



## Assessment of visceral pain associated with metritis in dairy cows

J. Stojkov,\* M. A. G. von Keyserlingk,\* J. N. Marchant-Forde,† and D. M. Weary\*<sup>1</sup>

\*Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, V6T 1Z4 Canada

†USDA-Agricultural Research Service Livestock Behavior Research Unit, West Lafayette, IN 47907-2042

### ABSTRACT

Metritis is a common disease in dairy cattle, but to our knowledge, no work has assessed pain associated with this disease. Tissue palpation is commonly used to assess pain in human and veterinary medicine. The objective of this study was to evaluate visceral pain responses during rectal palpation, with and without uterine palpation, in healthy cows and in cows diagnosed with metritis. A total of 49 Holstein dairy cows (mean  $\pm$  standard deviation parity of  $2.8 \pm 1.8$ ) were subjected to systematic health checks every 3 d after parturition for 21 d, scoring for vaginal discharge (0 to 4); 13 cows showed a discharge score  $\geq 2$  during at least 1 health check and were classified as metritic, whereas 29 cows were classified as healthy and showed no sign of this or any other disease (including mastitis and lameness). Back arch and heart rate variability before examination and during palpation were recorded using video and heart rate monitors. Back arch ( $\text{cm}^2$ ) on the day of diagnosis was greater in metritic versus healthy cows ( $1,034 \pm 72$  vs.  $612 \pm 48 \text{ cm}^2$ ), and greater during rectal palpation with uterine palpation versus rectal palpation without uterine palpation ( $869 \pm 45$  vs.  $777 \pm 45 \text{ cm}^2$ ). Heart rate frequency domain analysis showed that the low-frequency portion was higher in cows with metritis versus healthy cows ( $16.5 \pm 1.2$  vs.  $12.9 \pm 1.0$ ). Time domain analysis showed that the standard deviation between normal to normal interbeat intervals and the root mean square of successive differences both decreased during rectal palpation with uterine palpation versus rectal palpation without uterine palpation ( $1.9 \pm 0.1$  vs.  $2.5 \pm 0.1$  and  $1.3 \pm 0.1$  vs.  $1.7 \pm 0.1$ , respectively). Together, these results indicate that the inflammation associated with metritis is painful, and that the pain response can be detected during rectal palpation with and without uterine palpation. Rectal palpation with uterine palpation appears to be more aversive than rectal palpation without uterine palpa-

tion, suggesting that the former should be avoided when possible.

**Key words:** rectal palpation, back arch, heart rate variability, pain behavior

### INTRODUCTION

Physiological and pathological conditions can result in visceral pain in female reproductive organs. For example, humans sometimes report pelvic pain in the middle of the reproductive cycle, believed to be associated with ovarian follicle rupture and subsequent uterine contractions (Bonica et al., 1990). Pelvic pain is also associated with certain pathologies of the uterus, including postpartum metritis (Nelson et al., 1998), endometritis, and proliferation of the uterine mucosa (endometriosis; Bonica et al., 1990). Human patients diagnosed with deep infiltrated endometriosis also report a reduction in their subjective sense of wellbeing (Montanari et al., 2013).

Visceral pain in farm animals is mainly associated with severe gastrointestinal conditions, such as equine colic or traumatic reticuloperitonitis, and distension of abomasum or intestines in cattle (Radostits et al., 2007; Rialland et al., 2014). In cattle, back arch is considered as an indicator of hoof lesions (Flower and Weary, 2006) and as a diagnostic indicator for abdominal pain (Radostits et al., 2007). Parturition in cattle, thought to cause visceral pain in the dilatation phase and somatic pain in the expulsion phase (Lowe, 2002; Mainau and Manteca, 2011), is also associated with back arching (Haupt, 1991).

Responses to noxious stimuli provide one method of assessing visceral pain. Behavioral and physiological responses, such as flexion or withdrawal reflex, agitation while lying, generalized or regional muscle contractions, facial expression changes, cardiovascular, and respiratory changes, are associated with visceral pain (Ness and Gebhart, 1990; Rialland et al., 2014). Lumen distension or inflammation of certain hollow internal organs, such as the colon, bladder, and uterus, can initiate pain responses, but visceral organs are generally unresponsive to other damaging stimuli, such as cutting and burning (Cervero, 1994). Induced inflammation of the uterus

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<sup>1</sup>Corresponding author: danweary@mail.ubc.ca

evoked arching of the back and licking of the abdomen in mice (Wesselmann et al., 1998). Similar behavioral responses were reported in rats following inflammation of the colon (Laird et al., 2001). Collectively these studies suggest that uterine inflammation is likely to be painful in cattle.

Noxious stimulus of the uterine region is known to provoke cardiovascular and visceromotor responses (Ness and Gebhart, 1990). Visceral pain has been associated with cardiovascular responses, for example during colorectal distension in rabbits (Shafford and Schadt, 2008). Heart rate variability (**HRV**) changes in response to pain in sheep (Stubsjøen et al., 2009) and in response to internal and external stressors in cattle (Mohr et al., 2002). Heart rate variability can be used to assess the balance between sympathetic and parasympathetic activity of the autonomic nervous system and is considered a well-established, noninvasive method for detecting pain, stress, and pathological conditions in humans and other animals (von Borell et al., 2007).

Metritis is a common disease in cattle, with reported incidence ranging from 10 to 30% (Giuliodori et al., 2013). Culling rates in multiparous cows that develop metritis are approximately 30% higher compared with healthy cows (Wittrock et al., 2011). Metritis is known to induce behavioral and physiological changes including decreased feed intake, reduced milk production, and reduced competitiveness at the feeding bunk (Huzzey et al., 2007). To our knowledge, no study has assessed pain or even considered visceral pain as an underlying cause for these changes.

Tissue palpation, applied on a broad surface of an inflamed organ (Cervero, 1994), is a well-established method of evoking and thereby assessing pain (Ness and Gebhart, 1990; Radostits et al., 2007). Therefore, the objective of our study was to examine behavioral and physiological responses of dairy cattle during rectal palpation with and without uterine palpation in the days after calving. We hypothesized that transrectal uterine palpation would increase visceral pain in cows with metritis and that this pain would be associated with physiological and behavioral changes, specifically HRV and back arch. We predicted that the visceral pain provoked by palpation would increase the back arch in cows with metritis and would shift HRV toward sympathetic dominance, characteristic of painful conditions.

## MATERIALS AND METHODS

The experiment was conducted at The University of British Columbia Dairy Education and Research Center (Agassiz, British Columbia, Canada). Care of the

animals was according to the guidelines published by the Canadian Council on Animal Care (CCAC, 2009).

### ***Animals, Housing, and Management***

From July to September 2013, 49 Holstein dairy cows with mean ( $\pm$ SD) parity of 2.8 ( $\pm$ 1.8) were enrolled in the study. The average BW for the multiparous cows was (mean  $\pm$  SD) 751  $\pm$  95 kg and for the primiparous cows was 662  $\pm$  82 kg. All cows calved in an individual maternity pen but were then moved to a group pen where they were kept for 21 d. The postpartum pen had 20 lying stalls, arranged in 2 rows. The stocking density throughout the study was maintained at 100%. Each stall was fitted with a mattress and covered with 5 cm of sand. The postpartum pen had vulcanized rubber floors on the alleys and was equipped with 12 Insentec feed bins (Insentec, Marknesse, the Netherlands). Cows had ad libitum access to a TMR from the feed bins that were refilled twice daily at approximately 0800 and 1600 h. Water was also provided ad libitum from 2 self-filling water troughs. Cows were milked twice daily in a double-12 parallel milking parlor at approximately 0700 and 1700 h.

### ***Data Collection Using Videos and Heart Rate Monitors***

Cows were subjected to systematic health checks starting 3 d after parturition and continuing every 3 d for 21 d. Immediately after the morning milking, cows were moved into the sorting area for health check. The sorting area was equipped with 1 water trough and cows were restrained using self-locking headlocks. Once the cow was restrained, colored wax markings (Livestock Paint Crayon, Carmel, Montreal, QC, Canada) were placed on her spine to monitor changes in back arch. A video camera (HDR-PJ380, Sony Corporation, Tokyo, Japan) recorded the cow at 60 frames per second, from a distance of 3 m, and at a height of 1.45 m with an inclination (tilt) of 10°.

Heart rate monitors (**HRM**; Polar Equine RS800CX, Polar Electro Oy, Kempele, Finland) were used to measure the HRV of the cattle before and during the health checks, and were set to record interbeat intervals (R-R recording rate). The Polar HRM records heart rate from a distance and was previously validated for use in cows (Hopster and Blokhuis, 1994). Each HRM consists of an elastic strap, Wearlink transmitter, and watch receiver. The elastic strap had 2 incorporated electrodes and removable Wearlink transmitter attached, and was placed around the chest of each cow. Immediately before fitting the HRM, the left side of the cow's thorax and the lower part of the chest were soaked with warm

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