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## Assessment of daily activity patterns and biomarkers of pain, inflammation, and stress in lactating dairy cows diagnosed with clinical metritis

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### ABSTRACT

The objectives of the present case-control study were to assess (1) daily activity patterns (lying time, number of steps, number of lying bouts, and lying bout duration), and (2) circulating concentrations of biomarkers of pain (substance P), inflammation (haptoglobin), and stress (cortisol) in lactating dairy cows diagnosed with clinical metritis. Lactating dairy cows ( $n = 200$ ) from 2 commercial dairy herds were enrolled in the present study. Cows diagnosed with clinical metritis ( $n = 100$ ) at  $7 \pm 3$  d in milk were matched according to lactation and days in milk to cows without clinical metritis (NO-CM;  $n = 100$ ). On study d 1, clinical metritis was diagnosed (using a Metricheck device, Simcro Tech Ltd., Hamilton, New Zealand) by the presence of watery, reddish, or brownish foul-smelling vaginal discharge, and blood samples were collected for assessment of circulating concentration of substance P, haptoglobin, cortisol, total calcium,  $\beta$ -hydroxybutyrate, and blood cells. In addition, on study d 1 body condition of cows was visually assessed, and activity monitors were placed on the hind leg of a subset of cows (CM,  $n = 56$ ; CON,  $n = 56$ ) and were kept until study d 7. Cows showing any other signs of other diseases were not included in the study. Cows with clinical metritis tended to spend more time lying (CM = 628.92 min/d; NO-CM = 591.23 min/d) compared with NO-CM cows. Activity analysis by parity revealed that primiparous cows with clinical metritis spent more time lying compared with primiparous cows without clinical metritis. However, no differences in daily lying time were observed between multiparous cows with and without clinical metritis. Furthermore, cows in the CM group had a higher circulating concentration of substance P (CM = 47.15 pg/mL; NO-CM = 37.73 pg/mL) and haptoglobin (CM = 233.00  $\mu$ g/

mL; NO-CM = 99.98  $\mu$ g/mL) when compared with NO-CM cows. Cows with clinical metritis had lower body condition score, and a greater proportion of cows in this group had hypocalcemia when compared with cows without clinical metritis. The circulating concentration of leukocytes and erythrocytes were decreased in cows with clinical metritis compared with cows without clinical metritis. Results from this study showed that concentrations of markers of inflammation, stress, pain, and activity were affected in cows diagnosed with clinical metritis; thus, strategies aimed to minimize the negative effects associated with clinical metritis may be required to improve the welfare of dairy cows.

**Key words:** dairy cattle, substance P, haptoglobin

### INTRODUCTION

Clinical metritis is an inflammatory process that affects all layers of the uterus during the early postpartum period in lactating dairy cows (Bondurant, 1999; Sheldon et al., 2006). This condition is characterized by the presence of an abnormally enlarged uterus with fetid red-brownish uterine discharge, with or without systemic signs of illness such as depression, anorexia, decreased milk yield, and pyrexia within 21 d after parturition (Sheldon et al., 2006). The incidence of clinical metritis ranges from 15 to 20%, although it could be higher depending on the herd (Gilbert, 2016). Clinical metritis negatively affects profitability due to reduced milk yield (Rajala and Gröhn, 1998), delayed time to conception (Fourichon et al., 2000), and increased culling rates (Gröhn et al., 2003). Furthermore, a recent study reported that cows with clinical metritis experienced visceral pain in response to palpation of the uterus (Stojkov et al., 2015). Thus, in addition to the negative economic implications, clinical metritis could be regarded as a welfare concern.

Early identification, examination, and treatment of sick animals are crucial to ensure animal welfare in food animal production systems. However, animals such as

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cattle are stoic and physical and behavioral changes associated with diseases and pain can be subtle, making their identification challenging (Fitzpatrick, 2002). Activity monitors have been used to assess the effects of common health events on dairy cattle behavior. Huzzey et al. (2007) showed that prepartum DMI and feeding behavior were altered in cows that developed clinical metritis after calving. Liboreiro et al. (2015) reported that cows diagnosed with clinical metritis spent less time ruminating and had reduced activity compared with cows without clinical metritis. More recently, Stangaferro et al. (2016a,b) showed that the combination of rumination and activity accurately identified cows at the onset of diseases such as displaced abomasum and ketosis; however, it lacked sensitivity to identify cows with clinical metritis. Therefore, the association of clinical metritis and activity patterns in dairy cows warrants further research.

Biomarkers of inflammation and stress such as haptoglobin (**HP**) and cortisol have been used to assess inflammation and stress in dairy cows with clinical metritis and other postpartum diseases (Huzzey et al., 2009, 2011; Burnett et al., 2015). More recently, substance P (**SP**) has emerged as a potential marker of nociception and inflammation in cattle. Substance P is an undecapeptide released from the dorsal horn of the spinal cord that regulates the excitability of nociceptive neurons and is associated with the transmission and modulation of pain; furthermore, SP is involved in the integration of inflammation, stress, and anxiety (DeVane, 2001; Datar et al., 2004; Perl, 2011). Studies have used the circulating concentration of SP, among other parameters, to assess inflammation and nociception in dairy and beef calves following castration and dehorning (Coetzee et al., 2012; Allen et al., 2013; Repenning et al., 2013). Overall, circulating concentrations of SP were increased in calves following dehorning and castration, and some studies demonstrated a reduction in SP following pain management (Coetzee et al., 2012; Allen et al., 2013). However, more research is required to better understand the association of postpartum diseases such as clinical metritis and circulating concentrations of SP in lactating dairy cows.

The objectives of this study were to assess (1) daily activity patterns, and (2) circulating concentrations of SP, HP, and cortisol in lactating dairy cows diagnosed with clinical metritis. Furthermore, determination of BHB, total calcium, white blood cells, milk yield, and reproductive data were collected for a more comprehensive assessment of clinical metritis in dairy cattle. We hypothesized that cows that experience clinical metritis will have altered activity patterns, and higher circulating concentration of SP, HP, and cortisol compared with cows without clinical metritis.

## MATERIALS AND METHODS

### *Animals, Facilities, and Feeding*

During the present case-control study, lactating Holstein dairy cows ( $n = 200$ ) that calved between August 2014 and October 2015 in 2 commercial dairy herds in South Central Ohio were enrolled. Herd A milked approximately 1,100 cows and had a yearly rolling herd average milk yield of 9,796 kg, and herd B milked approximately 2,100 cows with a yearly rolling herd average milk yield of 10,547 kg. Pregnant animals were allocated to prepartum freestall pens with sand bedding at approximately 21 d prior to their expected calving date. Pre-parturient heifers and cows displaying imminent signs of calving were moved into individual (herd A) or grouped (3–4 cows, herd B) loose straw calving pens. Following parturition, primiparous (**PRIM**) and multiparous (**MULT**) cows were comingled in a postpartum pen where they remained for approximately 21 d. Thereafter, cows were moved to pens according to lactation number and reproductive status for the remaining of their lactation. All cows were housed in naturally ventilated 6-row freestall barns bedded with sand and had free access to feed and water. Postpartum cows (up to 21 DIM) were milked 3 and 4 times daily in herds A and B, respectively. After being moved from the postpartum pen, all cows were milked 3 times daily at approximately 8 h intervals in both herds. The mean stall stocking densities in the fresh pen were 89.78 and 105.29% for farm A and B, respectively. Feed was delivered once daily, and feed push up was performed at approximately 2-h intervals. Both dairy herds kept individual animal health, reproductive, and productive records using computerized record-keeping systems (Dairy Comp 305, Valley Ag Software, Tulare, CA). The procedures described below were reviewed and approved by the Institutional Animal Care Use Committee at The Ohio State University (protocol number: 2016A00000057).

### *Animal Enrollment and Diagnosis of Clinical Metritis*

Weekly, a list of cows for diagnosis of clinical metritis was obtained using on-farm computer records based on calving dates. The cows that presented history of disease or medical treatment were excluded from the list. The vaginal discharge of lactating dairy cows was screened for diagnosis of clinical metritis using a Metricheck device (Simcro Tech Ltd., Hamilton, New Zealand) at  $7 \pm 3$  d after parturition (study d 1). Briefly, the vulva was cleaned with a paper towel and the Metricheck device was introduced through the vulva into the cranial portion of the vagina; then the

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