Abstract

Metritis is a common disease in dairy cattle but little 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 and uterine palpation in healthy cows and in cows diagnosed with clinical signs of metritis. A total of 49 Holstein dairy cows (mean ± SD parity = 2.8 ± 1.8) were subjected to systematic health checks starting 3 d after parturition and continuing every 3 d for 21 d. Cows were scored for vaginal discharge (0 to 4); 13 cows showed a discharge score ≥ 2 during at least one health check and were classified as metritic and 29 cows were classified as ‘healthy’ all showing no sign of any other disease (including mastitis and lameness). Back arch and heart rate variability (HRV) before examination and during palpation were recorded using video and heart rate monitors. Back arch (cm²) on the day of diagnosis was greater in metritic versus healthy cows (1034.3 ± 72.7 cm² vs. 612.8 ± 48.7 cm²), and greater during uterine versus rectal palpation (869.2 ± 45.0 cm² vs. 777.9 ± 45.0 cm²). Heart rate frequency analysis showed that the low frequency portion (LF %) was higher in cows with metritis versus healthy cows (16.5 ± 1.2 vs. 12.9±1.0). The SD between normal to normal inter beat intervals and the root mean square of successive differences both decreased during uterine versus rectal palpation (1.9 ± 0.1 vs. 2.5 ± 0.1 and 1.3 ± 0.1 vs. 1.7 ± 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 and uterine palpation. Uterine palpation appears to be more aversive than rectal palpation, suggesting that the former should be avoided when possible.
Preface

The research for this thesis was conducted at the University of British Columbia Dairy Education and Research Centre in Agassiz, BC. The research project and the methodology were approved by the University of British Columbia Animal Care Committee (application #A13-0148).

The material in Chapter 2 was submitted for publication under the title: Assessment of visceral pain associated with metritis in dairy cows by Stojkov, J., M.A.G. von Keyserlingk, J.N. Marchant-Forde and D.M. Weary. The manuscript was co-authored by my primary supervisor D.M. Weary, my co-supervisor, M.A.G. von Keyserlingk, and external collaborator J.N. Marchant-Forde from USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN. The co-authors supervised and assisted interpreting the data and provided comments on manuscript drafts. The methodology of the study was developed in consultation with D.M. Weary and M.A.G. von Keyserlingk. Jane Stojkov, the lead investigator established the main ideas of the study, conducted data collection, statistical analysis and manuscript composition.
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List of Abbreviations

ANOVA = analysis of variance
ANS = autonomic nervous system. Portion of the nervous system that controls visceral functions of the body
AVN = atrioventricular node
ARM = autoregressive modeling
Bpm = beats per minute
CPS = composite pain scale
DIM = days in milk
DMI = dry matter intake
FFT = fast Fourier transformation
HF = high frequency
HGS = horse grimace scale
HR = heart rate
HRM = heart rate monitors
HRV = heart rate variability
IASP = International Association for the Study of Pain
IBI = inter-beats interval. Time interval between two consecutive heart beats
LF = low frequency
LF/HF = low and high frequency ratio
LS = least-squares means
MGS = mouse grimace scale
NN50 = number of differences between two successive IBIs greater than 50 ms
NN interval = Normal-to-Normal interval = IBI
NRS = numerical rating system
NSAID = non-steroidal anti-inflammatory drug
PNS = parasympathetic nervous system
PRP = passive rectal palpation
PSD = power spectral density
RGS = rat grimace scale
RMSSD = square root of the mean of the sum of the squares of differences between successive IBIs
SDNN = standard deviation of normal-to-normal intervals
SN = sinoatrial node
SNS = sympathetic nervous system
UP = uterine palpation
VLF = very low frequency
Glossary

Algogenic = inducing pain
Allodynia = pain due to a stimulus that does not normally provoke pain
Appendicitis = inflammation of the appendix
Dystocia = obstructed (difficult) labour
Dysmenorrhea = pain during menstruation that interferes with daily activities
Hyperalgesia = increased sensitivity
Metritis = inflammation of the uterus
Neurovegetative = related to the vegetative (autonomic) nervous system
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To my supportive parents Ivanka and Todor, inspiring sisters Aleksandra and Svetlana, and my loving wife, Biljana
Chapter 1: General Introduction

1.1 Introduction

Animal welfare encompasses three major types of concern (Fraser et al., 1997): 1) natural behaviour - can animals express natural behaviours, 2) affective states – are animals free from negative states such as pain and fear, and also able to experience positive emotional states, and 3) biological functioning – are animals free from diseases and injuries, and in a normal range of physiological and behavioural functioning. Producers often focus on the animals' health and nutrition, which they see contributing to high productivity (Spooner et al., 2012). Similarly, veterinarians emphasize the importance of the animals’ health, with concerns focused primarily on prevention and treatment of diseases, injuries, and reproductive problems. In contrast, the general public perspective often focuses on the affective states of animals such as suffering from pain, fear, and hunger, and the opportunity to express natural behaviours (Vanhonacker et al., 2008; Spooner et al., 2012).

Dairy cattle experience routine husbandry practices that are likely painful, (e.g., tail docking, dehorning, and castration). In addition, cattle may experience numerous (production related) physiological and pathological painful conditions such as lameness, mastitis, displaced abomasum and parturition (Huxley and Whay, 2006). For instance, parturition is considered to cause visceral pain in the dilatation phase and sharp somatic pain in the expulsion phase (Mainau and Manteca, 2011), but is rarely treated by veterinarians when parturition is unassisted (eutocia) (Huxley and Whay, 2006). In comparison, humans rank labour pain as one of the most painful events, described as more painful than back pain, cancer, and arthritis (Melzack et al., 1981). In cattle, conditions such as acute metritis and left displaced abomasum are also considered painful (Huxley and Whay, 2006) as these conditions are accompanied with
inflammation and ischemia; well established noxious visceral stimuli (Ness and Gebhart, 1990). Despite this acknowledgement that these conditions are painful, analgesics are rarely used to mitigate the pain in cattle (Huxley and Whay, 2006).

1.1.1 Definition, classification, and mechanism of pain

The International Association for the Study of Pain (IASP) defines pain as, “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1994). This definition acknowledges different types of pain: cutaneous or superficial, somatic or musculoskeletal, and visceral. Cutaneous or superficial pain results from stimulation of the pain receptors in the skin e.g., fire burns, infected surgical wounds, and conjunctivitis. Somatic or musculoskeletal pain arises from underlying structures like muscles, joints, tendons, periosteum, and ligaments e.g., fractures, arthritis, and joint dislocations. Visceral pain arises from the receptors in the viscera, e.g., inflammation as in nephritis and enteritis; distension of viscera, such as the stomach, intestines, uterus and bladder (Radostits, 2007).

In general, the pain receptors (nociceptors) located on the endings of nerve fibres (A-delta and C fibres) encode the noxious stimulus, and as electrical impulses are transmitted via the dorsal horn of the spinal cord to the brain (Anderson and Muir, 2005b). Specialized nerve endings of the afferent neurons, also called peripheral nociceptors, are responsible for perceiving the noxious stimulus (Radostits, 2007). There are five classes of nociceptors: 1) thermal, 2) mechanoheat, 3) polymodal, 4) visceral, and 5) silent nociceptors (Radostits, 2007). When mechanical or thermal nociceptors are sensitized, the information travels through the large-diameter (fast conducting) thinly myelinated A-delta fibres, which are responsible for the ‘first pain’ or the ‘physiological pain’ (Anderson and Muir, 2005b; Radostits, 2007). Physiological pain is induced by minimal or potential tissue damage and represents a warning sign to the
organism of potential harm. Physiological pain is also responsible for triggering the body’s protective, physiologic and avoidance responses such as the ‘withdrawal reflex’ (Muir et al., 2001). The small-diameter unmyelinated slow-conducting C fibres are responsible for the ‘second pain’ or the slow response to the noxious stimuli that persists after the initial painful sensation ends (Radostits, 2007). This pain is perceived as dull and diffuse, and motivates the animal to decrease its movements to minimize pain and support recovery (Anil et al., 2002).

1.1.2 Characteristics of visceral pain

Visceral pain is associated with disorders and pathological conditions of the internal organs (e.g., intestines, urinary bladder and uterus). There is relatively little understanding of the mechanisms of visceral pain. Knowledge regarding visceral pain mainly derives from the experimental studies of somatic pain (Cervero and Laird, 1999; Cervero, 2010). Visceral pain is usually considered as a variant of somatic pain, suggesting that somatic and visceral pain have the same neurological mechanism (Cervero, 2010). However, newer work has revealed important differences in the mechanism of visceral pain between individual visceral organs and organ systems (Cervero and Laird, 1999). According to Cervero (2010), there are two main principles that apply to visceral pain: 1) visceral pain has different neurological mechanism than somatic pain, and 2) there is a difference in perception and psychological processing of visceral and somatic pain (Cervero and Laird, 1999; Cervero, 2010).

Cervero and Laird (1999) also describe five characteristics that distinguish visceral from somatic pain: 1) Visceral pain cannot be evoked from all visceral organs. Some visceral organs lack sensory receptors (e.g., liver, most solid visceral organs and lung parenchyma) and are thus not sensitive to pain; 2) Visceral injury does not always cause pain; e.g., cutting and burning the intestines does not cause pain. In contrast, stretching of the bladder or colon can cause pain. Both of these unique features of visceral pain are due to the nature of the peripheral
receptors of the nerves that innervate the visceral organs; 3) Visceral pain causes diffuse and poorly localized painful sensations; 4) Visceral pain is referred to other locations such as the body wall or other viscera and, 5) Visceral pain is accompanied with motor and autonomic reflexes, such as vomiting, increased abdominal muscle tension, and arched posture e.g., appendicitis, renal colic. The latter three features of visceral pain may be explained by the relative scarcity of peripheral afferent fibers in the visceral organs and the lack of separate visceral sensory pathways in the spinal cord and the central nervous system (Cervero and Laird, 1999; Cervero, 2010; Al-Chaer and Traub, 2002).

1.1.3 Inflammation mechanism

Inflammation is a complex series of responses by the organism to pathogens or other irritants (Lees et al., 2004). Tissue damage arising from infection initiates an inflammatory response by the body, including production of hydrogen and potassium ions, prostaglandins, histamine, bradykinin, growth factors, cytokines, and chemokines (Anderson and Muir, 2005b; Muir and Woolf, 2001). The mixture of mediators called ‘sensitizing or inflammatory soup’ act as nociceptor activators. This soup modifies the high-threshold into low-threshold nociceptors and contributes to the activation of the silent or sleeping nociceptors. This peripheral sensitization causes a condition called primary hyperalgesia (Anderson and Muir, 2005b; Muir and Woolf, 2001). The hypersensitivity amplifies the responses to noxious stimuli and reduces the intensity of the stimulus necessary to initiate pain (Muir and Woolf, 2001). Further excitability of the dorsal horn neurons results in central sensitization and development of secondary hyperalgesia and allodynia (Anderson and Muir, 2005a; Muir and Woolf, 2001). This condition increases the sensitivity of the neighbouring or remote (somatic and/or visceral) areas to the inflammation (Cervero, 2010).
Conditions associated with tissue inflammation are common causes of pain in farm animals (i.e., lameness, mastitis) (Sheldon et al., 2006; Dolan and Nolan, 2000). Recognizing and assessing pain associated with inflammatory diseases may be used to improve animal husbandry practices, and is essential for establishing pain management protocols.

1.2 Painful conditions in cattle

Cattle may experience numerous painful conditions that are often related to routine husbandry procedures or other pathological conditions.

1.2.1 Procedural related pain

Pain in farm animals has been studied for some routine husbandry procedures such as castration and dehorning. Physiological measures (heart rate, respiration rate and cortisol concentration levels), and behavioural measures (vocalization, head and ear movement, rearing, tripping, feet stomping, licking at the site, and tail wagging) have been used as pain indicators during or following the painful procedure (Molony et al., 1995; Faulkner and Weary, 2000; Stafford, 2007).

There is limited research on pain related to surgical procedures such as displaced abomasum surgery and caesarean section in farm animals (Walker et al., 2011). To our knowledge only one study reported pain related to rumen fistulation in dairy cattle and pain mitigation using a NSAID after surgery (Newby et al., 2014). However, studies on rats (Roughan and Flecknell, 2003), cats (Waran et al., 2007) and sea lions (Walker et al., 2009) described back arching, writhing, twitching and crouching as behaviours that occur in the hours after abdominal surgery.

Most of the pain related studies in farm animals are concentrated on assessing somatic pain; very little work has assessed visceral pain. Moreover, the literature that does refer to
visceral pain has been combined with somatic pain, mainly described as part of the castration process (Molony et al., 1995) or during induced traumatic reticuloperitonitis (Rialland et al., 2014). Both of these models used to assess visceral pain include trauma to the peritoneum that has somatic innervation (Giamberardino, 2009).

1.2.2 Pain related to disease

Lameness in farm animals has most likely been studied because of the painful nature, multifactorial etiology, and the high incidence (Grohn et al., 2003; Huxley and Whay 2006; von Keyserlingk et al., 2012). Algometers that measure the pressure that provokes foot withdrawal response were used to assess pain on the claw and soft tissues (Dyer et al., 2007). Studies have also assessed the pain related to hoof pathologies with and without analgesic (Dyer et al., 2007). One commonly used pain scale in cattle is the numerical rating system (NRS) for lameness scoring (Sprecher et al., 1997; Flower and Weary, 2006). In this scale gait and body posture, especially the back arch, are assessed to assess pain associated with hoof and leg pathologies. NRS ranges from 1 to 5, where 1 stands for normal gait and level back posture (normal) and 5 for severe lame cows with reluctance to bear weight on one or more feet and shows arched back posture (Sprecher et al., 1997). The NRS has been validated for detecting lameness in dairy cattle (Flower and Weary, 2006), and is established as a lameness assessment tool in the Code of Practice for Dairy Cattle (National Farm Animal Care Council, 2009).

Mastitis is a common condition in dairy cows. According to Huxley and Whay (2006) most veterinarians consider mastitis painful, especially acute mastitis caused by \textit{E. Coli}. However, few studies have assessed the pain related to mastitis. Behavioural changes associated with mastitis such as reduced lying time and restlessness during milking were reported by Medrano-Galarza et al. (2012). Using an algometer, Fitzpatrick et al. (2013)
assessed the increased udder sensitivity after induced clinical mastitis. Similarly, Milne et al. (2003) found increased sensitivity to pressure in the leg adjacent to the mastitic quarter, compared to the sensitivity on the opposite leg. These studies indicated that even moderate cases of mastitis are painful.

1.3 Metritis, general knowledge of the disease

Bacterial contamination of the uterus after parturition causes uterine disease (Sheldon et al., 2006). A uterus contaminated with pathogenic bacteria is typically inflamed, has histological lesions of the endometrium, and delayed uterine involution (Sheldon et al., 2002). Although metritis is not generally a life threatening infection, it is associated with impaired productive and reproductive performance (Borsberry and Dobson, 1989). Metritis is an acute inflammation of the uterus that usually occurs in the first 21 days after calving. Clinical signs include foul-smelling red-brown watery uterine discharge, dullness, inappetance, increased heart rate and reduced milk yield described as puerperal metritis (Sheldon et al., 2006). Metritis can also occur in a milder form where animals lack the symptoms of systematic illness but have an enlarged uterus and purulent uterine discharge (described as clinical metritis) (Sheldon et al., 2006). This condition is related to retained placenta, dystocia, stillbirth or twins, conditions that promote bacterial contamination in the uterus (Sheldon et al., 2006; LaBlanc et al., 2008). Although there is little evidence on the relationship between clinical signs and histopathology, the presence of pus proves presence of pathogenic bacteria in the uterus (Williams et al., 2005).

Timely diagnosis of the uterine infection is important for applying appropriate treatment and assessing the severity of the disease (Sheldon et al., 2006). A recent review reported that studies dealing with metritis applied 9 different diagnostic methods, illustrating the lack of a gold standard for metritis diagnosis. Vaginal discharge inspection, trans-rectal palpation and rectal
temperature were the most frequently used methods for metritis diagnosis (Sannmann et al., 2012).

The various methods used for metritis diagnosis likely explain discrepancies in the reported incidence. Incidence of metritis varies among studies from 7.6% (Grohn et al., 1995) to 69% (Urton et al., 2005). The incidence for puerperal and clinical metritis has been reported to be around 10% and 30%, respectively (Huzzey et al., 2007; Giuliodori et al., 2013).

Decline of reproductive performance is one important consequence of uterine disease. The negative effects of metritis on the reproductive performances in cattle include increased calving to conception intervals, delays in return to cyclicity after calving and changes in the uterine environment (Sheldon et al., 2002; Sheldon and Dobson, 2004; Giuliodori et al., 2013). In addition, culling decisions are often based on the reproductive status. Multiparous cows that develop metritis are at approximately 30% higher risk of culling compared to healthy cows (Grohn et al., 2003; Wittrock et al., 2011). Milk production declines in cows with metritis, which is related to the decreased feed intake and decreased energy for milk production (Wittrock et al., 2011; Giuliodori et al., 2013). Dry matter intake (DMI) and feeding behaviour decrease in the week before parturition, and are reported as good predictors for identifying cows at risk for developing metritis (Huzzey et al., 2007). Another behavior that modifies in cows at risk for metritis is feed competitiveness, which declines in the week before parturition (Huzzey et al., 2007).

Following metritis diagnosis cows spent less time feeding and have reduced DMI and water intake compared to the healthy cows (Huzzey et al., 2007). These changes may be associated with pain that these cows experience due to metritis. However, the fewer aggressive interactions during feeding before parturition suggest that avoiding competition at the feeding bunk is not associated with pain due to the disease.
Due to the difference in mechanisms between visceral and somatic pain the intensity of tissue damage cannot be easily assessed using spontaneous behavioural reactions (Foreman, 1999), suggesting that more sophisticated approaches are required. However, to date no study has directly assessed the pain associated with this condition in cattle. Below I review evidence from other species on responses to visceral pain associated with uterine infection and other conditions.

1.4 Assessment of pain in animals

There are three main methods used by pain researchers to assess pain in animals (Weary et al., 2006): 1) measures that reflect general body functioning (e.g., food and water intake, and weight gain), 2) physiological responses (e.g., heart rate, respiratory rate, and cortisol concentration), and 3) behaviour responses (e.g., vocalization, body posture, and gait assessment). Although food and water intake are easily quantifiable measures, they are more useful when assessing delayed changes in the body functioning, but not immediate responses to acute pain. Physiological measures for assessing pain are based on measuring responses of the autonomic nervous system (sympathetic activation), such as heart rate, and responses of the hypothalamic - pituitary - adrenocortical system, such as concentrations of cortisol. Heart rate variability provides more detailed information about responses of the autonomic nervous system, and has been increasingly used for assessing pain and stress in animals (von Borell et al., 2007). Behavioural measures involve responses such as vocalization (Taylor and Weary, 2000), restlessness (Molony and Kent, 1997), changes in the body posture (back arch) (Giamberardino et al., 1995; Roughan and Flecknell, 2003), and changes in the gait (lameness) (Sprecher et al., 1997, Flower and Weary, 2006), etc. Some animals mask behavioural responses to pain until their condition exacerbates suggesting that a combination of measures may be useful. In the following section of my thesis I will present and elaborate some frequently used pain related responses from behavioural and physiological nature.
1.4.1 **Back arching behaviour**

Back arching in cattle has been described in different physiological and pathological conditions. Physiologically, cows arch their back during defecation, urination, and parturition (Pilz et al., 2012; Houpt, 1991). In the arching process animals curve their back (spine). The abdominal muscular contractions and the posture is thought to assist in eliminating excretes from the body. Similarly, during parturition the uterine contractions and the abdominal muscles contribute in the process of expelling the foetus from the uterus/birth canal. Back arch has been reported as a response to vaginal examination in cows. However, it is unclear if the arching behaviour is a reflective attempt to expel the hand from the vagina or reaction to pain associated with the examination (Pilz et al., 2012).

Back arch has been reported as a symptom of several pathological conditions in cattle. Lameness in cattle is painful and associated with back arch (Sprecher et al., 1997, Flower and Weary, 2006). Abdominal pain in cattle due to reticuloperitonitis and abomasal volvulus is associated with back arch (Radostits, 2007). Back arching is considered a response to the abdominal pain associated with diarrhea in calves (Todd et al., 2010; Millman, 2013). Back arching behaviour also has been reported in laboratory animals as response to visceral pain and after surgery. Mice and rats with induced inflammation of the uterus and colon showed changes in the body posture such as hunching, hump-backed position, stretching the body and licking the lower abdomen (Wesselmann et al., 1998; Laird et al., 2001). Also, back arching and writhing have been described in rats with visceral pain caused by artificial ureteral calculosis (Giamberardino et al., 1995) and in rats after abdominal surgery (Roughan and Flecknell, 2003).

1.4.2 **Facial expressions**

Facial changes related to pain and other emotions in humans have been thoroughly studied (Ekman and Friesen, 1978). Facial expressions scales also have been developed and
used as a pain assessment tool in patients with limited communication capabilities (Williams, 2002). More recently, studies have described certain facial expressions in mice and rats related to painful stimuli (Langford et al., 2010; Leach et al., 2012; Sotocinal et al., 2011). Facial actions included orbital tightening, nose bulge, cheek bulge, ear position, and whisker change. Mouse and rat grimace scales were developed by coding these facial actions. Both scales were able to quantify pain of moderate duration and detect weak analgesic effects (Langford et al., 2010; Leach et al., 2012). Sotocinal et al. (2011) described the differences in facial expressions between rats and mice and developed a rodent face finder software for automated scoring of both mouse and rat faces.

Few studies have investigated facial expressions in farm animals in connection to their emotions. In cattle and sheep facial expressions during negative emotions such as anxiety, stress and frustration are described as protruded eyes, larger visibility of the eye white, flared nostrils, and flattened ears (Tate et al., 2006; Reefmann et al., 2009; Sandem et al., 2002). Recently, a horse grimace scale (HGS) was developed based on the mouse and rat grimace scales (MGS and RGS), involving 6 facial actions (Costa et al., 2014). The authors reported that HGS had average accuracy of 73.3% when assessing pain in horses subjected to castration.

1.4.3 Heart rate variability (HRV)

The heart beat activity is determined by the rate of depolarization of the cardiac pacemaker tissue, found in the sinoatrial node (SN), atrioventricular node (AV) and the Purkinje tissue. The SN is considered as the primary pulse generator because of its fast depolarization rate (Hainsworth, 1995). In healthy individuals, the heart rate at any time represents the net effect between the parasympathetic (vagus) nerves that decrease the HR and the sympathetic nerves that increase HR. The vagal dominance is present at rest while physical activity increases sympathetic dominance and decreases vagal activity. An increase in the HR could be
caused by: 1) sympathetic dominance, 2) decreased vagal activity, or 3) from simultaneous modifications in both sympathetic and parasympathetic regulatory systems (Hainsworth, 1995). The capacity of the two branches of the ANS to behave independently or in synchrony illustrates the complex interaction between the branches; simple addition or subtraction cannot be used to determine the effect of the two branches of the ANS. Detailed analysis of short term variation in the inter-beat interval (IBI) has been used to assess ANS regulation of cardiovascular function. Moreover, HRV analysis allows precise assessment of the balance between sympathetic and parasympathetic activity of the ANS. Also, HRV is considered a well-established, non-invasive method for detecting pain, stress and pathological conditions in humans and animals (von Borell et al., 2007). HRV analysis typically involves time domain and frequency domain analysis.

1.4.3.1 Time domain measures

The simplest parameters used to analyze HRV are from the time domain. These measures can be divided into two classes (Malik et al., 1996; von Borell et al., 2007): 1) measures of variability derived from IBI data; and, 2) measures of variability derived from differences between adjacent IBIs. The first class time domain measures include the mean IBI and the HR. Both of these measures are easy to calculate but less sensitive and not very informative regarding the sympathovagal balance. The standard deviation of normal to normal intervals (SDNN) can be observed as variations of all IBIs during a 24 h observation or during a single observed period (e.g. 5 min). The SDNN is measured in milliseconds and is considered to be a good indicator of the overall heart rate variability, as it reflects all the variability components (Malik et al., 1996). Because the HRV increases with the recorded length it is necessary to compare the SDNN obtained from segments with same IBI duration. These parameters indicate both sympathetic and parasympathetic activity and reflect the long term variability of cardiac activity (Kleiger et al., 1995; Malik et al., 1996; von Borell et al., 2007). The most informative parameter in the second class of time domain measures is the root mean square of successive
differences (RMSSD). The RMSSD estimates the high frequency beat-to-beat variations that represent vagal activity. Another parameter that uses beat-to-beat variations, is the number of neighbouring IBIs that differ more than 50 ms or NN50, and the pNN50, the proportion of beats differing by 50ms (NN50/total number of IBIs). These parameters are independent from diurnal or other long term trends and are indicators of vagal activity (von Borell et al., 2007; Kleiger et al., 1995).

The time domain measures, especially the RMSSD is considered reliable even when using intervals as short as 10 s (Thong et al., 2003). This might be explained by the vagal regulation that initiates prompt changes in HR. The HR changes associated with PNS occur within 5 s, while those associated with SNS occur slowly with delay of 5-20 s (von Borell et al., 2007). This is explained by the fast reacting acetylcholine mechanisms of the PNS, and the slow SNS regulation mediated by norepinephrine release from the sympathetic nerves (Malik et al., 1996).

The time domain measures are useful when identifying pain and stress in humans and animals (von Borell et al., 2007). Decline of SDNN has been reported as an indicator of pain in several studies, while reduced RMSSD has been primarily associated with stress (Hirsch et al., 1995). For instance, in sheep subjected to ischemic noxious stimulus both SDNN and RMSSD decreased compared to the sheep treated with analgesics (Stubsjøen et al., 2009). In laboratory mice a reduction of SDNN was reported 24 h after laparotomy (Arreas et al., 2007). Both SDNN and RMSSD were also reduced in calves with induced diarrhea (Mohr et al., 2002). In humans SDNN has also been reported to decrease following the application of a thermal painful stimulus (Meeuse et al., 2013), and in patients with angina (Ruggeri et al., 1996).
### 1.4.3.2 Frequency domain measures

For a deeper understanding of the dynamics caused by the beat-to-beat variations and how the overall variance is divided into different frequencies, methods such as the fast Fourier transformation (FFT) or autoregressive modelling (ARM) must be applied (Cerutti et al., 1995). The HR signal consists of harmonic waves, which when put together compose a complete waveform. The previously mentioned methods transform the HR waveform into power spectral density (PSD) (von Borell et al., 2007; Cerutti et al., 1995). There are three main portions that contribute to the total power. 1) Very low frequency (VLF) range ≤0.04 Hz, contains the long period rhythms. VLF is related to long term regulation mechanisms such as thermoregulation, reninangiotensin system and other humoral factors (Cerutti et al., 1995). VLF is considered to reflect both sympathetic and vagal activity, and therefore its physiological meaning is not clear (von Borell et al., 2007). 2) The low frequency (LF) ranges 0.04-0.15 Hz (generally centered around 0.1 Hz). Although there are controversies regarding the sympathetic and parasympathetic involvement in LF power, an increase in LF power has been considered as a marker of sympathetic activation (rest-tilt maneuver, mental stress, hemorrhage, coronary occlusion, etc.) (Cerutti et al., 1995; Kamath and Fallen, 1992). The high frequency (HF) component range 0.15-0.4 Hz is synchronous with the respiratory rate. This range corresponds with the respiratory rate of the species (e.g., in cattle HF ranges 0.20-0.58 Hz, adjusted for the respiratory rate of 12-35/min) (von Borell et al., 2007; Cerutti et al., 1995). HF is widely accepted as a marker of parasympathetic activity. LF/HF ratio is a measure of sympathovagal balance, but can also be influenced by other physiological functions. LF and HF power can be expressed in absolute values (seconds²) or as normalized units (nu) as proportion of the total power (e.g., LF/total power) (von Borell et al., 2007; Cerutti et al., 1995).

The HF component reflects the vagal activity and has been used to assess stress responses in humans and animals. Studies in humans reported decreased HF power in
response to high stress in the morning and increased HF power in the evening when the mental
stress was lower (Orsila et al., 2008). The opposite was established for the LF power in the
same study (Orsila et al., 2008). HF power was reported to increase in horses during rest but
was reduced when walking and trotting (Physick-Sheard et al., 2000). Increased LF component
has been primarily associated with painful conditions in humans and animals. Increase of LF
power was observed in volunteers that were subjected to painful stimulus (Terkelson et al.,
2005). Laminitis, a painful condition in horses, caused LF power to increase and HF to
decrease. Following analgesic treatment this pattern was reversed (Rietmann et al., 2004).

1.4.4 Veterinary approach to assessing pain

In clinical settings pain is a valuable symptom and used by veterinarians when
assessing an animal’s health. Veterinary clinicians have relied on various pain scales for
assessing pain. For example, the visual analog scale and simple descriptive scale have been
used to describe pain in animals (Anil et al., 2002; Bufalari et al., 2007). Recently, the composite
pain scale (CPS) was validated for assessing somatic and visceral pain in horses (van Loon et
al., 2010). The CPS combines behavioural data (appearance of the animal, sweating, kicking at
the abdomen, pawing on the floor, posture, head movement, appetite, and response to
observer), responses to stimuli (interaction, responses to palpation), and physiological data
(heart rate, respiration rate, digestive sounds, and rectal temperature) for pain assessment.
Results from a survey of animal health professionals identified the need for scoring systems for
pain assessment in cattle (Fitzpatrick et al., 2002).

Few studies have reported the attitudes and approaches of veterinarians toward pain
assessment and management in farm animals. A survey on the attitudes of U.K. veterinarians
showed that pain was typically ranked higher by female veterinarians and recent graduates
(Huxley and Whay, 2006). Veterinarians that showed more empathy towards animal pain also
used pain mitigation frequently (Huxley and Whay, 2006). Similar findings were reported for veterinarians from Scandinavian countries regarding use of analgesics in cattle; younger graduates and female veterinary practitioners agreed that using analgesics improves recovery (Thomsen et al., 2010). As the pain literature grows and the knowledge about pain accumulates, the recognition and treatment of pain in farm animals will likely improve.

1.5 Assessing visceral pain in humans and animal models

The characteristics of visceral pain and the difficulty in defining adequate noxious visceral stimuli requires the development of appropriate methods and models for assessing visceral pain.

1.5.1 Historical overview of visceral pain studies

By the beginning of the 20th century it was recognized that internal organs sometimes lack sensitivity to normal painful stimuli such as cutting, scratching or pinching, but other work showed sensitivity to other stimuli, including electrical, mechanical and chemical stimulation, and ischemia (Ness and Gebhart, 1990). Distension of easily accessible hollow organs was commonly used to provoke visceral pain in the gastrointestinal tract in humans. Painful distension of the esophagus was reported by Payne and Poulton (1923) and described as “burning” at low pressures and “gripping” when larger pressures were applied. Goligher and Hughes (1951), using an inflated balloon, found that patients reported pain in the colon and rectum at 80 and 62 mm Hg, respectively. Other studies have reported presence of mechanoreceptors on the surface of the uterus and the broad ligament in cats (Floyd et al., 1976). These mechanoreceptors respond to manual compression of the uterus and to intense uterine distension of up to 100 mmHg of pressure (Abrahams and Teare, 1969). Collectively, these studies provide evidence that normal (healthy) visceral organs are capable of painful sensations, particularly in situations where the organ wall is stretched.
Visceral pain associated with inflammation of internal organs was first described in cases of appendicitis. Acute appendicitis was described to be extremely sensitive, even to the normal intestinal motility (Kinsella, 1940). More recently, inflammation of the uterus associated with post-partum metritis (Nelson et al., 1998), endometritis, and proliferation of the uterine mucosa (endometriosis) has been described as painful (Bonica, 1990).

1.5.2 Pelvic pain and innervation of the uterus

Female reproductive organs can be divided to internal and external genitalia. The external female genitalia have somatic innervations, including labia major and minor, clitoris and vestibule, responding to sensations such as touch, temperature and pain. Pain is the only sensation that can be evoked from the internal genitalia (Cervero, 1994). The innervation of the female internal genitalia consists of afferent fibres running in sympathetic and parasympathetic nerves. The afferents in the hypogastric nerves innervate the ovaries, fallopian tubes, uterus and the uterine portion of the cervix, and project to the lower thoracic and upper lumbar segments of the spinal cord. Afferents in the pelvic nerves innervate the vagina and enter the sacral segments of the cord (Cervero, 1994).

In humans, certain physiological conditions can induce painful sensations from the internal genitalia. Pelvic pain has been associated with rupture of the ovarian follicle, and followed by uterine contractions stimulated by the follicular fluid release (after ovulation). This syndrome that occurs in the middle of the cycle and followed with pelvic pain is known as “Mittelschmerz” (Bonica, 1990). In contrast, parturition is commonly accompanied by pain from the uterus. The dilatation phase is frequently associated with visceral pain arising from the cervical widening, uterine contractions, and distension of the uterus (Lowe, 2002). Certain pathological conditions such as ovarian cysts, torsion of the fallopian tubes caused by tumours,
inflammatory processes of the uterus and proliferation of the uterine mucosa are considered to cause pelvic pain with different intensity (Bonica, 1990; Cervero, 1994).

Despite the findings summarized above there is a lack of understanding of the functional roles of afferent fibres from the internal genitalia (Cervero, 1994). Studies have shown that blocking the sympathetic innervation of the uterus eliminates the visceromotor reflexes activated by uterine distension in animals, and labour pain in women (Cervero, 1994). Moreover, section of the hypogastric plexus entirely alleviated primary dysmenorrhea in women, without interference of the reproductive functions (Cervero, 1994). Collectively these studies, and the substantial clinical observations, suggest that sympathetic afferents mediate uterine pain (Cervero, 1994; Bonica, 1990).

1.5.3 Visceral pain

Visceral pain is considered as a major reported symptom in internal medicine and one of the most frequent reasons patients seek medical attention (Bonica, 1990). There are two phases of the visceral pain symptom: 1) true visceral pain and 2) referred pain (Giamberardino et al., 2001; 2002). The first phase or ‘true visceral pain’ consists of vague, diffuse and poorly localized sensation, associated with neurovegetative signs such as nausea, vomiting, sweating, changes in heart rate and blood pressure, and emotional reactions such as anxiety and anguish (Giamberardino et al., 2001; 2002). Although the intensity of the true visceral pain can vary from slight to unbearable, it has no relationship with the extent of internal tissue damage. For example, silent myocardial infarction can cause extensive damage to the myocardium and cause only mild pain while other less damaging ischemic episodes in some forms of angina can cause major suffering in the patients (Foreman, 1999). The second phase begins when the visceral pain becomes referred to somatic structures and becomes sharper, more localized and defined, similar to somatic pain. The subsequent hyperalgesia involves the skin, the
subcutaneous tissue, and the muscle layer (Giamberardino et al., 2002). This phenomenon of hyperalgesia also affects the visceral organs, mainly due to inflammation and prolonged stimulation (visceral hyperalgesia) (Giamberardino et al., 2001, 2002).

Another interesting phenomenon of hyperalgesia is the interaction between two different visceral structures, called ‘viscero-visceral hyperalgesia’. This term describes the clinical manifestation of an algogenic condition from one to another viscus whose afferent innervation is partially overlapping (Giamberardino et al., 2001, 2002). For example, patients with conditions of ischemic heart disease and gallbladder calculosis complain of a higher number of painful episodes than patients who suffer from ischemic heart disease only (innervation of gallbladder and heart partially overlap) (Giamberardino et al., 2001). Similar findings are established in patients with urinary calculosis and pelvic inflammatory conditions, such as dysmenorrhea (female reproductive organs and urinary tract innervation overlap) (Giamberardino et al., 2001).

Visceral pain originating from the chest, abdomen or the pelvic region is experienced by a large portion of the population in the modern society (Halder and Locke, 2009). These conditions are usually described as non-specific abdominal pain, irritable bowel syndrome (chronic gastrointestinal disorder accompanied by recurrent abdominal pain), non-cardiac chest pain, pelvic pain, etc. Visceral pain conditions contribute to diminished quality of life, increased medical expenses and contribute to the decline of productivity in the workplace (Halder and Locke, 2009).

1.5.4 Visceral pain models

Various animal models have been used to investigate visceral pain. There are three classes of visceral models (Cervero and Laird, 2009): 1) simple models of visceral pain, 2) mechanism based models of visceral pain, and 3) disease based models of visceral pain. The ‘writhing test’ is the oldest of the simple models of visceral pain. This test consists of applying an
intra-peritoneal injection of certain irritants and observing the acute behavioural responses such as writhes or abdominal contractions. This test has been criticized, as it involves the peritoneum, which has somatic afferents. Another test is colo-rectal distension (CRD), which uses rapid distension of the colorectal regions via inflation of a balloon and recording the related behavioral responses. Similar approaches have been applied to other hollow internal organs such as stomach, duodenum, gallbladder, ureter, urinary bladder and uterus (Cervero and Laird, 2009).

Mechanism based models of visceral pain include models of primary and secondary hyperalgesia. Primary hyperalgesia models are based on inducing inflammation in internal organ using substances such as turpentine and mustard oil. The inflammation sensitizes the nociceptors and generates primary hyperalgesia (Cervero and Laird, 2009). These experiments have shown increased pain sensitivity and lower pain thresholds of the afferent fibers in the gastrointestinal tract, urinary tract, reproductive organs and other organ systems during inflammation (Traub, 2003; Giamberardino et al., 2002).

Models of secondary (referred) hyperalgesia use substances such as capsaicin that can generate central sensitization (Cervero and Laird, 2009). Using these models scientists can investigate the viscero-somatic and viscero-visceral convergence of the neural pathways and have found evidence of convergence of pelvic visceral afferents involving colon/rectum, urinary bladder and uterus (Ness and Gebhart, 1990; Berkley et al., 1993). In addition, the secondary hyperalgesia has been studied showing tactile hypersensitivity occurring near the incision site after surgeries (Zahn et al., 1999).

Disease based models of visceral pain are valuable for studying the specific mechanisms related to diseases, including models of gastrointestinal diseases, urogenital diseases, and models of functional pain (Cervero and Laird, 2009). Models that reproduce the
natural complexity of the visceral pain and hyperalgesia are required to address the complexity of the associated visceral pain.

1.5.5 Recommendations for assessing visceral pain

Experimental studies concerning visceral pain have had difficulties in properly defining an adequate noxious stimulus (Ness and Gebhart, 1990). A noxious stimulus is typically defined as one that produces tissue damage or potential tissue damage (Sherrington, 1903). Although this definition is considered valid for the cutaneous structures, it cannot be readily applied to visceral organs that are insensitive to cutting and burning stimuli. However, numerous studies have established that pain can originate from the viscera, if an adequate stimulus is applied. An early example of evoked visceral pain was that caused by squeezing the entire length of inflamed appendices (Kinsella, 1940). However, pinching the appendices of the same patients with forceps (2-3 mm width) was painless (Ness and Gebhart, 1990; Kinsella, 1940). Noxious visceral stimuli are preferably natural stimuli that can induce visceral pain and behavioural responses typically associated with tissue damaging stimuli such as escape and avoidance. In addition, an adequate noxious visceral stimulus should be reproducible, easily controlled, quantifiable, and minimally invasive (Ness and Gebhart, 1990).

Inflammation, ischemia and mechanical stimuli (e.g., traction of the mesentery, distension of hollow organs, stretching of serosal tissues and compression of some organs) are well-established adequate noxious visceral stimuli. These stimuli are expected to provoke responses including flexion/withdrawal, head turning, grimacing, vocalization, cardiovascular changes, respiratory changes and generalized or regional muscle contractions (Ness and Gebhart, 1990). These responses cease when the noxious stimulus is terminated.

According to Ness and Gebhart (1990) recommendations for a valid adequate noxious visceral stimulus (in humans and animals) are the following: (1) the stimulus must produce pain
(in humans) and ideally be related to pathological pain; (2) the stimulus must alter the behaviour of the experimental subject in a way consistent with an interpretation that the stimulus is aversive (i.e., the animal changes behaviour to avoid the onset or continuation of the stimulus); (3) the stimulus must evoke basic physiological 'pseudoaffective' reflexes consistent with what occurs in humans in response to visceral pain; and, (4) responses to the stimulus must be modulated by anti-nociceptive manipulations (e.g., morphine) in a way consistent with the clinical effects of the same manipulations in humans experiencing visceral pain (Ness and Gebhart, 1990).

1.6 Objective and hypothesis

The objective of this study was to examine behavioural and physiological responses of dairy cattle during rectal and uterine palpation in the days after calving, and to determine if cows with clinical signs of metritis show stronger pain responses. We hypothesized that trans-rectal uterine palpation would increase visceral pain in cows with metritis and that this pain would be associated with physiological and behavioural changes, specifically heart rate variability and back arch.
Chapter 2: Assessment of visceral pain associated with metritis in dairy cows

2.1 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 post-partum metritis (Nelson et al., 1998), endometritis, and proliferation of the uterine mucosa (endometriosis). Human patients diagnosed with deep infiltrated endometriosis also report a reduction in their subjective sense of well-being (Montanari et al., 2013).

Pain arising from the visceral organs is rarely studied in animals. Visceral pain in farm animals is mainly associated with severe gastrointestinal conditions, such as the equine colic, and distension of abomasum or intestines in cattle (Radostits, 2007). In cattle, back arch is considered as an indicator of hoof lesions (Flower and Weary, 2006), and as a diagnostic for abdominal pain (Radostits, 2007). Parturition in cattle, thought to cause visceral pain in the dilatation phase and somatic pain in the expulsion phase (Mainau and Manteca, 2011; Lowe, 2002), is associated with behaviours including back arching (Houpt, 1991).

Responses to noxious stimuli provide one method of assessing visceral pain. Responses such as flexion/withdrawal reflex, generalized or regional muscle contractions, facial expression changes, cardiovascular, and respiratory changes are associated with visceral pain (Ness and Gebhart, 1990). Lumen distension or inflammation of certain hollow internal organs, such as the colon, bladder, and uterus can initiate pain responses, but the same visceral organs are generally unresponsive to other damaging stimuli, such as cutting and burning (Cervero, 1994). Induced inflammation of the uterus evoked arching of the back and licking of the
abdomen in mice (Wesselmann et al., 1998). Similar behavioural responses were reported in rats following inflammation of the colon (Laird et al., 2001). Collectively these studies suggest that uterine inflammation may also 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 also 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 (Stubsojen et al., 2009) and in response to internal and external stressors in cattle (Mohr et al., 2002). HRV can be used to assess the balance between sympathetic and parasympathetic activity of the autonomic nervous system and is considered to be a well-established, non-invasive 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 to healthy cows (Wittrock et al., 2010). Metritis is known to induce behavioural 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 specifically assessed the pain or even considered visceral pain as an underlying cause for these changes.

Tissue palpation, applied on a broad surface of an inflamed uterus (Cervero, 1994), is a well-established method of evoking pain (Ness and Gebhart, 1990; Radostits, 2007). The objective of this study was to examine behavioural and physiological responses of dairy cattle during rectal and uterine palpation in the days after calving, and to determine if cows with clinical signs of metritis show stronger pain responses. We hypothesized that trans-rectal
uterine palpation would increase visceral pain in cows with metritis and that this pain would be associated with physiological and behavioural changes, specifically heart rate variability and back arch.

2.2 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).

2.2.1 Animals, housing and management

From July to September 2013, 49 Holstein dairy cows with mean (±SD) parity of 2.8 (±1.8) were enrolled in this study. The average body weight for the multiparous cows was (mean ± SD) 751 ± 95 kg and for the primiparous cows was 662 ± 82 kg. All cows calved in an individual maternity pen but were then moved to a group pen where they were kept for 21 days. The post-partum pen had 20 lying stalls, arranged in two 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 feed bins. Cows had ad libitum access to a total mixed ration (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 parlour at approximately 0700 and 1700 h.

2.2.2 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 one water trough and cows
were restrained using self-locking headlocks. Once restrained, coloured wax markings (Livestock Paint Crayon, Carmel, Montreal, QC) were placed on the cow’s 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 heart rate variation of the cattle prior to and during the health checks, and were set to record inter-beat intervals (R-R recording rate). The Polar HRM records heart rate variability from a distance, and was previously validated for use in cows (Hopster and Blokhuis, 1994). Each HRM consists of elastic strap, Wearlink® transmitter and Wearlink® watch. The elastic strap had two incorporated electrodes and removable Wearlink® transmitter attached, and was placed around the chest of each cow. Immediately before fitting of the HRM, the left side of the cow’s chest and the lower part were soaked with warm water. The upper electrode was located behind the left shoulder blade and the lower electrode placed near the sternum. After placing the elastic strap around the cow’s chest, the Wearlink® watch was started. Previous studies have reported a period of 5-10 min as sufficient for animals to adjust to the HRM equipment (von Borell et al., 2007; Moher et al., 2002). In this study cows were allowed to habituate to the equipment for approximately 10 min. The trained examiner used the Wearlink® watch to digitally mark each step of the health check procedure.

### 2.2.3 Health check description

The health checks were undertaken by a trained veterinarian and involved rectal cleaning, passive rectal palpation, uterine palpation and lastly, vaginal discharge scoring. The veterinarian entered the rectal cavity with a lubricated hand (General Lube, First Priority, Inc., Elgin, IL, USA), and evacuated the feces from the rectum (mean duration ± SD of this step: 34.0
± 5.5 s). Cows were left undisturbed for approximately 1 min after the hand was removed. The examiner then re-entered the rectal cavity and placed the palm of his hand in a stand-still position against the uterine wall (passive rectal palpation) for 20 s. The examiner then applied constant force for 20 s on the uterus in the direction of the pelvic wall (active phase). To estimate the force applied during the uterine palpation, the event was replicated under laboratory conditions using a Vernier Force Plate and Logger pro software (Vernier Software & Technology, Beaverton, OR, USA); force averaged (±SD) 96 ± 11 N. Finally, vaginal discharge (VD) was collected and scored following method described by Huzzey and colleagues (2007); briefly, no mucus or clear mucus, no odor = 0; cloudy mucus, with some pus or blood, no odour = 1; mucopurulent (less than 50% pus) and foul smell = 2; purulent (more than 50% pus) and foul smell = 3; and putrid (red/brown color, watery, foul smell) = 4. Cows with VD scores of 0 or 1 during the entire 21 d observation period were considered healthy. Cows with VD scores of 2, 3 and 4 were classified as having metritis. Following diagnosis, cows were treated with antibiotics and analgesics, and not subjected to any additional health checks. Cows that developed lameness, displaced abomasum, severe vaginal or vulvar tears, mastitis, or that were treated with antibiotics or analgesics before the exam were excluded from the trial (n=7).

2.2.4 Back arch

Back arch was defined as the two-dimensional semicircular shape created by curving the spine during the passive rectal and uterine palpation. The back arch was quantified using the wax body-marks on the highest thoracic vertebra and on the first coccygeal vertebra. Two lines that connected the body-marks were used to mark the area of back arch (Fig. 1). The area of back arch was selected and measured from photos using Adobe Photoshop CS6 (Adobe Systems, San Jose, CA, USA). The pixels from the selected two-dimensional semicircular area from the photo were converted into cm² that corresponded with the actual area of back arch on the cow. Back arch was assessed during passive rectal palpation using 4 snapshots taken 4, 8,
12, and 16 s, from the onset of this phase; these four values were then averaged. This procedure was repeated for the uterine palpation phase. Back arch before examination was assessed from 2 snapshots at 10 s and 20 s before rectal cleaning commenced, these values were also averaged. Back arch was quantified for 42 cows (15 primiparous and 27 multiparous); of these, 29 were healthy and 13 metritic.

Intra- and inter-observer agreement for back arch was calculated using 20 randomly selected photos. We used the methods described by Bland and Altman (2003) to calculate the difference between observers and limits of agreement. Intra-observer difference was $2.8 \pm 27.8$ cm$^2$ (mean ± SD) and limits of agreement (57.4 cm$^2$ to -51.7 cm$^2$). For the inter-observer difference, the mean (±SD) was $-26.8 \pm 36.7$ cm$^2$ and the limits of agreement ranged from 45.1 cm$^2$ to -98.7 cm$^2$.

2.2.5 Heart rate variability (HRV)

The data from the Wearlink® watch were downloaded using the Polar Pro Trainer 5 Equine Edition software (Polar Electro Oy, Kempele, Finland) and HRV measures were extracted using the graphical output. HRV data were visually inspected for presence of the five types of anomalies as described by Marchant-Forde and colleagues (2004). Visual inspection was conducted before and after the error correction filter was applied. The anomalies were corrected using the automatic Polar Pro Trainer 5 error correction filter set at moderate mode (default setting). However, the setting was changed to very high mode to edit specific artifacts that were not successfully handled using the default setting (n=10). Files that contained more than 5% anomalies were excluded from the analysis (von Borell et al., 2007). The frequency bands were set to 0.003-0.04 Hz for the very low frequency (VLF), 0.04-0.25 Hz for the low frequency (LF) and 0.25-0.50 Hz for the high frequency (HF), adjusted for the respiratory rate in cattle (Kamath and Fallen, 1993; Mohr et al., 2002; von Borell et al., 2007). Two different time
periods were selected to analyze the HRV measures. The period before examination started 10 min after the HRM were attached and consisted of 512 inter beat intervals (IBI). The average duration (mean ± SD) of these segments was 6.1 ± 0.3 min. The cows were in a stationary position and no examination was conducted at this point. Both time and frequency domain measures were extracted and used in the analysis for HRV prior to examination. In the time domain, SD between normal to normal inter beat intervals (SDNN), root of the mean square of successive differences (RMSSD) and average IBI were extracted. The frequency domain measures included very low frequency (VLF), low frequency (LF), high frequency (HF), and the LF/HF ratio. VLF, LF and HF were expressed in normalized units as percentage of total power (e.g. LF/total power); VLF%, LF% and HF% (von Borell et al., 2007). The second period (during passive rectal and uterine palpation), contained 2 sections of 20 IBI each selected during passive rectal and uterine palpation. Segments were selected starting at 4 s from the onset of the procedure and lasted approximately (mean ± SD) 14 ± 1 s. Due to the short duration of these recordings only time domain analysis was conducted and included SDNN, RMSSD, and average IBI (Thong et al., 2003). HRV measures during examination were calculated and analyzed for 38 cows (29 healthy and 9 metritic), consisting of 12 primiparous and 26 multiparous. Cows that showed anomalies in the HR data after the error correction filter was applied were excluded from the analysis (n=4). The sample for HRV measures before examination included an additional 15 cows. Cows that did not provide 512 IBI’s or showed anomalies in the data after the error correction filter was applied were excluded from the analysis (n=10). In total, HRV measures before examination were calculated and analyzed for 47 cows (30 healthy and 17 metritic), consisting of 16 primiparous and 31 multiparous cows.

2.2.6 Statistical analysis

Statistical analyses were conducted using SAS (version 9.3, SAS Institute Inc., Cary, NC). The UNIVARIATE procedure was used to scan for normality and outliers. Two different
models were used. Two-way ANOVA was used to determine the effect of health status (healthy versus metritic) and parity (primiparous versus multiparous) on the back arch and HRV measures prior to examination. The LS Means were calculated and adjusted using the Tukey adjustment statement, and were compared using the PDIFF statement.

The effects of health status, examination method, parity and DIM (days in milk) on back arch and HRV measures during passive rectal and uterine palpation were tested using MIXED model, where cow was set as a random variable. As the effect of parity and DIM were not significant, the final model included only the health status (healthy cows versus cows with metritis), type of exam (passive rectal versus uterine palpation), and the interaction. Residuals from each model were checked for homoscedasticity and normality.

The LF/HF ratio and normalized values of VLF, LF, HF from the frequency domain, and SDNN, RMSSD and average IBI from the time domain were used in the analyses. The presence of outliers and skewness of HF% and LF/HF ratio measures in the HRV before examination required that these data be log transformed. Similarly, SDNN and RMSSD from the HRV during passive rectal and uterine palpation were log transformed to normalize errors.

2.3 Results

Back arch before examination tended to be higher in metritic versus healthy cows (119 ± 48 cm² vs. 16 ± 33 cm²; F₁,₄₀= 3.2; P= 0.08). During the examination, back arch was greater in cows with metritis than in healthy cows (1034 ± 73 cm² vs. 613 ± 49 cm²; F₁,₄₀= 23.2; P< 0.001; Fig. 2). Regardless of health status, the back arch was also greater during the uterine palpation compared to the rectal palpation (869 ± 45 cm² versus 778 ± 45 cm²; F₁,₄₀= 18.4; P= 0.001). The interaction between type of exam and health status had no effect on the back arch.
Before examination, parity affected both time and frequency domain HRV measures (IBI, VLF% and LF%). Health status only affected frequency domain HRV measures (Table 1). IBI and LF% were 47.7 ms and 5.4 units higher in multiparous cow. In contrast, VLF% was 5.1 units lower in multiparous cows. Differences between healthy and metritic cows were observed for VLF% and LF%. LF% was 3.6 units higher in metritic cows and VLF% was 3.9 units lower in metritic cows. Interaction between health status and parity had no effect on HRV.

Time domain measures of HRV were lower during the uterine palpation relative to the passive rectal palpation (SDNN $F_{1,36} = 9.6; P < 0.01$, and RMSSD $F_{1,36} = 9.8; P < 0.01$; Fig.3). Uterine palpation decreased SDNN and RMSSD by about 25%, compared to passive rectal palpation. There was no difference in IBI during passive rectal versus uterine palpation (706.8 ± 13.6 ms vs. 708.6 ± 13.6 ms, respectively), and no interaction between parity and health status for any HRV measure. Health status had no significant influence on the HRV time domain measures during examination. SDNN for healthy versus metritic cows averaged 2.2 ± 0.1 ms versus 2.3 ± 0.2 ms, RMSSD averaged 1.4 ± 0.1 ms versus 1.6 ± 0.1 ms, respectively, and IBI averaged 718.4 ± 13.1 ms versus 696.9 ± 23.6 ms.

2.4 Discussion

Back arch in response to visceral pain has been previously reported in studies in laboratory rats with induced uterine inflammation (Wesselmann et al., 1998), and mice with induced colon inflammation (Laird et al., 2001). To our knowledge, this is the first study that objectively measured back arch in response to visceral pain associated with metritis, and the first to use this measure to assess pain response in naturally occurring cases of disease in any species. Cows with metritis responded more to both rectal and uterine palpations. This more pronounced back arch is likely associated with pain that accompanies the inflammation of the uterine wall; inflammation and ischemia of the viscera are considered to be noxious visceral
stimuli (Ness and Gebhart, 1990). The results from this study suggest that metritic cows have uterine hyperalgesia. Inflammation of the internal organs may activate previously unresponsive receptors, contributing to increased sensitivity during palpation (Cervero and Laird, 1999). Similarly, pain associated with appendicitis can lower pain thresholds to the extent that the motility of the surrounding intestines becomes painful (Cervero, 1994).

Back arching in cattle is associated with both physiological and pathological conditions. From a physiological perspective, back arching is associated with urination, defecation and parturition (Pilz et al., 2012; Houpt, 1991). Pathologically, back arching is associated with hoof lesions and has been incorporated into scales used to assess lameness (Flower and Weary, 2006). Back arch is also used for diagnosis of abdominal pain caused by peritonitis, traumatic reticuloperitonitis, and abomasal volvulus (Radostits, 2007). The presence of back arch in cattle with inflammation of the peritoneum and the adjacent visceral organs supports the assumption that inflammation of the viscera causes back arching. Back arch has also been reported during vaginal examination (Pilz et al., 2012). In other species, back arch has also been reported as a pain response following abdominal surgery in sea lions (Walker et al., 2009) and rats (Roughan and Flecknell, 2001). The latter authors have advocated including back arch into a scoring system for abdominal pain.

Although the majority of the fecal material within the rectum had been removed, insertion of the hand and arm may have induced a defecation response that is often accompanied by an arched back. However, differences between healthy and metritic cows cannot be accounted for by the defecation reflex (Pilz et al., 2012). We suggest that the observed differences are a consequence of the palpation stimulating visceral pain in cows with an inflamed uterus. Visceral pain is often projected to other regions of the body (‘referred pain’; Wesselmann and Lai, 1997). Referred visceral pain was previously reported in studies in humans (Giamberardino et al., 1997) and lab animals (Wesselmann and Lai, 1997). Rats with an inflamed uterus show
evidence of referred pain around the abdomen, groin, lower back, thighs and perineal area (Wesselmann and Lai, 1997). Similarly, studies have found that visceral pain stimulates the adjacent viscera, inducing viscero-visceral hyperalgesia (Giamberardino et al., 2001, 2010; Cervero, 2000). Brinkert and colleagues (2007) showed that menstrual pain causes hyperalgesia in the sigmoid colon and the rectum. Considering the proximity of the rectal segment involved in the palpation and the inflamed uterus, we suggest that the back arching during passive rectal palpation in metritic cows can be attributed to the visceral hyperalgesia of the rectum.

The low frequency portion of HRV reflects both sympathetic and parasympathetic branch of the autonomic nervous system (Malik and Camm, 1995). However, LF in normalized units or LF% is considered to reflect mainly sympathetic activity (Malik et al., 1996). Uterine pain is mediated by sympathetic nerves and blocking the sympathetic innervations of the uterus reduces visceromotor reflexes caused by uterine distension in animals and pain during labour in women (review by Cervero, 1994). Sympathetic activation projected as increased HR and decreased HRV has been related to post-laparotomy pain in mice (Arras et al., 2007), noxious ischemic stimulus in sheep (Stubsjoen et al., 2009), and visceral pain in rabbits (Shafford and Schadt, 2008). Moreover, an increase in LF in response to acute pain is reported in studies with human subjects (Terkelsen et al., 2004). Increased LF% in the present study suggests that the pain in cows with metritis contributes to sympathetic activation and cardiovascular changes that shift the sympathovagal balance toward sympathetic dominance (Janing, 1995; Terkelsen et al., 2004). The VLF component of the HRV is associated with both sympathetic and parasympathetic making these differences more difficult to interpret (Malik et al., 1996; von Borell et al., 2007).

Before the examination, IBI and LF% were lower in primiparous versus multiparous cows. Studies in humans have found increased HRV with ageing, suggesting a shift in the
sympathovagal balance towards parasympathetic dominance, due to maturation of the autonomic nervous system (Silvetti et al., 2001). Overall, the activity of both branches of the autonomic nervous system is expected to decline with age (Shimazu et al., 2005; Yukishita et al., 2010). One possible explanation for the results in this study is the presence of cows in early post-partum lactating condition in the analyzed population, with different metabolic needs that influence the sympathovagal balance. Further studies are required to determine the changes in the sympathovagal balance caused by autonomic nervous system maturation in cattle.

Two time domain measures of heart rate variability, SDNN and RMSSD, differed with the type of examination performed. SDNN reflects the long-term variability of HRV and is considered an indicator of both sympathetic and vagal activity (von Borell et al., 2007; Moher et al., 2002). RMSSD indicates short-term variability and primarily reflects vagal activity (von Borell et al., 2007). RMSSD is also considered as a reliable ultra-short HRV measure even when using intervals as short as 10 s (Thong et al., 2003). In this study, both measures decreased during the uterine palpation in comparison to the rectal palpation, suggesting that the uterine palpation was more painful. Previous studies reported that sympathetic afferents mediate painful sensations from the uterus (Cervero, 1994), and that uterine palpation can result in cardiovascular and visceromotor responses (Ness and Gebhart, 1990). The decreased SDNN and RMSSD suggest that the overall HR variability is decreased and that the sympathovagal balance shifted towards sympathetic dominance. Previous studies related with stress, visceral and somatic pain are consistent with the current results. Induced stress in calves decreased both SDNN and RMSSD (the Poly-Vagal Theory by Porges, 1995; Moher et al., 2002). In addition, an internal stress factor (diarrhea), which might be accompanied by abdominal pain, resulted in a more intense decline of these measures (Moher et al., 2002). In humans, heart related pain is associated with a reduction in HRV, suggesting that cardiac pain shifts the autonomic balance towards the sympathetic dominance (Ruggerri et al., 1996). Similarly, Yap
and colleagues (2000) found that the sympathovagal balance shifts towards sympathetic dominance when the HRV is reduced. SDNN is also decreased in humans subjected to pain (Meeuse et al., 2013). In summary, these results suggest that pain and stress are higher during uterine palpation compared to passive rectal palpation in both healthy and metritic cows.

2.5 Conclusion

Back arch during passive rectal exam and uterine palpation can be used to assess visceral pain in dairy cattle. The spectral analysis of heart rate variability indicates that metritis is painful, contributing to increased sympathetic activity. Time domain measures also indicate that uterine palpation is more painful than passive rectal exam, suggesting that the former should be favoured for pain assessment. To our knowledge this is the first study to show evidence of visceral pain in cows with metritis. The examination methods used in the study may be used to identify cows that are experiencing pain associated with metritis, and thus identify animals that might likely benefit from treatment with analgesics.
Table 1: Heart rate variability measures in the period before examination

Means ± SE heart rate variability before examination, shown separately for primiparous (n=16) and multiparous (n=31) and for healthy (n=30) and metritic cows (n=17). Time domain measures standard deviation of normal to normal inter beat intervals (SDNN) and root mean square of successive differences (RMSSD) are presented in milliseconds (ms); VLF, LF and HF were expressed in normalized units as percentage of total power, other measures are log transformed (ln).

<table>
<thead>
<tr>
<th>HRV parameter</th>
<th>Primiparous cows</th>
<th>Multiparous cows</th>
<th>Healthy cows</th>
<th>Metritic cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (ms)</td>
<td>15.9 ± 1.4</td>
<td>18.4 ± 1.1</td>
<td>17.2 ± 1.2</td>
<td>17.0 ± 1.4</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>4.4 ± 0.5</td>
<td>4.9 ± 0.4</td>
<td>4.4 ± 0.4</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Average IBI (ms)</td>
<td>689.6 ± 15.7</td>
<td>737.4 ± 12.5</td>
<td>719.2 ± 13.1</td>
<td>707.8 ± 15.4</td>
</tr>
<tr>
<td>VLF%</td>
<td>86.9 ± 1.3</td>
<td>81.8 ± 1.0</td>
<td>86.3 ± 1.1</td>
<td>82.4 ± 1.3</td>
</tr>
<tr>
<td>LF%</td>
<td>12.0 ± 1.3</td>
<td>17.4 ± 1.0</td>
<td>12.9 ± 1.0</td>
<td>16.5 ± 1.2</td>
</tr>
<tr>
<td>lnHF%</td>
<td>-0.5 ± 0.2</td>
<td>-0.4 ± 0.2</td>
<td>-0.8 ± 0.2</td>
<td>-0.2 ± 0.2</td>
</tr>
<tr>
<td>lnLF/HF</td>
<td>7.5 ± 0.2</td>
<td>7.9 ± 0.2</td>
<td>7.8 ± 0.2</td>
<td>7.6 ± 0.2</td>
</tr>
</tbody>
</table>
**Figure 1**: Calculation of back arch before and during examination

Back arch before examination and during passive rectal and uterine palpation. Two wax body-marks were placed on the spine of each cow. The first mark (A) was placed on the shoulder region (highest thoracic vertebrae) and the second mark on the first coccygeal vertebrae (B). Straight line C (from A to B) created the base of the semicircle. The second line D followed the spine curving and reconnected body-marks A and B, thus forming the curved part of the semicircle. By connecting the body marks A and B, using lines C and D a semi-circular shape was formed that followed the arching of the spine. The selected back arch area was then converted into cm².
Figure 2: Back arch during passive rectal and uterine palpation

Means ± SE of back arch during passive rectal and uterine palpation for healthy cows (n=29) and those diagnosed with metritis (n=13).
**Figure 3:** Time domain measures during passive rectal and uterine palpation

Means ± SE of two temporal measures of heart rate variability: SDNN (log transformed standard deviation of normal to normal inter beat intervals) and RMSSD (log transformed root mean square of successive differences). Results are shown separately for (n=38) cows undergoing passive rectal exam versus uterine palpation.
Chapter 3: General discussion

3.1 Summary

Cattle experience numerous production related disorders that may cause visceral pain, including metritis, enteritis, intestinal volvulus, displacement of abomasum and others conditions that are associated with inflammation or ischemia. These stimuli are well-established noxious visceral stimuli proven to initiate pain from visceral origin (Ness and Gebhart, 1990). The limited knowledge of farmers and bovine practitioners regarding visceral pain creates difficulties in recognizing and assessing this pain, and applying appropriate pain management.

The objective of my thesis was to evaluate the behavioral and physiological pain responses in cows associated with metritis. Trans-rectal palpation was used to amplify the pain responses of the inflamed internal organs. Palpation as an instrument for pain amplification and assessment has been used by clinicians and researchers in humans and animal studies (Rutherford, 2002; Ness and Gebhart, 1990). Dealing with stoic animals that can mask the pain until the condition exacerbates, and the nature of visceral pain, were two reasons to use trans-rectal palpation to amplify the pain responses.

Cows with metritis had a more pronounced back arch during the trans-rectal palpation compared to the healthy cows. The back arch was larger in metritic cows during both the passive rectal exam and uterine palpation. Back arch was higher in metritic cows likely due to the hypersensitivity of the uterus caused by the inflammation, lowering the threshold of the high-threshold nociceptors and activating the silent nociceptors (Anderson and Muir, 2005; Muir and Woolf, 2001). The back arch response of metritic cows during passive rectal palpation is indicative of central sensation and the viscero-visceral hyperalgesia caused by the overlapping pathways of the afferents of the uterus and the colon/rectum (Giamberardino et al., 2001; Anderson and Muir, 2005; Muir and Woolf, 2001). The HRV measures taken before palpation
showed an increased LF % portion or sympathetic dominance in metritic cows compared to healthy cows. SDNN and RMSSD decreased during uterine palpation compared to passive rectal palpation in both healthy and metritic cows. The decline in both parameters suggests shifting the sympathovagal balance towards the sympathetic dominance, describing uterine palpation as more painful. In conclusion, this is the first study to assess visceral pain in naturally occurring cases of metritis in dairy cows.

3.2 Strengths and limitations of the study

One advantage of this study was the measurement technique used to quantify the back arch. Adobe Photoshop CS6 software (Adobe Systems, San Jose, CA, USA) allowed for precise quantification of back arch. Pain assessment using changes of the body posture such as back arch is usually assessed using clinical scales. However, this introduces an element of subjectivity likely contributing to poor intra- and inter-observer reliability (Weary et al., 2006). Back arch has been assessed and often considered as a pain related behavior, including in lameness scoring scale in cattle (Sprecher et al., 1997), in response to vaginal examination in cattle (Pilz et al., 2012), as result of induced inflammation of the uterus and colon in mice and rats (Wesselmann et al., 1998; Laird et al., 2001), and after abdominal surgery in rats (Roughan and Flecknell, 2003) and sea lions (Walker et al., 2009). In addition, the use of image analysis software, as pioneered in the current study, may be useful in future work in helping to improve reliability of this measure.

An additional strength of this study was integration of physiological measures to assess the pain associated with metritis. HRV analysis provides detailed information about the relationship of the two branches of the ANS and the overall sympathovagal balance. Moreover, HRV is a well-established and non-invasive method for detecting pain, stress and pathological conditions in humans and animals (von Borell et al., 2007). Combinations of physiological
measures (e.g., HRV) and behavior (e.g., back arch) may be particularly useful when assessing pain in stoic animals such as cattle.

Portions of the methodology of my study might be considered problematic. For example, the short duration of observations during palpations limited the use of spectral HRV analysis. Spectral analysis requires a minimum of 512 IBIs (von Borell et al., 2007). However, we were able to use the time domain analysis, especially the RMSSD, which is considered reliable even when using intervals as short as 10 s (Thong et al., 2003). Time and frequency domain measures are considered to be correlated (Kautzner and Hnatkova, 1995).

A second limitation of my study is the absence of validation of the pain response measures. Validating response measures involves observing the responses in conditions with and without pain and with and without analgesics that alleviates the pain (Rutherford et al., 2002). In this study, we compared the response measures between healthy and metritic cows during trans-rectal palpation, without using analgesics. Comparing healthy and metritic cows, we established that the changes in the behavior are associated with the disease but cannot be certain that the responses were due to pain per se.

### 3.3 Future research

This research provided evidence that the trans-rectal palpation initiates a more pronounced back arch response in cows with metritis. Moreover, both passive rectal palpation and uterine palpation evoked larger back arch in metritic cows. This response is likely due to the inflammation that caused primary hyperalgesia of the uterus and secondary hyperalgesia and allodynia to the colon/rectum. I conclude that back arch can be considered useful when assessing visceral pain associated with metritis in cattle. Passive rectal palpation alone can discriminate healthy form metritic cows. Therefore in future studies concerning visceral pain, I suggest the use of only PRP, perhaps combined with the use of other appropriate instrument to
substitute the examiner’s arm (e.g., pressure balloons). This approach could provide longer intervals (256 or 512 IBI’s) suitable for spectral analysis and will enable the examiner to control the pressure applied to the rectal wall. Additionally, this method may be useful in establishing the range of stimuli that initiates pain in this condition, and assess the sensitivity of back arch as a measure to visceral pain. Overall this approach will provide more accurate assessment of the SNS response, the most relevant component when assessing pain (Malik et al., 1996; von Borell et al., 2007).

If, as suggested by the results of this thesis, uterine inflammation causes secondary hyperalgesia and allodynia to the colon/rectum region, an interesting follow-up experiment would be to assess changes in posture during defecation. Mild painful or innocuous stimulus such as passage of feces or gas may become painful in metritic cows and thus cause pain responses (e.g., back arch, HRV). Similarly, an innocuous stimulus such as motility of the surrounding intestines has been reported to cause pain in patients with appendicitis (Bonica, 1990). Moreover, defecation is reported to cause pain in patients with irritable bowel syndrome (Halder and Locke, 2009). Using this approach we will avoid subjecting the animals to additional stressful manipulation that might provoke stress and influence the results.

Although the strength of the current study was the objective analysis of back arch, these methods could be improved. For example, continuous analysis of video may improve the sensitivity of the measure. Automated methods to assess back arching may also be useful for early detection of other conditions including lameness. Recent studies have started using image analysis and developing algorithms for automatic lameness detection using back posture in cattle (Poursaberi et al., 2010; Viazzi et al., 2013). Future studies should concentrate on development of algorithm that could automatically analyze each image of a video recording, and quantify the back arch.
Future research should concentrate on analyzing the effect of analgesics on visceral pain in metritic cows and how this affects recovery. This approach will assist in validating back arch as pain-specific measure for visceral pain associated with metritis. Moreover, back arch as pain-specific measure may be used as a diagnostic tool for pain related conditions of the internal organs including metritis. Another aspect is assessing the effects of analgesics on recovery from the inflammation caused by the disease. Bednarek et al. (2003) reported that a combination of NSAIDs and antimicrobial treatment in calves with bronchopneumonia improved recovery.

Back arch was larger during the uterine palpation compared to the passive rectal palpation. However, during the uterine palpation the rectal wall was involved as a mediator and the force applied on the uterus was through the rectal wall (trans-rectal palpation). The applied force on the rectum during the uterine palpation might contribute to the increased back arch. Thus the back arch during uterine palpation should be considered as the combined response to uterine and rectal hypersensitivity. Future studies could quantify how much the rectum contributes to the back arch by applying similar force on the rectum in other directions then that of the uterus and assessing the changes of the back arch.

Future research may also consider analyzing the facial expressions in cattle. Studies in mice, rats and horses facial expressions are associated with pain and reliable grimace scales have been developed (Langford et al., 2010; Leach et al., 2011; Sotocinal et al., 2011; Costa et al., 2014). However, grimace scales in laboratory animals and horses have primarily assessed somatic and post-operative pain. To my knowledge there are no studies that analyzed the facial expressions due to visceral pain. Recent findings suggest that the facial expressions may indicate the emotional component of pain in animals (Langford et al., 2010). Moreover, lesions of the rostral anterior insula (involved in the emotional component of pain in humans) lowered the facial expression intensity but did not affect the abdominal contractions ‘behavior associated
with abdominal pain’ (Langford et al., 2010). Future research might assess the relationship between grimacing and back arching in cows with visceral pain associated with metritis to analyze both the sensory and emotional component of pain.

Future studies might consider analyzing the social behavior such as competition for resources (e.g., feed, stalls) in cows with metritis. Huzzey and colleagues (2007) have already reported reduced feed competitiveness the week before calving as a predictor to metritis. However, no study to date has observed the social behavior in cows with metritis or related this behavior to the pain associated with this disease. These behaviors may represent an emotional component of pain associated with metritis; as the animals avoid aggressive and potentially painful behavior, and seek pain-relieving environment that will promote recovery (Anil et al., 2002).

3.4 Conclusion and recommendations

The aim of this research was to create appropriate methodology and incorporate behavioural and physiological measures in the process of assessing visceral pain in cows with metritis. Recent progress in understanding visceral pain mechanisms, and differences from somatic pain, has come from the human literature and experiments on laboratory animals. Before the work described in this thesis, no study has assessed visceral pain in cattle associated with normally occurring diseases. A difficulty when assessing visceral pain is selecting an adequate noxious visceral stimulus. In this study I created a methodology to amplify mild visceral pain using trans-rectal palpation of the uterus. In addition, image analysis software was used for the precise measurement of back arch behaviour and HRV measures were used to describe changes in ANS responses related to pain.

The current study demonstrated that back arch is a sensitive behavioural measure. Using changes in back arch I was able to discriminate between healthy and metritic cows during
passive rectal and uterine palpation. The uterine hyperalgesia and the secondary hyperalgesia and allodynia of the rectum likely increased the sensitivity and amplified the pain responses in metritic cows. The HRV measures indicated increased sympathetic activity in metritic cows in the period before examination, again suggesting that this disease is painful. Due to the short duration of the trans-rectal palpation only time domain analysis was used, with no difference between healthy and metritic cows. However, HRV was lower in both healthy and metritic cows during uterine palpation, suggesting that this palpation is more painful than the passive rectal palpation.

Visceral pain is characterized with vague and diffuse sensation and emotional reactions such as anxiety; as such pain due to metritis is not easily recognized. The results from this study indicate that metritis is a painful condition. Moreover, the changes in behaviour were consistent in mild and severe cases of metritis. Therefore, regardless of the severity, metritis should be considered as a condition that causes pain and should be treated accordingly.

On the basis of the findings of this thesis I recommend a more systematic approach in dealing with metritis in dairy herds. One key element is recognizing the importance of this condition from welfare and production perspective. Regular diagnosis of this condition in the post-partum period as part of the on-farm protocols would assist in early detection of metritis and provide reliable information regarding the incidence of the disease. Rectal palpation, commonly used for examination of reproductive health, may also be used for pain assessment related to the reproductive organs. I encourage dairy producers and bovine practitioners to use back arch in identifying animals that may be experiencing pain due to metritis. Moreover, I recommend including NSAIDs in the treatment of metritis cases; this treatment will likely alleviate pain associated with this disease and improve recovery.
Bibliography


