Procedure. In Exp. 1, 4 weaned Holstein steers (77 ± 2.1 kg) were used. The steers were moved from outdoor housing to an indoor temperature-controlled facility and housed in 2 rooms with each room housing 2 steers. The rooms measured 2.75 × 3.36 m and the flooring was rubber-coated expanded metal with a manure collection pit under the flooring. Inside each room, the steers were kept in individual stalls measuring 0.92 × 1.75 m. Air exchange in each room was approximately 15 changes/h, and the rooms were kept between 18 and 21°C. Steers were fed according to NRC requirements for maintenance and growth (NRC, 2001), which included 0.9 kg/d of grain on an as-fed basis and ad libitum access to water and alfalfa cubes. Because many alfalfa cubes were taken out of the feed buckets by the animals and dropped through the grates into the manure pit, an exact intake could not be calculated. At 1,000 h daily, the grain was fed, fresh alfalfa and water were offered, rooms were cleaned, and the manure collection pit was emptied.

Upon entering the facility, steers were implanted with 3 Biothermo microchips (Digital Angel Corp., Saint Paul, MN) that were part of a passive full-duplex, ISO 11784 and 11785 compliant system operating at 134.2-kHz radio frequency. The microchips were 2 × 12 mm and delivered via a prepackaged sterile positive displacement syringe to 3 implant locations including under the umbilical fold (UT), subcutaneous on the midline posterior to the poll (PT), and under the scutiform cartilage of the left ear (ET). The steers were then allowed 1 wk to acclimate to the facility and observed daily for inflammation around injection sites; no inflammation was observed. Photoperiod was initially set at 12 h of light and 12 h of dark. The animals were also used in another study examining photoperiodic effects on immune function during LPS challenge. On d 7, steers were placed on either short-day photoperiod (8 h light:16 h dark) or long-day photoperiod (16 h light: 8 h dark), and photoperiod treatments were reversed on d 22.

Rectal temperatures were measured with a digital thermometer with an 8.6-cm long, round-end probe connected to a digital readout (model TM99A, Cooper-Atkins, Middlefield, CT). Microchip queries were performed with a hand-held scanner that provided microchip identification number and current temperature reading (Biothermo Pocket EX Reader, Digital Angel Corp.).

During Period 1, an initial set of temperatures was recorded on d 7 to serve as a baseline before photoperiod change; however, those data are not presented because photoperiod did not influence BT. The first baseline temperature recording for this study began at 0800 h on d 21. Rectal temperature was recorded, and each microchip was scanned and recorded every 5 min from 0800 h until 1400 h, every 15 min from 1400 to 2000 h, and every 30 min from 2000 to 0800 h the next day. The same temperature measurement schedule was again followed on d 22, and LPS was administered into the jugular vessel at 1000 h at a rate of 0.1 μg/kg BW (E. coli 055:B5; reconstituted with 0.9% sterile saline solution to 10 μg/mL; Sigma-Aldrich, St. Louis, MO). Temperature collection ceased at 0800 h on d 23 and steers were allowed a 2-wk recovery period. A second replicate was performed with the same 4 steers during Period 2 that followed the same protocol, including baseline day and LPS challenge, starting on d 36 and ending on d 38, although photoperiod treatments were reversed in that replicate.

Statistical Analysis. A statistical analysis using PROC MIXED (SAS Inst. Inc., Cary, NC) was performed to compare d 7 baseline temperatures with d 21 temperatures using time as the repeated measure and animal as a random effect. The effect of photoperiod on BT was not significant in the model. The LPS challenge was not analyzed as a treatment effect, but those data
collected during LPS challenge were used to determine the correlation between RT and ET, PT, and UT. Rectal temperature and microchip data were analyzed using the PROC MEANS and PROC CORR functions in SAS with ET vs. RT, PT vs. RT, and UT vs. RT submitted to PROC CORR. To determine correlations, data during the baseline measurement periods were analyzed as a complete set, and data during the LPS challenge were divided into prechallenge (0800 to 1000 h), challenge (1000 to 1400 h), and postchallenge (1400 to 0800 h the next day). Results are expressed as Pearson’s correlation coefficients. A P-value < 0.10 was considered a likely correlation and a P-value < 0.05 was considered significant.

Experiment 2

Animal Description and Sampling Procedure. In Exp. 2, 4 weaned Holstein steers (127 ± 7.3 kg) were used. The steers were moved from outdoor housing to an indoor environmentally controlled facility and housed in 2 chambers; each chamber accommodated 2 steers. The chambers were 2.74 m long × 2.16 m wide × 2.41 m high, and each was equipped with a ventilation fan, 3 circulating fans, and a cooling unit. Supplemental heat during heat-stress periods was provided using two 1,500-W electric space heaters positioned near the circulating fans. Inside each chamber, the steers were housed in painted wood crates measuring 0.8 × 1.5 m and were 1.4 m high. Each crate had a grate in the rear for manure to pass through into a collection vessel below. At 0800 h daily, fresh feed and water were provided and manure was removed. The steers were allowed ad libitum access to alfalfa hay pellets and water and were each fed 2.3 kg/d on an as-fed basis of a grain mix to meet nutrient requirements for maintenance and growth (NRC, 2001). As in Exp. 1, alfalfa cubes were taken out of the feed buckets by the animals and that precluded calculation of exact feed intake.

Upon transfer to the chambers, the steers received a Biothermo microchip, as previously described, at the base of the left ear under the scutiform cartilage (ET). The steers were observed daily for 1 wk for inflammation around injection sites; no inflammation was observed. The steers were allowed 14 d to acclimate to a thermoneutral temperature (20.7 ± 0.7°C).

A crossover design was utilized, and, after the initial acclimation period, the steers in Chamber 1 were exposed to increasing ambient temperature starting at 0900 h continuing for 47 h. The ambient temperature was maintained at 34 ± 1.2°C until 1900 h when it was decreased (27.1 ± 1.1°C) to simulate night-time cooling, while still keeping the temperature increased. The temperature was again increased starting at 0900 h the next day and remained increased until 0000 h when it was returned to thermoneutral. The steers in Chamber 2 were maintained at thermoneutral temperature throughout Period 1. The steers remained at thermoneutrality for 14 d to reacclimate and reduce any residual effects of high ambient temperature. The treatments were then switched for each chamber following the same protocol as in Period 1. Throughout the study, ambient temperature and humidity were logged every 5 min using HOBO data loggers with onboard sensors (Model H08-006-04; Onset Corp., Pocasset, MA).

Rectal temperatures were collected with a digital thermometer with a 10-cm long, round-end probe connected to a digital readout (model M500, GLA Agricultural Products, San Luis Obispo, CA). Microchip queries were performed with a handheld scanner that provided chip identification number and current temperature reading (Biothermo Pocket EX Reader). Temperature collection (RT and ET) started at 0700 h at the beginning of each period and continued for 48 h. Data collected from 0700 to 0800 h the first day was not used in the analysis. The first hour was used to acclimate steers to the temperature collection process. The frequency of collection was every 5 min from 0800 to 1300 h, every 15 min from 1300 to 2000 h, and every 30 min from 2000 to 0800 h the next morning. On d 2 of the period, temperature collection frequency was the same as d 1, and LPS was administered into the jugular vessel at 1000 h at a dose of 0.1 μg/kg BW (E. coli 055:B5; reconstituted with 0.9% sterile saline solution to 10 μg/mL; Sigma-Aldrich). Respiration rate was assessed visually and recorded as breaths per minute, and skin-tent duration, eyeball recession (as described by Constable et al.; 1998), and recumbancy, assessed visually and recorded as standing or lying, were also monitored every 4 h from 1000 h on d 1 to 1800 h on d 2 to monitor heat stress and hydration status.

Statistical Analysis. Rectal temperature and microchip temperature at the ear were analyzed using the PROC MEANS, PROC MIXED, and PROC CORR functions in SAS. The analysis of the crossover design was performed using PROC MIXED with repeated measures with time as the repeated measure, chamber as the experimental unit, and animal as a random effect. The model included time of day, period of study, presence of heat stress, and the interaction of period × heat stress as fixed effects. In the crossover portion of the study, a P-value < 0.10 was considered significant. The LPS challenge was not analyzed as a treatment effect, but those data collected during LPS challenge were used to determine the correlation between RT and ET, PT, and UT. To determine correlations during each period between ET and RT, the data set was broken into periods (i.e., thermoneutral and no LPS challenge, thermoneutral with LPS challenge, heat stress and no LPS challenge, and
heat stress with LPS challenge). The correlation results are expressed as Pearson’s correlation coefficients. A P-value < 0.10 was considered a likely correlation and a P-value < 0.05 was considered a significant correlation.

**RESULTS**

**Experiment 1**

**Period 1.** Data for the baseline day are shown for clarity using a 3-point running average in Figure 1. Temperatures for RT, ET, PT, and UT during that 24-h period averaged 38.7, 37.0, 36.5, and 36.1°C with a SEM of 0.2, 1.0, 0.6, and 1.2°C, respectively. During the first 24-h baseline period, correlations between RT and ET, RT and PT, and RT and UT were all positive (r = 0.21, 0.16, and 0.16, respectively; P < 0.01).

Data from the first day of LPS challenge from 0800 to 0000 h are shown using a 3-point running average in Figure 2. The correlations between RT and ET, RT and PT, and RT and UT were 0.53, 0.61, and 0.43, respectively (P < 0.01) for the 2 h preceding the LPS challenge period, −0.40 (P < 0.01), 0.30 (P < 0.01), and 0.03 (P = 0.66), respectively, for the 4-h period after LPS injection, and −0.12 (P = 0.22), 0.53 (P < 0.01), and 0.21 (P < 0.03), respectively, from 1400 to 0800 h the next day. An inverse biphasic response was observed between the rectal and RFI temperatures during the LPS challenge period.

**Period 2.** Mean baseline temperatures were 38.7, 37.3, 36.7, and 36.5°C with SEM of 0.2, 0.7, 0.5, and 0.7 for RT, ET, PT, and UT, respectively. Correlations between RT and ET, RT and PT, and RT and UT were all positive (r = 0.11, 0.36, and 0.22, respectively; P < 0.03).

On the second day of LPS challenge (data not shown), correlations for RT and ET, RT and PT, and RT and UT were −0.19 (P = 0.07), −0.02 (P = 0.87), and −0.07 (P = 0.47), respectively, for the prechallenge period, −0.48, 0.36, and 0.38 (P < 0.01), respectively, for the 4-h period after the LPS injection, and −0.25 (P < 0.01), 0.53 (P < 0.01), and 0.17 (P < 0.08), respectively, from 1400 to 0800 h the next day. There was a similar inverse biphasic response of rectal and microchip temperatures during the second LPS challenge when compared with the first LPS challenge.

**Experiment 2**

For the duration of the study, steers in thermoneutral conditions averaged 15 ± 2 breaths/min and did not show any signs of dehydration. When under high ambient temperature, steers averaged 25 ± 2 breaths/min, had skin tent durations of 2 s, and showed some signs of eyeball recession (approximate 2 mm) by the end of 2 d of heat stress. All indicators of heat stress returned to normal within 4 h after reducing ambient temperature to a thermoneutral range.

Mean temperatures with SE for the thermoneutral and high ambient temperature periods are shown in Figure 3. Temperatures during the thermoneutral period without...
LPS challenge were 38.4 (± 0.2) and 39.1°C (± 0.1) for ET and RT, respectively. Mean temperatures during the high ambient-temperature period without LPS challenge for ET and RT were 39.2 (± 0.2) and 40.0°C (± 0.1), respectively. The mean ET and RT for the period before LPS administration (0800 to 1000 h) were 38.5 (± 0.5) and 38.9°C (± 0.3), respectively, under thermoneutral conditions. Rectal temperature and ET were also positively correlated during that period (r = 0.34, P < 0.01). During the LPS challenge under thermoneutral conditions (1000 to 1200 h), mean RT and ET temperatures were 39.4 (± 0.4) and 37.6°C (± 0.9), respectively, and were negatively correlated (r = −0.42, P < 0.01). The mean RT and ET temperatures were 39.4 (± 0.1) and 38.6°C (± 0.2), respectively, under thermoneutral conditions after the LPS challenge (1200 to 0800 h the next day). The mean RT during the thermoneutral period was greater (P = 0.09) during the LPS phase than was the pre-LPS phase RT (Figure 3). Rectal temperature was also greater (P = 0.05) than ET during the LPS phase under high ambient temperature conditions (Figure 3).

**DISCUSSION**

The ability to identify sick animals quickly, provide needed treatment, and reduce morbidity and mortality is important to animal producers. Systems aiding in that process have received new interest as the size of animal units have grown and producers have tried to offer individual care in large group settings. The rectum has been used as an easily accessible point to monitor fluctuations in BT. However, labor input to monitor RT is time consuming and expensive, especially for animals not in group housing. In addition, the use of RT as a diagnostic tool requires a database to identify animal-specific fluctuations. When using only a single temperature reading per day, it is difficult to adjust for natural circadian changes in animal BT (Araki et al., 1984), as well as changes attributed to ambient temperatures and solar radiation (e.g., increased RT during periods of heat stress; Legates et al., 1991; Gaughan et al., 2010; Scharf et al., 2011). The purpose of the present study was to evaluate an available system that utilizes implantable microchips that offer individual identification, as well as real-time temperature monitoring.

Identifying an ideal location for radio-frequency devices has been the topic of debate. The first consideration for placement in the animal is the ability to recover the implant to prevent introduction into the food chain and also to eliminate the reuse of devices in systems where unique numbers are needed (Sheridan, 1991). The second consideration for placement in the animal is ease of use under production settings without harming the animal. Dorn (1987) recommended that implants be placed subcutaneously on the neck just cranial to the shoulder on the left side. Further evaluation of implantation sites by Merks and Lambooij (1989) included subcutaneously at the front of the head, at the base of the ear, intramuscularly in the neck, and at the lateral side of the neck, cranial to the shoulder. A site under the scutiform cartilage of the ear was described by Fallon and Rogers (1999) and was also determined to be a suitable placement site for implants by Hasker et al. (1992) because of the ability to recover implants easily at slaughter. In the present study, the position under the scutiform cartilage was determined to be ideal from the recommendations of previous reports and the prospect of having a deeper implantation that would be less affected by external temperature fluctuations, but the 2 other sites were included to test the performance of the implants in areas that...
were closer to the ambient environment relative to the base of the ear where the implant was insulated by the scutiform cartilage. The umbilical implantation site potentially offers some benefit to dairy producers because the implants could be read from milking units attached ventrally. Microchips implanted at the UT, PT, and ET were all positively correlated with RT when steers were maintained in a controlled, thermoneutral environment, but all correlations were small (i.e., $r = 0.11$ to 0.36), suggesting that many factors may be altering temperature data, including a potential lag period in microchip temperature after LPS injection. A second collection of temperatures in this experiment confirmed the positive correlation between each chip and RT at thermoneutral ambient temperatures. The mean temperatures for the implant sites were less than RT, but expressed a similar pattern. These observations support the concept that implantable devices that measure temperature provide consistent, reliable indications of BT similar to traditional RT responses at thermoneutral ambient temperature.

During an experimentally induced fever caused by LPS injection, RT increased in a similar bimodal fashion as shown in previous research (Davis et al., 2003; Alzahal et al., 2011; Rose-Dye et al., 2011). In contrast to RT, which increased over a 5-h period after LPS injection, temperatures at the 3 microchip locations were reduced in a biphasic pattern. Whereas a negative correlation was exhibited only between RT and ET, the pattern of the temperature change was similar for all 3 microchip locations. The hypothesis that the temperature recorded by the microchips at all locations would be positively correlated to RT was rejected. The drop in implant temperature compared with the increase in RT was unexpected, and the negative correlation was especially robust for the ET implants. Gordon et al. (2002) reported a decrease in tail temperature when steers were heat stressed, and that ET generally reflected the BT of the animal.

The biphasic temperature patterns collected from the implants that were synchronized with biphasic responses in RT suggest that microchips have value in a system used to monitor animals for fluctuation in BT, specifically under thermoneutral ambient temperatures. The consistency of results obtained with the location at the base of the ear, along with the popularity of that location with other forms of identification indicate that it would be a viable place to further study the performance of the microchips. In addition, the results of Exp. 2 suggest that ET can be used to collect data potentially useful to identify animals experiencing heat stress and to use that information to manage heat mitigation strategies. Radio-frequency temperature collection also seems to be a promising technology for identifying animals experiencing a fever event, but these results emphasize the importance of individual data collection because individual animals may respond to environmental and pathogenic challenges in unique ways, including a possible lag relative to RT. The duration of the negative correlation between ET and RT (shorter duration under heat stress) should be considered when determining optimal data collection frequency. The larger deviation from the mean in ET compared with RT could be caused by larger fluctuations in blood flow at the ear relative to the rectum, but could also be influenced by environmental factors. An increase in the variability in the temperature readings of the implants was also noted during the 0800- to 1600-h period. This is the timeframe when feeding and pen cleaning occurred and could mean that activity of the animal could affect BT patterns. Future research...
is needed to determine how ET responds to various naturally occurring pathogenic insults. More research also is needed to determine the optimal frequency of data collection to identify animals during disease events and how environmental changes may impact the response. There is also a need to determine the specific effects of solar radiation, decreased ambient temperatures, management variables (e.g., sprinklers), animal size, and animal activity on temperatures collected with these microchips.

LITERATURE CITED


