INVESTIGATION OF PAIN IN EQUINE PATIENTS USING HEART RATE VARIABILITY, SALIVARY CORTISOL CONCENTRATION, AND BEHAVIOURAL PAIN SCORES

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ABSTRACT

Equine pain assessment is challenging as horses minimize the display of pain and are unable to self-report. Current methods of pain assessment are subjective and there is no gold standard or universally accepted pain assessment tool in equine patients. It is vital that veterinarians can recognize pain to adequately treat painful conditions and maintain animal welfare. Heart rate variability (HRV) is a non-invasive measure that reflects autonomic nervous system maintenance of cardiovascular homeostasis and may be used as an indicator of physiological stress and pain. This study had two objectives, first to compare measures of HRV, salivary cortisol, and pain scores in the assessment of pain among equine patients upon referral hospital admission; and to compare physiological and behavioral responses within a cohort of equine patients undergoing surgical treatment. It was hypothesized that there would be correlation between physiological and behavioural measures of pain. Data collected included HRV measurements, salivary cortisol concentration, and behavioural pain scores using three published pain scales. Horses were classified on admission as painful or not painful by a priori classification based on clinical perception. Data was collected on the day of hospital admission (T1), and twice postoperatively in surgical patients: T2, anesthetic recovery; and T3, prior to postoperative analgesia. Statistical analysis included descriptive statistics, and t-test comparisons between groups of painful versus not painful horses. Pearson's correlation determined measures of association between pain scores and physiological variables, and ANOVA compared perioperative time points. A total of 59 horses were included in the study, with 39 horses undergoing various surgical procedures. On hospital admission, painful horses had higher pain scores, standard

deviation of the normal RR interval (SDNN) and salivary cortisol concentrations than horses classified as not painful. All pain scales positively correlated with SDNN, and one scale each correlated with mean heart rate (MnHR) and salivary cortisol. Variables significantly altered perioperatively were the mean RR interval (MnRR), MnHR, and salivary cortisol. Anesthetic recovery (T2) was most significantly different perioperatively with the highest salivary cortisol and MnHR, and lowest MnRR; this indicates higher physiological stress at this time. This may be due to general anesthesia or the recovery experience, however pain scores also trended towards being elevated postoperatively, suggesting pain at this time. Study limitations include the high variability of the equine patients and clinical conditions, and the inability to separate physiological stress from pain. The lack of a generally accepted gold standard pain assessment tool also restricted pain evaluation to a subjective scale. Both SDNN and salivary cortisol were significantly higher in the painful group of horses, suggesting that these parameters may be useful in detecting pain. Heart rate appears too insensitive to be a reliable indicator of pain in the horse. Additionally, a pain scale was selected that appears robust in this population of equine patients. Having objective non-invasive measures of pain could improve accurate recognition and treatment of equine pain, with the goal of benefiting equine patient welfare. Further studies are required to better define these relationships and determine clinically significant cut-off values.

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DEDICATION

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
EQUUS COMPASS	Equine Utrecht University Scale for Composite Pain Assessment
EPS	Equine Pain Scale
Group 1	Not painful group of horses
Group 2	Painful group of horses
HF	High frequency
HGS	Horse Grimace Scale
HR	Heart rate
HRV	Heart rate variability
ISAP	International Association for the Study of Pain
LF	Low frequency
LF/HF	Ratio of low frequency power to high frequency power
MnHR	Mean heart rate
MnRR	Mean RR interval
ms	Milliseconds
ns	No significant difference
Pain1	Non-standardized pain scores for Pain Scale 1
Pain2	Non-standardized pain scores for Pain Scale 2
Pain3	Non-standardized pain scores for Pain Scale 3

painPCT	Standardized pain rating score for each pain scale, denoted as scale 1, 2, or 3. Average pain score expressed as a proportion (percent) of the total possible score for the individual pain scale.
PNS	Parasympathetic nervous system
PS1	Pain Scale 1
PS2	Pain Scale 2
PS3	Pain Scale 3
R1	Rater 1
R2	Rater 2
R3	Rater 3
RMSSD	Root mean square of the successive differences
SD	Standard deviation
SDNN	Standard deviation of normal RR intervals
SNS	Sympathetic nervous system
T1	Data collected on the day of hospital admission; all horses
$T1_{SX}$	Data collected on the day of hospital admission; surgical patients
T2	Data collected following recovery from anesthesia
T3	Data collected within 2 hours prior to routine post-surgery analgesia
μg/dL	Micrograms per decilitre

CHAPTER 1. GENERAL INTRODUCTION

1.1. Overview

Pain is necessary to recognize stimuli that can cause tissue damage so behaviours can be carried out to avoid it (1, 2, 3). Pain causes a catabolic state, reduces appetite, can modulate and suppress the immune system response, promotes inflammation, and can increase anesthetic requirements during surgery (1, 2, 3). This leads to increased morbidity, reduced welfare and can increase the cost of hospital care (1, 2, 4). Therefore, it is vital in veterinary medicine that veterinarians can recognize pain to adequately treat painful conditions and maintain animal welfare (5, 6). Pain has been recognized as the fifth vital sign (following body temperature, heart rate, respiratory rate, and blood pressure) in human medicine for years, and this is starting to be recognized in veterinary medicine also (7, 8). In the concept of pain as a vital sign, pain should be regularly assessed and recorded in the medical record, together with the other classic vital signs (7). Pain assessment is challenging, as pain can only be experienced by an individual, and direct measurement of subjective experiences is not possible (1, 3, 5, 9). No single measured parameter is a pathognomonic indicator of pain (1, 10). Behavioural responses to pain depend on the species, breed, age, and disease process (1, 11). Additionally, different sources and types of pain can manifest differently, such as lameness as a sign of musculoskeletal pain compared to rolling and flank-watching as signs of abdominal pain (1). Pain assessment in horses is particularly challenging. Horses are unable to self-report (6, 10) and as herbivores, horses minimize the display of pain to reduce attraction from potential predators (1, 3, 5, 10, 12).

1.2. Definition of pain

Pain has been defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” (13). In the article publishing this recently revised definition of pain, pain is also described as a personal experience, and the authors state that the “inability to communicate does not negate the possibility that a human or a non-human animal experiences pain” (13). Physiologically, pain is the result of detection of tissue injury by the nervous system, followed by conscious perception, resulting in behavioural responses or changes (14). Nociception, the detection of noxious stimuli by the nervous system, is the result of five processes: transduction, transmission, modulation, projection and perception (14, 15). Pain receptors, which are free nerve endings, are stimulated by mechanical, thermal and chemical pain stimuli (15, 16). Painful stimuli are transduced into electrical impulses, also called action potentials, that are transmitted to the spinal cord via Aδ or C fibers (14-16). These signals are modulated in the dorsal horn of the spinal cord before transmission to the brain stem and somatosensory cortex, where pain is perceived and physical and behavioural responses are initiated (14, 16).

Pain is necessary and protective; it is important to recognize and subsequently avoid stimuli that can cause tissue damage (3, 14, 15). It is also important in veterinary medicine to be able to recognize pain and adequately treat painful conditions to maintain animal welfare (5, 6, 8). Both trauma and surgery result in a stress response and need for tissue repair, leading to increased energy requirements (3). Untreated pain activates sympathoneuroadrenal pathways, causing elevations in cortisol, epinephrine,

norepinephrine, and decreased insulin (14). This can lead to a catabolic state and cause increased morbidity and mortality (2, 14). Additionally, painful horses tend to be less social, lie down less, and are restless and uninterested in food, which suggests increased stress levels and decreased quality of life (14). Therefore, pain relief is beneficial as it can improve appetite, aid in recovery, and improve quality of life (3, 4, 14).

1.3. Challenges and methods of pain assessment

1.3.1. Overview

Assessment of pain is challenging in both humans and animals. Pain can only be experienced by an individual, and direct measurement of subjective experiences is not possible (3, 5, 9). Pain assessment in horses is particularly challenging. They are unable to self-report (6, 10) and as herbivores, horses minimize the display of pain to reduce attraction from predators (1, 3, 5, 10, 12). Different types and sources of pain can also manifest differently, such as lameness caused by musculoskeletal pain compared to rolling and flank-watching caused by abdominal pain (1, 17). Additionally, handling experience and prior exposure of the horse to examination can influence a horse's responses to examination and pain assessment (5), and pain expression has been shown to be influenced by the personality and individuality of the horse (18). This suggests significant individual variation in response to examination of painful horses (10). Current methods of pain assessment are varied and involve various behavioural assessments, as well as physiological parameters which primarily include vital parameters and circulating hormone levels (5, 19).

1.3.2. Physiological assessments of pain

1.3.3. Vital parameters

Heart rate (HR) and respiratory rate are vital parameters that have been correlated with pain in horses and are the most commonly used method of pain assessment in clinical practice (5, 19, 20). These parameters are quick and easy to measure; however, they are non-specific for the presence and severity of pain in horses (3, 5, 19, 21). For example, stress, shock, endotoxemia, dehydration and sedative agents can significantly alter vital parameters (3, 19). Similarly, pain may be present in animals without elevations in heart rate and respiratory rate (3). Overall, the association of vital parameters with pain have mixed results in literature (5,19), with some studies showing strong correlation of pain scores with heart rate or respiratory rate (20, 22, 23), and others either fail to establish a direct relationship or find the relationship to be weak (9, 19, 21, 25).

1.3.3.1. Cortisol concentration and circulating hormones

Circulating hormone levels that may be altered in pain states include catecholamines, endorphins, and cortisol, and these have been used as indirect measures of pain in horses (3, 19, 20). Pain sensation is transmitted via the brain stem to the median eminence of the hypothalamus, resulting in secretion of corticotropin-releasing factor (26). This is secreted and carried to the anterior pituitary gland, where it induces adrenocorticotropic hormone (ACTH) secretion (26). ACTH then causes the adrenal cortex to increase cortisol secretion (26). Both physical and mental stress can lead to increased ACTH and cortisol secretion within minutes (26). Similar to vital parameters,

hormone levels are not specific to pain status, and various types of stress can cause elevated cortisol, such as trauma, infection, debilitating disease, or use of sympathomimetic drugs (19, 26, 27). Systemic cortisol levels can be assessed from plasma or saliva. Saliva can be collected easily and repeatedly, and is non-invasive (28, 29). Salivary cortisol represents the unbound fraction of total plasma cortisol that is biologically active (28, 29). Increases in serum cortisol levels during acute stress are largely made up of free (unbound) cortisol, and therefore it is more relevant and useful to measure free cortisol than total cortisol in serum (30). There is a diurnal pattern to equine cortisol levels (26, 28, 31, 32) with the highest concentrations occurring in the morning and decreasing throughout the day (28). Salivary cortisol has been used to determine stress levels in horses, such as when undergoing training (33) and transport (27). There are varying results in literature surrounding cortisol and pain conditions, with some studies utilizing serum cortisol and others salivary cortisol concentrations. Plasma cortisol levels have been correlated in some studies with pain states (10, 34), such as elevation of plasma cortisol in horses that received abdominal surgery in comparison to horses that received only anesthesia without surgical treatment (20). However, neither plasma cortisol nor catecholamine levels were significantly different between horses receiving analgesics and those without following arthroscopic surgery (24). Salivary cortisol levels were increased in horses following microchipping and branding procedures, which could be considered both painful and stressful (35). In laminitic horses, there was no significant change in serum cortisol when horses received analgesic treatment, however the chronic nature of this disease could influence these results (6). In a study assessing horses with colic, serum cortisol was elevated, and this

elevation was twice as high at the recorded most painful time point compared to all other data points (10). Elevations of systemic hormone levels in some horses compared to others may be related to the extent of surgical injury or the underlying disease condition in addition to postoperative pain (20). Currently, hormone analysis results are generally retrospective, due to the delay caused by laboratory analysis. This makes hormone analysis not clinically useful in real-time assessment of pain in equine patients (3, 19, 34).

1.3.3.2. Heart rate variability (HRV) analysis

Heart rate variability (HRV) describes fluctuations in heart inter-beat intervals (36) and is a non-invasive measure (27). Variation in heart inter-beat intervals is normal and considered healthy (37). These variations reflect the effect of the autonomic and neuroendocrine input on the heart to maintain cardiovascular homeostasis and normal blood pressure (38). Homeostasis is primarily maintained by the continuous variations in heart rate and vascular resistance by autonomic nervous system influences of the sympathetic and parasympathetic nervous systems (16, 38). The autonomic nervous system (ANS) functions without conscious effort and is controlled by centers in the spinal cord, brain stem, and hypothalamus (39). Autonomic neurons are activated by local stimuli to make appropriate modulatory responses (40). The parasympathetic nervous system (PNS) decreases heart rate and contractility and is more active under restful conditions (39, 41). The sympathetic nervous system (SNS) increases heart rate, contractility, and conduction velocity, which prepares the body for energy expenditure and stressful situations (39, 41). The SNS releases excitatory messengers, such as epinephrine and norepinephrine, in states of exercise, excitation, and some pathological

conditions (such as heart disease and acute gastrointestinal colic) (39, 42). The balance between these branches of the ANS within the heart is due to neuromodulators (natriuretic peptides, angiotensin II) released from the myocardium and coronary vessels, and co-transmitters between sympathetic and parasympathetic nerve endings (neuropeptide Y, vasoactive intestinal polypeptide) (43).

Heart rate variability measures, derived from the heart rate, detect subtle changes in heart rate caused by the ANS. Heart rate variability measures are determined from measuring inter-beat intervals, or the interval between consecutive R-waves (RR intervals) on an electrocardiogram (44). Inter-beat intervals are best determined from a recorded electrocardiogram and extraction of normal R-to-R wave intervals (RR intervals, also called normal-to-normal RR intervals) (36, 38). Commercial heart rate monitors automatically detect RR intervals without recording an electrocardiogram (44). These monitors have been shown to be reliable and comparable to electrocardiography in horses (45-47). Both methods of measuring heart rate variability are non-invasive (27).

Once inter-beat intervals are recorded, statistical analysis is performed using software to evaluate the autonomic input on the heart (38). The main methods of HRV data analysis are time domain methods and frequency domain (power spectral) methods (36, 38). Time domain methods of heart rate variability analysis describe HRV as a function of time and give a global view of HRV and autonomic control of the heart (38). These are the simplest methods of HRV analysis and include measures of variability derived from the inter-beat interval data as well as those derived from the differences between adjacent inter-beat intervals (44). Time domain measures include the mean RR

interval (MnRR), mean heart rate (MnHR), standard deviation of the normal RR interval (SDNN), and the root mean square of successive differences (RMSSD) (36). The SDNN is considered an estimate of overall HRV, while RMSSD is an estimate of short-term, or high-frequency, components of HRV (36, 38). Frequency domain methods of analysis describe HRV as a sum of oscillatory components defined by their frequency and amplitude (48). It represents the signal series by sum of the sinusoidal components of different amplitude, frequency and phase values (49). This is also known as power spectral density, which describes how power distributes as a function of frequency (36). Spectral analysis shows the effect (power) of each influence over a range of frequencies (38). The amount of power in different frequency bands can be assigned to underlying physiological functions (50). There are three main spectral components that have been defined: very low frequency (VLF), low frequency (LF) and high frequency (HF) (51). The physiological significance of the VLF band is not well defined, and its physiological correlate is under debate (36, 50). Low frequency power is considered a marker of sympathetic excitation while high frequency power is mainly mediated by the parasympathetic nervous system (48). And finally, the low frequency to high frequency ratio (LF/HF ratio) is an indication of overall sympathovagal balance (36). For example, an increase in the LF/HF ratio is thought to represent a shift towards sympathetic nervous system activity (50).

In horses, HRV is currently primarily used for research purposes rather than clinical or performance reasons (38). Research has involved reliability and repeatability (46, 47), exercise and training (52, 53), and stress (27, 33, 54, 55). Heart rate variability measures may also have potential applications in disease states, but there is limited

research in equine medicine. Two studies have been published investigating HRV alterations in horses with colic (42, 56). Research is particularly growing in HRV for assessment of stress and temperament (27, 50). Heart rate variability has been shown to be altered in horses when comparing between basal conditions and psychological stress (27, 54, 55). Horses in transport, which is thought to be a stressful activity, were found to have increased cortisol, increased heart rate, and decreased HRV parameters (RMSSD) that were indicative of stress (27). In response to a novel object test, horses showed a more pronounced decrease in HRV measures and increased average HR compared to horses that had received prior training (55). Heart rate variability measures (SDNN and RMSSD) decreased with stress during initial training of young sport horses, with the most pronounced response being the mounting of the rider (33). Recently, a Parasympathetic Tone Activity (PTA) index was created in dogs and horses, similar to the human analgesia/nociceptive index (ANI) in humans (57, 58). The aim of the index is to assess analgesia/nociception balance, and in one study evaluating the PTA in horses, the index predicted mean arterial pressure changes (57). This study also found that horses undergoing emergency surgery for colic had lower PTA values compared to horses undergoing elective surgery, presumably due to dominance of the SNS caused by a stress response to colic in these horses (57).

Studies in human literature have shown correlation of changes in HRV parameters with self-reported pain scores (59, 60). Human patients following minor spinal surgery were shown to have changes in HRV power spectral measurements, specifically decreased LF and LF/HF ratio (59). These HRV changes were correlated with self-reported pain on a numeric rating scale (NRS) (59). This study also determined

specific cut-points for LF and LF/HF ratio that correlated with NRS scores above three (out of a total possible score of ten) and were determined to denote acute pain (59). Another study showed that HRV parameters were lower in patients with both spinal cord injury and neuropathic pain (61). Patients with spinal cord injury alone had comparable HRV parameters to control patients (61). Similarly, HF power has been found to be decreased in people with induced pain states compared to rest states (62). It has also been shown that individuals effectively taking analgesics for pain control showed greater HRV parameters than those that were ineffectively taking analgesics (63). An analgesia/nociceptive index (ANI) has also been created and is derived from calculations of heart rate variability (53). This index was proposed to reflect the analgesia/nociception balance during general anesthesia and was found to correlate with patient reported NRS pain scores in the immediate postoperative period (58). Analgesia/nociceptive index measurements were significantly associated with pain intensity upon arousal in postoperative patients (58, 64). The advantage of the ANI is that measurements only require non-invasive echocardiogram recordings (58). Heart rate variability parameters have also been shown to correlate with self-reported pain scores in the Short Form McGill Pain Questionnaire (SF-MPQ) after abdominal surgery (65). The total SF-MPQ score positively correlated with the LF/HF ratio (65). These studies suggest that HRV is a useful tool for assessment of pain in humans and could bring about new knowledge for better pain intervention (60).

There are fewer studies in HRV with respect to pain in animals. In laboratory mice undergoing laparotomy with or without pain relief, animals not receiving analgesics exhibited decreased heart rate variability, suggestive of sympathetic

activation (22). These mice also showed increased HR, elevated temperature, and reduced food intake compared with control mice that did not undergo laparotomy (22). No pain symptoms were found by observation of the mice, and there was no influence of locomotor activity levels (22). In adult cattle, HF power was found to be lower in lame cows compared to non-lame cows (66). In one study investigating castration in calves, RMSSD increased in castration groups compared to controls, and more so in calves not receiving local anesthetic (23). In calves not receiving local analgesia, HF power increased compared to decreased LF power in calves receiving local anesthetic (23). Another study evaluating castration in calves showed that older calves (6 months of age) post-castration had lower HF power and higher LF power and LF/HF ratio compared to younger calves (8 weeks of age); this suggested that older calves may experience more distress during the castration procedure (67).

Research in HRV and pain in horses has varied results. One study measuring HRV parameters in horses with laminitis showed limited correlation between HRV and pain when compared to control horses (68). The authors of this study questioned if the results were affected by the chronicity of the laminitis pain (68). A study by a different group of researchers investigated power spectral analysis of HRV before and after horses with laminitis received analgesic therapy (6). Low frequency power and HF power increased in response to administration of non-steroidal anti-inflammatories (6). The HRV parameters also correlated with a clinical orthopedic index of laminitis pain (6). This study suggests an association of alterations in HRV parameters with pain in horses, as well as demonstrating efficacy of pain management in these horses suffering from laminitis. A study investigating anxiety and pain interactions in horses found pain

caused increased SDNN compared to anxiety and anxiety-pain states (25). Heart rate alone did not distinguish between pain states, as HR was only increased in horses with anxiety and anxiety-pain, not in horses experiencing mild somatic pain alone (25). In horses with colic, serum cortisol concentration correlated positively with LF/HF ratio, suggesting reduced parasympathetic activation in painful conditions (69). Mean SDNN increased over time with treatment in horses surviving from colic (69). However, in young horses undergoing branding and microchip implantation, procedures that can be considered both painful and stressful, measured HRV parameters did not change over time, but HR and salivary cortisol levels were elevated (35).

Based on the literature available, HRV measurements appear to correlate with assessment of pain in humans, mice, cows, and horses under some circumstances. Heart rate variability may be used as an indicator of physiological stress and has the potential to complement the subjective behaviour assessments of pain in horses, offering objective measurements (5, 6, 25). While a promising parameter for pain assessment, further studies are needed to validate HRV parameters as an indicator of pain in the horse. The creation of cut-points would also be beneficial for transitioning HRV measurements from research to clinical use.

1.3.3.3. Conclusions regarding physiological assessments of pain

Physiologic responses to pain are commonly used and provide better sensitivity and a quantitative assessment (19). However, these parameters also lack specificity for pain (19). Using physiological measures alone could cause inaccurate judgment of pain levels as many other stimuli may increase circulating stress hormones and elevate heart rate (19, 24, 34).

1.3.4. Behavioural assessments of pain

Behavioural assessments are often used in equine pain assessment and are based on observing subjects for subtle or overt changes in behaviour that occur as a result of pain (19). Several studies have established non-specific behavioural indicators of pain in horses, including facial expressions (14, 17, 70), demeanor, postures, interactions, and gait (10, 17, 19, 34). Behavioural changes are also considered nonspecific, as they are influenced by other factors such as breed, temperament, age, sex, environment, and disease process (1, 11, 19). Pain expression can also be influenced by the personality and individuality of the horse (18). There are also other limitations, including the need for trained and experienced observers, the time required to conduct assessments, and some assessment measures require observing response to palpation of the painful area (12, 17, 34, 71).

Formalized pain scoring systems have been used in human and veterinary medicine for decades. Pain scoring systems attempt to quantify pain by assigning a number rating or relative score to the assessed level of pain (70). Commonly used scales in human medicine are the visual analogue scale (VAS) and the numerical rating scale (NRS), in which the subject self-reports or is assigned a score of global perceived pain (70). The VAS is a continuous scale where the patient or observer marks the amount of perceived pain on a ten-centimeter line, which corresponds to no pain at the beginning (left) of the line, up to the worst imaginable pain on the right (70). The NRS is a numeric scale from zero (no pain) to ten (worst imaginable pain), and the patient or observer selects the number closest to the perceived amount of pain (70). These scales can be applied to horses but only include definitions of the two endpoints of the scale,

and ultimately are dependent on the observer's subjective perception of behaviour and pain (70). Visual analogue scales are thought to be more sensitive to identifying small changes in pain, while the NRS tends to be more repeatable and have better inter-observer reliability than the VAS (19). Overall, these scales have poor inter-observer agreement (70).

Composite pain scales are of high interest in equine pain assessment and use a combination of behavioural and physiological parameters scored individually and summed to create an overall pain score (19, 70). Multiple composite pain scales have been developed for horses, but these have been designed for specific conditions or types of pain, such as lameness, laminitis, ocular or abdominal pain (10, 12, 17, 19, 34, 70, 72). One type of composite pain scale originally used in rodents and rabbits is the grimace scale (12). A few pain scales have been designed assessing equine facial expressions, and research has been promising in use of facial pain scoring in identifying pain in horses (12, 17, 70, 73). Several composite pain scoring systems have been created using various behavioural and physiological parameters, and some scales have weighted scoring of these parameters (19). Studies in development of these pain scales appear promising in pain detection, though very few pain scales have undergone rigorous validation in the horse (70). These published studies demonstrate good to excellent inter-observer reliability (12, 17, 34, 70, 72). The experience and training of the observer dictate their ability to interpret scoring systems accurately and in an unbiased manner (5, 71). Additionally, the maximum score of these scales is rarely approached in the studies validating their use (19, 74), and few scales have identified cut-off values to distinguish between overtly painful horses and control horses (17, 74).

While these scales have not been tested in subtle pain conditions, there is concern that many pain scales may not be sensitive enough to detect subtle pain in horses (17, 19). Overall, pain scores are promising in systematic pain recognition and grading and have been found to successfully detect pain in multiple studies (10, 12, 17, 19, 20, 70, 72, 73). Further research is needed to validate these pain scales in various clinical conditions and to assign appropriate cut-off values.

1.4. Implications of pain assessment

While pain is a physiological necessity, it has adverse effects on function and welfare (2, 13). Pain causes a catabolic state, reduces appetite, suppresses the immune system response, promotes inflammation, and during surgery increases the amount of anesthetic medications required (1, 2, 3). This leads to increased morbidity, reduced welfare and can increase the cost of hospital care (1, 2, 4). Appropriate treatment of pain, with treatment of the underlying cause and analgesic medications, results in improved appetite and maintenance of body weight, aids recovery and can shorten hospitalization stay (2, 4). In human hospitals, the main reported cause of undertreated pain is the lack of pain assessment and subsequent recording in the patient chart (7). Pain was implemented as the fifth vital sign in patient assessment and documentation in many medical associations, with the aim of identifying and treating previously undertreated pain and reducing patient suffering (7). This concept is starting to be implemented in veterinary medicine due to concern for under-recognition and treatment of pain in animals (8). However, evaluation of pain is only as effective as the methods used and interpretation by trained professionals, and over-estimation and treatment of

pain can also cause morbidity (75). Improved recognition of equine pain could improve treatment and welfare of horses experiencing pain that otherwise was undetected. The first step is determining adequate methods of pain evaluation in horses, followed by training of veterinary professionals.

Pain assessment in horses is challenging and current methods of pain assessment are subjective and non-specific. Heart rate variability is a relatively new measurement to veterinary medicine that has the benefit of being non-invasive (27). Heart rate variability has been shown to be indicative of stress in humans and animals (36) and based on early research HRV may also be an indicator of pain in the horse. Having an objective non-invasive measure of pain could be of advantage to veterinarians and horse owners in improving pain recognition and management in equine patients.

Given the result of previous studies and the lack of standardized measures to assess equine pain, the primary goal of this study was to consider time and frequency domain estimates of heart rate variability as objective measures of pain in equine patients. In addition, the study intended to determine the association between heart rate variability estimates and concomitant measures of salivary cortisol and behavioural pain scores in equine patients. It was hypothesized that there would be a correlation between physiological and behavioural measures of pain.

CHAPTER 2. MATERIALS AND METHODS

2.1. Experimental animals

This study was approved by the University of Prince Edward Island Animal Care Committee (AUP#17-052) and complies with all institutional, provincial, and national regulations pertaining to the use of animals in research.

Owners of horses admitted to the Atlantic Veterinary College Veterinary Teaching Hospital gave consent for enrollment of their horses in the research study. Horses presented for routine veterinary treatment of naturally occurring health conditions or for voluntary husbandry purposes (castration). The study did not affect the diagnostic assessment or treatment of horses during hospitalization. Exclusion criteria included horses under one year of age, weighing less than 300kg, and horses exhibiting cardiovascular disease or arrhythmia upon admission. A convenience sample of 60 horses was obtained over 1.5 years.

Signalment, including breed, sex, age, and weight, vital parameters, and reason for hospital admission were recorded. Horses were allowed minimally 1 hour of acclimation to the hospital environment prior to sample and data collection. All data was collected simultaneously at approximately the same time of day in all horses. Horses were placed in a box stall (10 x 10 feet) and were left undisturbed during data collection.

Horses received routine analgesia as per the attending clinician on a case-by-case basis. Due to welfare concerns, horses that required immediate pain management upon hospital admission were excluded from the study. No horse received analgesics at the hospital prior to the first time (T1) of data collection.

2.2. A priori pain classification on admission to the hospital

On admission to the hospital, horses were classified as either 'not painful' (Group 1: n=18) or 'painful' (Group 2: n=18) based on a priori classification on admission. A priori parameters were set to determine this classification, based on terms used commonly in this hospital to determine clinical pain in equine patients (Table 2.1). To be classified as painful, horses had to be either lame at the walk, have colic signs, signs of ocular pain, or have a combination of at least two of the other clinical signs listed in Table 2.1. Elevated heart rate alone did not put a horse into the 'painful' category.

Table 2.1. Parameters used to determine pain classification of horses on hospital admission

Group 1: Not Painful	**Group 2: Painful**
Heart rate < 50bpm	Heart rate > 50bpm
Sound at the walk	Lameness visible at the walk or non-weight bearing
Good appetite	Poor appetite / No interest in food
Normal/bright and alert mentation	Altered mentation, depressed
Interactive with the environment	Not interactive
Absence of abnormal behaviours	Pawing or stomping
Absence of colic signs	Colic signs: flank watching, rolling, tucked abdomen, kicking, yawning
No sweating	Sweating
Not restless or agitated	Restless or agitated
Absence of ocular pain	Ocular pain: miosis, blepharospasm, epiphora

2.3. Data collection timeline

All horses had data collected on the day of hospital admission (T1) in the late afternoon. For horses undergoing surgical treatment, data was collected simultaneously at three time points: $T1_{SX}$, admission day; T2, anesthetic recovery; and T3, just prior to the first postoperative analgesia. In horses receiving surgery, admission day data was

designated as $T1_{SX}$ to distinguish surgical patient data results from the group of all horses on admission day, designated as T1. Horses not receiving surgical treatment had data collected at T1 only. In patients undergoing surgical treatment, surgery was performed on the second day of hospitalization and horses received routine preoperative analgesia. Data was collected at T2 following recovery from anesthesia, upon return to the horse's hospital stall. Data was then collected at T3 within 2 hours prior to routinely scheduled post-surgery analgesia (a non-steroidal anti-inflammatory drug (NSAID) at 10-12 hours post-anesthetic induction). A typical scenario is shown in Figure 2.1, in which the horse arrived at the hospital in the morning on the day prior to surgery and data collection at T1 was started between 16:00-18:00h on the first day in hospital. Surgery was performed the morning of the second day in hospital. T2 data collection started between 12:00-14:00h, and T3 was collected between 18:00-20:00h. The intention was to obtain admission-day data to assess pain of various conditions pre-surgery, and two data points post-surgery. The post-surgery data points were scheduled in an attempt to capture the time of best analgesia (T2, immediately post-anesthesia) and at the time of least analgesic treatment (T3, immediately prior to obtaining the next scheduled analgesic medication).

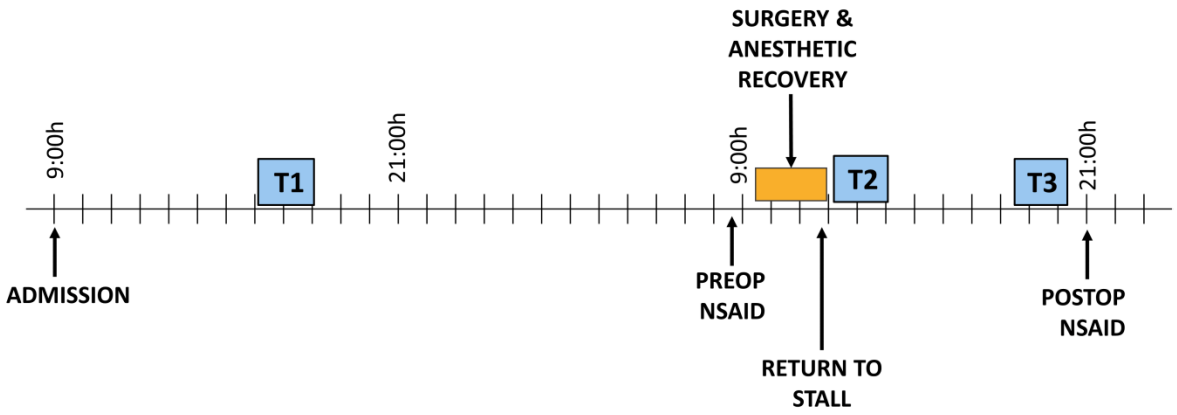

Figure 2.1. Example perioperative data collection timeline

2.4. Determination of salivary cortisol concentration

To determine salivary cortisol concentration, saliva was collected immediately prior to heart rate monitor application and video recording. Equine salivary cortisol levels follow a diurnal pattern (28, 31, 32) with the highest concentrations occurring in the morning and decreasing throughout the day (28). Because of this, measurements were taken at approximately the same time of day in all horses. A 4x4 gauze square was held in the horse's mouth for 60 seconds with hemostatic forceps. The gauze was then placed within a 12ml syringe and compressed to obtain saliva. Saliva samples were frozen at -20˚C until testing could be performed (76). Salivary cortisol concentration was measured via enzyme-linked immunosorbent assay (ELISA) (Salimetrics Salivary Cortisol Enzyme Immunoassay Kit, Item No 1-3002, Salimetrics, State College, PA, United States). Samples were analyzed in duplicate. Only samples with a coefficient of variation (CV) of less than or equal to 10% were used in the analyses (77). The assay was repeated for any saliva samples resulting in CV of greater than 10% between

duplicate samples. If there were no saliva sample remaining to allow for repeat testing the data point was excluded from statistical analysis.

2.5. Determination of heart rate variability

Heart rate variability measurements were obtained by use of a Polar Equine Heart Rate Monitor, (Polar Equine V800 Science, Polar Electro Oy, Kempele, Finland), placed on each horse during stall confinement. Absence of arrhythmias or heart murmur was confirmed via cardiac auscultation immediately prior to placement of the heart rate monitor. Heart rate variability calculations were based on time domain and autoregressive frequency domain measurements using the Kubios® HRV Standard software (Kubios HRV software [version 3.1.0], Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). R-R intervals were recorded for 15-minutes. Five-minute segments were chosen for HRV analysis, leaving a minimum of 2 minutes at the start of recording to allow for habituation of the horse to the heart rate monitor. Medium threshold-based artifact correction was used and detrending was not applied. All HRV analyses were reviewed for percent of artifacts corrected. Initially, for consistency, a 5-minute segment was selected starting at 5 minutes of recording; if there were excessive artifacts, the next available 5-minute segment with allowable artifacts was selected. Only RR interval segments with less than 10% artifact correction were used in analyses (78). Time domain measures included mean RR interval (MnRR), mean heart rate (MnHR), standard deviation of the normal RR interval (SDNN), and root mean square of successive differences (RMSSD). Frequency domain variables included low-frequency (LF) power (normalized units

(nu)), high-frequency (HF) power (nu), and low-frequency to high-frequency ratio (LF/HF). Frequency component thresholds for power spectral analysis were set at 0.01-0.15 Hz for low frequency and 0.15-0.5 Hz for high frequency (33).

2.6. Behavioural pain scoring

Horse behaviour in the stall was recorded on video via in-stall cameras. Two cameras (1080p HD Weatherproof Night-Vision Security Camera, Lorex Corporation, Markham, ON, Canada) were placed on opposite sides of the stall at maximum/ceiling height to avoid horse interference with the camera. Recordings were edited to one, 5-minute segment, corresponding to the time of HRV recording. Two still photos of each horse's face were acquired from the video to assist in facial pain scoring. Videos and photos were randomized using a random number generator and behavioural pain scoring was performed by three blinded veterinarians using three published composite pain scales: Pain Scale 1 (PS1), Horse Grimace Scale (HGS) (12); Pain Scale 2 (PS2), Equine Utrecht University Scale for Composite Pain Assessment (EQUUS COMPASS) (17); and Pain Scale 3 (PS3), The Equine Pain Scale (EPS) (70). Pain Scale 2 had two parameters removed from evaluation ("pain sounds" and "reaction to palpation of painful area in the flank") as video recording did not allow for sound analysis and the study design precluded human interaction during behavioural analysis. Every video was scored by the raters using all three pain scales.

Given that each of the three composite pain scales included a different number of variables, an approach for standardizing behavior scores across scales was used. First,

the pain scores for each scale were converted to percentages based on the score from each rater divided by the number of items in the scale. Next the sum of percent responses for each rater was added and divided by the number of raters (n = 3). This procedure created the percent pain rating score for each scale, as shown in Appendix A. The standardized responses on the three scales (painPCT1, painPCT2, and painPCT3) were then compared across the two sample groups: painful versus not painful horses based on clinical judgement.

Pain Scale 3 was chosen for assessment of pain in surgical patients based on good inter-rater reliability, and correlation with physiological parameters (SDNN, cortisol), as found in horses on admission to the hospital (T1) (Table 3.3 and Table 3.4). Pain scores from Pain Scale 3 in surgical patients were presented as non-standardized average scores across raters.

2.7. Statistical analyses

This study had two objectives, first to compare measures of heart rate variability, salivary cortisol, and observed behavior in the assessment of pain among equine patients; and secondly, to compare physiological and behavioral responses within a cohort of equine patients following selected surgeries. The analyses of data began with a summary of descriptive statistics.

Descriptive statistics were calculated for HRV measures, salivary cortisol, and pain rating scores, for the total group on admission and subsequently for the cohort of equine patients that underwent surgery.

For data at T1 on admission day, two group t-test comparisons using Satterthwaite test were used to determine significant differences between means for horses classified as not painful (Group 1) versus painful (Group 2) for measures of HRV, salivary cortisol concentration, and standardized pain rating scores. An alpha level of $p < 0.05$ was set as the significance level for decision making related to the null hypothesis that H_0: Group1 (scores) = Group2 (scores). Pearson Product Moment Coefficients were calculated to determine measures of association between standardized pain scores and physiological variables.

For horses undergoing surgical treatment, ANOVA with post hoc Scheffe tests were used to compare $T1_{SX}$, T2, and T3. Pearson's correlation was performed between non-standardized pain scores and physiological measures. Significance was set at $p < 0.05$.

To determine the consistency in ratings of pain, Pearson correlation coefficients were calculated between non-standardized average pain score ratings for each rater in each of the pain scales. This was performed in the total group of horses in admission, as well as in the surgical cohort of patients at each data time point. Significance was set at $p < 0.05$.

CHAPTER 3. RESULTS

3.1. Description of horses

3.1.1. Excluded data points

Sixty horses were enrolled in the study. One horse was initially admitted and subsequently developed second-degree atrioventricular block during the measurement period and was excluded from the data analysis, resulting in a total of 59 horses included in data analyses.

Out of the total 59 horses included in the study, 5 horses did not have admission data due to exclusion from temporary second-degree atrioventricular block, or inability to collect pre-operative data due to the horse's need for emergency surgery. The final sample size collected on the day of hospital admission (T1) used for data analyses consisted of 54 horses.

Of the 59 horses, 39 received surgical treatment under general anesthesia (n_{SX} = 39 horses). Nine horses had one missing data point due to inability to collect data or intermittent second-degree atrioventricular heart block: $T1_{SX}$ n = 34; T2 n = 36; T3 n = 38.

3.1.2. Horse signalment and presenting clinical conditions

The patient signalment data are normally distributed and are presented as mean and standard deviation (SD). Of the 59 horses included in the study, the average horse weight was 529.02 kilograms (kg) (SD 120.48 kg). There were 27 geldings (45.76%), 21 mares (35.59%) and 11 stallions (18.64%). Average age was 7.65 years (SD 6.10 years).

The most common breeds presented included Quarter Horse (n = 22, 37.29%), Standardbred (n = 18, 30.51%), and draft breeds (n = 12, 20.4%; Clydesdale, Belgian, Friesian, Percheron, and draft-cross breeds). Other breeds presented included: Arabian (n = 1), Appaloosa (n = 1), Thoroughbred (n = 2), and Warmblood (n = 3).

Horses presented with a variety of clinical conditions, including varying levels of lameness due to musculoskeletal conditions (n = 6), laceration (n = 4), septic or aseptic synovitis (n = 4), colic (n = 7), subchondral bone cyst (n = 2), fracture (first phalanx fracture, n = 1; sesamoid or osteochondral fracture, n = 4), dental conditions (n = 5), ocular conditions (n = 4), and for conditions requiring soft tissue surgery (cystolith (n = 2), tumor (n = 1), scirrhous cord (n = 1)). There were also systemically healthy or asymptomatic horses (n = 18) presenting for elective procedures, including castration (n = 8) and arthroscopy for osteochondritis dissecans (n = 7). The majority of stallions presented for castration (n = 8).

3.2. Evaluation of HRV, HR, salivary cortisol concentration and pain scores on hospital admission (T1)

The average and standard deviations of heart rate variability parameters, salivary cortisol concentration, and standardized pain scores across all horses on admission (n = 54) are presented in Table 3.1.

Table 3.1. Summary of heart rate variability, salivary cortisol concentration, and standardized pain scores for all horses on admission (n = 54)

	Mean	± SD	Range
MnHR (beats/min)	45.37	8.68	31 – 70
MnRR (ms)	1358.71	244.93	858 – 1915
SDNN (ms)	114.92	52.34	41.10 – 298.50
RMSSD (ms)	64.16	23.12	14.50 – 116.90
LF power (nu)	66.23	13.50	25.70 – 94.89
HF power (nu)	33.74	13.49	5.11 – 74.30
LF/HF ratio	2.69	2.62	0.35 – 18.56
Salivary cortisol (µg/dL)	0.19	0.10	0.038 – 0.38
painPCT1	16.20	11.21	0 – 52.78
painPCT2	10.12	6.19	0 – 31.48
painPCT3	9.57	12.20	0 – 64.44

SD – standard deviation
ms – milliseconds
nu – normalized units
µg/dL – micrograms per decilitre
MnHR – mean heart rate
MnRR – mean R-R interval
SDNN – standard deviation of the normal RR interval
RMSSD – root mean square of the successive differences
LF power – low frequency power
HF power – high frequency power
LF/HF ratio – the ratio of low frequency to high frequency power
painPCT – standardized percent pain rating score for each pain scale, denoted as scale 1, 2, or 3 (below)
PS1 – Pain Scale 1 – Horse Grimace Scale (8)
PS2 – Pain Scale 2 – EQUUS-COMPASS (12)
PS3 – Pain Scale 3 – Equine Pain Scale (60)

On admission to the hospital, 36 horses were classified as not painful (Group 1), and 18 horses were classified as painful (Group 2) based on clinical perception (Table 2.1). The average and standard deviation of HRV parameters, salivary cortisol concentration, and standardized pain scores in both pain categories are listed in Table 3.2.

A t-test comparison between Group 1 and Group 2 showed a significant difference in SDNN (standard deviation of the normal RR interval; $t = -2.20$; $df = 27$; $p < 0.04$) and salivary cortisol ($t = -3.68$; $df = 43$; $p < 0.01$). SDNN and cortisol were significantly higher in painful horses compared to not painful horses. Significant

differences were also observed between groups for each of the standardized pain rating scores, with higher average pain scores in the painful group of horses (painPCT1: $t = -2.44$; $df = 20$; $p < 0.03$. painPCT2: $t = -2.54$; $df = 20$; $p < 0.02$. painPCT3: $t = -3.88$; $df = 19$; $p < 0.01$). The highest achieved score for each pain scale, averaged over the three raters, was 52.78% of the maximum score for Pain Scale 1, and 31.48% and 64.44% of the maximum score for Pain Scale 2 and Pain Scale 3, respectively. All other variables, including heart rate, were not significantly different between groups as a result of the large variance between groups as noted in Table 3.2.

Table 3.2. Summary of heart rate variability, salivary cortisol concentration, and pain scores for all horses by pain categorization on admission

	Group 1: Not Painful (n = 36)			**Group 2: Painful (n=18)**			**Two group t-test comparison**
	Mean	**SD**	**Range**	**Mean**	**SD**	**Range**	
MnHR (beats/min)	44.61	7.68	33.00 – 66.00	46.91	10.47	31.00 – 70.00	n.s.
MnRR (ms)	1370.89	224.87	906 .00– 1818.00	1334.35	286.32	858.00 – 1915.00	n.s.
SDNN (ms)	103.31*****	45.12	41.10 – 217.20	138.13*****	59.11	49.70 – 298.50	t = -2.20
RMSSD (ms)	61.01	22.14	14.50 – 104.10	70.47	24.40	24.20 – 116.20	n.s.
LF power (nu)	65.60	13.80	25.70 – 87.74	67.49	13.16	46.17 – 94.89	n.s.
HF power (nu)	34.37	13.80	12.26 – 74.30	32.49	13.16	5.11-53.81	n.s.
LF/HF ratio	2.40	1.42	0.35 – 7.16	3.26	4.09	0.89 – 18.56	n s.
Salivary cortisol (µg/dL)	0.16*****	0.10	0.038 – 0.38	0.25*****	0.077	0.12 – 0.34	t = -3.68
painPCT1	13.04*****	9.71	0 – 38.89	22.53*****	11.59	5.56 – 52.78	t = -2.44
painPCT2	8.69*****	4.37	0 – 17.59	12.96*****	8.21	0.92 – 31.48	t = -2.54
painPCT3	5.49*****	6.62	0 – 25.56	17.71*****	16.38	1.11 – 64.44	t = -3.88

*Significant difference between Group 1 and Group 2 ($p < 0.05$) (n.s. refers to no significant difference).

The statistical association between pain scores and physiological measures based on Pearson product-moment correlation coefficients are presented in Table 3.3. Only limited correlations were statistically significant as noted in the table with the symbol (*). The HRV time domain measure SDNN was positively correlated with each of the three pain scale average, non-standardized scores (Pain1: $p = 0.0054$. Pain2: $p = 0.0077$. Pain3: $p < 0.0001$). Pain Scale 2 non-standardized pain scores (Pain2) were positively correlated with the HRV measure MnHR (mean heart rate; $p = 0.048$), while Pain Scale 3 non-standardized pain scores (Pain3) correlated positively with salivary cortisol concentration ($p = 0.012$). In addition, non-standardized pain scores from Pain Scale 3 positively correlated with non-standardized scores from both Pain Scale 1 (Pain1: $p=0.0005$) and Pain Scale 2 (Pain2; $p < 0.0001$).

Table 3.3. Pearson correlation coefficients (r values) between pain scores and physiological parameters for all horses on admission

	Pain1	**Pain2**	**Pain3**
MnHR	-0.17	0.27*****	-0.11
MnRR	0.26	-0.21	0.15
SDNN	0.37*****	0.36*****	0.51*****
RMSSD	0.13	-0.050	0.12
LF power	-0.025	-0.15	-0.24
HF power	0.025	0.15	0.24
LF/HF ratio	0.065	0.017	0.019
Salivary cortisol	0.20	0.12	0.34*****
Pain1	-	0.25	0.45*
Pain2	-	-	0.58*

*Significant correlation ($p < 0.05$).
Pain1 = non-standardized pain scores for Pain Scale 1
Pain2 = non-standardized pain scores for Pain Scale 2
Pain3 = non-standardized pain scores for Pain Scale 3

In order to determine the consistency in ratings of pain for the observed horses, Pearson correlation coefficients were calculated between non-standardized average pain score ratings in each of the pain scales. For Pain Scale 1, the overall ratings for Rater 1 (R1) were not correlated to the overall rating of pain by Rater 2 (R2). However, significant correlations albeit weak were observed for Rater 1 and Rater 3 (R3) ($p < 0.0001$) and for Rater 2 and Rater 3 ($p = 0.026$). For Pain Scale 2, there were strong significant correlations between all raters (R1 and R2: $p < 0.0001$; R1 and R3: $p < 0.0001$; R2 and R3: $p < 0.0001$). Significant correlations were also observed for all raters using Pain Scale 3 (R1 and R2: $p < 0.0001$; R1 and R3: $p < 0.0001$; R2 and R3: $p < 0.0001$).

Table 3.4. Pearson correlation coefficients (r values) between pain score raters for each pain scale on admission (T1)

	Pain Scale 1		**Pain Scale 2**		**Pain Scale 3**	
	R2	**R3**	**R2**	**R3**	**R2**	**R3**
R1	0.15	0.58*****	0.76*****	0.78*****	0.67*****	0.79*****
R2	-	0.30*****	-	0.83*****	-	0.79*****

*Significant correlation ($p < 0.05$).
R1 – Rater 1
R2 – Rater 2
R3 – Rater 3

3.3. Evaluation of HRV, HR, salivary cortisol concentration and pain scores perioperatively ($T1_{SX}$, T2, T3)

Pain Scale 3 was chosen for comparison of pain rating across time perioperatively. This scale was chosen based on the findings that Pain Scale 3 had high inter-rater reliability, and significant correlation of non-standardized scores with SDNN and salivary cortisol concentration. Descriptive data for HRV, salivary cortisol

concentration, and pain scores perioperatively are shown in Table 3.5 and in Figures 3.1-3.7. The variables that changed significantly at $p < 0.05$, perioperatively, were the MnRR interval (significant difference between T2 and T3), the MnHR values from the Kubios software ($T1_{SX}$ and T2; T2 and T3), and salivary cortisol concentration (T2 and T3; $T1_{SX}$ and T2).

Data collected at T2 (following recovery from anesthesia) appeared to be the most different data time point. Heart rate increased from $T1_{SX}$ to T2 and then decreased to preoperative levels, and salivary cortisol concentration followed the same pattern. Although not statistically significant, MnRR decreased from $T1_{SX}$ to T2 and then increased significantly from T2 to T3. The LF/HF ratio on average also trended towards decreasing at T2 although this was not statistically significant. Non-standardized pain scores did not change significantly over time but trended towards post-operative mean scores being higher than pre-operative scores.

Table 3.5. Average heart rate variability, salivary cortisol concentration, and pain scores perioperatively in equine veterinary teaching hospital patients

	T1$_{SX}$ (n = 34)		**T2** (n = 36)		**T3** (n = 38)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
MnHR (beats/min)	44.40*****	7.92	49.72***$^{+}$**	9.47	43.10^{+}	6.92
MnRR (ms)	1379.71	117.77	1241.76^{+}	230.79	1411.66^{+}	229.31
SDNN (ms)	116.98	47.90	96.54	50.66	103.45	39.60
RMSSD (ms)	63.81	22.09	61.67	35.13	70.21	30.09
LF power (nu)	67.12	12.48	62.77	15.28	64.38	16.40
HF power (nu)	32.85	12.48	36.21	16.12	35.60	16.39
LF/HF	2.58	1.60	2.19	1.35	2.76	3.28
Salivary cortisol (µg/dL)	0.17*****	0.10	0.34***$^{+}$**	0.17	0.22^{+}	0.11
Pain3	1.97	3.49	2.93	2.99	2.78	2.59

*****Significant difference between T1 and T2 ($p < 0.05$).
$^{+}$Significant difference between T2 and T3 ($p < 0.05$).
°Significant difference between T1 and T3 ($p < 0.05$).

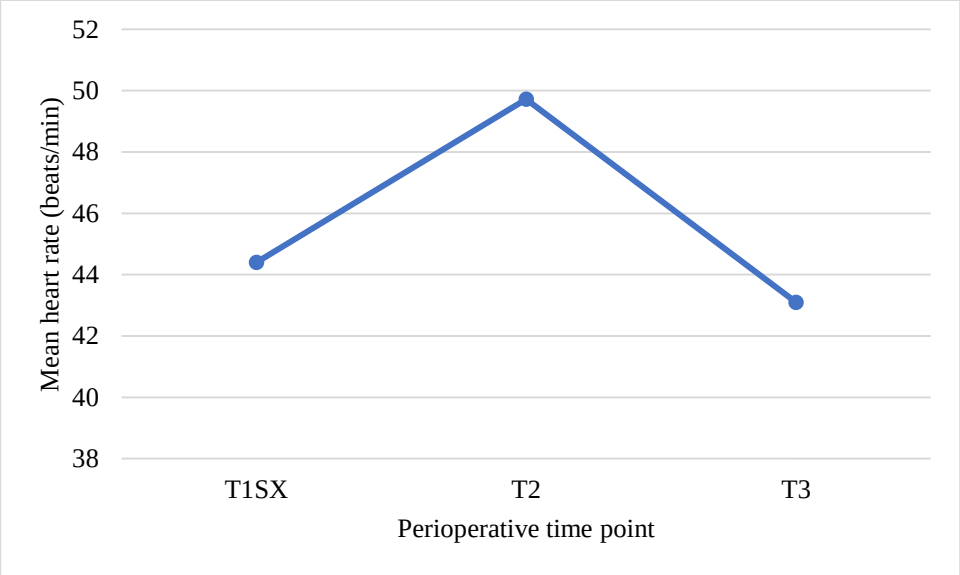

Figure 3.1. Change in MnHR (beats/minute) perioperatively

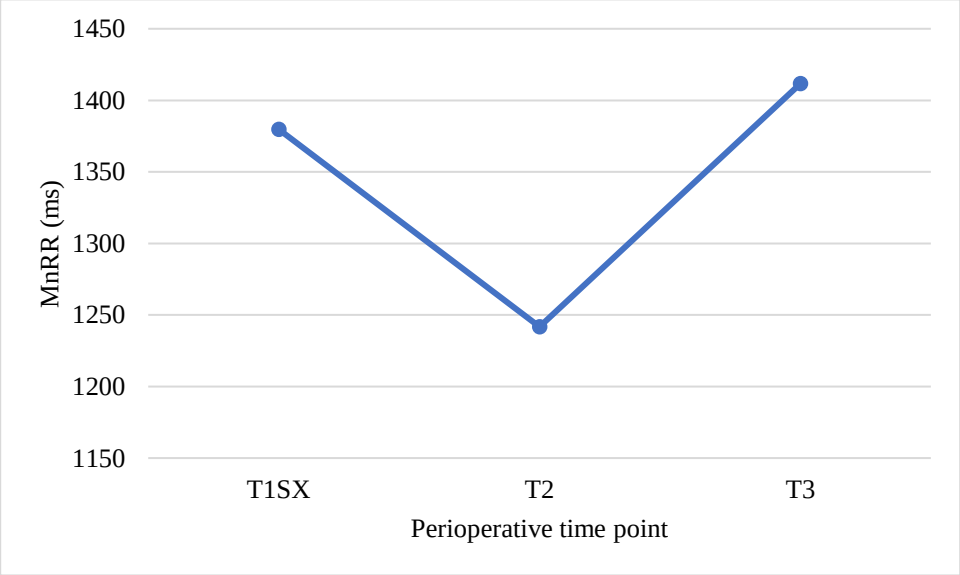

Figure 3.2. Change in MnRR (ms) perioperatively

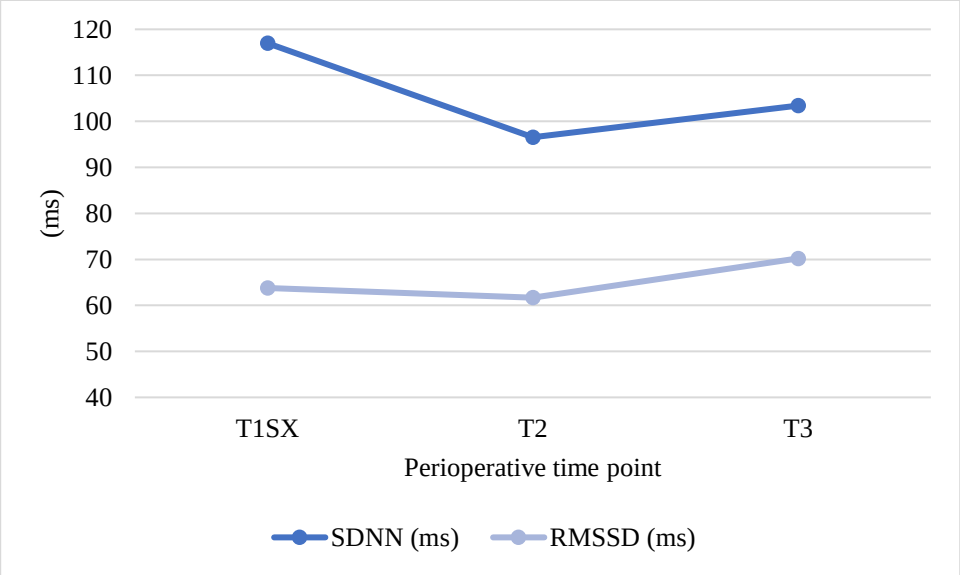

Figure 3.3. Change in SDNN (ms) and RMSSD (ms) perioperatively

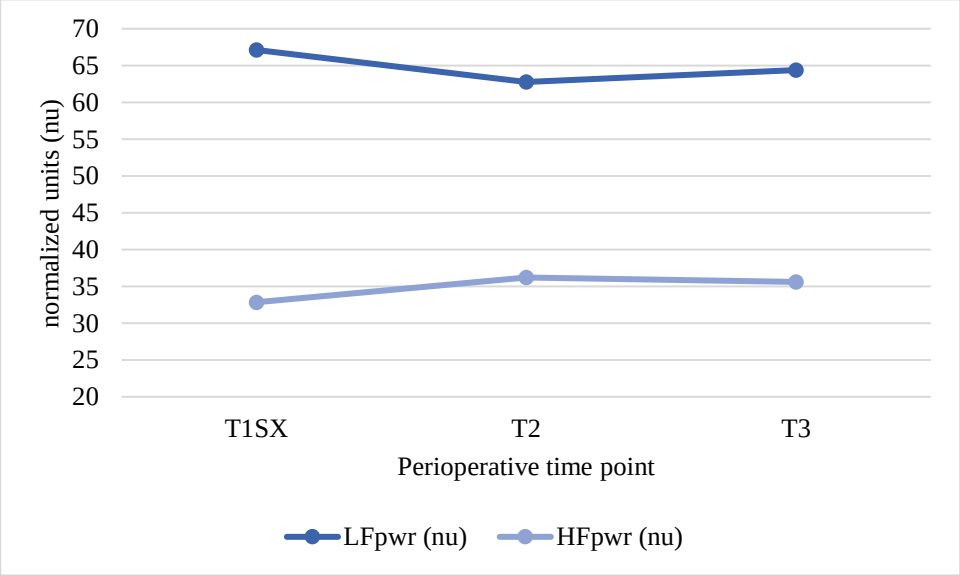

Figure 3.4. Change in LF power (nu) and HF power (nu) perioperatively

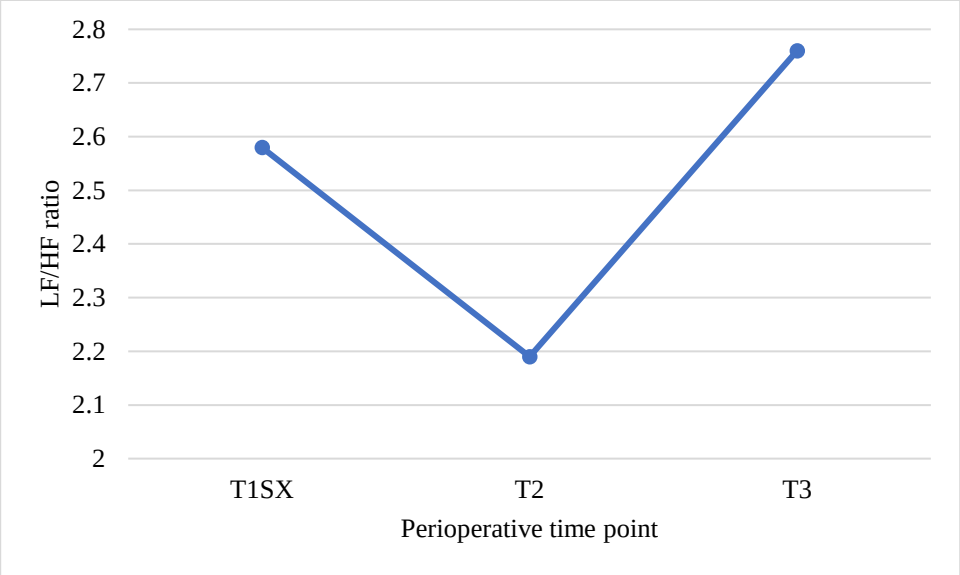

Figure 3.5. Change in LF/HF ratio perioperatively

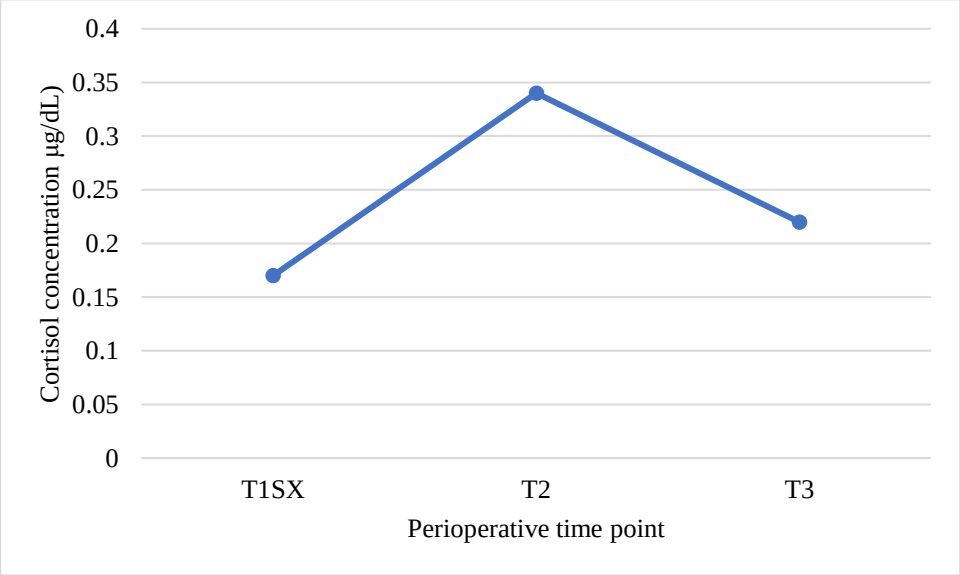

Figure 3.6. Change in salivary cortisol (µg/dL) perioperatively

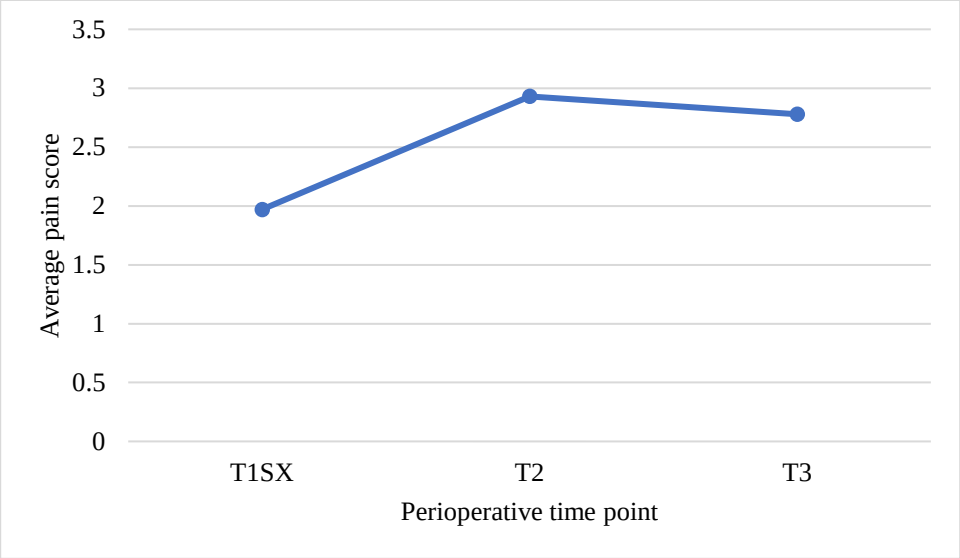

Figure 3.7. Change in Pain Scale 3 non-standardized scores perioperatively

Table 3.6 shows the r values of Pearson's correlation results between pain scores and physiological pain measures. At $T1_{SX}$, Pain Scale 3 non-standardized scores positively correlated with MnRR ($p = 0.027$). At both post-operative time points (T2 and T3), there were no significant correlations of physiological parameters with non-standardized pain scores.

Table 3.6. Pearson correlation coefficients (r values) of Pain Scale 3 non-standardized pain scores and physiological pain measures perioperatively

	Pain3		
	$T1_{SX}$	**T2**	**T3**
MnHR	-0.33	-0.10	0.28
MnRR	0.38*	0.096	-0.18
SDNN	0.32	0.23	0.017
RMSSD	0.15	-0.022	0.041
LFpwr (nu)	-0.17	0.25	0.0012
HFpwr (nu)	0.17	-0.17	-0.0017
LF/HF	-0.10	0.21	0.28
Salivary cortisol	0.25	0.24	0.080

*Significant correlation ($p < 0.05$).

Table 3.7 shows Pearson correlation coefficients between pain score raters using Pain Scale 3, for surgery patients perioperatively. All raters correlated significantly across perioperative time points. $T1_{SX}$ inter-rater correlations were weaker (R1 and R2; $p = 0.0036$. R1 and R3; $p = 0.0001$. R2 and R3; $p < 0.0001$) compared to postoperative correlations at T2 (R1 and R2; $p < 0.0001$. R1 and R3; $p < 0.0001$. R2 and R3; $p < 0.0001$) and T3 (R1 and R2; $p < 0.0001$. R1 and R3; $p < 0.0001$. R2 and R3; $p = 0.0066$).

Table 3.7. Pearson correlation coefficients (r values) between pain score raters for Pain Scale 3 perioperatively

	$T1_{SX}$		**T2**		**T3**	
	R2	**R3**	**R2**	**R3**	**R2**	**R3**
R1	0.49*****	0.61*****	0.72*****	0.73*****	0.63*****	0.71*****
R2	-	0.65*****	-	0.68*****	-	0.43*****

*Significant correlation ($p < 0.05$).

CHAPTER 4. DISCUSSION

4.1. Differences in pain assessment variables between painful and not painful horses on the day of hospital admission (T1)

This study described heart rate variability parameters, salivary cortisol concentration, and pain scores of horses admitted to a referral hospital. In addition, the study described these variables perioperatively in a cohort of horses receiving surgical treatment. Pain assessment in the hospital was based on clinical expertise and consisted of a combination of behavioural and physiological assessments: heart rate, respiratory rate, lameness evaluation, mentation, appetite, interaction with the hospital environment, presence of abnormal behaviours, sweating, agitation, and other specific localized pain parameters (such as blepharospasm in ocular pain, rolling and kicking in abdominal pain). This assessment method was initially used to categorize horses into painful or not painful groups, as there is no gold standard pain assessment tool, or universally accepted pain scale for multiple clinical conditions. There were significant differences in some measures of interest between horses in the two pain categories, and these variables were found to change perioperatively. Overall, salivary cortisol was increased in painful horses on admission and increased postoperatively in the cohort of surgical patients. Only one HRV parameter, SDNN, was affected by pain status in all horses on admission to the hospital, however this was not altered in the surgical cohort of horses perioperatively. In the surgical patients, the only HRV parameters significantly affected over time were MnHR and MnRR.

4.1.1. Heart rate variability

One HRV parameter, SDNN, was significantly different based on pain status when considering the total group of horses on the day of admission to the hospital (T1). SDNN was significantly higher in the painful group of horses, suggesting this parameter could be useful in pain detection. Further support of this is the significant positive correlation of SDNN with all three pain scales. SDNN represents an estimation of overall heart rate variability and autonomic nervous system function (36, 38). Increased SDNN suggests increased parasympathetic stimulation, contrary to the expectation of sympathetic activation in states of pain and stress (22). However, SDNN has also been found to be a significant parameter in other pain studies. Similar to the present study, mild somatic pain caused an increase in SDNN compared to states of anxiety and the combination of anxiety and pain in horses (25). SDNN also transiently increased in horses following microchipping and branding procedures (35). However, in a study by Rietmann et al., SDNN increased with analgesic treatment of horses with laminitis pain; in these horses HR also decreased with treatment (6). This is converse to the present study where SDNN increased with pain states in horses on admission, but it is possible that chronic pain conditions such as laminitis result in differences in autonomic physiologic response. Decreased SDNN in response to pain (laparotomy) was seen in laboratory mice for 24 hours after surgery (22). These mice, which did not receive analgesics, also had decreased RR interval and elevated HR, and none of these changes were seen in mice that received laparotomy with analgesia (22). In the mice receiving analgesics, these parameters remained unchanged, suggesting that these aberrations were related to pain and sympathetic activation (22).

The reason for discrepancy in increased versus decreased SDNN levels with pain states in these studies is unclear, and in some studies SDNN was unaffected by pain state (69). The species, environments, and types of pain involved are not consistent between studies, and changes in SDNN could be related to the type of disease state (i.e., systemic illness), the type of pain (i.e., musculoskeletal versus visceral) or the degree of pain (i.e., mild versus severe pain). In two studies assessing horses with colic, SDNN was higher in horses that survived to discharge, and SDNN was lower in horses with ischemic causes of colic (42, 56). It is possible that the types of clinical conditions may have affected the results of HRV parameters in the current study or were too variable to allow for further consistent patterns to be found.

4.1.2. Heart rate

In the study, average heart rate was not significantly different between painful and not painful horses on the day of admission to the hospital, demonstrating that this parameter alone is not sensitive enough to detect pain in the hospital population. In the study by Reid et al., heart rate increased in horses with anxiety and anxiety-pain but was not significantly different from baseline in horses with mild pain alone (25). This suggested that heart rate is more affected by anxiety in horses than it is by mild pain (25). Heart rate was found to be altered by pain in horses with laminitis and in mice after abdominal surgery (6, 22). Heart rate also increased in horses undergoing microchipping and branding procedures (35); and finally, horses surviving colic showed decreased heart rate over time with treatment, with no significant change in HR in non-surviving horses (69). Interestingly, 8-week-old calves showed increased HR for 10 to 20 minutes

post-castration, but this was not seen in the older 6-month-old calves (67). Heart rate was also increased following surgical castration of calves in another study, and this elevation was greater in calves not receiving local anesthetic (23). Studies in which heart rate was affected by pain states may have had more moderate to severe pain conditions in comparison to the present study. Stress can also elevate heart rate and may have been a factor in some of these studies (3, 19). While heart rate is one of the most common methods of pain evaluation by veterinarians (5, 19), this parameter does not appear sensitive enough to determine mild to moderate pain in horses.

4.1.3. Salivary cortisol concentration

There are varying results in literature surrounding cortisol and pain conditions, with some studies utilizing serum cortisol and others salivary cortisol concentrations. Saliva can be collected easily and repeatedly and is non-invasive (28). Salivary cortisol measures the unbound, biologically active fraction of total plasma cortisol (28). Increases in serum cortisol levels during acute stress are largely made up of free (unbound) cortisol, and therefore it is more useful and relevant to measure free cortisol than total cortisol in serum (30). Salivary cortisol has been used to determine stress levels in horses, such as when undergoing training (33) and transport (27). In the present study, salivary cortisol concentration was significantly increased in the painful group of horses, suggesting this may be used to evaluate pain in horses. In previous studies, salivary cortisol was shown to increase after young horses received either branding or microchip implantation procedures, which could be considered both painful and

stressful (35). Elevated serum cortisol has also been found in horses with colic, and when trended over time with pain assessments, this serum cortisol elevation was twice as high at the most painful time point (highest composite pain score) recorded compared to all other data points (10). Serum cortisol also differed between types of colic, with lower serum cortisol levels on admission in horses receiving conservative treatment compared to those requiring surgical treatment or were euthanized (69). Serum cortisol was also increased in calves undergoing surgical castration and increased to a greater degree in calves that did not receive local anesthesia (23). These studies suggest that cortisol is elevated in states of pain. However, in laminitic horses, there was no significant change in serum cortisol when horses received analgesic treatment, though the chronic nature of laminitis could influence these results (6). The present findings generally agree with the literature and suggest that salivary cortisol may be used as an indicator of pain in the horse.

4.1.4. Behavioural assessments of pain

Composite pain scales reported in the literature were used to assess pain in horses admitted to the hospital using a more objective method. Three veterinarians rated pain in horses using a single blind protocol and the results found that horses in the painful (Group 2) category had higher pain scores compared to not painful (Group 1) horses across three pain scales. These results suggest that pain can be detected in equine patients on admission to the hospital by use of these pain scales.

Despite a significant statistical difference between groups, the average pain scores in the current study did not reach the reported 'painful' cut-off for Pain Scale 1 (HGS, Horse Grimace Scale) or Pain Scale 2 (EQUUS COMPASS, Equine Utrecht University Scale for Composite Pain Assessment) (12, 17). There is no reported cut-off for Pain Scale 3 (EPS, Equine Pain Scale) (70). There is also a large overlap in the range of pain scores between groups. This may be indicative of a low level of sensitivity of the pain scales for mild pain states. It is worth considering that the studies assessing these pain scales, and determining cut-off values, involve specific circumstances, such as colic or castration. This contrasts with the variety of conditions and subsequently pain states of patients in the present study, without a gold standard pain assessment tool to determine truly painful versus truly not painful horses. None of the horses in the present study had severe pain due to requirement of data collection prior to administration of analgesics. As a result of welfare concerns, horses that required immediate pain management upon admission to the hospital were not included in the study.

4.1.5. Variability of patient data on hospital admission (T1) by pain state

The overall ranges of both pain scores and physiological parameters had overlap between the two groups on the day of hospital admission (T1). Subjectively, the standard deviations were higher in the painful group of horses for time-domain HRV parameters, HR, LF/HF ratio, and pain scores. This suggests greater overall variability in the painful group of horses and more homogeneity of these parameters in the not painful group. This may be due to differences in severity of the clinical conditions of

these patients or differences in individual pain expression between horses (18). In a study assessing stress in horses during road-transport, the authors found within-group variability to be generally high and suggested this may be due to differences between individual horses (27). The variability amongst painful horses may obscure the ability to distinguish further differences between groups.

4.2. Perioperative changes in HRV, HR, salivary cortisol, and pain scores

This study also aimed to describe perioperative trends in HRV parameters, salivary cortisol, and behavioural pain scores. The variables that were significantly altered over time perioperatively were MnRR, MnHR, and salivary cortisol. The alterations in MnRR were only significant between T2 and T3 post-operatively but trended toward the lowest value at T2, when the highest average values for MnHR, cortisol, and pain scores occurred. Decreased MnRR corresponds with an elevated HR (22), which is consistent with the findings in this study. Other studies have shown alterations in MnRR with applied treatments or stressors (6, 22, 27, 69). MnRR was decreased significantly in horses during road transport (27). MnRR also decreased in mice after laparotomy for 24 hours in mice not receiving analgesia, with no change in mice receiving anesthesia alone (22). In horses with colic, MnRR was lowest in horses that did not survive to hospital discharge (69). In horses that required surgery and survived to discharge, MnRR was shown to increase from admission to the day after admission (69). The RR interval also increased in horses being treated for laminitis pain, which followed decreased heart rate in these horses (6). While MnRR had some significant changes perioperatively, the lack of significant difference between pain

groups on the day of hospital admission (T1) suggests that this parameter may not be useful as a parameter for pain assessment in horses.

While MnHR was not significantly different between pain groups on the day of hospital admission, there were changes noted perioperatively with higher heart rate after anesthetic recovery (T2). Other studies show changes in heart rate perioperatively or over time. In horses surviving from colic, both medical and surgical treatment resulted in a decrease in heart rate; this change did not occur in horses that did not survive (69). Heart rate was significantly higher one hour after surgery than preoperative and operative time points in horses undergoing enucleation surgery (79). Elevated heart rate was also seen in mice undergoing laparotomy for 24 hours after surgery, however, it was only in the mice not receiving perioperative analgesia (22). Mice receiving analgesia and laparotomy also had elevated heart rate at 1-3 hours post-laparotomy but then HR normalized and was no longer elevated at 18-24 hours postoperatively (22). However, HR can also be affected by other factors than pain, as seen in horses undergoing surgical treatment for colic, where admission heart rate and postoperative heart rate were greater in horses with ischemic causes of colic, compared to the non-ischemic and control groups (42). Heart rate appears to be elevated in some pain conditions and circumstances, and this appears to be the case in our horse population only immediately post-recovery from surgery. In our population, heart rate alone is not consistently able to detect pain.

In the present study, cortisol levels were highest immediately post-anesthetic recovery, and remained higher postoperatively. This suggests an elevated stress response post-surgery. This may be indicative of pain caused by surgery or may be a stress

response from general anesthesia. There are few studies showing changes in cortisol levels over time with respect to surgical pain. In one colic study, cortisol decreased with treatment over time in all groups of colic patients, but without statistical significance in the group of non-surviving horses (69). In calves undergoing surgical castration, serum cortisol increased, and increased to a greater degree in calves that did not receive local anesthesia (23).

The average scores from Pain Scale 3 did not change significantly over time, but postoperative scores trended towards being higher than preoperative scores. This may be due to preoperative pain caused by the presenting medical condition in some patients causing preoperative and postoperative behavioural measures to be similar on average. It also may be due to standard perioperative analgesia offering adequate postoperative pain control. However, with other parameters showing significant alterations at T2 and the average pain scores being elevated but not statistically significant, it may suggest insensitivity of the pain scale for mild pain conditions. This pain scale was used in a previous study that evaluated horses with colic and also a group of horses with a wide range of conditions similar to the present study (10). The pain scale had excellent inter-observer reliability and was found to be reliable for a range of conditions, including both medical and surgical colic (10). The authors did modify the scale to include physiological parameters (heart rate, respiratory rate), however even without these parameters included, pain scores still correlated with serum cortisol concentration (10). This study only evaluated the inter-rater reliability and correlations of the pain scores with serum cortisol, and the pain scores amongst patients were not published (10).

Overall, perioperative alterations in physiological parameters were generally only significant when comparing $T1_{SX}$ and T2, and T2 to T3, suggesting that T2 (immediately post-anesthetic recovery) was the most significantly different time point perioperatively. Average MnRR was lowest, and average salivary cortisol and MnHR were highest at this time point. These findings are suggestive of higher physiological stress following anesthetic recovery (26, 50). While not significantly different from other time points, SDNN trended towards the lowest value at T2. Low SDNN is suggestive of increased influence of the sympathetic nervous system (22, 55). These trends could be the result of effects from general anesthesia or stress from the recovery experience, however, the trend of higher pain scores postoperatively suggests increased pain at this time. A lack of significant difference between $T1_{SX}$ and T3 could also be due to preoperative pain in some patients being similar to postoperative pain (T3) on average. HRV has been shown to be altered during general anesthesia in dogs (80) and humans (81). In dogs, HR increased with increasing inhalant anesthetic concentration, with corresponding decreases in SDNN and HF power (80). This represents a negative influence on vagal activity (69, 80). In humans, level of consciousness was found to be negatively correlated with LF power, and the complexity of the operation was also negatively correlated with LF and HF power (81). Frequency domain HRV parameters were not significantly affected by pain or over time in surgical patients in the present study. In the laparotomy study on mice, control mice receiving anesthesia without laparotomy did not show alteration in heart rate or RR interval (22). This suggests that the elevated heart rate in laparotomy patients was related to pain or stress from surgical intervention and not from the anesthesia itself. One study investigating the use of

retrobulbar nerve block during enucleation in anesthetized horses also found that horses had higher HR at 1 hour after surgery compared to preoperatively (79). However, there were no control patients undergoing anesthesia without surgery to determine if this elevation in heart rate was related to anesthesia or surgery. Horses undergoing surgery for treatment of colic had higher heart rate than horses undergoing anesthesia for MRI (no surgical intervention) and control horses receiving no intervention (20). There was also no difference between the anesthesia cases and control horses that did not undergo anesthesia (20). The horses undergoing surgery also had elevated plasma cortisol while there was no significant difference between the control and anesthesia groups (20). There was a decline in plasma cortisol in the anesthesia group of horses over 24 hours, suggesting the MRI procedure and general anesthesia did cause a stress response (20). A previous study on the equine stress response to anesthesia found that plasma cortisol increased during anesthesia, but there was no significant difference between pre-anesthetic and post-anesthetic levels (82). Heart rate was not altered post-anesthesia in this study (82). While there is some HRV and cortisol data with horses under general anesthesia, there are few studies with controlled patients receiving anesthesia without other intervention. There are no data thoroughly investigating the transition from anesthesia to early post-anesthetic period to determine when the effects of general anesthesia would no longer affect HRV. Our patients appear to have had higher post-operative pain in the immediate post-operative period following recovery compared to later in the postoperative period.

4.3. Correlation of behavioural and physiological estimates of pain

The study intended to determine the association between heart rate variability estimates and concomitant measures of salivary cortisol and behavioural pain scores in equine patients. It was hypothesized that there would be a correlation between physiological and behavioural measures of pain, and in the total group of horses on hospital admission (T1) there were limited relationships found between the objective physiological parameters (HRV, HR, cortisol concentration) and the pain scales. All pain scales correlated positively with SDNN. This is contrast to human patients undergoing abdominal surgery, in which pain scores correlated negatively with SDNN (65). On admission, MnHR correlated with Pain Scale 2, however mean heart rate was not significantly different between the groups of painful and not painful horses in this study. Pain Scale 3 correlated with salivary cortisol on hospital admission. In the study by Lawson et al., assessing horses with colic, serum cortisol was positively correlated with an adaptation of Pain Scale 3 used in the present study (10). Salivary cortisol only correlated with Pain Scale 3 in the present study, however, salivary cortisol is still very promising as an estimate of pain in the horse. Pain Scale 3 was also highly correlated with scales 1 and 2. It is possible that some physiological parameters do not change in proportion to increasing pain, as would be expected in a pain scale, which may explain lack of correlation of more parameters across pain scales.

There were few associations between physiological and behavioural pain parameters when only surgical patients were considered. Only MnRR was positively correlated with Pain Scale 3 on admission day, with no significant associations postoperatively. The associations between physiological and behavioural pain

parameters were different in the cohort of surgical patients compared to the total group on hospital admission, and this suggests that these relationships may differ depending on the perioperative time point. Potentially the high variability in the types of conditions, and subsequent different surgical procedures performed may have resulted in reduced ability to discern patterns in this cohort of patients.

4.4. Assessment of behavioural pain scores used in equine teaching hospital patients

On the day of hospital admission, Pain Scale 1 had poorer inter-rater reliability compared to Pain Scale 2 and Pain Scale 3. This may have been related to difficulties with performing facial pain scoring from video and still images. In the paper developing this pain scale, the authors reported more difficulty scoring facial pictures of dark coloured horses compared to those with light coloured coats (12); similar difficulty was occasionally encountered in the present study. Higher image quality with contrasting background has been recommended to improve this (12). Scoring patients in-person from outside the stall would likely resolve this issue, however, studies have shown that equine pain behaviour is affected by the presence of an observer and this may affect pain scoring results (83, 84). Facial pain can also be affected by other emotional states (74). It has also been suggested that Pain Scale 1 (the Horse Grimace Scale) requires standardized training of observers; one study showed that 30 minutes of training of observers that do not have horse experience did not allow for good agreement between observers (71). Pain Scale 2 and Pain Scale 3 had good inter-rater reliability. Pain Scale 3 had more significant correlations with physiological parameters (SDNN and salivary

cortisol concentration) in addition to being correlated with both other pain scales. Because of this, Pain Scale 3 was considered the most robust pain scale in our population of horses on the day of hospital admission.

There is a lack of published data regarding changes in pain scores in equine patients perioperatively or over time, and most publications regarding equine pain scales involve scale development and determination of scale reliability. Pain Scale 1 in this study, the Horse Grimace Scale (HGS), has been shown to have improvement in pain scores following treatment for laminitis pain in horses, with good inter-observer reliability (85). When this pain scale was used in horses undergoing castration, there were increased scores postoperatively, indicating increased pain, with no change in scores in control horses only receiving anesthesia (12). This shows lack of effect of anesthesia on the HGS (12). Pain Scale 2 had one study involving horses with colic in which conservatively treated horses showed a decrease in average pain scores comparing admission values to 12-24 hours post-admission (86). There are no published studies evaluating the effect of time or intervention on pain scores from Pain Scale 3.

There is no gold standard measure for pain with which to compare and validate composite pain scores. Equine pain scales have been undergoing validation most commonly by inter-rater and intra-rater reliability (9, 10, 12, 17, 34, 72, 85, 87). It is also common to validate pain scores by comparison of scores between groups of known painful subjects versus controls (17, 34, 72, 73. 87). Further methods used in validation of pain scales include correlation with other known pain scales (9, 12, 85), in addition to changes in the pain scores over time and with intervention or treatment (17, 34. 72, 87). Some studies additionally assess for association of pain scores with physiological

parameters, similar to the present study (10, 34). Despite various methods used, most equine pain scales have not had rigorous clinical validation (19). The majority of published equine pain scales have only been validated under specific experimental or clinical conditions, and these validation studies state that further study and validation is required to not only further refine the scales, but to also validate their use under other circumstances (9, 10, 12, 34, 72). Pain scales should ideally be validated for each pain state that they are going to be applied to (19), and it has also been suggested that pain scales should be validated using similar methods to practical application in clinical practice (83). Validation of a pain scale under one circumstance, such as a small group of horses undergoing surgical castration, does not mean that the scale will be applicable in other circumstances or other hospital settings. With a growing number of pain scales available, the determination of a universal pain scale that can be used across clinical settings would be of great value to equine veterinarians. This will require multiple studies across different equine patients, clinical conditions, and veterinary hospitals. The present study suggests the Equine Pain Scale (70) as one option for such a pain scale. Further validation is required to determine if this pain scale is sensitive enough for mild pain states and useful across pain states in equine patients.

4.5. Limitations

There are limitations to consider in the present study. One limitation is the high variability of subjects and clinical conditions in this study. The clinical caseload at this institution did not allow for selection of one breed or age-range of horses, nor specific clinical conditions. It is possible that the types of clinical conditions may have affected

the results in the current study or were too variable to allow for consistent patterns to be found. With various clinical conditions also comes variability in the types of surgical procedures and medications. While most horses followed a standardized protocol of preoperative non-steroidal anti-inflammatory medication and routine anesthetic regimen, some horses received other medications, such as antibiotics. It was not possible in this observational study to standardize medications. However, this study is representative of the type of cases presented at this institution, representing the variety of patients presented to a referral hospital in this region of practice. Exclusions included horses under one year of age, weighing less than 300kg, or exhibiting cardiovascular disease or arrhythmia upon admission. These exclusions aimed to reduce potential factors that could affect heart rate variability measures. Cardiovascular disease and arrhythmia are known to affect heart rate variability (36, 38). Further study of the relationship of these parameters in controlled, refined clinical conditions (i.e., restricted to one clinical pathology or procedure such as colic, castration, arthroscopy) may help strengthen evidence of relationship. However, creation of pain assessment tools only for specific clinical conditions creates a wide variety and large number of different pain tools that may not be similarly used or equal across varying types of horses and clinical conditions.

Another limitation is the inability to separate stress from pain. Not only does pain inherently cause physiological stress (1), but it is expected that transport (27) and admittance to a hospital could also cause stress. Horses were allowed an acclimatization period upon entrance to the hospital; however, it is unknown if the time given was sufficient in all subjects due to differences in horse temperament. Baseline values prior

to the injury or illness causing pain were also not able to be obtained, and stress cannot be distinguished from pain in a hospital study setting (3, 19, 35). An attempt was made to reduce the confounding effect of stress by recording HRV and behaviours with the horses left unrestrained and undisturbed in the box stall.

In addition, the lack of a generally accepted gold standard pain assessment tool restricted pain evaluation to a subjective pain scale which could risk classification of patients as painful versus not painful. The lack of a generally accepted pain scale or gold standard pain assessment tool also restricts our ability to determine the true association of HRV and cortisol with pain conditions in horses.

4.6. Implications and further directions

It is vital in veterinary medicine to be able to recognize pain and adequately treat painful conditions to maintain animal welfare (5, 6, 8). Both trauma and surgery result in a stress response and need for tissue repair (3). This leads to increased energy requirements and can cause increased morbidity and mortality (2, 3, 14). Additionally, painful horses generally have increased stress levels and decreased quality of life (14). Therefore, pain relief is not only necessary for animal welfare, but it also can improve appetite and aid in recovery (3, 4, 14). Improving veterinarians' recognition of equine pain could improve treatment of previously unrecognized pain and in turn benefit patient welfare.

There is no gold standard measure, nor any single pathognomonic measure to use as an indicator of pain (1, 10). There are numerous methods of pain assessment

currently used in clinical equine veterinary practice, including physiological and behavioural assessments, but these methods also have their limitations in their use and interpretation of results (19, 70). Heart rate variability is a relatively new tool to veterinary medicine that has the benefit of being non-invasive and may be an additional tool for equine pain assessment (5, 6, 25, 27, 36). The purpose of this study was to not only describe measures of heart rate variability, salivary cortisol, and behavioural pain scores in equine veterinary teaching hospital patients, but also to assess these parameters as measures of pain in this population of horses. The intent was to consider HRV parameters as objective measures of pain and determine associations between physiological and behavioural pain scores. Heart rate, salivary cortisol, and some HRV parameters are associated with the pain scales used in this study, and a robust pain scale (Pain Scale 3, the Equine Pain Scale (70)) was selected in this population of horses. However, relationships between these parameters appear dependent on the perioperative time point, and there are currently no cut-offs with respect to HRV parameters, salivary cortisol concentration, or the Equine Pain Scale to make these parameters immediately clinically useful. Further validation of these parameters as measures of pain is required before they may be used reliably and routinely in equine veterinary patients. Repeated studies in other hospital populations will also validate their use in other populations of horses. A proposed next step involves a controlled study evaluating two groups of horses: 1) a painful group of horses with similar clinical conditions requiring surgical therapy; 2) a control groups of horses free of pain and undergoing anesthesia for diagnostic purposes (i.e., for MRI or CT imaging). This would help further investigate and define the relationships between pain, behavioural and physiological measures of

pain. Potentially, clinically relevant cut-offs for these parameters could be created from such a study.

4.7. Conclusions

Overall, heart rate variability is a relatively new measurement to veterinary medicine that is non-invasive (27, 36, 58) and has been shown to be indicative of stress in humans and animals (36). Pain assessment is challenging in animals such as horses, and while there are numerous methods of pain assessment in clinical use, these are limited by subjectivity, interpretation and are generally considered insensitive for mild pain conditions (5, 24, 88). Heart rate variability has shown correlation with pain scores in human medicine, and preliminary studies in horses also show promising results (6, 25, 59, 65). In the present study, both SDNN and salivary cortisol were significantly higher in the painful group of horses, suggesting that these parameters may be useful in detecting pain. Additionally, a pain scale was selected (Pain Scale 3, the Equine Pain Scale; 70) that appears to be robust in this population of equine patients. Heart rate appears too insensitive to be a reliable indicator of mild to moderate pain states in the horse. Based on correlating the objective physiological parameters (HRV, cortisol) there are limited relationships between pain rating scales and these objective measures. Heart rate variability is a potential indicator of pain in the horse and having an objective non-invasive measure of pain would be of advantage to veterinarians and horse owners in improving pain recognition and management in equine patients. Further studies are required to better define these relationships and determine clinically significant cut-off values.

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APPENDIX A. PAIN SCORING ALGORITHM

PS1R1A=(PS1R1/12)*100

PS1R2B=(PS1R2/12)*100

PS1R3C=(PS1R3/12)*100

PS2R1A=(PS2R1/36)*100

PS2R2B=(PS2R2/36)*100

PS2R3C=(PS2R3/36)*100

PS3R1A=(PS3R1/30)*100

PS3R2B=(PS3R2/30)*100

PS3R3C=(PS3R3/30)*100

painPCT1=(PS1R1A+PS1R2B+PS1R3C)/3

painPCT2=(PS2R1A+PS2R2B+PS2R3C)/3

painPCT3=(PS3R1A+PS3R2B+PS3R3C)/3